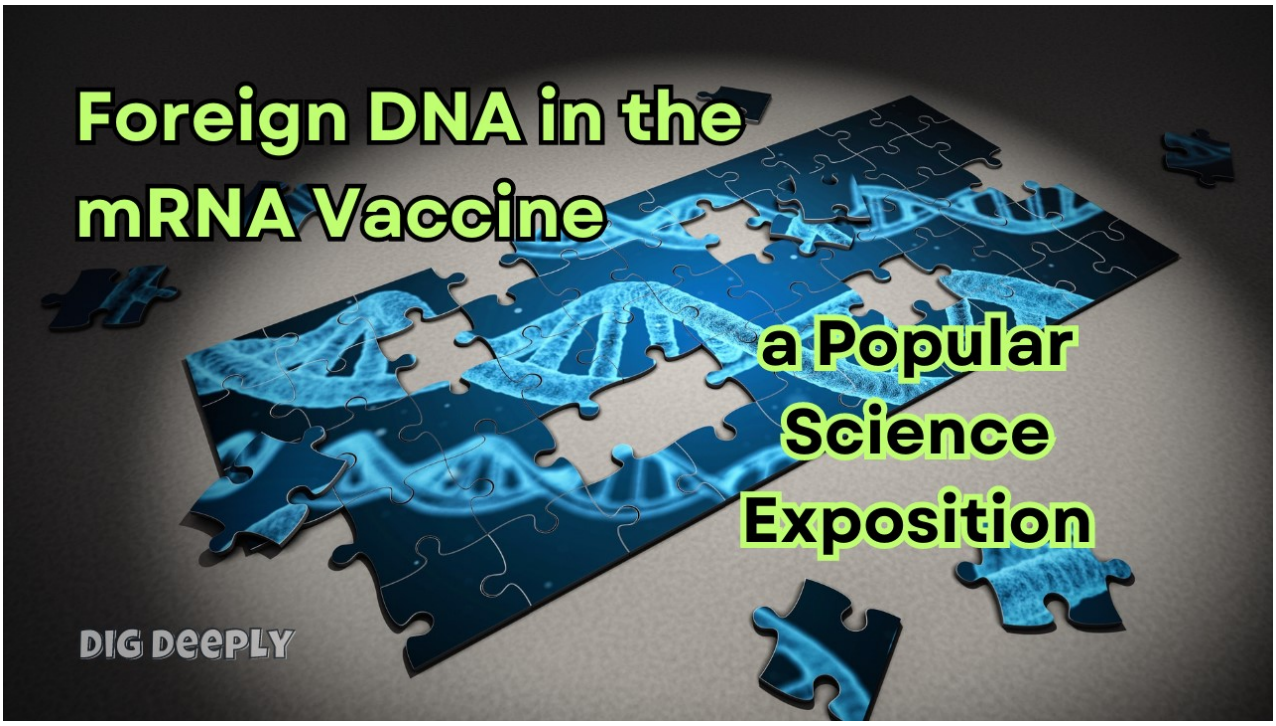
# Dig Deeply



## Foreign DNA in the mRNA Vaccine




In recent years, the development and application of mRNA vaccines have experienced significant progress. What was once only present in the laboratory has now become a reality for many of us, as we have been vaccinated multiple times with mRNA vaccines. This technology is now viewed as a promising tool for future medical interventions. It is expected that in the near future, more mRNA-based vaccines will enter the market to combat various diseases. An overview of this can be found here.

Currently, six mRNA vaccines for Covid-19 vaccination are approved worldwide, with two of them approved in the EU. The European Medicines Agency (EMA) has classified the safety profile of these approved mRNA vaccines as very reassuring. However, recent media reports dealing with the issue of „foreign DNA in corona vaccines" have led to controversial discussions and attracted increased attention.

**Sources:**

- DNA Contaminants in COVID Vaccines Are 'Beyond the Pale': Florida Surgeon General Explains the Call for Vaccine Halt
- Fact Check: No evidence for vaccine DNA risk raised by Florida Surgeon General
- False claims recirculate on harmful DNA in Covid-19 vaccines
- DNA Contamination of COVID Vaccines Explored in Journal of American Physicians and Surgeons
- Fact Check: No Evidence Cancer-Causing „Green Monkey DNA" Found In mRNA COVID Vaccines
- Genomics Expert Who Discovered DNA Contamination in mRNA Shots Accuses Regulators of Lying About Cancer Risks
- Mood-mongering with alleged DNA impurities

Given the essential importance of mRNA vaccines for our health, it is interesting to take a closer look at the precise background and the critical viewpoints.

In public debates, scientific arguments are often taken out of context and mixed with general descriptions. Particularly when political and economic interests are involved, objectivity in public discourse seems to be increasingly neglected. Clarifying this issue is not only important from a scientific perspective but also relevant in the socio-political context.

This paper aims to build a bridge between the highly specialized scientific explanations in the specialist literature and the generally understandable descriptions in the media. By conveying detailed knowledge on the topic of „foreign DNA in vaccines", the goal is to help make complex processes understandable to a broader audience.

It is therefore suggested that the individual sections of this article be read in consecutive order. This approach allows the information to systematically build upon each other, enabling a more comprehensive understanding of the topic.

Engaging deeply with the subject allows interested readers to expand their know-

ledge base and develop a deeper understanding of the underlying connections. In this context, knowledge becomes a crucial resource. Individuals who have a better understanding of the processes involved in vaccine development are more likely to make informed decisions, as opposed to those without this knowledge who may rely on the statements and beliefs of others.

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# 1. Discovery of foreign DNA in the vaccine

According to a **study** published on the OSF Preprints platform on April 11, 2023, batches of the Pfizer and Moderna COVID-19 mRNA vaccines were found to contain **DNA contaminations exceeding the limits set by the European Medicines Agency (EMA) of 330 ng/mg and the FDA of 10 ng/dose**. These DNA contaminations are referred to as „foreign DNA", meaning genetic material not naturally present in the human body. This study marks the beginning of investigations into this topic.

The study was conducted under the direction of Kevin McKernan and his team. Dr. Kevin McKernan is an American geneticist and plant biologist. He initially worked as head of the research and development department of the **Human Genome Project** at the Massachusetts Institute of Technology (MIT). He later founded **Medicinal Genomics**, where he serves as CSO (Chief Scientific Officer). He has extensive experience in the biotechnology industry, particularly in DNA sequencing and genomics projects. His expertise extends to next-generation sequencing technologies and he has been instrumental in the development of methods and technologies for decoding DNA.

On October 19, 2023, another **study** was published that once again investigated DNA contamination in mRNA products from Pfizer and Moderna. The primary author of this study is Dr. David Speicher, with Dr. Kevin McKernan listed as a co-author.

According to **Speicher**, speaking to The Epoch Times, „In our study, we measured DNA copies of the Spike gene, ORI (Origin of Replication), and SV40 Enhancer genes. The loads of SV40 enhancer promoter, ORI and virus spike in Pfizer are up to 186 billion copies per dose."

Several million copies of the ORI and spike DNA were also found in the Moderna vials, but the SV40 enhancer gene was not detected there. [Study]

This initiated a debate about the presence of foreign DNA in the COVID-19 vaccines.

# 2. What is the explosive nature of the study results?

## 2.1. The significance of DNA for mRNA vaccines

As explained in the **BioNTech short video**, the production of mRNA vaccines begins with the identification of genetic information, specifically DNA, which forms the basis of the vaccine. In this case, it is the Spike protein DNA of the SARS-CoV-2 virus. To obtain sufficient genetic material for vaccine production, this DNA is first replicated. Various methods are available for this replication process. The replicated DNA is then used as a template to synthesize mRNA. The produced mRNA carries the genetic information for the Spike protein.

## 2.2. The manufacturing processes of the BioNTech mRNA vaccine

In the **European Medicines Agency (EMA) assessment report for the BioNTech mRNA vaccine**, two different manufacturing processes are mentioned on page 18: **Process 1**, which was used during the clinical trials, and **Process 2**, which is employed during large-scale production.

In **Process 1**, the replication of the Spike protein DNA is carried out using the (**polymerase chain reaction PCR)**. In **Process 2**, the Spike protein DNA is replicated using **bacteria**. Due to the urgency to rapidly address the demand for vaccines, ‚Process 2' has been established as the standard for large-scale production.

Joshua Guetzkow and Retsef Levi, Israeli researchers, were the first to bring this matter to public attention. They analyzed Pfizer documents that have been gradually released through legal proceedings (see here: lawsuit and court order). The findings of this analysis were published in May 2022 in the **British Medical Journal**. There you can read the following:

*„An October 2020 amendment to the protocol of the pivotal Pfizer/BioNTech BNT162b2 (Comirnaty) clinical trial (C4591001) indicates that nearly all vaccine doses used in the trial came from 'clinical batches' manufactured using what is referred to as 'Process 1'. However, in order to upscale production for large-scale distribution of 'emergency supply' after authorization, a new method was developed, 'Process 2'. The differences include changes to the DNA template used to transcribe the RNA and the purification phase, as well as the manufacturing process of the lipid nanoparticles. Notably, 'Process 2' batches were shown to have substantially lower mRNA integrity. The protocol amendment states that each lot of 'Process 2'-manufactured BNT162b2 would be administered to approximately 250 participants 16 to 55 years of age with comparative immunogenicity and safety analyses conducted with 250 randomly selected 'Process 1' batch recipients. To the best of our knowledge, there is no publicly available report on this comparison of 'Process 1' versus 'Process 2' doses."*

In other words, the approval process was based on product derived from the ‚Process 1' manufacturing process. These mRNA vaccines were administered to **22.000 volunteers** and the **efficacy and safety claims** are based on the data from these individuals.
However, ‚Process 2', a completely different manufacturing process, was used for the global deployment of the mRNA vaccines. The vaccine produced using this process was only tested on **250 volunteers**.

In this context, the EMA notes the following in the assessment report for the BioNTech mRNA vaccine on page 32:

„***Based on the differences observed between batches manufactured by active substance Process 1 and 2 for the CQA (Critical Quality Attribute) mRNA integrity and lack of characterisation data, a MO (Major Objection) was raised regarding comparability, characterisation and clinical qualification of the one proposed acceptance criteria.*** *Biological characterisation of the active substance was limited, and additional data and discussion were requested to address functionality."*

The investigation results by McKernan and Speicher pertain to vaccine batches manufactured using ‚Process 2'.

In determining the **safety profile of an mRNA vaccine**, certain characteristics of the vaccine are crucial, including its composition, **purity**, and stability. The purity of the vaccine is essential to ensure that it is free from potentially harmful contaminants. Contaminants could induce unwanted immune reactions or other side effects.

In ‚Process 2', the replication of the Spike protein DNA in bacteria involves subsequent purification to remove DNA fragments, bacterial residues, and process-related impurities.

It appears that Moderna also employs a similar process. [Study]

However, the study by Speicher DJ et al. suggests that the purification of the vaccine solution may not always be effective. The study states: „*These data demonstrate the presence of billions to hundreds of billions of DNA molecules per dose in these vaccines. Using fluorometry, all vaccines exceed the guidelines for residual DNA set by FDA and WHO of 10 ng/dose by 188 – 509-fold.*"

To gain a better understanding of this situation and its potential implications, the following chapters will provide a more detailed introduction to the manufacturing processes of mRNA vaccines as well as the methods for quality assurance.

# 3. The manufacturing processes in a nutchell

As already described, ‚Process 1' and ‚Process 2' mainly differ in the way the Spike protein DNA is replicated, which serves as a template for the production of mRNA containing the blueprint for the spike protein of the coronavirus.

‚Process 1' utilizes **PCR devices** for DNA replication, an **in-vitro procedure**, while ‚Process 2' employs **bacteria** for DNA replication, constituting an **in-vivo procedure**.

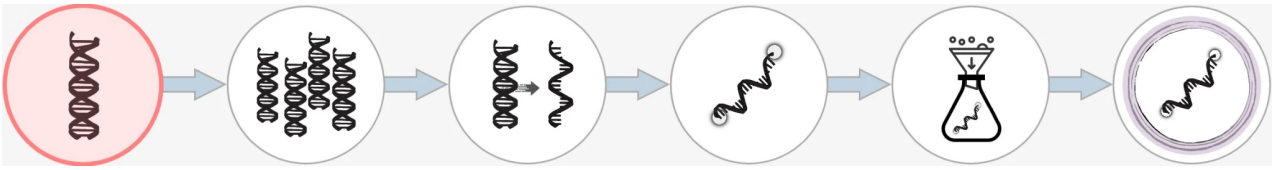
The following table broadly outlines the similarities and differences in the Pfizer production process, which may vary in reality.

| | Production Steps |
|---|---|
| **1.** | Extraction of the genetic information |
| **2.** | Replication of the spike protein DNA<br>**Process 1: using PCR**<br>**Process 2: using Bacteria** |
| **3.** | Transcription to produce the RNA |
| **4.** | RNA-Processing |
| **5.** | Purification of the synthetic mRNA<br>**Process 1: Magnetic bead purification** (*)<br>**Process 2: Proteinase K digestion and UFDF steps** (*) |
| **6.** | mRNA is packaged into lipid nanoparticles |

(*) Information on the manufacturing process according to the EMA „EPAR for the Covid-19 Vaccine Comirnaty“ (in Section 2.2., Page 32)



# 3.1. Extraction of the genetic information



The first step is the identification of the genetic information (DNA) for the desired protein, in this case, the Spike protein of the coronavirus.

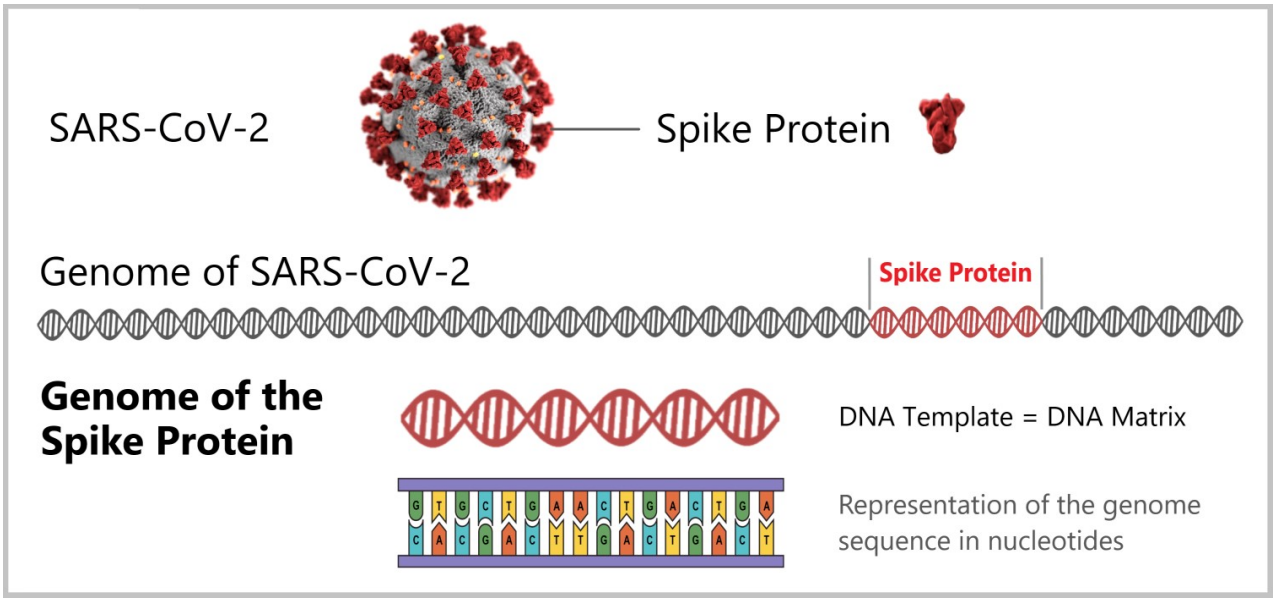


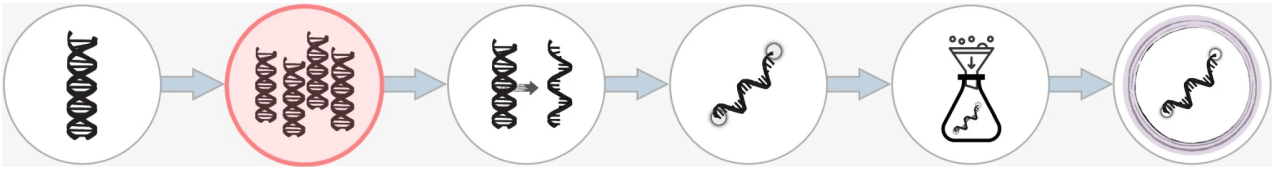

Fig. 1: The genetic information of the SARS-CoV-2 Spike protein serves as the DNA template

The identified DNA of the Spike protein serves as a template for mRNA synthesis in the manufacturing process. In this context, the identified DNA sequence is often referred to as the „DNA matrix" or „DNA template".

To access the DNA of the Spike protein, one must first isolate the virus from an infected sample, such as blood or tissue. Subsequently, the virus is sequenced to determine the exact sequence of nucleotides (the genetic ‚letters') in the virus DNA. The obtained genetic information is then entered into public databases. This allows researchers and biotechnicians to access previously published genetic data, even if they have not isolated the virus themselves. With the sequencing data of the virus, biotechnicians are able to synthetically produce the Spike protein.

It is common for research teams or companies to access previously published genetic data provided by other researchers in public databases. This approach is briefly described by Francis Aurelio deSouza, CEO of Illumina (2016 to 2023), in the discussion „World Economic Forum Panel on Preparing for the Next Pandemic" (Minute: 12:20-13:51) regarding SARS-CoV-2.

## 3.2. Replication of the spike protein DNA



### 3.2.1. Replication of the spike protein DNA – Process 1

‚Process 1' utilizes PCR devices for DNA replication, an in vitro procedure. The term ‚in vitro' refers to experiments or processes conducted outside of a living organism, typically in a test tube or a Petri dish. PCR mimics DNA replication as it occurs in living organisms.

Since the beginning of the COVID-19 pandemic, we are all familiar with the abbreviation PCR. However, very few people know what PCR is and how it works. Therefore, here is a brief overview.

**Polymerase Chain Reaction (PCR)**

PCR is the abbreviation for Polymerase Chain Reaction. With the help of the Polymerase Chain Reaction, many copies of a specific DNA segment can be produced in vitro.

To initiate a Polymerase Chain Reaction (PCR), various ingredients and equipment are required:

a) The **DNA-Template** to be replicated (see Fig. 1)

b) **Nucleotides:** To synthesize new DNA, many nucleotides are needed as they are the building blocks of DNA. There are four types of nucleotides in total, carrying the genetic information: Adenine (A), Thymine (T), Cytosine (C), and Guanine (G).

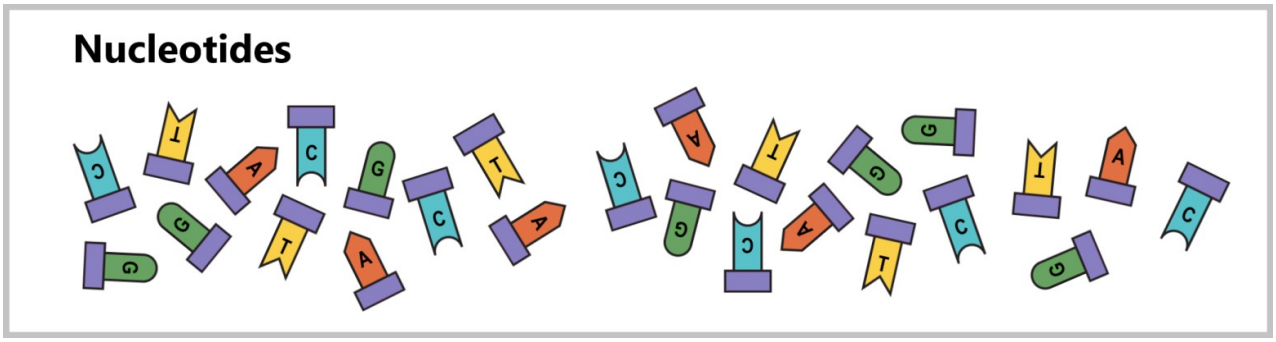



Fig. 2: Nucleotides

c) **DNA Polymerase:** DNA polymerase is an enzyme that plays a crucial role in DNA synthesis. It can be likened to a builder. DNA polymerase takes the existing DNA as a template and uses nucleotide building blocks to construct a new DNA strand. It assembles these building blocks in the correct sequence to ensure that the copy of the DNA looks exactly like the original.

d) **Primer:** A primer is a short DNA sequence that serves as a starting point for DNA synthesis. The primer acts as a guide for the DNA polymerase, indicating where it should start copying the DNA and in which direction it should proceed.

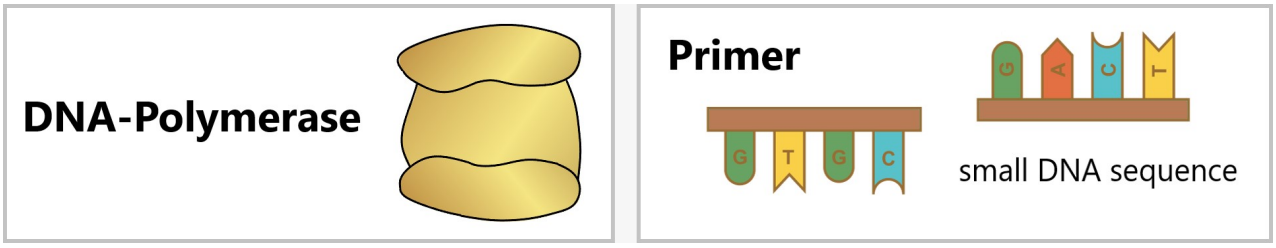



Fig. 3: Schematic representation of the DNA polymerase enzyme and the primer

e) **Buffer solution:** The buffer solution creates a stable and controlled environment for the PCR, allowing the DNA polymerase to work efficiently. It optimizes the pH, stabilizes enzyme activity, and ensures that the necessary ions (usually magnesium ions) are present to enable successful DNA synthesis.

Or to put it simply: The buffer solution in PCR is like the broth in a soup. Think of the PCR reaction as cooking a complex dish where all ingredients must interact perfectly. The buffer solution plays the role of the broth, not only influencing the taste but also ensuring that everything runs smoothly. In short, the buffer solution is like the „broth“ that makes the PCR a successful „dish“, with DNA polymerase being the main ingredient.

f) **Thermocycler:** A device that automatically performs PCR temperature cycles. It allows precise control of the temperature for the various PCR steps.

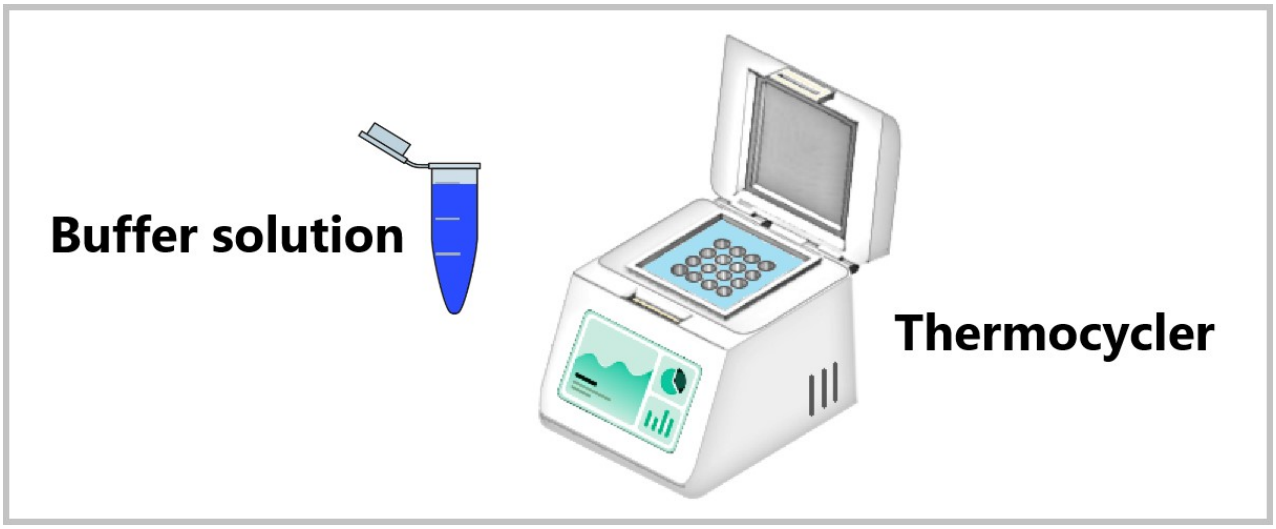



Fig. 4: Schematic representation of the buffer solution and a thermocycler

**Procedure of the Polymerase Chain Reaction**

All ingredients such as the DNA template, nucleotides, DNA polymerase, and primers are added to a tube containing buffer solution. The tube is then placed into the thermocycler. The basic steps of the procedure include denaturation, primer annealing, and DNA synthesis.

**Step 1 – Denaturation (splitting):** The PCR begins with heating the DNA template in the thermocycler. The DNA is heated to approximately 94-98°C for about 20-30 seconds. This process separates the two strands of DNA (denaturation) as the bonds between the nucleotides break apart. From a double-stranded DNA, two single-stranded DNA molecules are formed, which serve as templates for their replication.

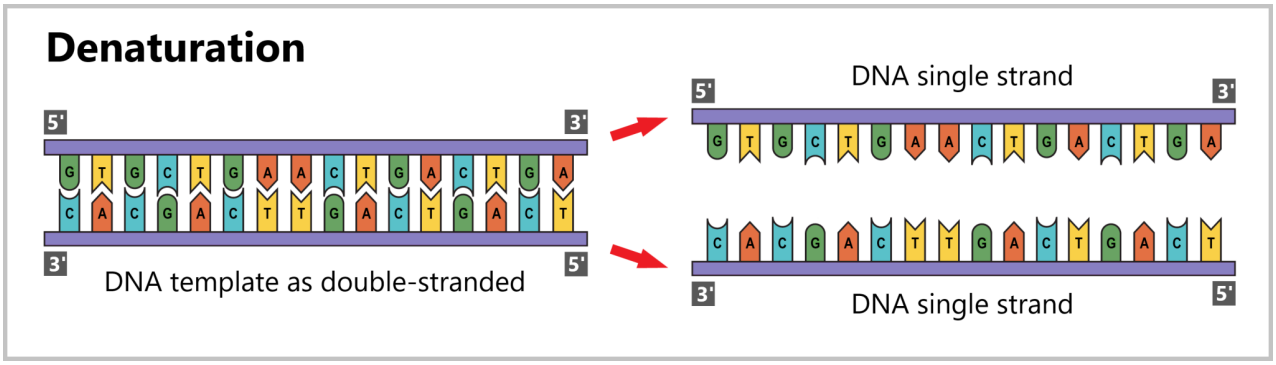



Fig.5: Denaturation

**Step 2 – Primer annealing:** In the second step, the reaction mixture is cooled to approximately 50-65°C. Now the primers bind to the respective individual strands of DNA. The primers serve as starting points for DNA synthesis. In PCR, two pri-

mers are used, one for each strand of DNA, to mark the desired region to be amplified. The principle of complementary base pairing applies (Adenine with Thymine and Guanine with Cytosine).

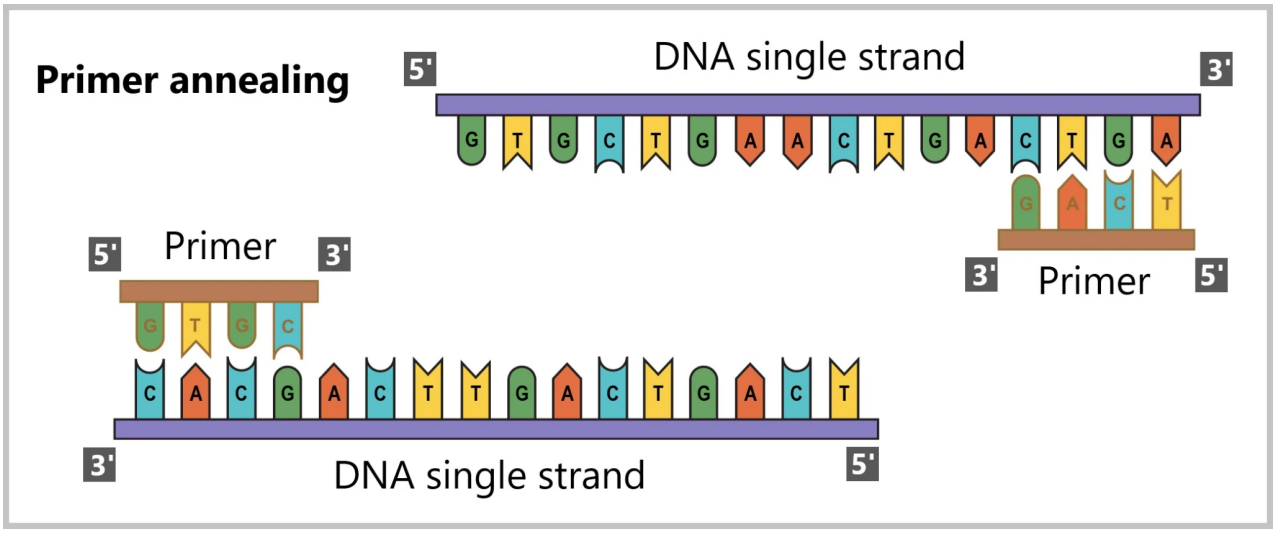



Fig. 6: Primer annealing

**Step 3 – DNA synthesis:** This step is also known as **amplification** (Latin amplificatio: amplification), **elongation** (Latin elongare: to lengthen) or **polymerization** (Greek poly: many; Greek meros: part). The temperature is set to the optimum working temperature of the DNA polymerase (approximately 70°C). The DNA polymerase binds to the primer and starts the synthesis of the new DNA strand. Meanwhile, it reads the single strand of the DNA template in the 3′ to 5′ direction and synthesizes the new DNA in the 5′ to 3′ direction. This is done using the base pairing rules (A with T and G with C), resulting in two new double strands.

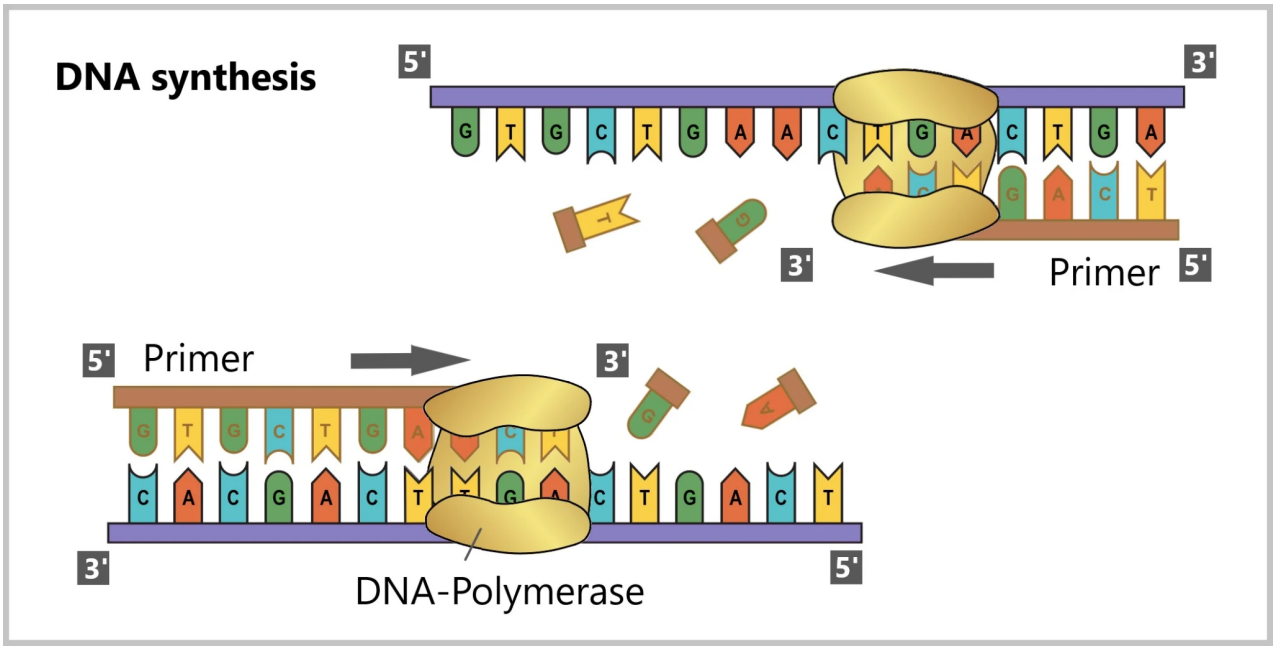



Fig. 7: DNA synthesis

**Cycle repetition**

The resulting DNA double strands serve as templates for the next cycle. Steps 1 to 3 are repeated until the required amount of DNA is achieved. Each cycle doubles the amount of DNA. The term „Polymerase Chain Reaction" describes the cyclic nature of this technique. It illustrates that the process is repeated in consecutive cycles, with each round leading to an exponential increase in DNA (1-2-4-8-16-32, etc.).

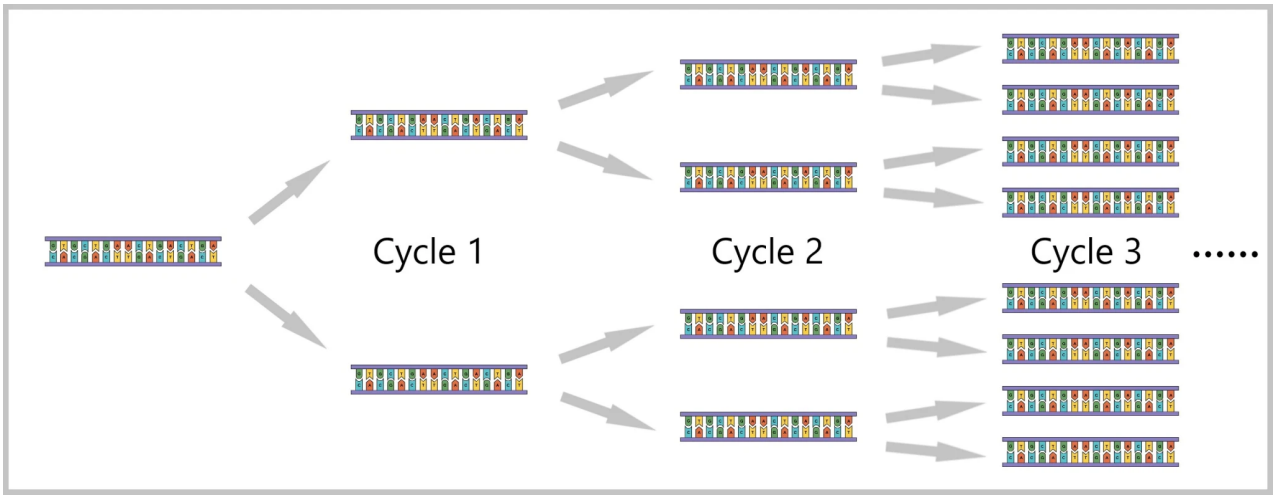



Fig. 8: Exponential Amplification of DNA

### 3.2.2. Replication of the spike protein DNA – Process 2

‚Process 2' utilizes living cells, specifically bacteria, for DNA replication, which is an in vivo procedure. **In vivo** (Latin for ‚within the living') refers in science to processes that occur within a living organism.

### a) Why bacteria?

The core of evolution lies in the continuous alteration of genetic material. This flexibility is crucial for organisms to successfully adapt to new environmental conditions over the course of generations. There are various ways in which living organisms modify their genetic material. Mutations change existing genetic elements, while **gene transfer** can add new elements.

For higher, multicellular organisms, genetic mixing typically occurs through the mating of individuals of the same species. The union of paternal and maternal germ cells results in the creation of a new organism with a unique genetic composition.

In contrast, single-celled organisms like bacteria do not exhibit sexual reproduction and instead reproduce through simple division. During division, the bacterial cell forms two identical daughter cells. This process begins with the duplication of genetic material within the cell, followed by the division of the cell itself.

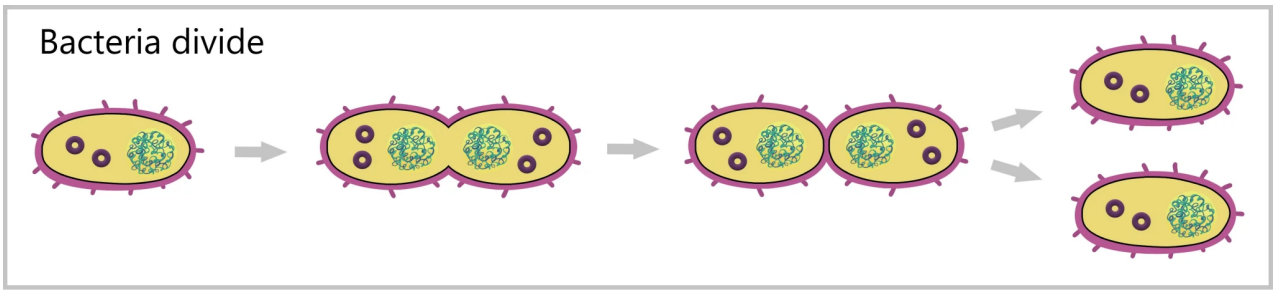



Fig. 9: Bacteria divide

In prokaryotes such as bacteria, everything necessary for survival, reproduction, and evolution occurs within the single cell. Nevertheless, they also strive for genetic exchange and are considered highly adaptable.

In contrast to eukaryotes like humans, the genetic material of bacteria, their DNA, is not packaged within a nucleus. It floats freely inside the cell, in the so-called cytoplasm.

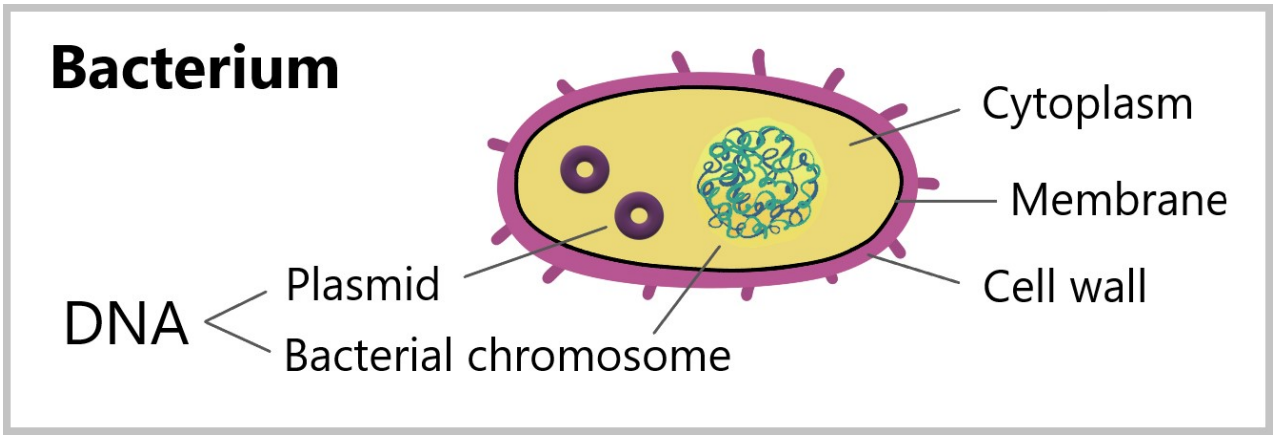



Fig. 10: Structure of a bacterium

The majority of the genetic material is stored in a single DNA molecule called the **bacterial chromosome**. Additionally, bacteria possess independent, ring-shaped DNA molecules known as **plasmids**. These plasmids play a significant role in gene transfer as they often carry important additional genetic information. [Gentechnik-ken]

Plasmids enable bacteria to exchange this additional information with each other. This gene transfer enables bacteria to rapidly acquire new genetic information, which can give them advantages in adapting to the environment, resistance to antibiotics or the ability to utilize new food sources. This mechanism grants bacteria the ability to effectively adapt to changing environmental conditions and survive successfully.

**The ability to introduce and manipulate genetic material in bacteria, particularly through the use of plasmids, has marked an important milestone in the development of genetic engineering.**

The birth of genetic engineering can be traced back to the experiment conducted by Stanley Cohen and Herbert Boyer in 1973. In this experiment, they introduced a plasmid into bacteria and demonstrated that the inserted gene could be expressed in the bacteria; meaning the gene was activated in the bacteria. This was the first successful attempt to transfer a gene from one species to another. The plasmid used carried antibiotic resistance genes, which allowed the scientists to distinguish the transformed bacteria from the non-transformed ones. This groundbreaking work laid the foundation for modern genetic engineering. [Das Cohen-Boyer-Experiment]

### b) The bacterium Escherichia coli (E. coli)

In science and research, the bacterium Escherichia coli (E. coli) is commonly used for several reasons:

- E. coli can be easily cultured and propagated in the laboratory.
- The replication time of E. coli is relatively short, typically ranging from 20 to 30 minutes.
- The genome of E. coli is well-researched and understood.
- E. coli is easily genetically manipulated.
- Cultivating E. coli is cost-effective compared to some other organisms.

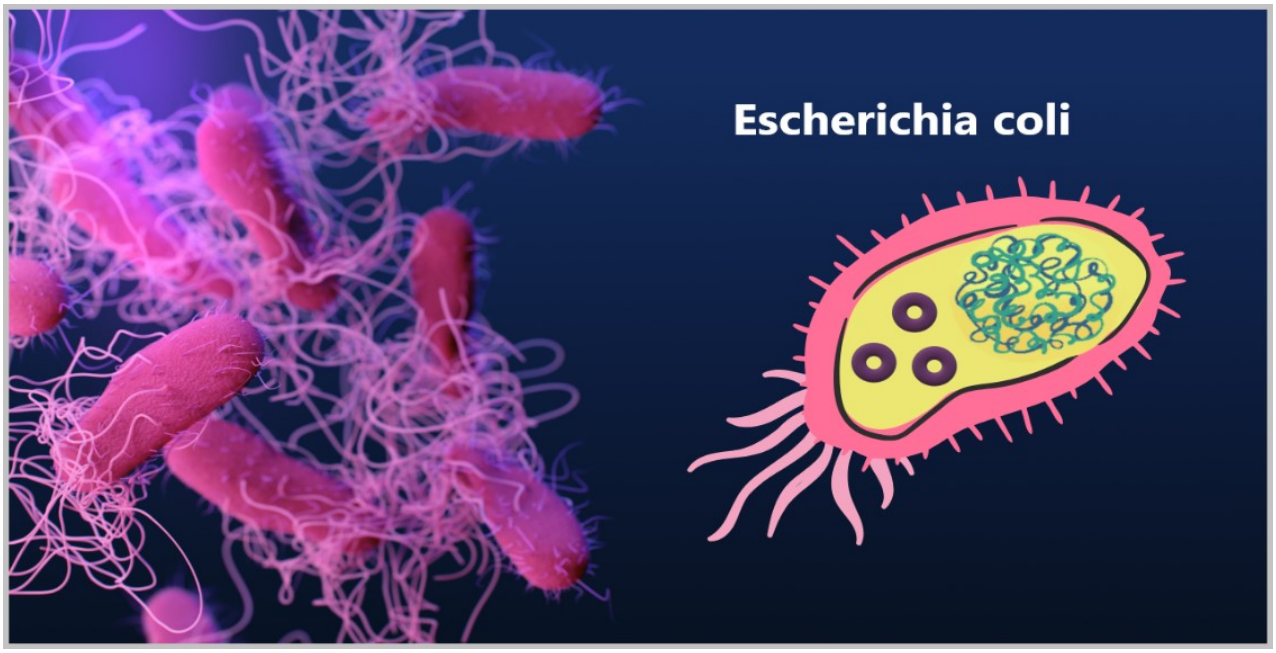



Fig. 11: Escherichia coli

**Escherichia coli** is the best-studied organism in terms of molecular biology and genetics and is therefore also known as the geneticist's pet.

For these reasons, E. coli is also used in vaccine development.

### c) Plasmids

Plasmids are additional DNA molecules in bacteria that exist independently of the bacterial chromosome. They are smaller than the bacterial chromosome and can vary in number within the cell. Plasmids are present as double-stranded DNA. They are usually ring-shaped, which means that the DNA is arranged in a closed circle.

This ring-shaped structure distinguishes plasmids from the linear DNA found in the cell nucleus of eukaryotes (such as humans).

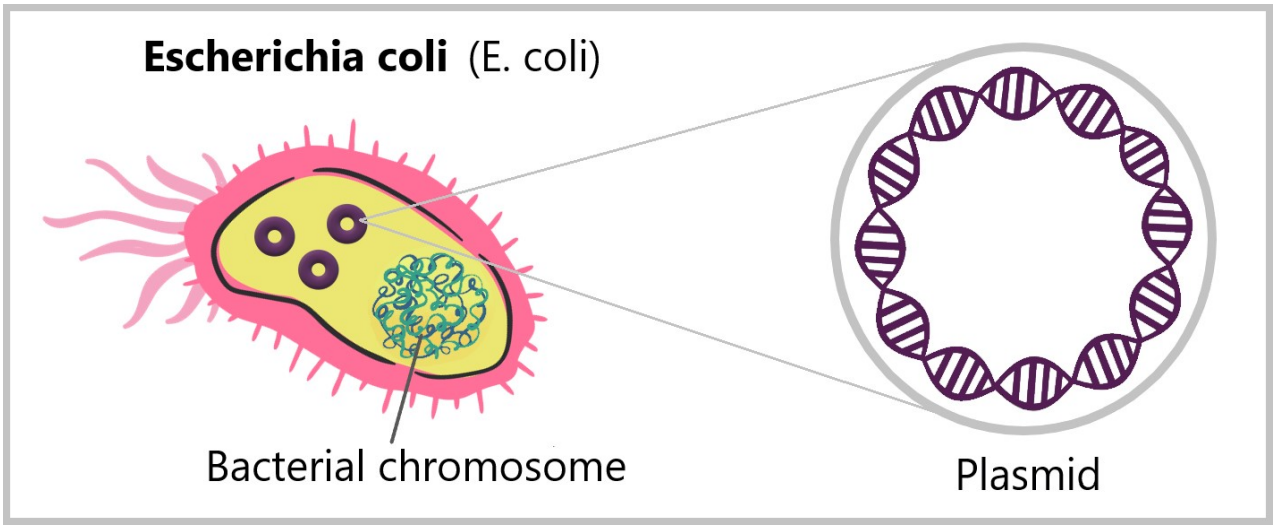



Fig. 12: Schematic representation of the DNA of Escherichia coli

A plasmid can carry various genetic information. For example, it might contain a gene for antibiotic resistance, a gene for the production of a specific protein, or other useful genes.

To enable scientists to see which genes are present on the plasmid and how they are arranged, they use so-called „plasmid maps“. The plasmid map is a schematic representation of the genetic structure of the plasmid DNA. The following figure aims to explain basic terms.

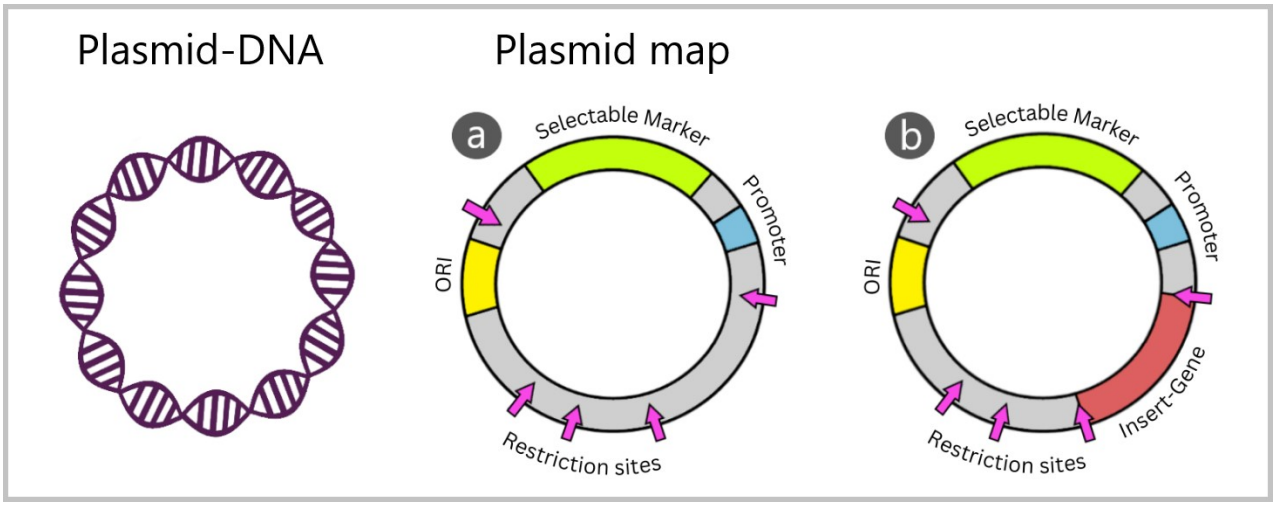



Fig. 13: Plasmid map – schematic representation of the plasmid genes

**ORI (Origin of Replication):** In the plasmid DNA, there is a special region known as the „origin of replication“ (ORI) or simply „ORI“. The origin of replication acts as the

starting point for the duplication of the plasmid. When the bacterium senses the need to reproduce (under favorable environmental conditions and internal signals), it can activate this starting point, and the plasmid makes a copy of itself. For successful replication of the plasmid, this ORI must match the bacterium, much like a key fitting into a lock. If the ORI and bacterium are compatible, the **plasmid can replicate independently**, even apart from cell division.

**Selectable Marker:** These are special gene segments that give the bacteria a survival advantage under certain conditions. These are often antibiotic resistance genes that allow the bacteria to survive in a medium with a specific antibiotic.

**Promoter:** The promoter is a specific DNA region that controls the activity of genes. It acts as a start signal that initiates gene expression, allowing proteins to be produced from the genes. The promoter is important because it influences the timing and quantity of protein synthesis. It regulates when and to what extent certain genes on the plasmid are transcribed, thereby controlling the production of the corresponding proteins.

**Restriction sites:** Restriction sites are specific DNA sequences on a plasmid that can be recognized and cut by restriction enzymes. Restriction enzymes are proteins that tend to cut DNA at specific sequences. Bacteria use restriction sites as part of their defense strategy. When a bacterium encounters foreign DNA that does not belong to it, it can use restriction enzymes to cut and destroy this DNA.

### d) Plasmids in genetic engineering

Although restriction enzymes are originally part of the bacterial defense against foreign DNA, scientists in genetic engineering use these enzymes specifically for gene transfer.

When a researcher wants to insert DNA into a plasmid, for example, they can use restriction enzymes. These enzymes act like tiny molecular scissors. They recognize a specific sequence of DNA letters (nucleotides) in the DNA. This sequence is called a restriction site. When the restriction enzyme encounters the specific DNA sequence, it binds to it and cuts the DNA at that specific location. It cuts both strands of the DNA, breaking the DNA at that site. This cut creates two separate DNA fragments. These fragments can be further used by inserting foreign DNA into

this gap and reconnecting the ends with an enzyme called DNA ligase.

This mechanism is called restriction digestion. It allows for the introduction of foreign DNA into a plasmid. It is a key technology in genetic engineering for performing targeted genetic manipulations and transferring genes into bacteria.

Figure 13-b shows a plasmid map with an inserted gene, also referred to as an insert gene.

**Insert Gene:** The insert gene is the actual gene or genetic sequence that is introduced into the plasmid. This can be a gene that codes for a specific protein or other genetic information that is to be examined or expressed.

A short, animated summary of what a plasmid is and what it is used for can be found here.

### e) Plasmids in vaccine production

The ability of the plasmid to replicate itself makes it very useful for research and biotechnology. It can be used to produce large quantities of a specific foreign DNA that has been introduced into the plasmid beforehand.

### f) Incorporation of the spike protein DNA into the plasmid

The DNA for the spike protein of SARS-CoV-2 is inserted into the plasmid; sometimes referred to as „cloning“. Special enzymes act as genetic scissors (restriction enzymes) and glues (ligases). They cut the plasmid at a specific site and insert the spike protein gene. Once the gene is in place, the plasmid is closed again.

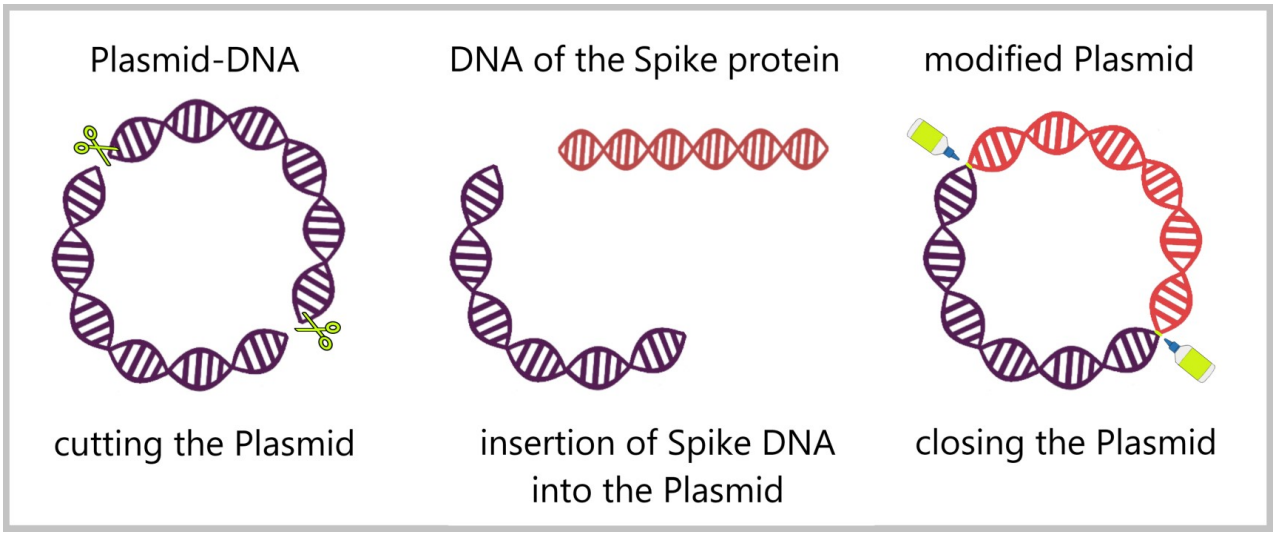



Fig. 14: Insertion of the SARS-CoV-2 spike protein gene into a plasmid

The spike DNA, however, is not introduced into the plasmid alone but along with other genetic elements.

As already mentioned at the beginning, Speicher et al. found other sequences during their investigation of the Pfizer and Moderna vaccines, including ORI, SV40 components, and an antibiotic resistance gene.

Let's take a look at a simplified representation of the Pfizer and Moderna plasmid cards. More detailed representation can be found here.

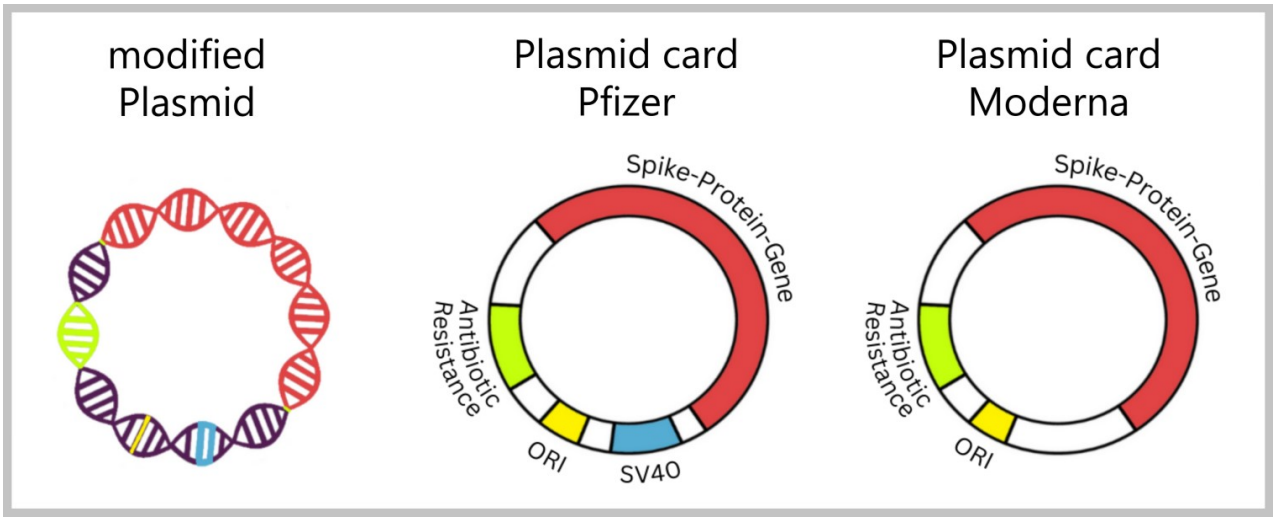



Fig. 15: Simplified representation of the Pfizer and Moderna plasmid cards

**ORI (Origin of Replication):** The ORI serves as the starting point for DNA replication. It provides signals that inform the replication machinery where to initiate DNA duplication.

**Spike-Protein-Gene:** The Spike protein gene encodes for the Spike protein, which is present on the surface of the SARS-CoV-2 virus and plays a crucial role in infecting host cells.

**SV40-Gene-Components:** SV40 stands for Simian Virus 40, a virus found in monkeys. SV40 can infect both monkeys and humans. The SV40 enhancer gene and the SV40 Origin of Replication (Ori) are commonly used tools in molecular biology to promote gene expression and DNA replication in cells. (Gene expression refers to the process where the genetic information of a gene is converted into a functional protein.) SV40 gene components were only found in the Pfizer.

**Antibiotic Resistance Gene:** This gene encodes a trait that protects bacteria from antibiotics. In genetics, it is often used as a selectable marker. Only those bacteria that have taken up the foreign gene via the plasmid survive in an environment with the corresponding antibiotic. This makes it possible to obtain only those bacteria in the production process that have successfully taken up the modified plasmid. Moderna uses the „Kanamycin resistance gene" and Pfizer the „Neo/Kan resistance gene". Neo/Kan resistance gene is an abbreviation for a gene that is responsible for resistance to the antibiotics neomycin and kanamycin.

### g) Transfer of the modified plasmids into bacteria

The modified plasmids, now carrying the Spike protein gene, are then introduced into the E. coli bacteria. This process is called transformation. The bacteria take up the plasmid and integrate it into their cellular structure.

In molecular biology and genetic engineering, **plasmids are often referred to as vectors** when they are used to transport and replicate foreign genetic information. Vectors serve as carriers for transferring foreign DNA into a host cell (bacterium).

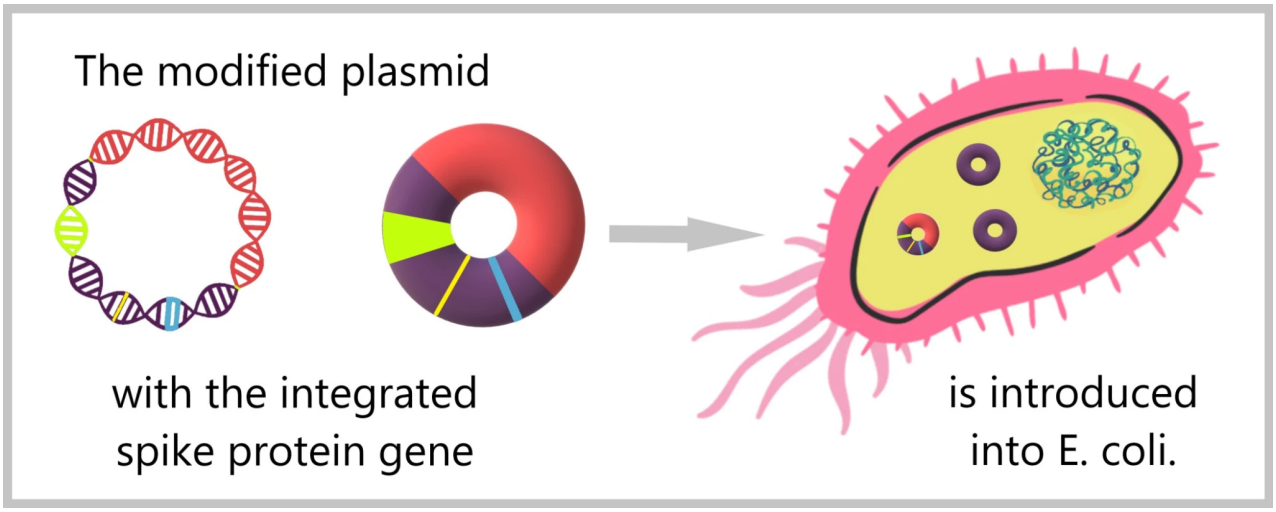



Fig. 16: The plasmid as a vector

## h) Multiplication of the bacteria

The bacteria are now placed in a **fermenter**. A fermenter, also known as a bioreactor, is a device used in biotechnology to produce products such as antibiotics, enzymes, vitamins or vaccines. They can be used for large-scale production. A fermenter enables the precise control of factors such as temperature, pH value, aeration, stirrer speed, nutrient supply and others.

The nutrient medium in the fermenter contains all the necessary nutrients for the bacteria. Under these favorable conditions, the bacteria begin to multiply. During this multiplication, the plasmids within the bacteria also replicate.

To ensure that only the bacteria that have taken up the plasmid with the spike protein gene multiply, the antibiotic is added to the bacteria in the fermenter. Only the bacteria that have successfully inserted the modified plasmid survive, as the spike protein gene is located in the same plasmid as the antibiotic resistance (see Figs. 15 and 16).

E. coli bacteria can divide once every 20-30 minutes. Within a few days, a colony of trillions of bacteria with modified plasmids carrying the genetic information of the spike protein has developed in the fermenter.

## i) Harvesting the bacteria

After the E. coli cells have produced the plasmid with the SARS-CoV-2 spike se-

quence, they are harvested. „Harvesting“ refers to the process by which cells are removed from the culture media and collected. Collection is usually done by centrifugation, which concentrates the cells at the bottom of a container.

### j) Modified plasmids isolation

The isolation of the modified plasmids is achieved by lysing the bacteria, followed by various purification steps to remove impurities. „Lysis“ refers to the process of deliberately breaking or destroying the cell membrane of E. coli cells to release the cellular contents.

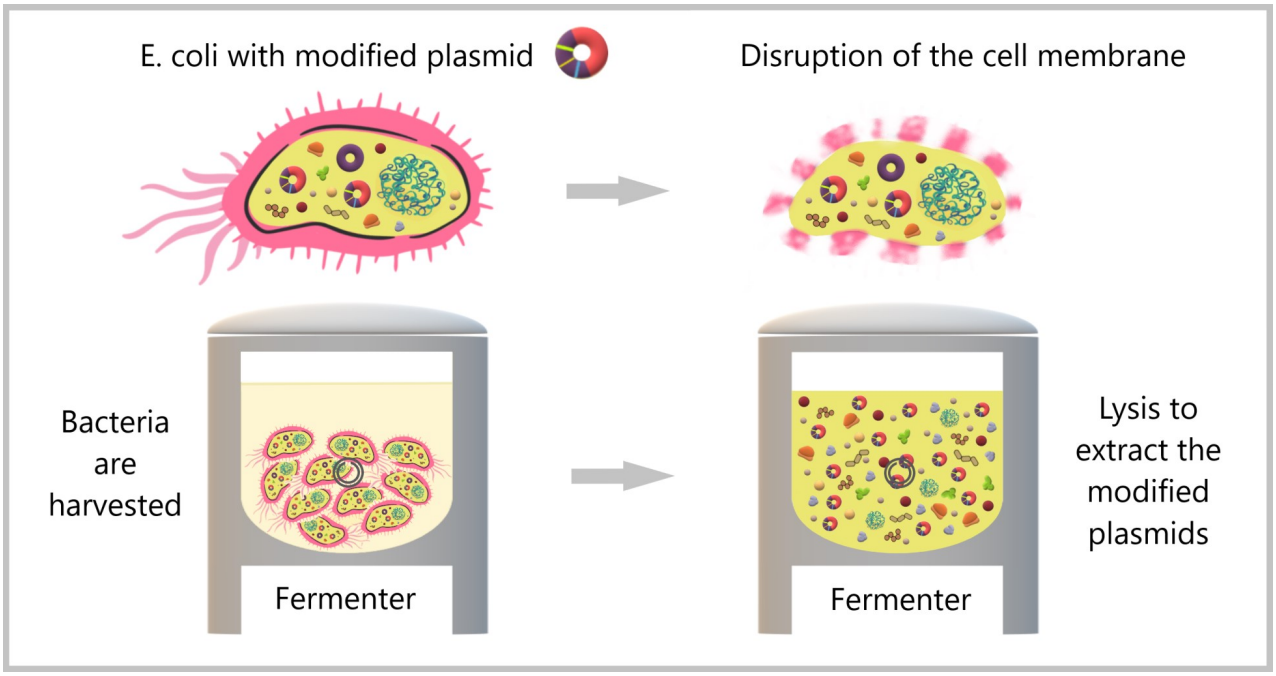



Fig. 17: Harvesting and lysis of the bacteria

The rupture of the cell membrane during the lysis process results in the entire cellular content, consisting of DNA, proteins and other molecules, being released. After lysis, the cellular content of the bacteria and the remaining part of the cell membrane are found in the solution (Figs. 17 and 18).

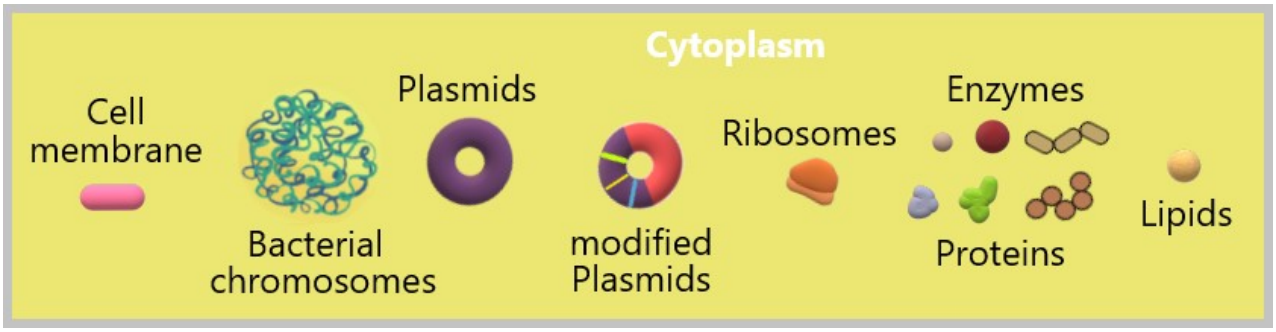


Fig. 18: Additional components of the solution after bacterial lysis

Therefore, after lysis, the extracted material is purified using various methods to isolate the modified plasmids. Purification steps remove unwanted impurities such as proteins and other cellular components (see Section 3.5.2. Purification of synthetic mRNA in Process 2).

At this stage, the plasmids exist in their circular structure.

### k) Linearization of the spike protein DNA

The isolated plasmid is now specifically cut using a restriction enzyme. This restriction enzyme recognizes the specific DNA sequence of the SARS-CoV-2 spike and cuts it out of the plasmid. The cut creates a linear form of the SARS-CoV-2 spike sequence, which is now more accessible for transcription. Linear DNA is generally better recognized by the enzymes responsible for transcription.

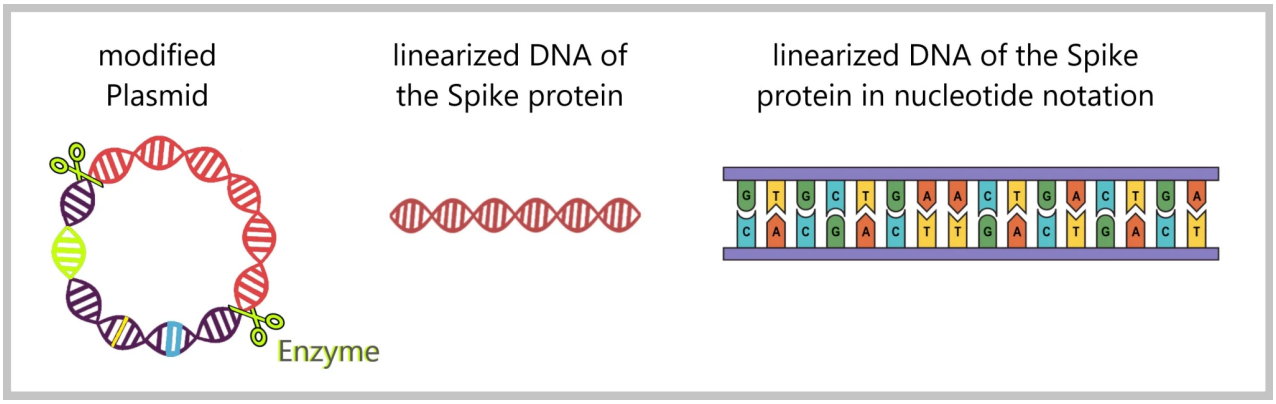
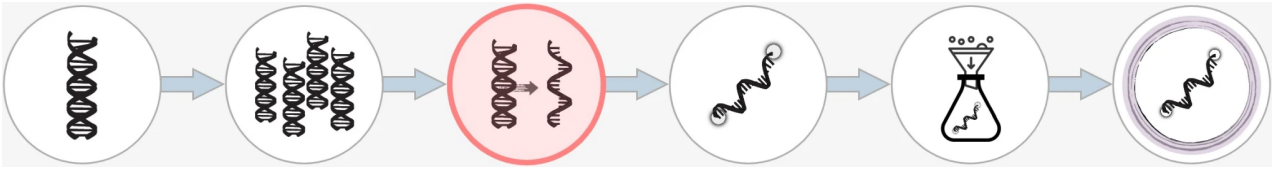



Fig. 19: Linearization of the Spike Protein DNA

## 3.3. Transcription to produce the RNA



Transcription is the process by which RNA is synthesized from DNA, or in other words, **transcription** is the „rewriting“ of DNA into RNA. In vitro transcription (IVT) is carried out using enzymes known as **RNA polymerases** (see Fig. 20).

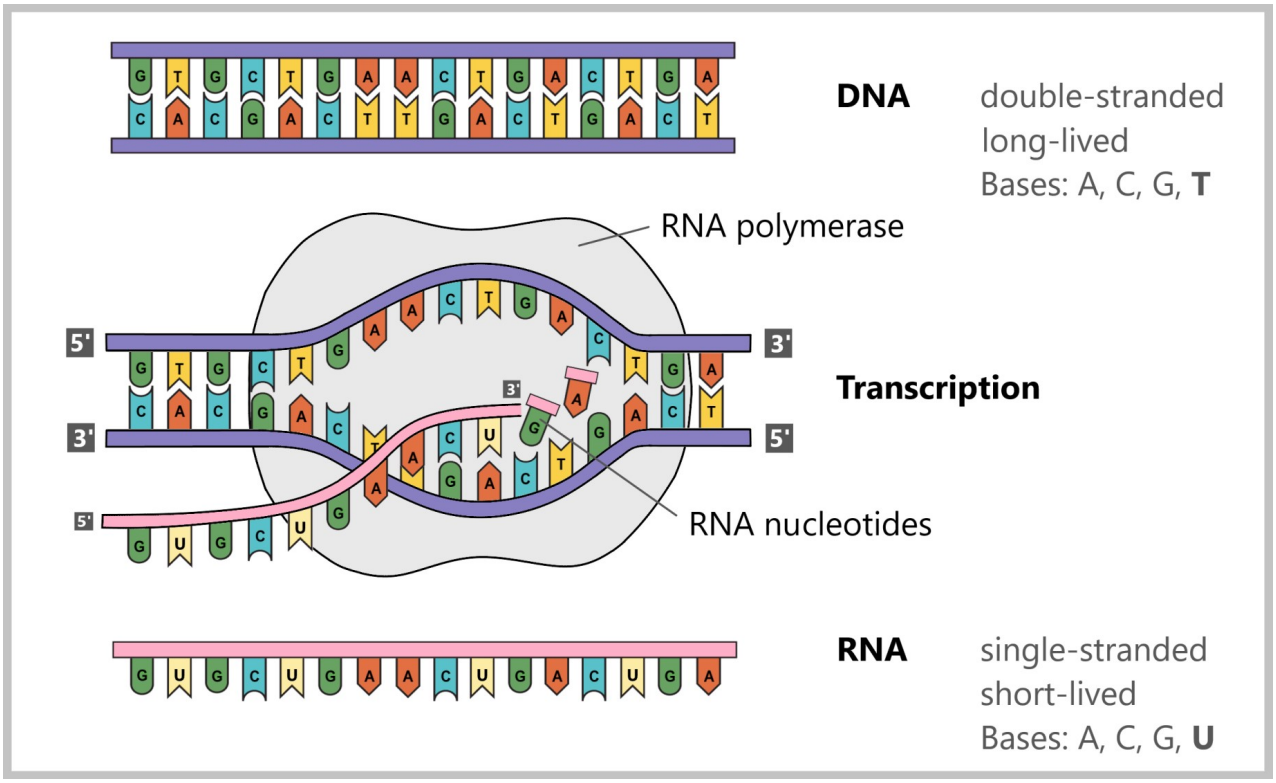



Fig. 20: Transcription – Rewriting DNA into RNA

The transcription process consists of three main phases:

**Initiation**: The RNA polymerase binds to the beginning of the DNA template. It recognizes specific DNA sequences known as promoters, which define the starting point of transcription. To initiate transcription, the DNA template is first denatured. This means that the double helix of DNA is separated to make the single-stranded DNA accessible for RNA synthesis.

**Elongation:** Now, the RNA polymerase moves along the DNA template and reads it. Polymerases move from 5′ to 3′ (more on this here). During reading, the RNA polymerase builds the RNA strand using RNA nucleotides (the building blocks of RNA). This is done by forming complementary base pairs between the RNA nucleotides and the bases of the DNA template. The base pairing takes place between adenine (A) and uracil (U), as well as between cytosine (C) and guanine (G).

**Termination:** Transcription ends at a specific stop signal on the DNA. The RNA polymerase separates from the DNA and the synthesized RNA is released.

A single-stranded RNA is formed from the double-stranded DNA. DNA and RNA are similar in composition and structure, but have significant differences:

**Function:** DNA contains the genetic information required for the structure and function of an organism. RNA is responsible for converting the genetic information into proteins.

**Structure:** DNA is normally arranged as a double helix, with two strands intertwined. RNA is usually present as a single strand, although it can adopt certain regional double helix structures.

**Bases:** Both contain adenine (A), cytosine (C), and guanine (G), but in DNA, thymine (T) is used, while in RNA, uracil (U) occurs instead of thymine. This means that base pairing in DNA is adenine-thymine (A-T) and cytosine-guanine (C-G), whereas in RNA, it is adenine-uracil (A-U) and cytosine-guanine (C-G).

**Stability and Lifespan:** DNA is typically more stable and has a longer lifespan than RNA. RNA molecules can be degraded more quickly in the cell, and this is due to the presence of **uracil**. Uracil is an unstable base that can be readily degraded by enzymes.

**Why does the organism use uracil in RNA?**
The rapid degradation of RNA helps the cell to adapt quickly to changes. The short lifespan of RNA enables the cell to swiftly alter its genetic information, which is especially important for processes such as cell division and adaptation to new environmental conditions. Uracil also allows the cell to quickly turn genes on or off, which is crucial for adapting to different conditions. In summary, uracil in RNA provides the cell with more flexibility and control over its genetic information, significantly influencing its adaptability and survival capability.

**Uracil is a component of uridine,** a compound that additionally contains sugar. Uridine is thus a molecule composed of uracil and sugar, both of which are constituents of RNA (see Fig. 21).

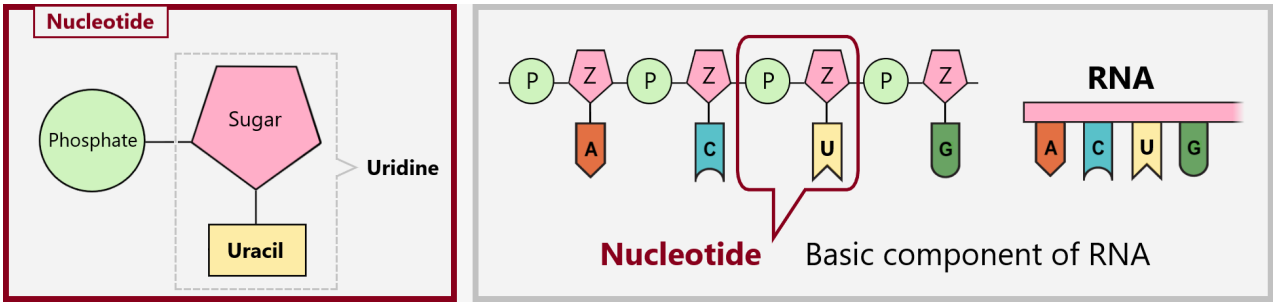



Fig. 21: Nucleotide – Basic Building Block of RNA

For the production of mRNA vaccines, **pseudouridine** is used instead of uridine to ensure stable RNA that is degraded more slowly. [Kariko et al.]

**Both the Pfizer and Moderna mRNA vaccines contain pseudouridine mψU instead of uridine.** [Science]

During transcription, the synthetic nucleoside **N-methyl-pseudouridine (mψU)** is incorporated into the artificial RNA instead of the natural nucleoside uridine (see Fig. 22).

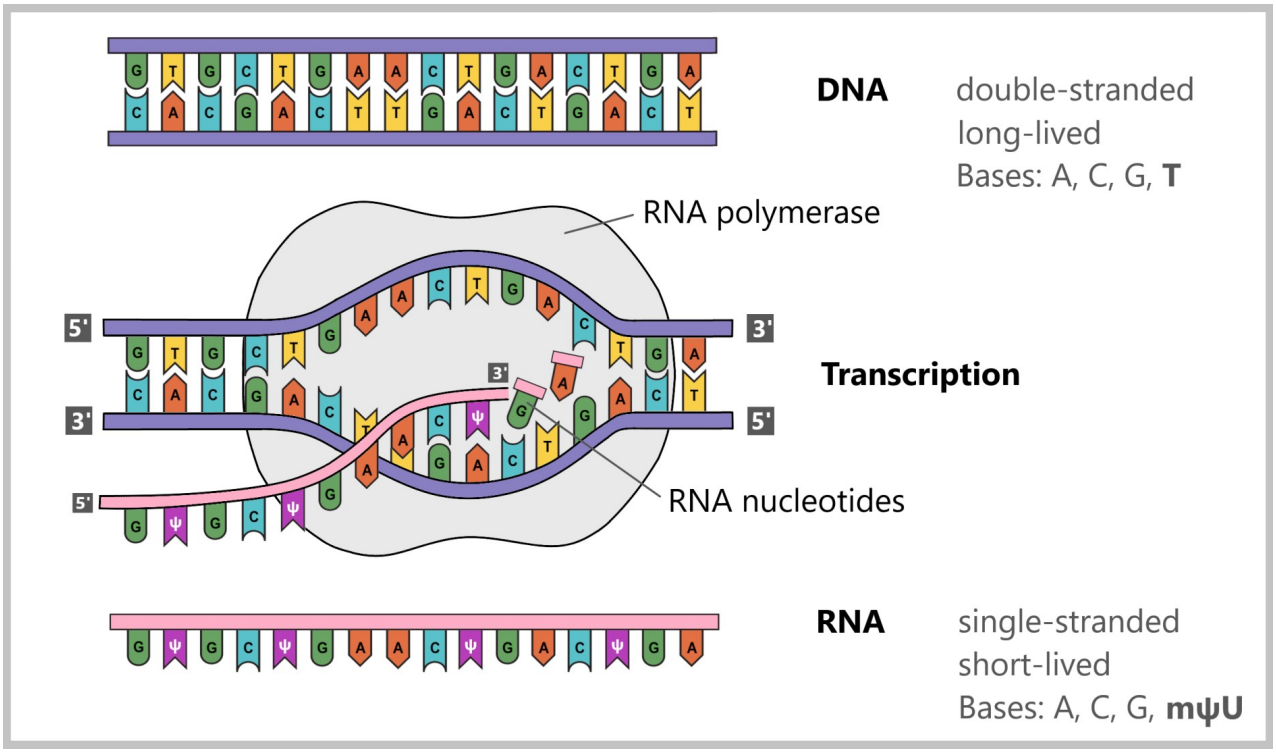



Fig. 22: Transcription – Rewriting DNA into RNA (pre-mRNA) with Pseudouridine instead of Uridine

During transcription, however, undesirable byproducts such as **DNA-RNA hybrids** and **double-stranded RNA (dsRNA)** can also be generated.

### DNA-RNA-Hybrids

The formation of DNA-RNA hybrids during transcription occurs when the emerging RNA strand binds to a single DNA strand. This byproduct consists of a portion of DNA and a portion of RNA, resulting in a hybrid molecule.

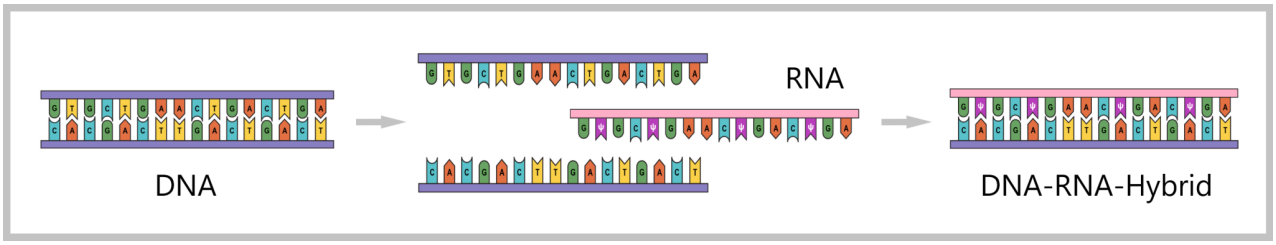



Fig. 23: DNA-RNA-Hybrid

After in-vitro transcription (IVT), the degradation of these DNA-RNA hybrids does not occur spontaneously as in living cells. To remove such byproducts, specific purification steps are carried out (see Section 3.5.2).

**Double-stranded RNA (dsRNA)**

The formation of double-stranded RNA (dsRNA) during in vitro transcription (IVT) of messenger RNA (mRNA) is also an undesirable byproduct. [NIH – Doppelsträngige RNA...]

As described above, transcription initiates once the DNA template is denatured, meaning it is separated into two individual strands. At this point, the RNA polymerase binds to the resulting single-stranded DNA template and initiates the synthesis of a complementary RNA strand. Under specific conditions, such as in a denaturing environment, the freshly synthesized RNA strands may tend to interact with each other and form a double-stranded structure. This can occur in two ways:

**Intermolecular Base Pairing:** Two RNA strands synthesized from different DNA templates can interact with each other and form base pairs.
**Intramolecular Base Pairing:** A single RNA strand can fold upon itself and form base pairs with itself.

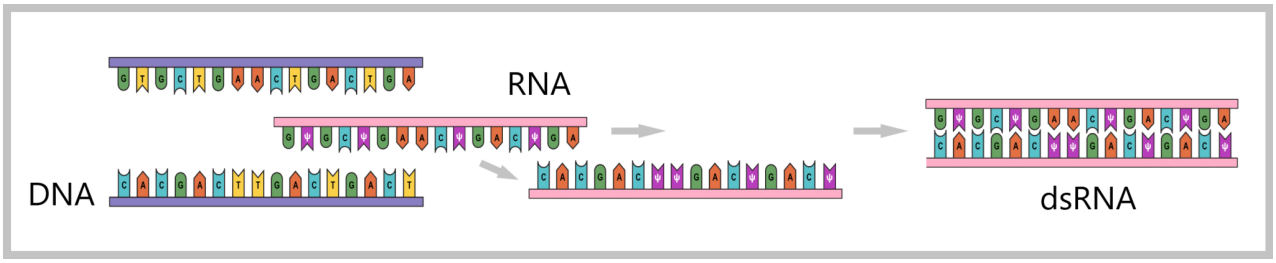
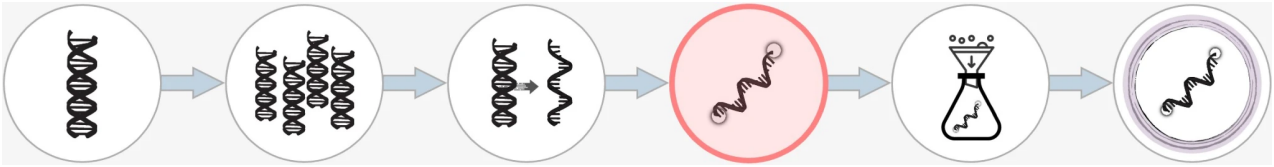


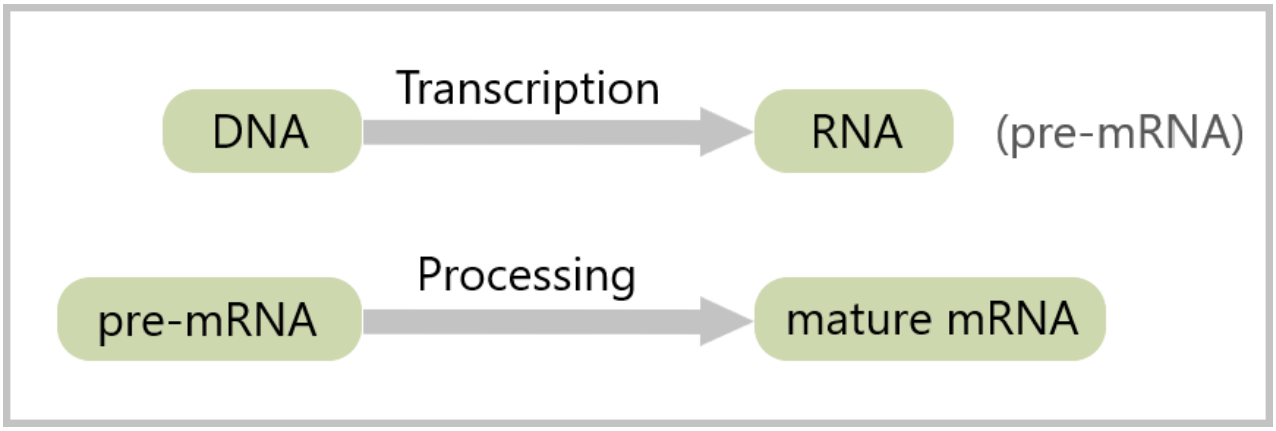

Fig. 24: Double-stranded RNA (dsRNA)

Specific purification steps (see Section 3.5.2) are employed to remove such byproducts.

# 3.4. RNA-Processing



RNA processing involves a series of modifications that occur after transcription to convert the RNA (pre-mRNA) into mature, functional mRNA.




In the production of mRNA for vaccines, efforts are made to mimic the natural processes of transcription and post-transcriptional modifications that typically occur in cells of eukaryotes (like humans). The synthetically produced mRNA is designed to replicate certain characteristics of naturally occurring mRNA.

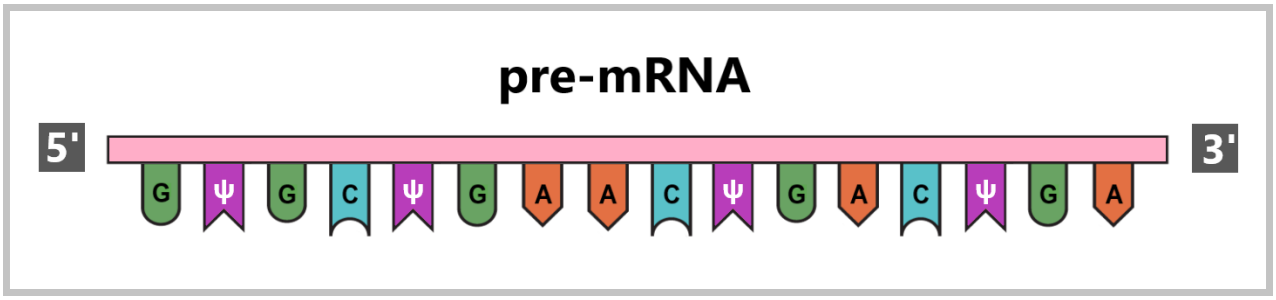



Fig. 25: pre-mRNA

A functional mRNA that serves as a vaccine must have the structure of a normal cellular mRNA, including a special protective cap at the 5′-end and a stabilization tail at the 3′-end. These modifications are important for the stability of the mRNA and its efficient translation into proteins.

Therefore, the modifications of the pre-mRNA include the addition of the protective

cap (mRNA-capping) and the stabilization tail (polyadenylation).

**mRNA-capping:** mRNA capping takes place during transcription, while the RNA polymerase reads the DNA strand and synthesizes the mRNA. As soon as the mRNA has synthesized a short piece, the protective cap, consisting of 7-methylguanosine, is added to the 5′-end of the mRNA. This modification protects the mRNA from degradation by nucleolytic enzymes.

**Polyadenylation:** Polyadenylation occurs shortly after transcription by binding a long chain of adenine nucleotides to the 3′-end of the mRNA. This poly-A tail increases the stability of the mature mRNA and protects it from degradation. The poly-A tail can be several hundred nucleotides long. The frequency of mRNA being read is regulated by the poly-A tail, as it gradually shortens during translation, the process by which proteins are synthesized based on the mRNA template. This continues until the poly-A tail is completely removed, leading to mRNA degradation.

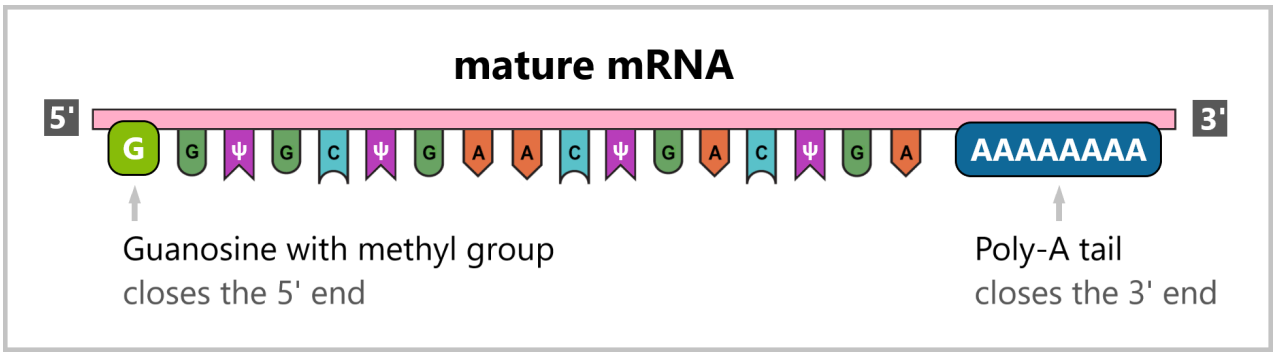
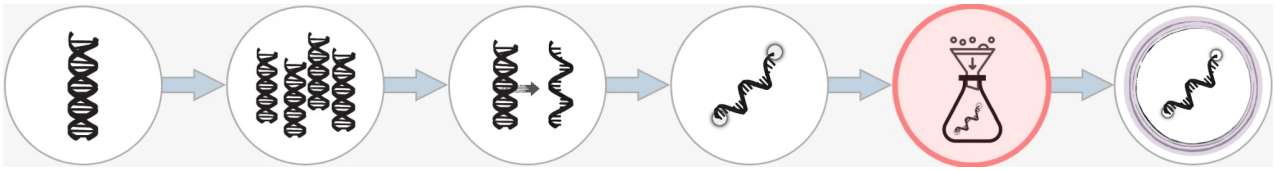



Fig. 26: mature mRNA

# 3.5. Purification of the synthetic mRNA





## 3.5.1. Purification of the synthetic mRNA – Process 1

The purification of PCR products and in vitro transcribed RNA is an important step

to remove contaminants such as unincorporated nucleotides, DNA templates, and enzymes.

Several methods are available for purification. According to information on the manufacturing process provided by the EMA „EPAR for the BioNTech/Pfizer COVID-19 Vaccine" (in Section 2.2. on page 32), purification using magnetic beads is used in ‚Process 1'.

**Magnetic bead purification**

For the purification of mRNA, magnetic beads are typically engineered to selectively bind to RNA. This can be achieved by using special probes that are attached to the surface of the beads. These probes consist of a sequence of thymines (T) that are complementary to the adenines (A) in the poly-A tail of the mRNA (see Fig.27).

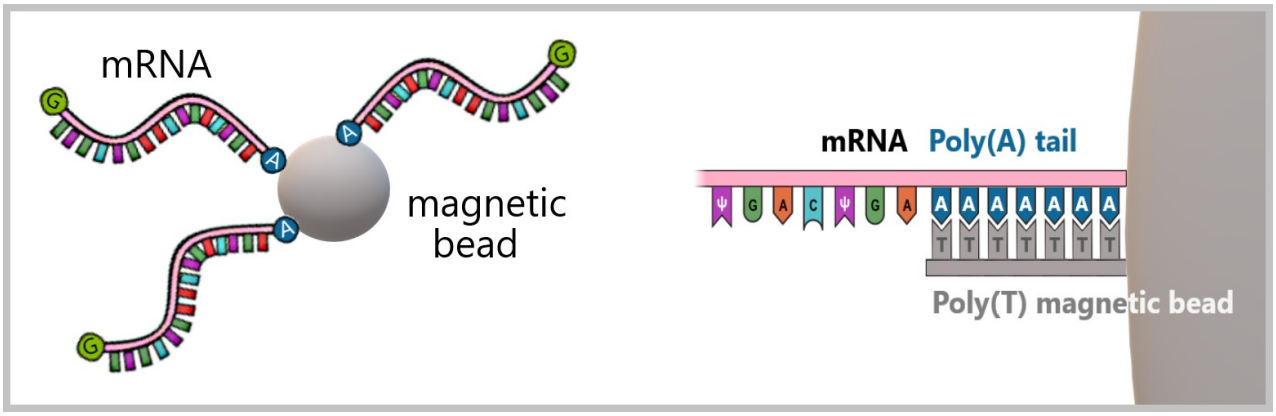



Fig. 27: Poly(A) RNA molecule bind to Poly(T) magnetic bead

The purification process then proceeds as follows (see Fig.28).

**(a)** The magnetic beads, coated with specific thymine molecules, are added to the vaccine solution containing the desired mRNA. **(b)** The molecules on the magnetic beads have an affinity for the target molecules of the mRNA, the adenines on the poly-A tail, and selectively bind to them. **(c)** After the magnetic beads have bound the target molecules, a magnetic field is applied. The magnetic beads with the bound mRNAs are attracted by the magnet and pulled to a specific area of the vessel. **(d)** Several washing steps are performed to remove unbound impurities, with fresh buffer solution added. A buffer solution is a liquid that ensures the conditions of the target molecules and the magnetic beads are just right for them to work together. At the same time, it helps to stabilize the pH of the liquid so that everything works well. **(e)** To separate the bound mRNA from the magnetic beads, a special

solution, an elution buffer, is added. The conditions in the elution buffer cause the magnetic beads to release the bound mRNAs. The released mRNAs can now be used in a concentrated form for subsequent processing steps.

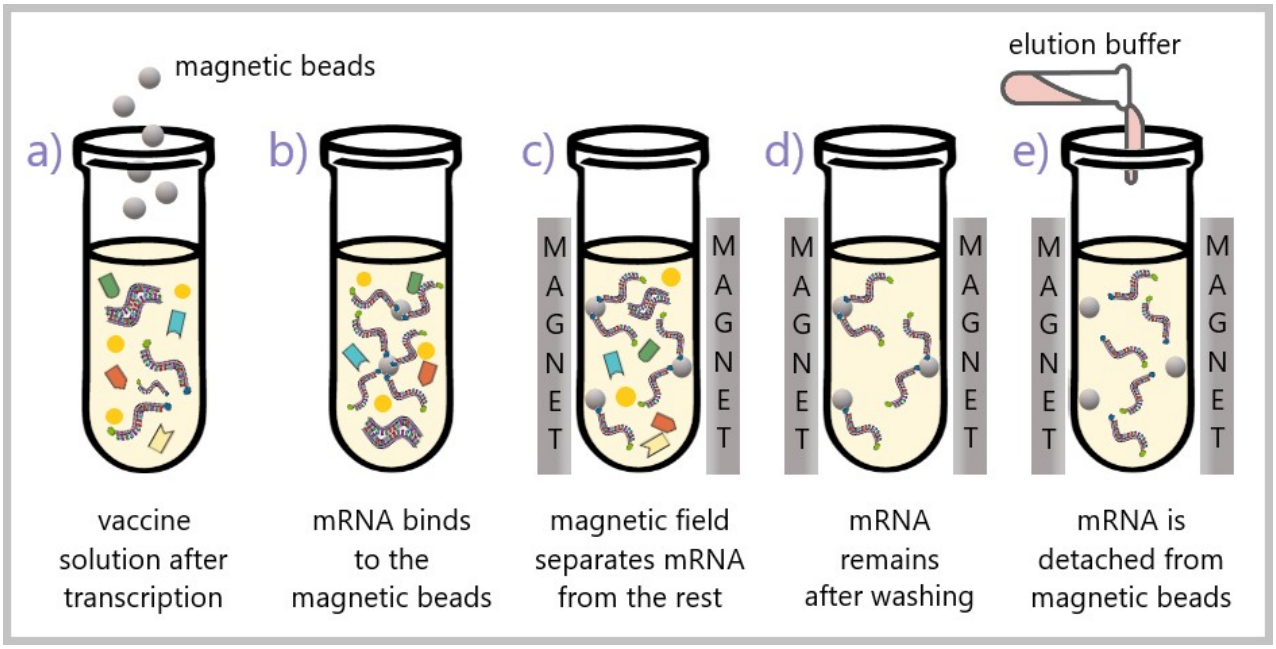



Fig. 28: Magnetic Bead Purification

Further detailed information on magnetic bead purification can be found here and here.

According to official information, magnetic bead purification is only carried out as part of ‚Process 1'. With the transition to large-scale production (Process 2), the purification method also changes. This change could be related to the magnetic force, which plays a crucial role in precise separation in large volumes. It is noted that simple magnets are considered limited as their magnetic force decreases rapidly with increasing distance. [sepmag]

### 3.5.2. Purification of the synthetic mRNA – Process 2

During the production of mRNA by plasmids and in vitro transcription, various impurities are introduced or generated. [ScienceDirect]

It is therefore necessary to purify the vaccine solution with the desired mRNA from potential contaminants and other materials. Potential contaminants include remnants of the DNA template, double-stranded RNA, DNA-RNA hybrids, endotoxins, excess RNA polymerase and basic impurities. [securecell]

**Endotoxins:** Endotoxins are toxic components of bacteria, more precisely of gram-negative bacteria such as the bacterium Escherichia coli (E. coli). These endotoxins are components of the outer membrane of bacteria and can cause strong immunological reactions in the organism.

**DNA templates:** The DNA templates (SARS-CoV-2 spike sequence) are read during transcription and serve as a template for RNA synthesis. The remnants of these DNA templates are still present in the vaccination solution and must therefore be carefully removed to ensure purity and safety.

**Double-stranded RNA (dsRNA):** During the transcription process, double-stranded RNA (dsRNA) can be formed as an intermediate product. [PubMed]
Double-stranded RNA is considered immunogenic because it is recognized as a potential indicator of viral infection in many organisms, including mammals. There are various types of receptors that recognize double-stranded RNA as foreign and can trigger an immune response.

**DNA-RNA hybrids:** During the transcription process, hybridization can occur between the DNA template and the newly synthesized RNA. The RNA can partially bind to the DNA and thus form a DNA-RNA hybrid. If such hybrids remain in the vaccine solution, they could potentially trigger undesirable immunological reactions or other unforeseen effects.

**RNA polymerase:** Excess RNA polymerase, the enzyme that carries out transcription, can be a potential contaminant. Overexpression or incomplete removal of this enzyme could lead to undesirable effects. (Enzymes belong to the proteins).

**Basic impurities:** This term refers to contaminants that can be introduced during the various manufacturing steps. These may include buffers, enzymes, proteins, or other chemical substances used during the manufacturing process.

Various methods are available to purify the transcribed mRNA. According to the information provided by the European Medicines Agency (EMA) in the „EPAR for the BioNTech/Pfizer COVID-19 Vaccine“ (Section 2.2, page 32), during the manufacturing process of ‚Process 2', **Proteinase K digestion** and **UFDF (Ultrafiltration/Diafiltration) steps** are applied. Another purification step mentioned in the EMA document (on pages 17 and 40) is **DNase I** treatment.

In addition, leading biotechnology companies such as MERCK and SARTORIUS mention **chromatography** as a further purification step in the production of mRNA vaccines.

The official documents do not provide detailed insights into the exact steps of purification throughout the entire manufacturing process of the current COVID-19 mRNA vaccines. Since the main goal of this article is to provide basic knowledge in this field in an understandable manner, the following section will only describe purification steps at a specific point in the process as an example. It is important to note that the exact composition and sequence of purification steps may vary under real production conditions and are often much more complex. [QdB] Further in-depth information on this topic can be found in the publications of MERCK (Herstellungsstrategien für mRNA-Impfstoffe und Therapeutika) and SARTORIUS (Overview of Sartorius Solutions serving mRNA Processing, Page 7).

In the following, we will consider the following purification steps:

a) DNase I treatment
b) Proteinase K digestion
c) UF/DF (Ultrafiltration/Diafiltration) steps
d) Chromatography

### a) DNase I treatment

DNases are enzymes capable of cutting DNA. In this context, terms like ‚cleaving' and ‚digesting' DNA are often used. DNases act like tiny scissors, cutting the DNA into smaller fragments. There are different types of DNases, which differ in their ability to cut DNA at specific or nonspecific sites.

DNase I, which is often obtained from bovine pancreas, cuts the DNA non-specifically, i.e. it can strike at different points on the DNA strand.

In vaccine production, DNase I is used to cleave the existing DNA in the solution into smaller fragments. These smaller DNA fragments can then be more easily removed by subsequent purification steps.

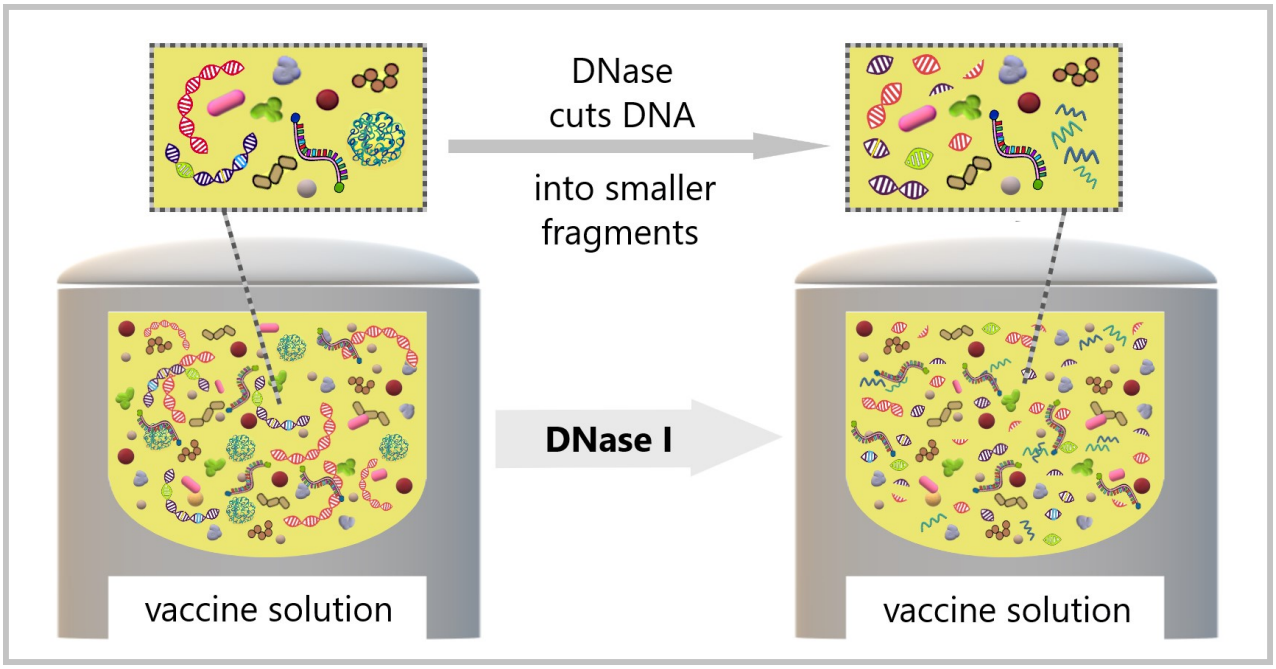



Fig. 29: Schematic representation of DNase I as a purification step

The effect of DNase I on DNA can vary depending on the presence of magnesium ions (Mg2+) and manganese ions (Mn2+). The type of ions influences the specific cutting patterns of DNase I, which can result in different types of DNA fragments, including both single-stranded (ssDNA) and double-stranded (dsDNA) DNA fragments. [bioswisstec, YEASEN]

**In the presence of Mg2+,** DNase I cuts the double-stranded DNA independently at randomly selected sites. This results in single-stranded DNA fragments **(ssDNA fragments).**

**In the presence of Mn2+,** DNase I cuts both dsDNA strands at approximately the same position. This can lead to the formation of double-stranded DNA fragments (**dsDNA fragments**) if the cut fragments overlap or have common areas. Single-stranded DNA fragments (ssDNA fragments) with overhanging ends can also be formed.

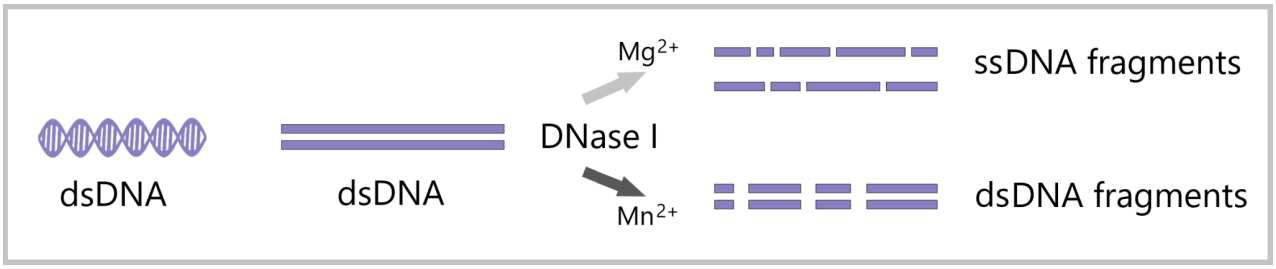



Fig. 30: Schematic representation of DNase I as a function of Mg and Mn

Pfizer-BioNTech supplier Thermo Fisher Scientific discusses the effectiveness of DNase I in an online article. The text makes it clear that the effectiveness of DNase I depends on various factors, such as the initial DNA contamination. It is explained that DNase I is able to cleave double-stranded DNA (dsDNA) efficiently, while its activity for single-stranded DNA (ssDNA) is 500-fold lower. When it comes to activity on RNA-DNA hybrids, it is less than 1-2% of the activity on dsDNA. The paper points out that it is most likely difficult to completely remove every single DNA molecular chain when preparing RNA samples.

### b) Proteinase K digestion

Proteinase K is an enzyme that belongs to the class of proteases. These enzymes have the ability to hydrolyze proteins, meaning they break them down into smaller fragments such as peptides or amino acids. The proteins in the vaccine solution may originate from bacterial components as well as from adjuvants and stabilizers used during the manufacturing process.

The mechanism of action of Proteinase K begins with its binding to the protein. Following this binding, Proteinase K cleaves the proteins into smaller fragments. Subsequently, the resulting fragments are released from the enzyme.

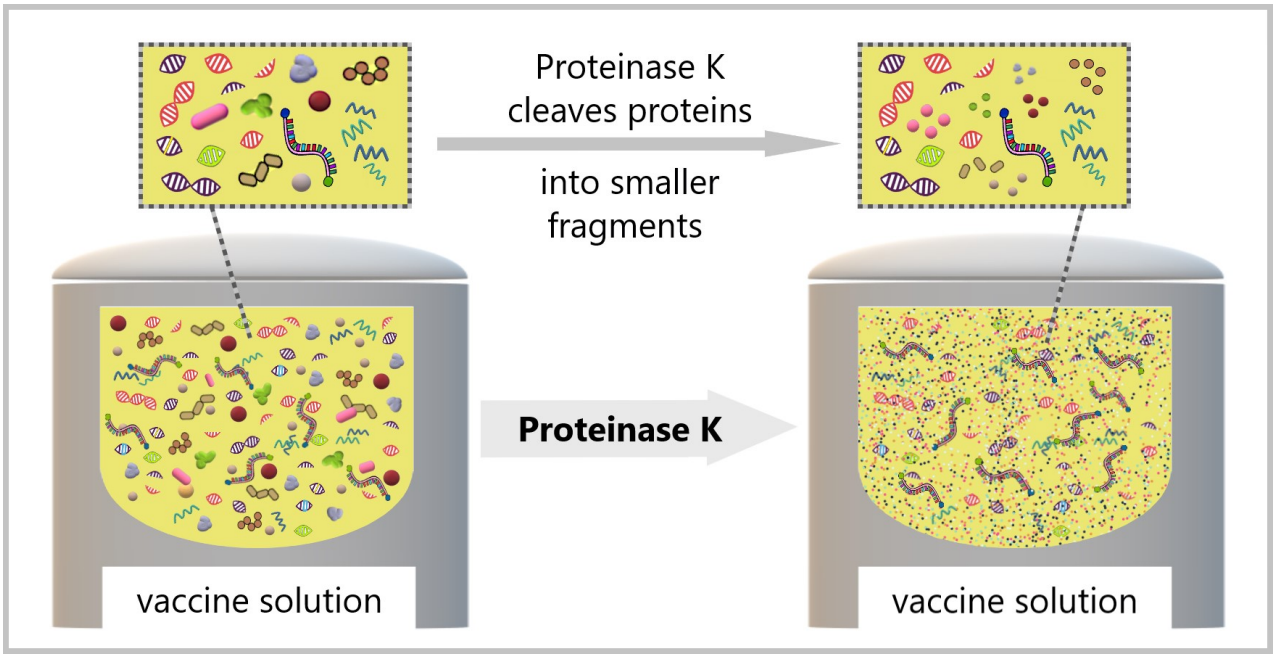



Fig. 31: Schematic representation of Proteinase K as a purification step

After inactivating Proteinase K, a purification of the vaccine solution is often carried out to remove the unwanted residues. Filtration steps can be employed to remove

the smaller fragments.

### c) Purification step: UF/DF

The abbreviations ‚UF' and ‚DF' stand for ‚Ultrafiltration' and ‚Diafiltration' respectively. Ultrafiltration separates molecules based on their size, while diafiltration helps to further remove impurities and concentrate the desired product. Together, they are referred to as the UF/DF process. The combination of Ultrafiltration/Diafiltration (UF/DF) is a common method for purifying biological products such as mRNA.

**Ultrafiltration** is a process that involves a specialized type of filtration using a semipermeable (partially permeable) membrane with defined pore sizes. Through this membrane, molecules can be separated based on their size. Smaller molecules can pass through the membrane, while larger molecules, such as mRNA, are retained.

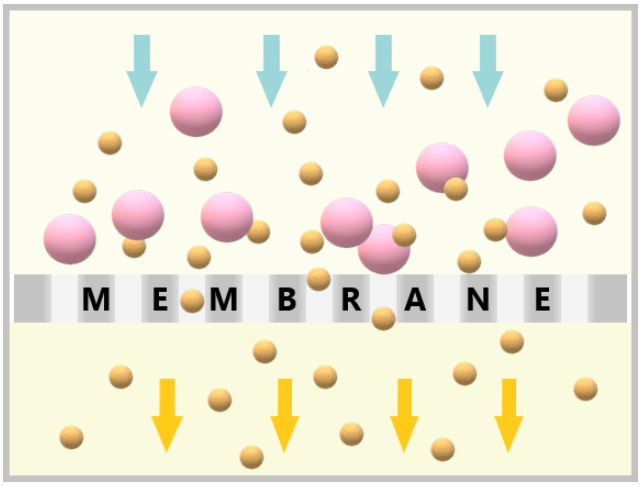



Fig. 32: Only smaller molecules pass through the membrane

During **diafiltration**, fresh buffer (a specialized liquid or vaccine solution) is continuously added during ultrafiltration. This helps further dilute impurities and, for example, concentrate the mRNA in an appropriate buffer solution.

The predominant technique for ultrafiltration/diafiltration (UF/DF) is tangential flow filtration (TFF). [ROCKER]

#### Tangential flow filtration

„The traditional filtration method, known as **dead-end filtration** or direct flow filtra-

tion, involves the vertical flow of the sample through the membrane, allowing small molecules to pass through and be separated. However, this method has the drawback of causing macromolecules to accumulate on the membrane surface and form a filter cake layer. With increasing filtration time, the layer becomes thicker, leading to reduced filtration efficiency and shorter membrane lifespan.

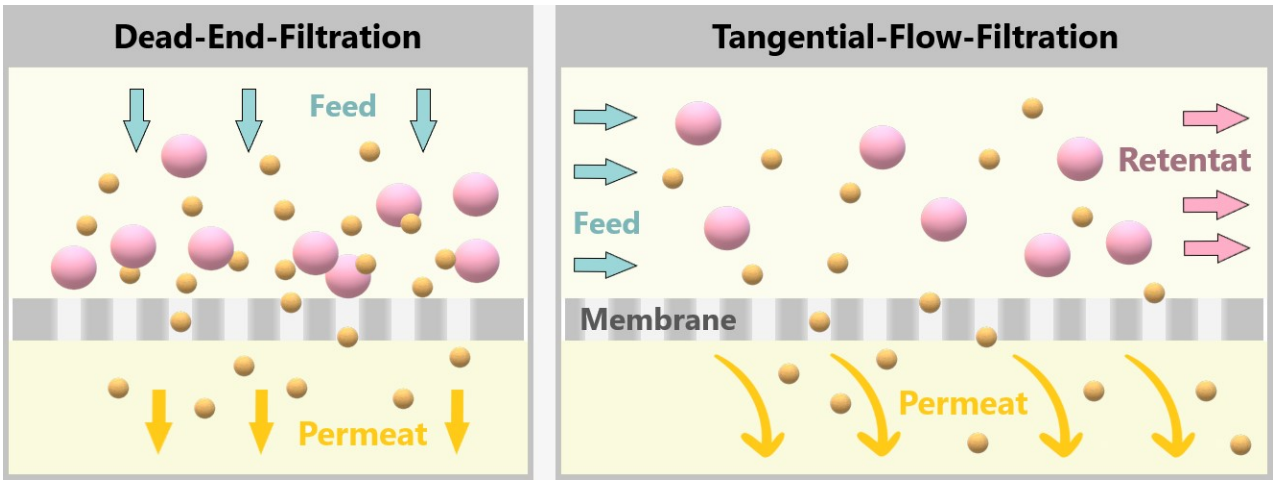



Fig. 33: Dead-End-Filtration vs Tangential-Flow-Filtration

In contrast, in tangential flow filtration (TFF), the sample flow moves horizontally at a tangential angle across the membrane surface. The sample flow is continuously filtered, and during circulation, it flushes the membrane surface, preventing the accumulation of macromolecules and reducing concentration polarization caused by decreased flow rates. This ensures a stable flow rate and effectively prolongs the lifespan of the filter membrane. Tangential flow filtration offers additional advantages as it enables both concentration and diafiltration simultaneously.“ [ROCKER]

Here are some definitions from the drawing:

**Retentate:** The retentate is the material that remains after filtration. In vaccine production, the retentate represents the desired substance, such as mRNA.

**Permeate:** The permeate is the material that passes through the filter. In vaccine manufacturing, the permeate represents the substances that are allowed to pass through the filter, which are undesired in the vaccine.

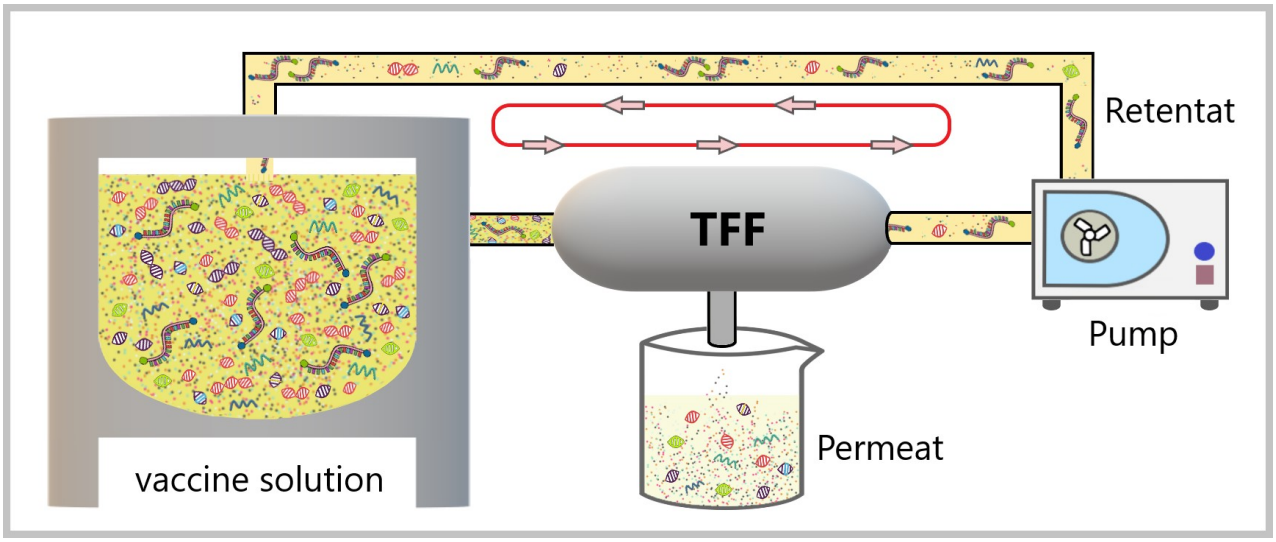



Fig. 34: Combination of ultrafiltration/diafiltration (UF/DF) with tangential flow filtration (TFF)

The figure depicts the process of ultrafiltration/diafiltration (UF/DF) using tangential flow filtration (TFF). The vaccine solution flows through the filtration system (referred to here as TFF). Ultrafiltration is employed to separate the mRNA from impurities using membranes with defined pore sizes that only allow molecules below a certain size to pass through. During ultrafiltration, diafiltration is conducted. In this step, the mRNA is washed with an appropriate buffer to further reduce the concentration of undesired impurities and concentrate the mRNA in a suitable buffer solution.

The result of this process is two products. Firstly, the permeate, which contains the undesirable substances removed from the vaccine solution. And secondly, the retentate, which contains the desired mRNA, mixed with the added buffer.

To maintain the cycle, a pump returns the retentate back into the container along with any remaining impurities. This allows for the process to be repeated, passing the solution through the UF/DF cycle again. This cycle is repeated until the desired purity and concentration of mRNA is achieved in the final product.

In an online post by MERCK on „Manufacturing Strategies for mRNA Vaccines and Therapeutics", tangential flow filtration (TFF) is discussed as a purification method. It is noted that when using TFF, small DNA fragments can hybridize with mRNA, causing additional impurities. However, this risk can be avoided, as explained in the post, by removing the DNA template through separation.

MERCK refers to a **patent** from 2014 by Stéphane Bancel, CEO of Moderna, which deals with „Manufacturing methods for the production of RNA transcripts". It states:

**[0079]** The linearized plasmid DNA template is removed from the in vitro transcription, e.g., the DNA template is separated from the RNA transcript. In one embodiment, the DNA template is removed **chromatographically** using an poly A capture, e.g., oligo dT, based **affinity purification step**.

**[0080]** It is typical to utilize DNase I to enzymatically digest DNA template immediately following in vitro transcription. In the methods of the invention, **DNase is not utilized**. **Whole plasmid removal is preferred to enzymatic digestion due to the fact that the risk of degraded DNA fragments hybridizing to the transcribed mRNA is mitigated.**

**[0082]** The method for production of an RNA transcript can include additional purification steps after the in vitro transcription, e.g., an ion exchange chromatography step.

For comprehensive purification, it is obviously necessary to include an additional chromatography step in addition to DNase I and TFF.

### d) Chromatography

Chromatography is a method for separating different substances in a mixture. In general, this process involves a mobile phase and a stationary phase. The mobile phase is the mixture of substances to be separated, which moves through the stationary phase. The substances in the mobile phase interact with those in the stationary phase. Due to these interactions, the individual components in the mixture move at different speeds or get stuck, allowing for the separation of the various substances from each other.

Figure 35 shows various chromatography techniques such as reversed-phase ion pair, anion exchange and affinity chromatography.

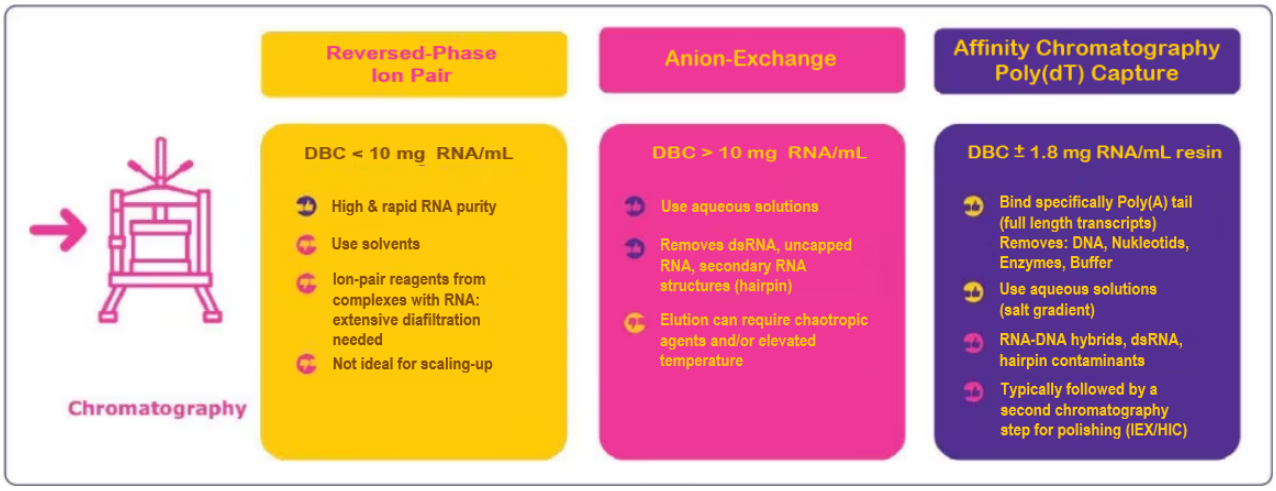



Fig. 35: Comparison of reversed-phase ion pair, anion exchange and affinity chromatography for mRNA purification. [MERCK]

According to MERCK, poly(dT) affinity chromatography is particularly emphasized for large-scale production. Therefore, the principle of affinity chromatography is explained in more detail in the following step.

### Poly(dT) affinity chromatography

In affinity chromatography, the idea is that molecules of interest (such as mRNA) have a specific affinity or attraction to a particular material. The attractive forces between the (mRNA) molecules (mobile phase) and the material (stationary phase) are utilized to isolate the desired molecules from a mixture.

mRNA contains a specific sequence known as Poly(A) (see Fig.36). In affinity chromatography, a column containing a material that includes Poly(dT) is used. Poly(dT) is a chain of nucleotides, all containing the base pair „Thymidine“. Poly(A), on the other hand, is a chain of nucleotides, all containing the base pair „Adenine“. Poly(dT) serves as the „counterpart“ to Poly(A) – they attract each other.

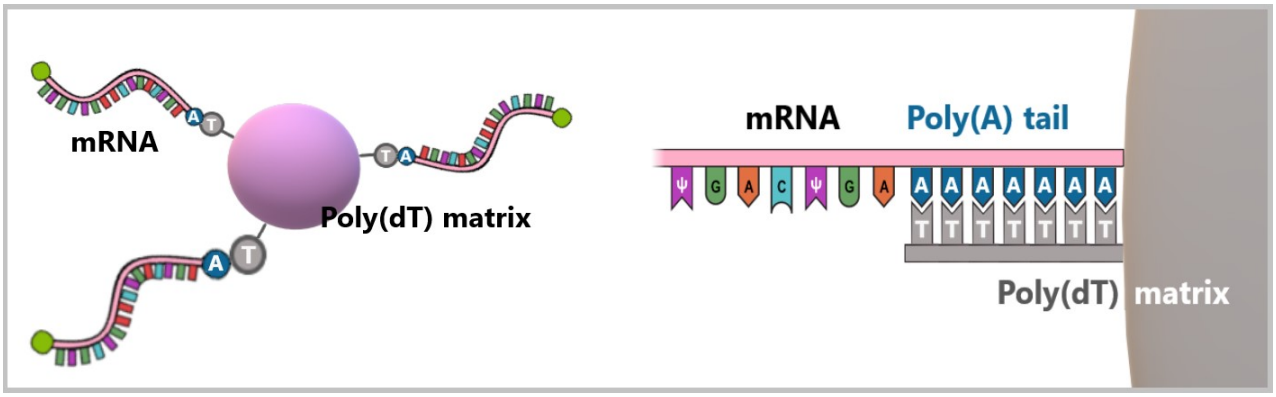



Fig. 36: Poly(A) of mRNA binds to Poly(dT) material

The process roughly follows this sequence: A Poly(dT) matrix is placed in a column. One can imagine the column as a cylindrical container filled with a specific Poly(dT) material.

The vaccine solution containing the mRNA to be purified (see Fig. 37), **a)** is passed through the column. **b)** The mRNA binds specifically to the poly(dT) matrix, **c)** while other components pass through unbound. This step separates the mRNA from the other components. **d)** The bound mRNA can then be released from the column by changing the conditions or adding specific elution buffers.

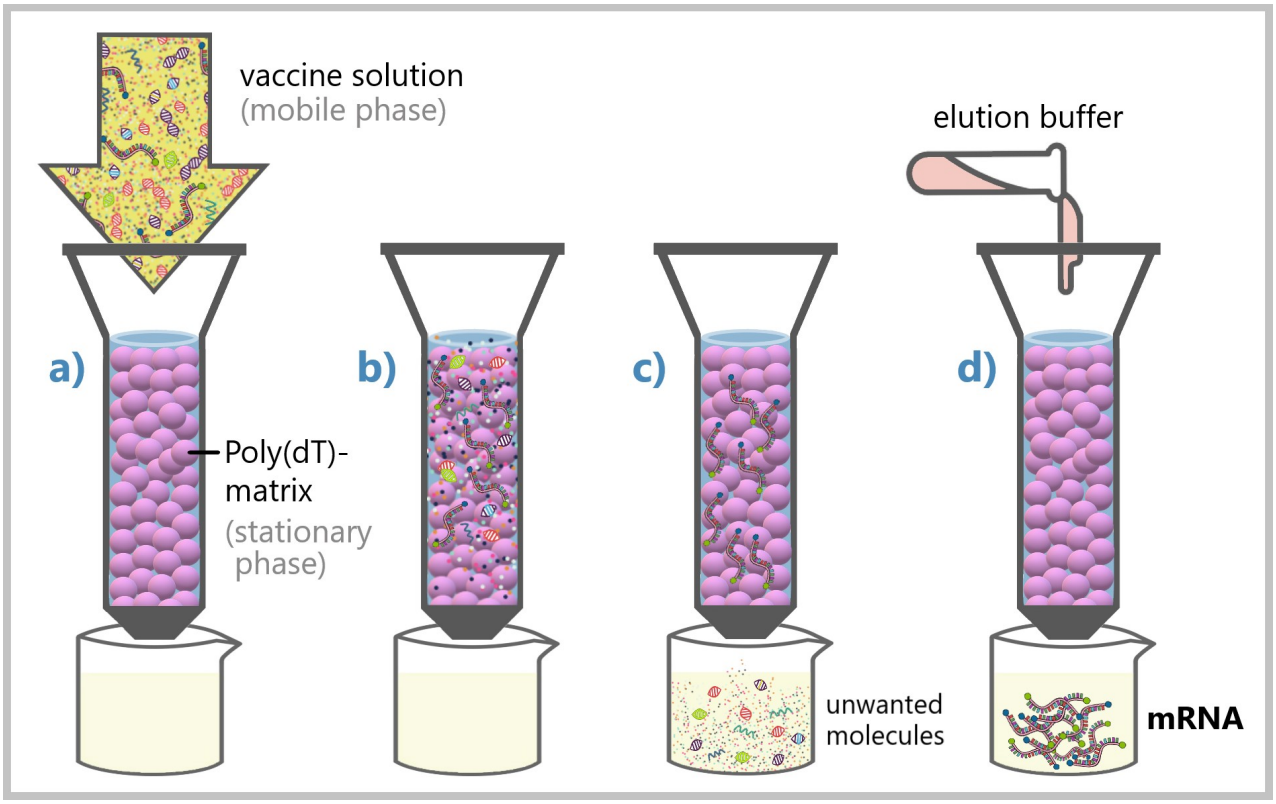



Fig. 37: Schematic representation of poly(dT) affinity chromatography

Due to the limitation of Poly(dT) affinity chromatography, which does not allow differentiation between double-stranded RNA (dsRNA) and single-stranded RNA (ssRNA), and also does not remove product-related impurities such as DNA fragments that have hybridized to the mRNA, MERCK recommends an additional chromatography step using anion exchange chromatography.

### Anion exchange chromatography

Anion exchange chromatography (AEX chromatography) is a chromatographic technique for separating molecules based on their different charges. It utilizes differences in net surface charge.

In this process, a positively charged anion exchange resin is used as the stationary phase to selectively bind and separate molecules with negative charges. This stationary phase typically consists of small beads or particles. The mobile phase, in this case the vaccine solution, contains the target molecule – mRNA, double-stranded RNA (dsRNA), and other potential impurities (see Fig.38).

The binding between the negatively charged molecules and the resin depends on the strength of the negative charge on the molecules. Molecules with higher negative charge bind more tightly to the resin, while those with lower negative charge have weaker interactions and may elute (detach) more easily.

**a)** The process is carried out in a column format in which the vaccine solution is applied to the column containing the positively charged anion exchange resin. Negatively charged molecules in the vaccine solution bind to the resin, **b)** while unbound molecules are washed away.

Since both mRNA and dsRNA carry negative charges due to their phosphate groups, the separation occurs based on their different structures and affinities to the anion exchange resin. Often, dsRNA binds more strongly to the resin than mRNA.

By changing the buffer conditions, such as increasing the ionic strength of the elution buffer or changing the pH value, the bound RNA molecules are gradually eluted from the column. The different affinities of mRNA and dsRNA to the anion exchange resin lead to different retention times (time required for a molecule to pass through the column), whereby **c)** dsRNA generally binds more strongly to the resin and **d)** therefore remains bound for longer.

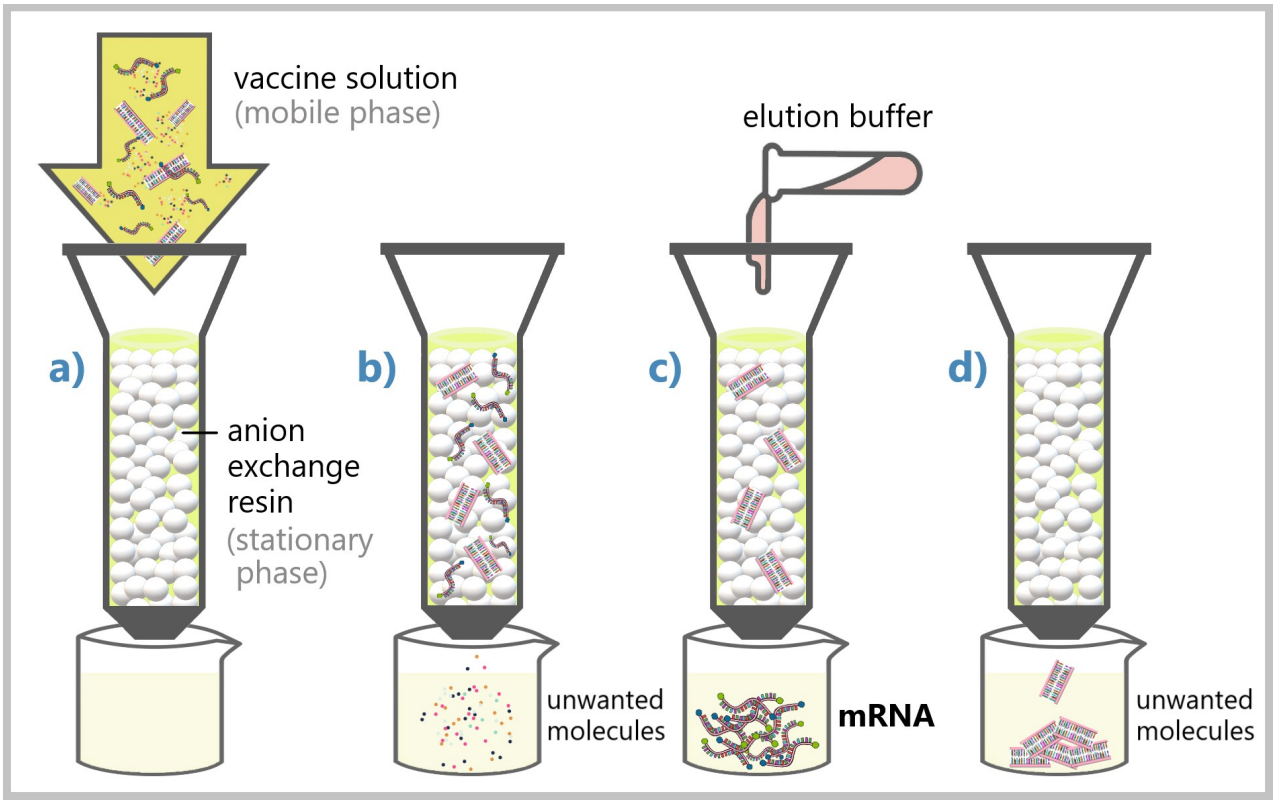
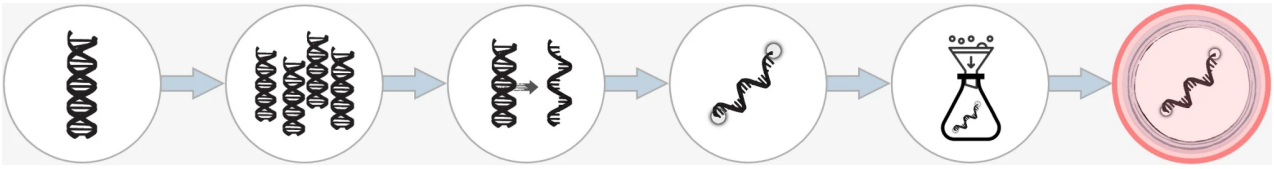



Fig. 38: Schematic representation of anion exchange chromatography

In the official EMA document for BioNTech, there are no explicit indications regarding the use of chromatography during the production process. However, „unofficial" working documents from EMA available online list chromatography as a purification step on page 29.

In a ScienceDirect article (an online platform for scientific journals), it is mentioned that Moderna's COVID-19 mRNA vaccines were prepared and purified using both anion exchange and Poly dT affinity chromatography.

## 3.6. mRNA is packaged into lipid nanoparticles



The purified mRNA is encapsulated in lipid nanoparticles. These lipid shells protect the mRNA from degradation in the body, promote its uptake into cells, and enable the release of mRNA into the cell interior. The formulated RNA vaccines are now ready for administration.

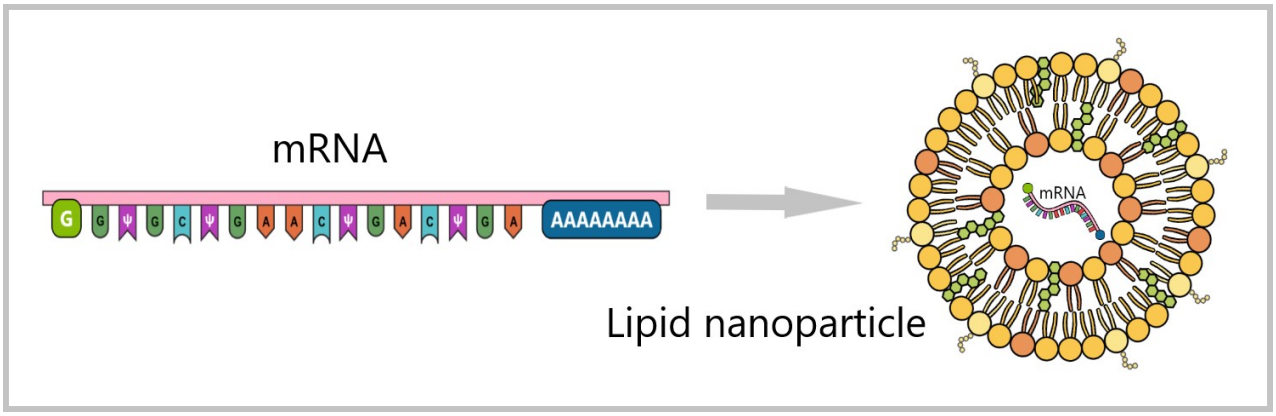



Fig. 39: mRNA is packaged into lipid nanoparticles

## 3.7. Few conclusions

The illustrative depiction of the production process in this chapter has not only provided a closer explanation of the structural steps involved in the manufacturing process of mRNA vaccines but has also roughly outlined the technological challenges faced by the manufacturers.

As indicated by the documents from MERCK, SARTORIUS, and Thermo Fisher Scientific, the research and development of the individual technological steps are still ongoing processes. The EMA documents show that the exchange of information between manufacturers and regulatory authorities regarding some manufacturing parameters is not yet complete. The critical report by Kevin McKernan et al. can be contextualized within this framework.

# 4. Methods for measuring DNA and RNA

Testing the RNA concentration and potential residual DNA (contaminants) is a crucial step in the production process of COVID-19 mRNA vaccines. This measure is of essential importance in the context of product monitoring and quality assurance. It ensures that the manufactured vaccines meet the requirements for purity and efficacy.

In this chapter, an overview of various measurement methods is provided, which serve as detection methods for RNA and DNA in mRNA vaccines, including:

**4.1. Fluorescence Assay**

These techniques are discussed in more detail to provide readers with an understanding of their principal operation and potential applications.

# 4.1. Fluorescence Assay

A fluorescence assay is a biochemical test in which fluorescence is used as an indicator of the presence or quantity of a specific target molecule. This may also involve the measurement of nucleic acids such as DNA or RNA, whereby fluorescence signals are generated through specific binding of fluorescent dyes or probes to the target nucleic acids.

A fluorescence assay is determined by criteria such as

- Specificity (to selectively detect the target molecule),
- Sensitivity (even at low concentrations of the target molecule),
- Stability and reproducibility,
- Robustness (resistant to small variations),
- Feasibility,
- Time and cost efficiency..

Fluorescence assay is an overarching term that encompasses various applications, including quantitative PCR (qPCR), fluorometry with DNA or RNA dyes, qubit fluorometry and many other fluorescence-based techniques. These methods all use the principle of fluorescence to detect, quantify or analyze specific molecules.

Typically, the results of fluorescence assays such as qPCR, fluorometry with DNA or RNA dyes, and Qubit fluorometry are reported as the amount of DNA per unit volume. The unit is typically nanograms per microliter (ng/µl), indicating the weight of DNA per volume unit.

### 4.1.1. quantitative Polymerase Chain Reaction – qPCR

Quantitative PCR (qPCR), also known as quantitative real-time PCR, is an extension of the conventional PCR technique that allows for the determination of the quantity of a target sequence in a sample. The basic steps of qPCR are identical to those of PCR, as described in the previous section (3.2.1).

In qPCR, a special dye known as a fluorescent reporter is used as a kind of „light signal" to measure the amount of DNA during the PCR process.

There are two different strategies in qPCR that make it possible to determine the amount of DNA: a) dye-based and b) probe-based qPCR.

**a) Dye-based qPCR**

In the analysis, a mixture of nucleotides, a primer pair (forward and reverse), a DNA polymerase, and a fluorophore dye is added to the sample under investigation. SYBR Green is a dye commonly used in quantitative PCR (qPCR) to quantify DNA.

**1)** The first step is denaturation, in which the double-stranded DNA is split into two individual strands by heating it to around 95 degrees Celsius. **2)** In a subsequent step, the primers bind to specific regions of the DNA target sequence at a lower temperature. **3)** In the third step, a new DNA strand is synthesized with the help of DNA polymerase. The dye binds to the newly synthesized double-stranded DNA, generating fluorescence. This fluorescence is measured in each PCR cycle. Typically, 35-40 cycles are run. As the DNA target sequence is amplified, more binding sites for the dye are created, thus the increase in fluorescence directly correlates with the amount of double-stranded DNA present.

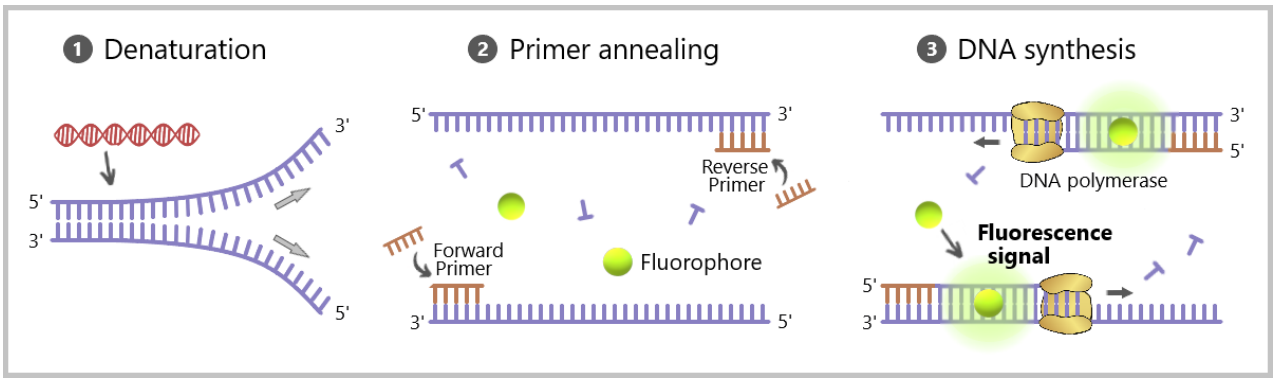



Fig. 40: Dye-based qPCR (schematic representation)

**The primers are crucial:** Amplification in dye-based qPCR requires the use of specifically designed primers. These primers bind selectively to specific sequences on

the individual strands of the target DNA. DNA synthesis begins only after primer binding occurs.

With the dye-based method, only one DNA target sequence can be detected at a time. As only one specific primer pair is required, this method is a fast and cost-effective option.

However, a disadvantage is that the dye may not exclusively bind to the target sequence, leading to inaccurate measurements. To ensure that only the intended DNA sequence was amplified during the polymerase chain reaction (PCR), a melting curve is typically generated after each experiment. This curve is important because each DNA sequence has its own characteristic melting point. A specific and expected melting curve indicates that the amplification was successful and specific.

**b) Probe-based qPCR**

In the analysis, a mixture of nucleotides, a primer pair, and a DNA polymerase is added to the sample under investigation. Additionally, a probe is introduced, which is labeled with a fluorophore (fluorescent dye) and a quencher (suppresses the fluorescence signal) (see Fig. 41). As long as the probe remains intact and is not degraded, the fluorescence signal is suppressed due to the spatial proximity to the quencher.

**1)** The first step is denaturation, where the double-stranded DNA is separated into two single strands by heating to about 95 degrees Celsius. **2)** In a subsequent step, both the primers and the specific probe bind to specific regions of the DNA target sequence at a lowered temperature. **3)** In the third step, facilitated by the DNA polymerase, synthesis of a new DNA strand occurs. During DNA synthesis by the DNA polymerase, the probe is degraded, leading to the separation of the fluorophore from the quencher and the release of the fluorescence signal. In qPCR, the quantity of fluorescent signals generated during DNA synthesis is measured in real-time.

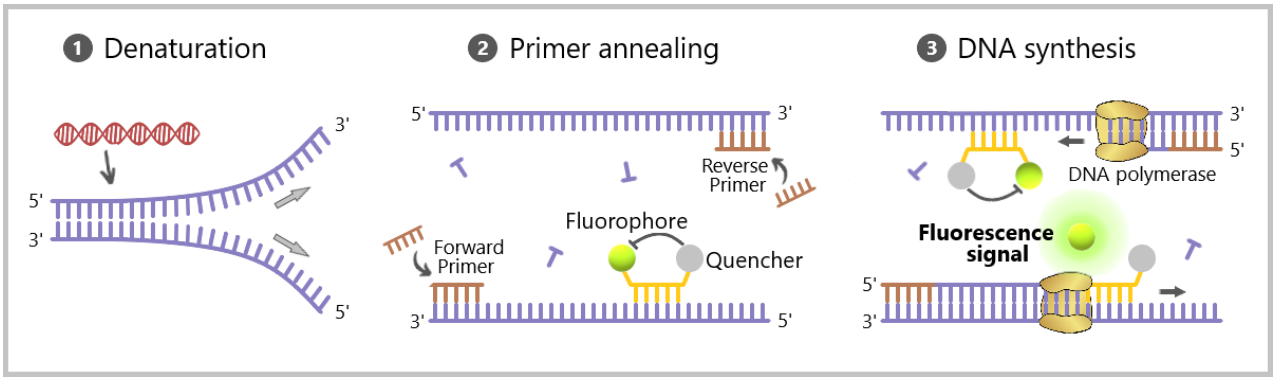



Fig. 41: Probe-based qPCR (schematic representation)

The increase in these light signals occurs in direct proportion to the amount of DNA that is amplified during each round of PCR. In other words, the measured fluorescence is proportional to the amount of DNA in the sample.

**Both primers and probes are crucial:** The primers bind to specific sequences on the individual strands of the target DNA. DNA synthesis only starts when the primer binds. The probes serve as an additional tool for recognizing and quantifying the target DNA. The probes in qPCR are designed to bind specifically to a particular DNA sequence. This sequence is selected so that it is unique and characteristic of the DNA fragment being searched for. The probes only bind to the target DNA if the sequence is present in the sample. This makes it possible to search specifically for a particular DNA sequence and to quantify its quantity. Therefore, the measurement in probe-based qPCR tends to be more accurate and specific compared to dye-based qPCR.

Detailed information on how the two qPCR methods work can be found here.

**qPCR can also be used for the quantification of RNA.**

This requires an additional step, the transcription of the RNA into complementary DNA (cDNA). This step is called reverse transcription (RT), and therefore the application of qPCR to RNA is called RT-qPCR or reverse transcription-qPCR. This cDNA then serves as a template for PCR amplification. The qPCR is then performed as described above to determine the amount of specific RNA in a sample.

**Limitations of qPCR**

In the polymerase chain reaction (PCR), the efficient amplification of DNA depends on its specific length. It is known that amplicons (amplified DNA fragments) of more

than 100 bp (base pairs) are preferred in quantitative PCR (qPCR). A base pair refers to the bond between two nucleotides (adenine-thymine and cytosine-guanine) within the DNA.

The reason is that longer amplicons enable more stable amplification and can improve the specific detection of the target gene. Amplicons with fewer than 100 base pairs (bp) may be less specific and tend to generate nonspecific products, which can lead to misinterpretations of qPCR results.

### 4.1.2. Fluorometry using DNA- or RNA- dyes

The fluorometric measurement of nucleic acids relies on the use of specific fluorescent dyes that selectively bind to DNA or RNA. These dyes emit a fluorescence signal only when they are bound to the target. The concentrations of nucleic acids are determined based on the fluorescence signals of the samples.

Basic steps of fluorometry for DNA and RNA quantification:

DNA or RNA is extracted from the sample and then purified. Contaminants such as proteins, lipids, or other chemical compounds can interfere with the binding of fluorescent dyes to the DNA or RNA or generate additional signals in fluorometry that do not originate from the nucleic acids.

A fluorescent dye is selectively chosen to enable specific binding to DNA or RNA. This binding marks the nucleic acids in the sample, facilitating the subsequent quantification of their quantity. The selection of the dye is based on its affinity for specific structures or properties of the nucleic acids. Examples include:

**RiboGreen for RNA:** RiboGreen is designed to bind specifically to ribonucleic acids (RNA). This dye does not differentiate between different types of RNA, but rather between RNA and DNA in general.

**PicoGreen for DNA:** PicoGreen is highly sensitive to DNA. This dye does not differentiate between different types of DNA, but rather between DNA and RNA in general.

**1)** After the specific dyes are added to the sample, they interact with the nucleic acids (DNA or RNA). **2)** The dye binds to the target nucleic acids in a specific

manner. This binding leads to the release of fluorescence, causing the labeled nucleic acids to emit light. **3)** The sample is loaded into the fluorometer, a device designed for fluorescence measurement. The fluorometer excites the dyes in the sample with light of a specific wavelength (λex). The fluorescent dye absorbs this light and emits light at a longer wavelength (λem). The emitted fluorescence is measured, and the **intensity of the signal** is recorded.

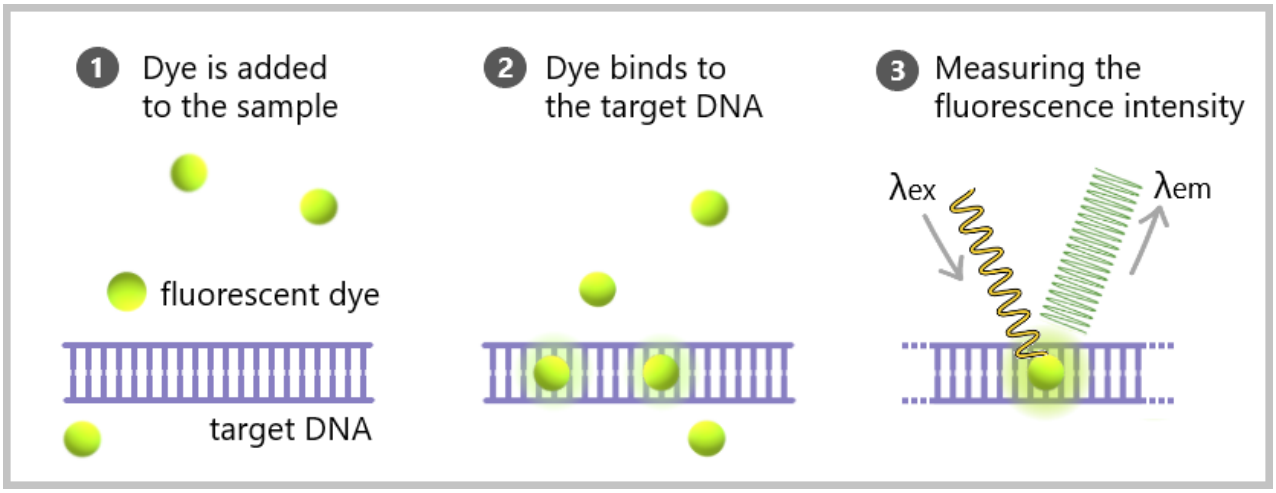



Fig. 42: Fluorometry with DNA Dyes (schematic representation)

To ensure accurate results, controls are carried out to ensure that the measured fluorescence originates exclusively from the target nucleic acid. A standard curve is often created in advance for quantification. The concentration of DNA or RNA in the sample is then calculated using this standard curve.

### 4.1.3. Qubit-Fluorometry

Qubit fluorometry is a specialized form of fluorometry used in the quantitative analysis of nucleic acids, particularly DNA and RNA. It relies on the use of fluorescent dyes that specifically interact with nucleic acids and emit a fluorescence signal when bound.

In contrast to conventional fluorometry techniques, which often rely on measuring fluorescence intensity, Qubit fluorometry uses the **measurement of fluorescence signals** emitted by dyes bound to nucleic acids. This enables highly sensitive and precise determination of DNA or RNA concentration in a sample, even at very low concentrations.

#### DNA measurement

For sample preparation, a nucleic acid-binding fluorescent dye is used that binds specifically to DNA and emits a fluorescent signal.

The Qubit fluorometer is calibrated to ensure it correctly responds to the fluorescence signals of the DNA dye solution and performs accurate measurements. A series of standard samples with known concentrations of DNA is prepared. These samples are used to create a standard curve representing the relationship between fluorescence intensity and DNA concentration.

**1)** The sample is mixed with the DNA dye solution and loaded into the Qubit fluorometer. **2)** The dye binds to the DNA and emits a fluorescence signal. **3)** The fluorometer measures the fluorescence signals of the samples and compares them with the standard curve to determine the concentration of DNA in the samples. The measured DNA concentrations are calculated using the standard curve and obtained as results of qubit fluorometry.

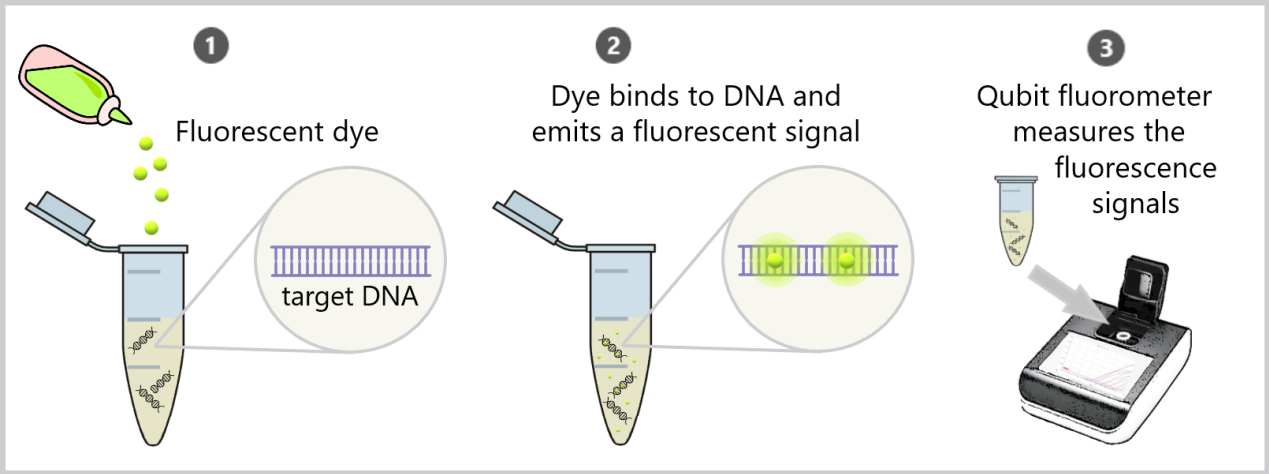



Fig. 43: Qubit Fluorometry (schematic representation)

The measurement of RNA is carried out similarly using a specific RNA fluorescent dye.

**Qubit fluorometry – a more accurate method for quantifying DNA or RNA**

Qubit fluorometry is often considered a more accurate method for quantifying DNA or RNA compared to other methods such as UV spectroscopy or gel electrophoresis. The main reason for the accuracy of qubit fluorometry is its ability to specifically and selectively measure the fluorescence of nucleic acids without interference from other substances in the sample. This enables precise quantification of nucleic acids, even at low concentrations and in complex samples.

Although qubit fluorometry is a precise method for measuring the total amount of DNA or RNA in a sample, it cannot distinguish between different sequences. It only measures the **total DNA concentration** or RNA concentration. If specific information about the DNA or RNA sequence is required, other methods such as qPCR must be used.

## 4.2. Oxford Nanopore Technology

Nanopore sequencing enables real-time analysis of long DNA or RNA fragments.

Nanopores are tiny openings that are only a few nanometers in size. They are embedded in a membrane made of a conductive material. This special membrane is placed in a chip.

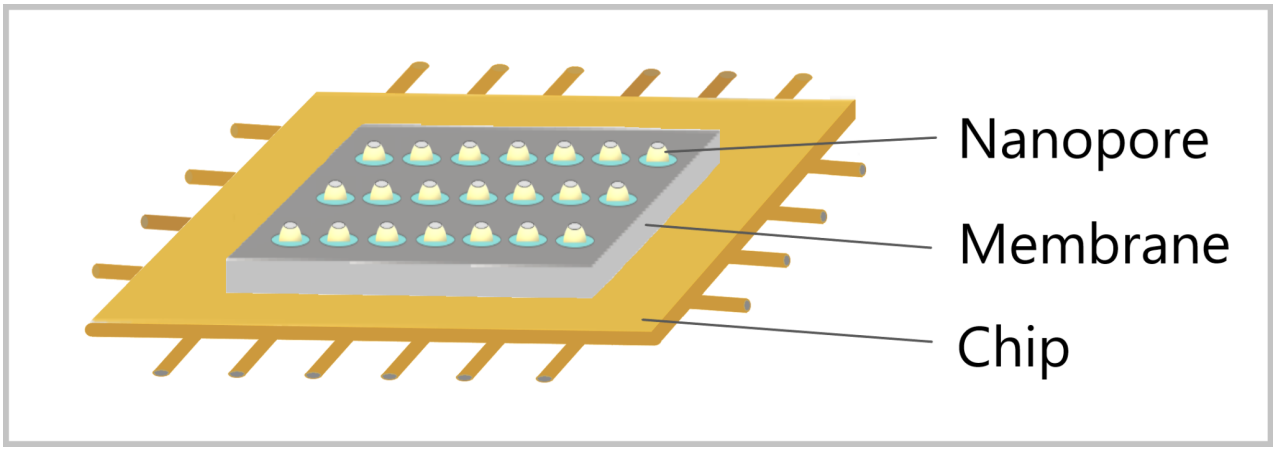



Fig. 44: Nanopores in conductive membrane on a chip (schematic representation)

The DNA or RNA sample is first fragmented into smaller pieces. This fragmentation facilitates the transport of the molecules through the nanopores. The fragmented DNA or RNA is then placed in a liquid called an electrolyte solution. This liquid contains dissolved salts and allows the charged molecules to move freely. The electrolyte solution is brought into contact with the nanopores to start the sequencing process.

A constant electrical voltage is applied to generate a current of ions through the nanopores. This causes the charged DNA or RNA molecules to migrate to the nanopores and pass through them (see Fig. 45).

Helicase, an enzyme, binds to the double strands of DNA, separates and unwinds

them into single-stranded forms to prepare them for transport through the nanopore. The action of helicase is not required for RNA, which is already single-stranded.

The motor protein, which is part of the helicase, then pushes the single-stranded DNA or RNA through the nanopore. As the DNA or RNA moves through the nanopore, it changes the electrical current flowing through the pore. This electrical signature depends on the sequence of the DNA that is passing through the pore. Each base in the DNA (Adenine, Cytosine, Thymine or Guanine) has a characteristic effect. This change in current is then perceived as a change in electrical voltage, which is measured at the nanopore. Software calculates the DNA sequence from the change in voltage.

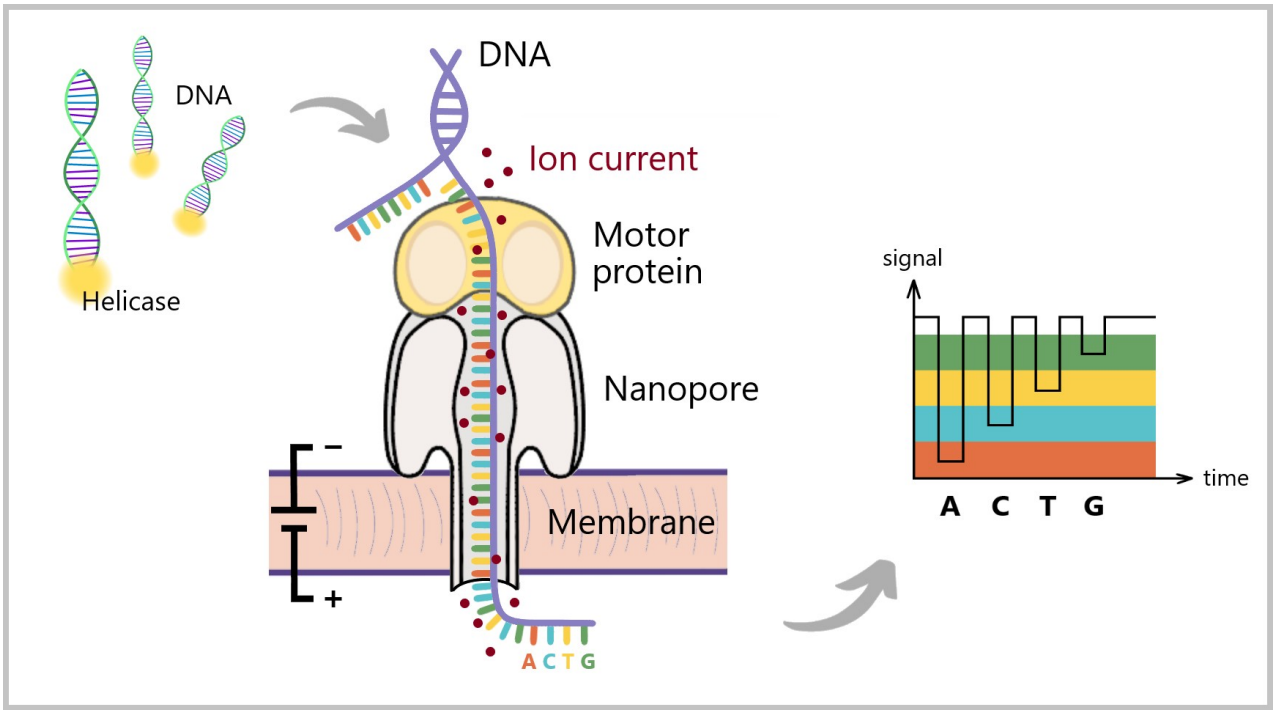



Fig. 45: Oxford Nanopore technology (schematic representation)

The result of the Oxford Nanopore technique is the sequence of the DNA or RNA sample examined. This sequence can provide information about the genetic composition, length or other important genetic characteristics.

This video is recommended for a better illustration of nanopore sequencing.

## 4.3. UV Spectroscopy

UV spectroscopy is a common technique for analyzing nucleic acids, which is used

to determine the concentration of DNA or RNA in a sample. The principle is based on the absorption of electromagnetic radiation by nucleic acids, especially in the UV range, which leads to characteristic absorption patterns. The following section explains the application of this technique to RNA.

In UV spectroscopy, special devices known as UV/VIS spectrophotometers are used. These comprise a radiation source, a monochromator consisting of a prism and a diaphragm, and a detector. The RNA to be analyzed is placed on a transparent sample carrier that allows UV light to pass through. During the measurement, the sample is placed between the monochromator and the detector. The monochromator ensures that only radiation of a certain wavelength hits the sample. RNA molecules absorb UV light at certain wavelengths, especially in the range of about 240 to 300 nm (nanometers), due to the presence of bases such as adenine, guanine, cytosine, and uracil.

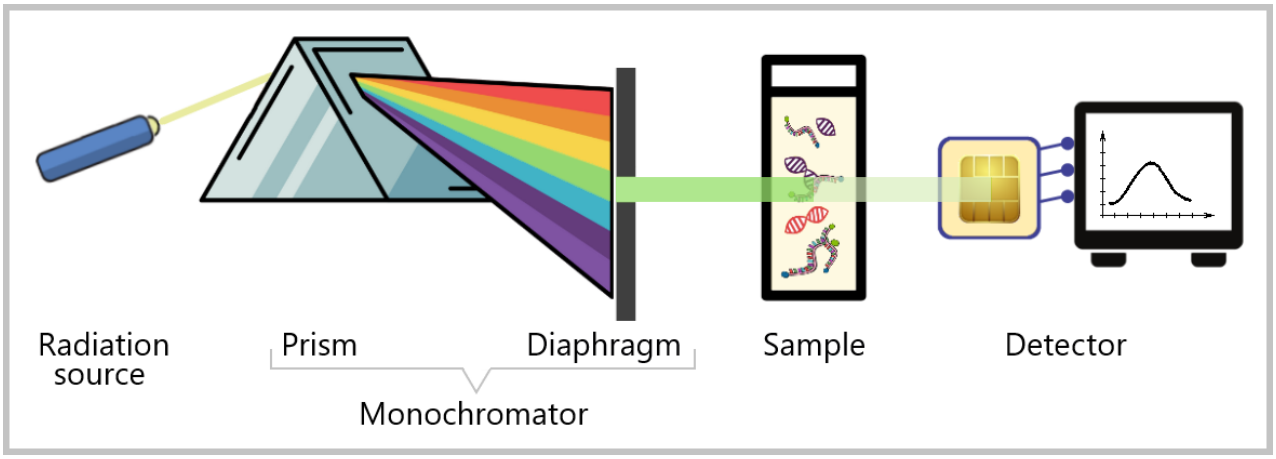



Fig. 46: UV-Spectroscopy (schematic representation)

The RNA solution is irradiated and absorbs part of the light, which attenuates the radiation. The more RNA it contains, the higher the absorption.

The absorption of UV light by the RNA molecules leads to a characteristic absorption pattern. By measuring the absorption at different wavelengths, UV spectroscopy can provide information about the concentration of RNA in the sample as well as its purity and structure.

Detailed information on this topic can be found here.

Although UV spectroscopy is an extremely useful technique for nucleic acid analysis, it also has some disadvantages, such as sensitivity to contaminants. To obtain

accurate results, UV spectroscopy requires high purity of the samples.

**If a sample contains both RNA and DNA, it can affect the measurement results of UV spectroscopy.** Since both RNA and DNA absorb UV light, the presence of DNA in the sample can lead to an overestimation of the measured RNA concentration. This is because the UV signal originates not only from the RNA but also from the DNA, resulting in an increased absorption value.

# 5. Measurement results and their interpretation

The detailed depiction of the fundamental production steps in the manufacturing and quality assurance of mRNA vaccines in Chapters 3 and 4 lays the groundwork for a deeper understanding of the analytical results of the independent laboratories and the resulting discussions.



## 5.1. Guidelines for limiting residual DNA in vaccines

Currently, the US FDA recommends that manufacturers of viral vaccines limit the amount of **residual DNA in the final product for parenteral vaccinations to below 10 ng/dose and restrict the size of the DNA to less than the size of a functional gene or ~200 base pairs.** [1] This is also in line with the recommendations of the World Health Organization (WHO). [2, 3] Previous guidelines set by the FDA in 1985 established residual DNA limits at 10 pg/dose (10 pg = 0.01 ng). A WHO study group concluded in 1986 that the risk is negligible up to 100 pg/dose, and in 1996, the WHO further increased the limits to up to 10 ng per dose. [2] [Study]

**[1]** https://www.fda.gov/media/113760/download
**[2]** https://pubmed.ncbi.nlm.nih.gov/17321940/
**[3]** https://cdn.who.int/media/docs/default-source/biologicals/documents/trs_978_annex_3.pdf

# 5.2. The measurement methods used by Pfizer

To ensure compliance with the limits defined by the World Health Organization (WHO) during the production process, Pfizer uses the quantitative Polymerase Chain Reaction (qPCR) measurement method to quantify residual DNA content (see Fig. 47).

The figure also indicates that the manufacturer employs „UV Spectroscopy“ and fluorescence assay methods to determine the RNA quantity. The term „fluorescence assay“ is a broad term encompassing various measurement methods based on fluorescence. It could be fluorometry with a specific fluorescent dye, as neither qPCR nor qubit fluorometry are explicitly mentioned in the protocol.

The manufacturer selectively uses different measurement methods to determine the RNA content and DNA content (see also here, here and here).

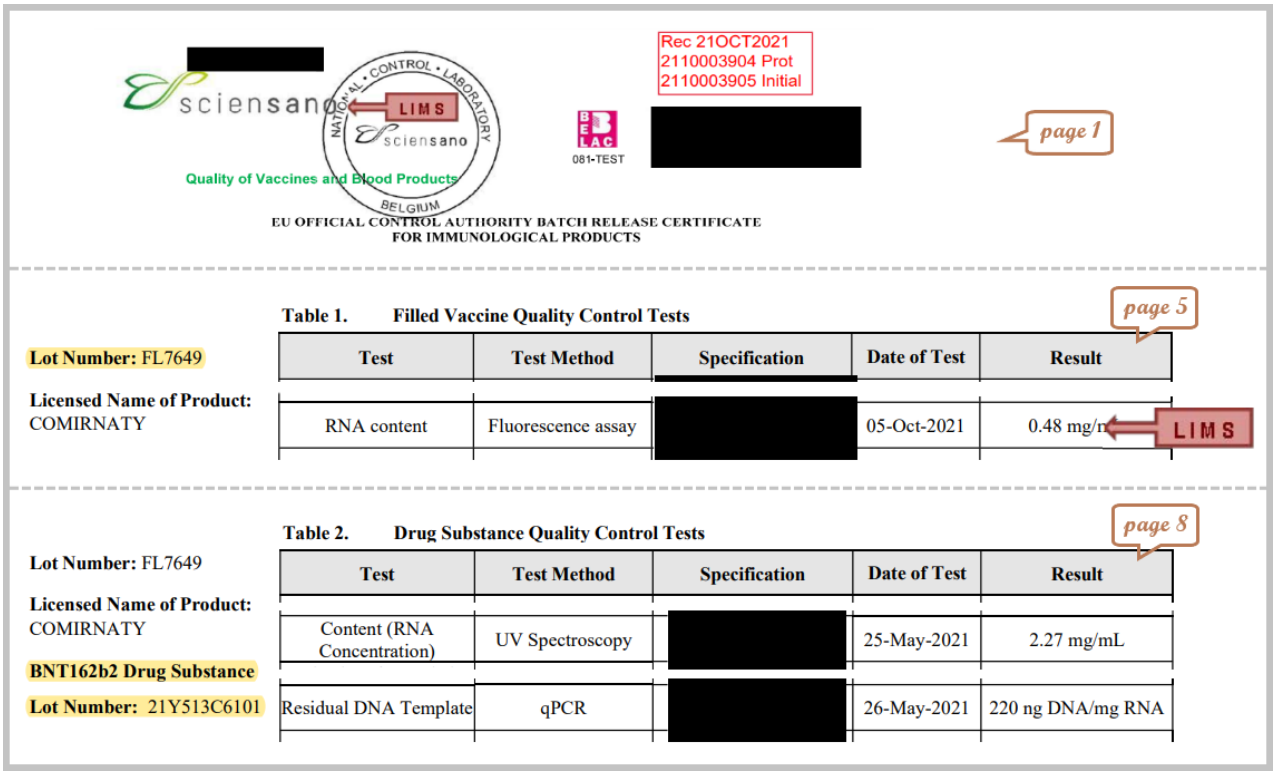



Fig. 47: EU Official Control – Quality control tests [Source]

It is important to note that the measured values for RNA and DNA content depend on the measurement method used. A higher measured RNA content and lower residual DNA quantity typically indicate higher product quality. It should be noted that there are no regulatory requirements specifying which measurement methods should be used for quality assurance.

## 5.3. DNA fragments detected in COVID-19 vaccines

In the study titled „**DNA fragments detected in COVID-19 vaccines in Canada**", RNA sequencing of COVID-19 mRNA vaccines was conducted. Dr. David Speicher is the lead author of this study, while Dr. Kevin McKernan is listed as a co-author. The investigation revealed DNA fragments originating from the plasmids used in the manufacturing process. All information provided here is from the study conducted by Speicher et al.

**The study examined** 27 COVID-19 vaccine vials originating from Canada and belonging to 12 different batches from Moderna and Pfizer. Here is a detailed list:

Number of vials per vaccine type:

- Pfizer-BioNTech BNT162b2: 8 vials (unopened, expired)
- Moderna Spikevax mRNA-1273: 16 vials (unopened, expired)
- Moderna XBB.1.5: 3 vials (used, not expired)

Details of the examined batches:

- 5 batches of monovalent Moderna vaccines for children/adults
- 1 batch of bivalent BA.4/5 vaccine for adults
- 1 batch of bivalent Wuhan-BA.1 vaccine for children and adults
- 1 batch of monovalent Moderna XBB.1.5 vaccine
- 3 batches of monovalent Pfizer vaccines for adults
- 1 batch of bivalent Wuhan-BA.4/5 Pfizer vaccine for adults

### 5.3.1. Measurement methods and results of the study

The study used established and standardized methods for the detection of DNA and RNA, including **quantitative polymerase chain reaction (qPCR)** and **qubit fluorometry techniques**, using previously published primer and probe sequences. **Oxford Nanopore sequencing** was used to determine the size distribution of DNA fragments. The methods used have already been presented in Chapter 4.

The measurement results are presented in the following tables, with the ranges of the measured values provided. The values in **ng/dose** (nanograms per dose) represent the measured quantities of DNA per vaccine dose.

**Pfizer**

| | qPCR | Qubit fluorometry | WHO limit |
|---|---|---|---|
| origin of replication (ori): | 0,28 – 4,27 ng/Dose | total: 1.896 – 3.720 ng/Dose | 10 ng/Dose |
| spike sequences: | 0,22 – 2,43 ng/Dose | | |

**Moderna**

| | qPCR | Qubit fluorometry | WHO limit |
|---|---|---|---|
| origin of replication (ori): | 0,01 – 0,34 ng/Dose | total: 3.270 – 5.100 ng/Dose | 10 ng/Dose |
| spike sequences: | 0,25 – 0,78 ng/Dose | | |

In qPCR, specific primers are used to amplify only the DNA sequences that are specific for the plasmid origin of replication (ori) and the spike sequences. Therefore, the values for each sequence can be measured and quantified separately.

Qubit fluorometry, on the other hand, measures the total amount of DNA in the sample, regardless of the specific sequence. Therefore, in qubit fluorometry, no distinction is made between the individual sequences, but the total amount of DNA in the sample is quantified.

### Conclusion

The available data indicate that in the tested mRNA COVID-19 vaccines, there are billions to hundreds of billions of DNA molecules per dose. **Using Qubit fluorometry, all tested vaccines exceed the WHO guidelines for residual DNA of 10 ng/dose by 188- to 509-fold**. Interestingly, the residual DNA content measured by the qPCR method in all tested products was below these guidelines. These results demonstrate the importance of clear and consistent interpretation of guidelines for DNA quantity. [Study]

At this point, it is important to note that the measured data strongly depend on the

measurement method used. Thus, the study highlights an existing weakness in regulatory guidelines, which allows for the selective use of different measurement methods to obtain desired results (see Fig. 47).

**In Patent US10077439B2 by Moderna**, authored among others by Stéphane Bancel, CEO of Moderna, it is explicitly noted that quantitative Polymerase Chain Reaction (qPCR) underestimates the total amount of existing DNA.

**[00103]** … **Quantitative PCR is often applied to measure the residual DNA but it only detects the DNA molecules that contain both qPCR primers thus does not measure all other smaller DNA molecules that are partially digested.** …

See also Chapter 4.1.1.c) Limitations of qPCR. For more information, the article „Fluorometry Deep Dive" by McKernan is recommended.

„*The important thing for regulators to know is that no one technology gives you the answer*", McKernan said in an interview with REBEL News Canada.

That means employing multiple measurement methods to determine DNA and RNA values would enhance the reliability of the results and identify potential discrepancies between the methods. The use of multiple methods allows for internal validation of the results and can help identify potential sources of error. For instance, the qPCR method could be used to measure DNA and RNA values, and the results could then be compared with the values obtained through the Qubit fluorometry measurement method. By comparing the results from different methods, potential differences or inconsistencies can be identified.

### 5.3.2. An unexpected finding: SV-40 promoter/enhancer

Speicher et al. found SV40-promoter-enhancer-ori-sequences and SV40-polyA-sequences in the Pfizer vaccines. See also Table 2 of the study.

SV40, which stands for Simian Virus 40, is a virus discovered in 1960 in kidney cells of monkeys. It can infect both monkeys and humans. SV40 is known for its use in biomedical research and as a contaminant in some earlier batches of polio vaccines.

„*It is not the full SV-40 virus as seen in polio vaccines.*" [McKernan]

**SV40** contains certain genetic sections that play an important role in how genes are activated. These sections include the SV40 enhancer and the SV40 promoter.

The **SV40 promoter** acts as a starting sequence for a gene. When a gene is expressed (active), it means that the genetic information of that gene is translated into a protein. The SV40 promoter is a particularly strong starting sequence capable of efficiently initiating gene expression. Imagine gene expression as starting a motor in a car. The SV40 promoter is like the ignition key that turns on the engine. When the ignition key is turned, the engine is activated, and the car starts moving.

The **SV40 enhancer** is located near the promoter and can increase the activity of the promoter. The SV40 enhancer can then be regarded as a turbo boost that gives the engine extra power. When the turbo boost is activated, the car accelerates faster and reaches a higher speed. Similarly, the SV40 enhancer boosts the activity of the SV40 promoter and increases the expression of the gene, similar to how the turbo boost increases the power of the engine.

McKernan: „*Another concerning discovery was the finding of an SV-40 promoter in the vials… This is something that helps promote the antibiotic resistance that is the backbone of these vectors. … If this DNA is at high levels inside these vaccines then that promoter could integrate into the genome and there's concern with injecting high amounts of SV-40 promoters because there is a risk it will be promoting the expression of genes inappropriate in the genome.*"

### SV40 components and regulatory authorities

The figure below shows Pfizer's plasmid map on the left, which was reconstructed using McKernan's measurement results. On the right is the plasmid map that Pfizer included in the approval documents for the European Medicines Agency (EMA). It can be clearly seen that the SV40 components are missing from the plasmid map on the right.

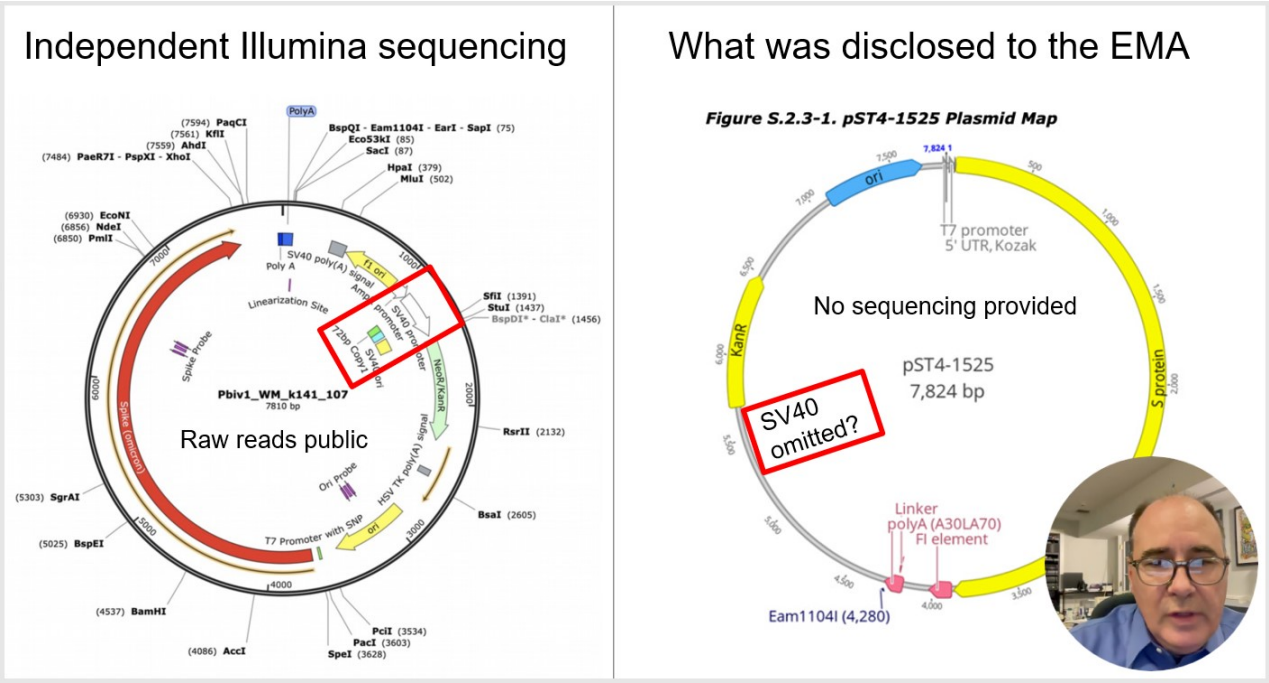



Fig. 48: Plasmid map (right) disclosed to the EMA (see Figure S.2.3-1. pST4-1525 Plasmid Map) [Powerpoint recording Massachusetts slides, slide 6]

Since the guidelines of the World Health Organization (WHO) and the U.S. Food and Drug Administration (FDA) mandate that all open reading frames and promoters, such as the SV40 components, must be disclosed, McKernan's team confronted the authorities with their measurement results. As a result, the European Medicines Agency (EMA), the FDA, and Health Canada confirmed that indeed SV40 components are used in the plasmid. [Powerpoint recording Massachusetts slides, Slide 17]

In an email to The Epoch Times (October 2023), Health Canada writes: „Although the full DNA sequence of the Pfizer plasmid was provided at the time of initial filing, the sponsor did not specifically identify the SV40 sequence."

Shortly afterwards, the European Medicines Agency (EMA) also informed The Epoch Times in an email: „While the full DNA sequence of the plasmid starting material was provided in the initial marketing authorization application for Comirnaty, the applicant did not specifically highlight the SV40 sequence. … BioNTech did not highlight the inclusion of the enhancer in its vaccine because „it was considered to be a non-functional part of the plasmid." They have since clarified this information in response to questions raised by EMA."

We remember that the **EMA recommended conditional approval** for the COVID-19 vaccine Comirnaty from BioNTech and Pfizer **at the end of 2020**. It was

only three years later that the authorities found the SV40 sequence in the Comirnaty marketing authorization application.

A **spokesperson for the European Medicines Agency declared** that the agency „can confirm that it has not seen any reliable evidence of residual DNA exceeding approved/safe levels" for the Pfizer/BioNTech or Moderna COVID-19 vaccines. Nor is the EMA „aware of scientific evidence showing that the very small amounts of residual DNA that may be present in vaccine batches could integrate into the DNA of vaccinated individuals".

The potential risks associated with residual DNA content are discussed in Chapter 7, „Are further investigations necessary?"

### 5.3.3. Size profile of residual DNA

To capture the different sizes or lengths of residual DNA molecules, Speicher DJ et al. sequenced the BNT162b2 vaccine using Oxford Nanopore (ONT) sequencing (Fig. 49).

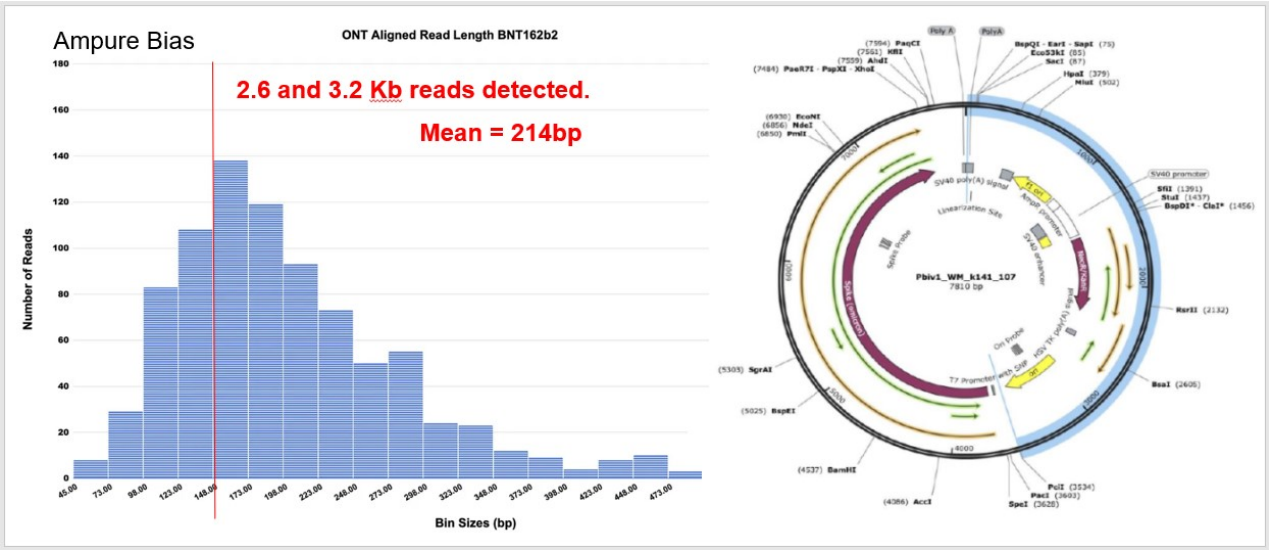



Fig. 49: Oxford Nanopore Sequencing [Study, left: Fig.10, right: Fig.11]

The sequencing revealed a total plasmid length of 7810 bp (base pairs). As shown in the figure (left), a large portion of the measured DNA molecules' mass is small. The analysis indicated an average DNA fragment length of 214 bp.

„One in fifty molecules was more than 1,000 bases long." [McKernan, Covid-19

Vaccine Expert Panel Briefing to the Massachusetts Legislature and Public Health Officials, 1:29:30]

They also found a fragment that was 3.2 kb (kilobase pairs) or 3200 bp (base pairs) long and covered the entire backside of the plasmid (see figure on the right, shown as a blue vector region). In an interview, McKernan explained that this 3,200 base pair fragment contained the gene for resistance to the antibiotics kanamycin and neomycin, including the corresponding SV40 promoter. He continued: „Other fragments of similar length included the sequence for the spike protein."

According to the guidelines of the World Health Organization (WHO), it is recommended to limit the size of the residual DNA to less than the size of a functional gene or about ~200 base pairs.

# 5.4. Reproduction of the study results

### 5.4.1. Dr. Phillip Buckhaults

McKernan and Speicher's findings were confirmed by Dr. Phillip Buckhaults, Professor at the University of South Carolina. Dr. Phillip Buckhaults is an experienced molecular biologist and cancer geneticist with extensive experience in next-gen sequencing applications for global gene expression analysis and gene mutation detection. He presented his findings on DNA contamination related to the mRNA COVID-19 vaccines at a South Carolina Senate hearing in September 2023. The original post has been removed from YouTube, but can still be found here and here.

Dr. Buckhaults and his colleagues identified foreign DNA in batches of Pfizer-BioNTech (Comirnaty) and Moderna (Spikevax). [@P_J_Buckhaults]

In the hearing, Dr. Buckhaults describes his findings:

*„So this is a picture of the sequencing read that the sequencing run that I did in the lab from a couple of batches of the Pfizer vaccine. And all those little bitty lines here (Fig. 50) are the little tiny pieces of DNA that are in the vaccine. They don't belong there. They are not part of the sales pitch or the marketing campaign. And there's a lot of them."*

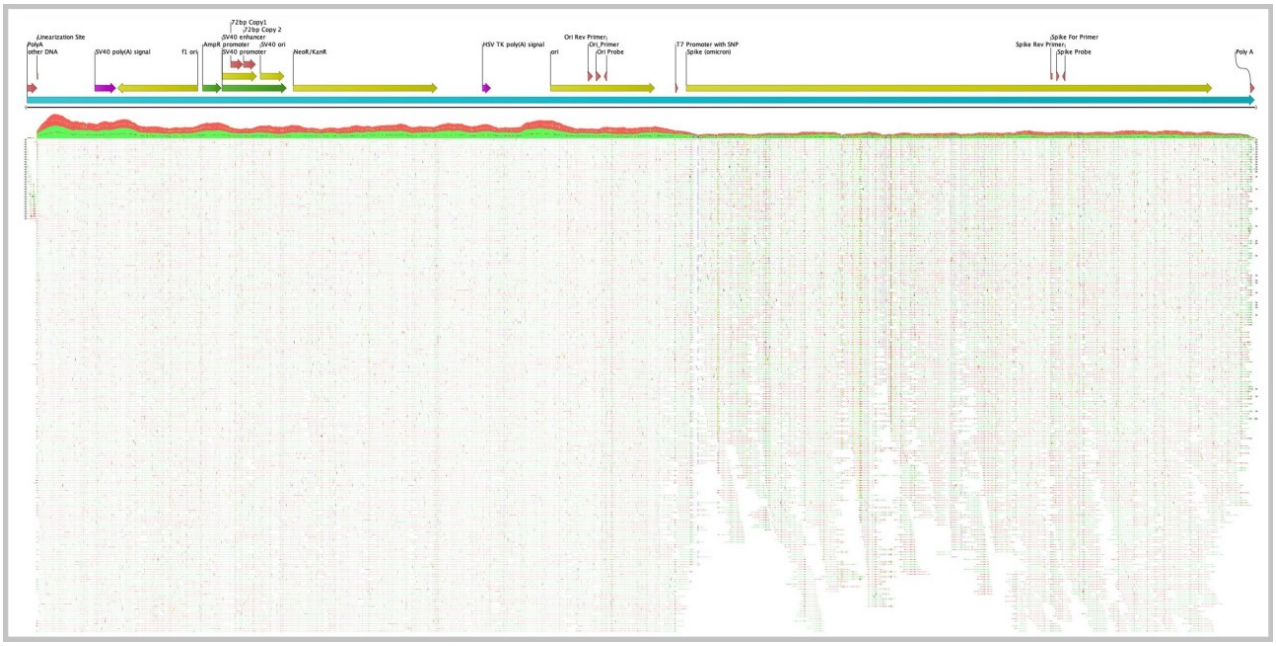


Fig. 50: DNA pieces found in the Pfizer vaccine [source]

*„This little graph here (Fig. 51, left) in the middle is the size distribution it Peaks around 100 base pairs 120 bas pairs. The DNA pieces that are in the vaccine are short little pieces 100 120 there's soma that are about 500 base pairs a few that are even 5.000 but most of them are around 100 base pairs. Why is this important? Because* ***the probability of a DNA piece of DNA integrating into the human genome is unrelated to its size. Your genome risk is just a function how many particles there are.*** *… All these little pieces of DNA that are in the vaccine many many thousands of opportunities to modify a cell of a vaccinated person. The pieces are very small because during the process they chopped them up to try to make them go away but they actually increased the hazard of genome modification in the process."*

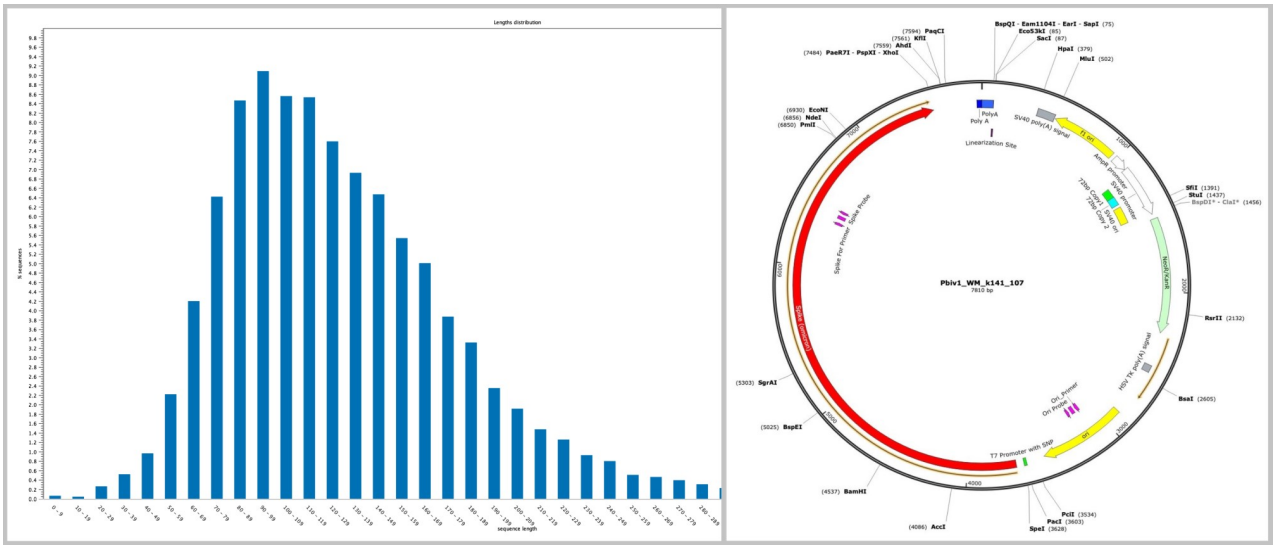


Fig.51: left) Distribution of the DNA pieces in the Pfizer vaccine according to the length of the ba-

se pairs; right) Replicated plasmid map based on the sample analysis [source]

*„So we took all these pieces of DNA and we used them to glue together, what the source DNA must have been. This is what we do in the lab all the time and all these little red and green lines here (Fig. 50) these are all independent little pieces of DNA. This must have had 100.000 pieces of DNA in this sequencing run and you can put them all back together and see what they came from is this circle over here (Fig. 51, right).“*

*„It's a plasmid that you can go shopping online to buy from Agilent. And it's clear that Pfizer took this plasmid and then they clon Spike into it and they used it for in a process called In-vitro- Transcription/Translation where you feed an RNA polymerase with this plasmid and it makes a whole bunch of mRNA copies for you.* ***And then you take this mRNA you mix it with the lipid nanoparticle transection reagent and now you've got your mRNA vaccine but they failed to get the DNA out before.*** *They did this****. So these little pieces they did make some effort to chop it up so all these little pieces of the plasma got packaged in with the RNA.*** *That's clear as day what happened just from the forensics of looking at the DNA sequencing.“*

*„We can measure the quantity of this stuff pretty easy in the lab. This is we're good at doing this kind of stuff … We can quantify exactly how much of this stuff is in a vaccine or any other tissue and you know I estimate that there were about two billion copies of the one piece that we're looking for in every dose. … But if you see 2 billion copies of this there's about 200 billion of everything else.* ***So what this means is that there's probably about 200 billion pieces of this plasma DNA in each dose of the vaccine and it's encapsulated in this lipid nano particle so it's ready to be delivered inside the cell. This is a bad idea.“***

### 5.4.2. Dr. Jürgen O. Kirchner and Prof. Dr. Brigitte König

The German biologist Dr. Jürgen O. Kirchner warned of the problem of DNA contamination of the BioNTech vaccine as early as 2022 in the book „The mRNA Machine“, which he published under the pseudonym David O. Fischer.

Kirchner is a PhD biologist with extensive professional experience in the pharmaceutical industry. He has worked for various pharmaceutical companies and has

been extensively involved in studies aimed at demonstrating the effectiveness of medications. In his role, he has also been responsible for marketing pharmaceuticals and obtaining their approval.

Dr. Kirchner sent batches of the BioNTech vaccine delivered to Germany for examination to the laboratory of Prof. Dr. Brigitte König in Magdeburg.

Prof. Dr. König studied biology, chemistry and medicine, habilitated in „Medical Microbiology and Infection Immunology" and is an external professor at the Faculty of Medicine at Otto von Guericke University in Magdeburg.

The following passages from Dr. Kirchner's interview with The Epoch Times provide insights into his findings.

*„The DNA contaminations in the mRNA vaccines are a very serious issue, which initially was not so apparent, although it is stated in the reports of the European Medicines Agency EMA as early as February 2021, that these vaccines are contaminated with DNA. The extent was not known. This was discovered by an American scientist, Kevin McKernan, and his team, and was published in April of this year (2023)."*

*„Subsequently, I found a laboratory and requested German batches of this vaccine to be examined to see how things stand with us. And the results were a disaster – highly significant contaminations with DNA."*

*„This vaccine – you asked where it comes from – is contaminated with DNA because it is produced with the help of bacteria. These bacteria provide the DNA template, which is then used to produce and multiply the mRNA. In the clinical trials, this was done without bacteria, which means that the vaccines in the clinical trials were not nearly as contaminated with DNA as the commercial products. This also means that the risk posed by the DNA in the vaccines was not clinically tested at all, because the vaccine in the clinical trials did not have this. So this whole issue is a huge black hole where nobody knows what is behind it. And that needs to be changed very quickly."*

*„Charges of the BioNTech vaccine were tested. Currently, we are using data from five batches, from which we were able to obtain sealed batches. The limit for contamination has been set by the WHO itself at 10 ng (nanograms) per dose. The lo-*

*west concentration found in one vaccine was 83 times higher, and the highest was 355 times higher. … We're not talking about slightly exceeding the limit here; rather, we're talking about having 3500 instead of 10. This is something one must simply consider."*

The results of their analyses are summarized in the following table.

| BioNTech Charge | DNA ng/µl | DNA ng/Dosis | Vielfaches von 10 ng ** | Plasmid-nachweis |
|---|---|---|---|---|
| **GH9715** | 9,45 | 2835 | **284fach** | **JA** |
| **FW1374** | 7,78 | 2334 | **233fach** | **JA** |
| **34396TB** | 3,38 | 1014 | **101fach** | **JA** |
| **ACB5317** | 11,80 | 3540 | **354fach** | **JA** |
| **FP1972** | 2,78 | 834 | **83fach** | **JA** |

** Laut WHO beträgt der Grenzwert für DNA in Parenteralia 10 ng pro Dosis (Gesamt-DNA).

The results were presented to the general public in an MDR report on December 12, 2023. However, the MDR report was removed from the MDR media library shortly afterwards. The report can still be viewed at this link.

In response to inquiries from The Epoch Times about why the MDR report was deleted, an MDR spokesperson provided the following response: „MDR broadcast a report on coronavirus vaccines on 12.12.2023 at 8.15 p.m. in the program ‚Umschau'. After careful internal review, it is clear that our journalistic due diligence criteria were not met. The report was depublished on 17.12.2023." However, MDR did not say exactly which „journalistic due diligence criteria" were not met in the report.

### 5.5. Brief summary

- The measured values for DNA and RNA depend on the specific measurement method used for analysis.
- There is no uniform regulatory standard from authorities prescribing which measurement methods must be used by manufacturers to test the RNA and DNA of a substance.
- Large quantities of small DNA fragments were found, exceeding the WHO recommended limit of 10 ng/dose.
- Occasionally, very long DNA molecules were also found, exceeding the WHO

recommended length of 200 base pairs.
- Functional components of the SV40 virus were found, including the SV40 promoter and enhancer.
- The residual DNA, which may be present during the packaging of mRNA into lipid nanoparticles, could also be packaged into the lipid nanoparticles. These lipid nanoparticles help increase the stability and protection of the encapsulated nucleic acids by shielding them from degradation by enzymes such as nucleases that are normally present in biological systems.

# 6. Official reactions and knowledge regarding foreign DNA



## 6.1. The European Medicines Agency (EMA)

### 6.1.1. What is the EMA and what is its role?

The European Medicines Agency (EMA) is an agency of the European Union based in Amsterdam, the Netherlands. The EMA was established to facilitate a standardized approval process for medicines across the European Union, ensuring faster access to new medicines for patients. Here are some of the main tasks of the EMA:

**Authorization of medicinal products:** The EMA plays a central role in evaluating applications for the approval of new medicines in the European Union. It assesses the quality, safety, and efficacy of medicines before they are made available on the European market.

**Monitoring the safety of medicinal products:** The EMA continuously monitors the safety of already authorized medicinal products. This includes the collection of data

on side effects and other safety-relevant information. If necessary, measures can be taken to ensure the safety of patients, such as the amendment of package leaflets or the revocation of marketing authorizations.

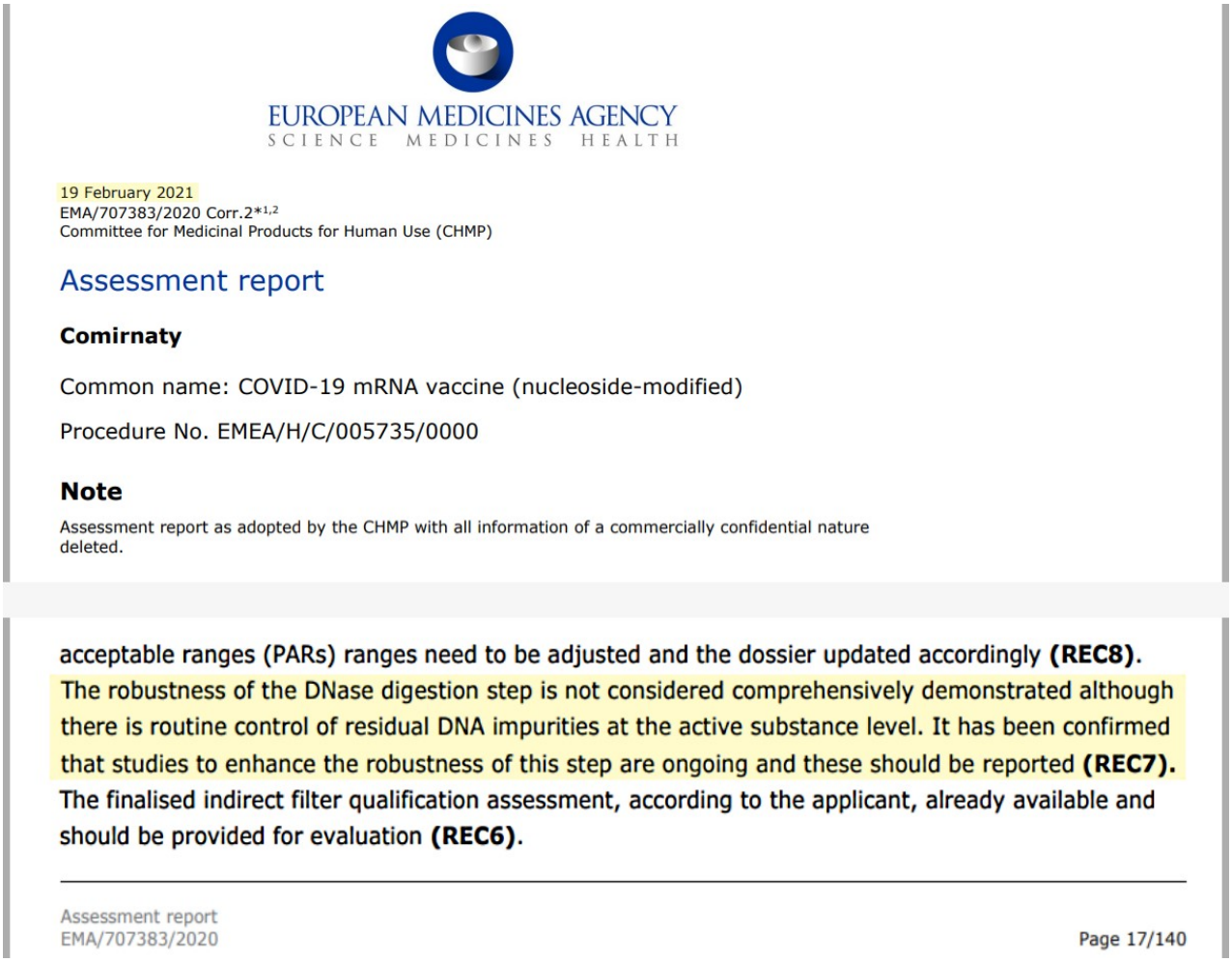
**Quality control:** The EMA assesses the quality of medicines and ensures they meet required standards. This encompasses the manufacturing, testing, and packaging of medicines.

### 6.1.2. What did the EMA know about the DNA residues?

The following is a review of some EMA documents along with corresponding explanations. The redacted areas in the documents were made by the EMA itself.

An „Assessment Report" is an evaluation report prepared by the EMA to document the assessment of a medicinal product by the Committee for Medicinal Products for Human Use (CHMP). This report includes a comprehensive analysis of all available data. Additionally, it may also include recommendations and requirements for further monitoring of the medicinal product after approval.

**EMA document from February 2021**



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

19 February 2021
EMA/707383/2020 Corr.2*[1,2]
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

**Comirnaty**

Common name: COVID-19 mRNA vaccine (nucleoside-modified)

Procedure No. EMEA/H/C/005735/0000

**Note**

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

acceptable ranges (PARs) ranges need to be adjusted and the dossier updated accordingly **(REC8)**. The robustness of the DNase digestion step is not considered comprehensively demonstrated although there is routine control of residual DNA impurities at the active substance level. It has been confirmed that studies to enhance the robustness of this step are ongoing and these should be reported **(REC7).** The finalised indirect filter qualification assessment, according to the applicant, already available and should be provided for evaluation **(REC6)**.

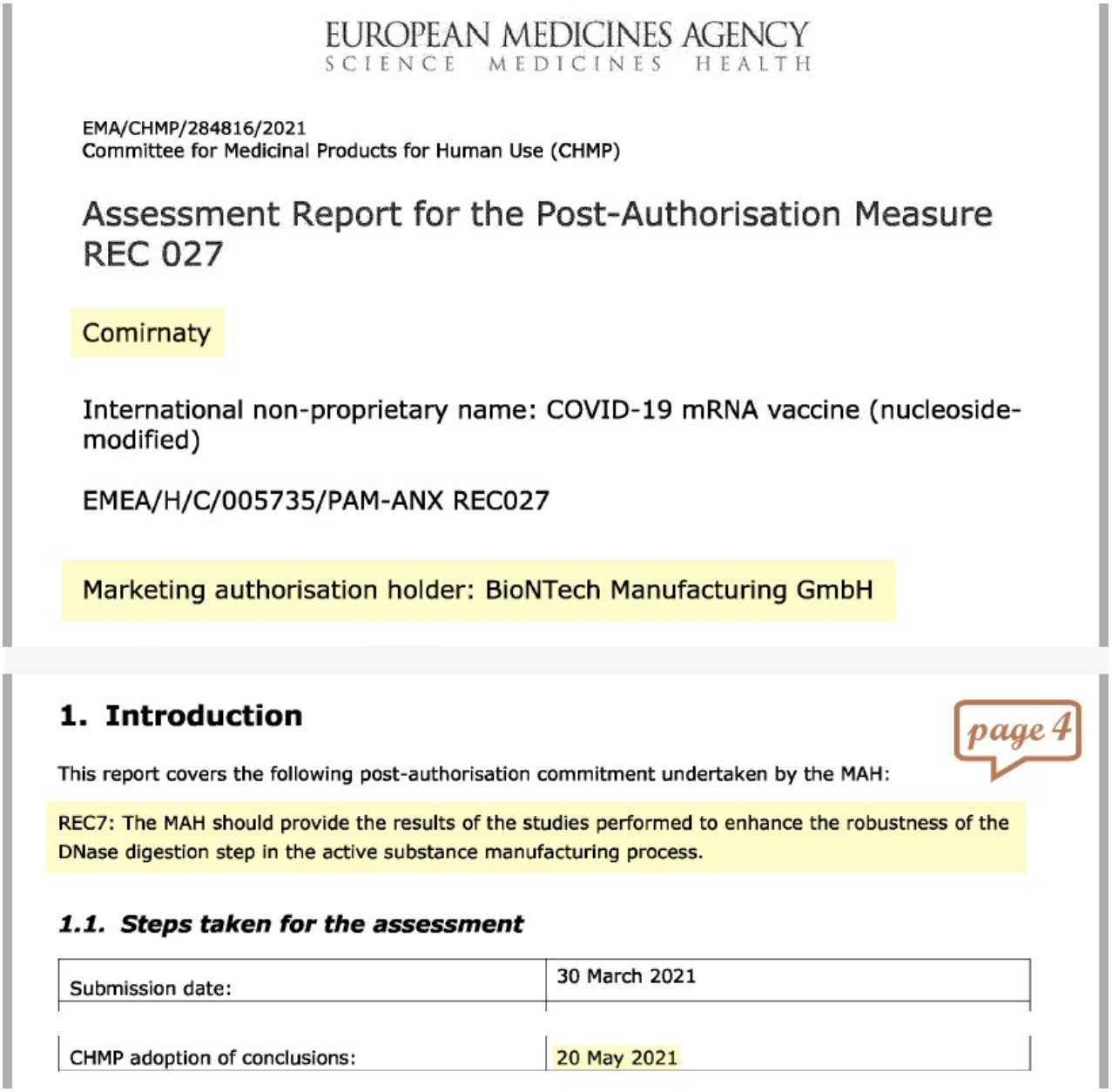
Assessment report
EMA/707383/2020 Page 17/140

EMA-Document, 19th February 2021

*„The robustness of the DNase digestion step is not considered comprehensively demonstrated although there is routine control of residual DNA impurities at the active substance level. It has been confirmed that studies to enhance the robustness of this step are ongoing and these should be reported (REC7)."*

In the EMA's Assessment Report from February 2021, it was noted that the robustness of the DNase digestion step had not been fully demonstrated, despite routine control of residual DNA impurities at the active substance level. It was confirmed that studies to improve the robustness of this step are ongoing and that these studies should be reported according to the recommendation (REC7) of the marketing authorization holder (BioNTech).

### EMA document from May 2021



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

EMA/CHMP/284816/2021
Committee for Medicinal Products for Human Use (CHMP)

Assessment Report for the Post-Authorisation Measure REC 027

Comirnaty

International non-proprietary name: COVID-19 mRNA vaccine (nucleoside-modified)

EMEA/H/C/005735/PAM-ANX REC027

Marketing authorisation holder: BioNTech Manufacturing GmbH

**1. Introduction**

page 4

This report covers the following post-authorisation commitment undertaken by the MAH:

REC7: The MAH should provide the results of the studies performed to enhance the robustness of the DNase digestion step in the active substance manufacturing process.

***1.1. Steps taken for the assessment***

| | |
|---|---|
| Submission date: | 30 March 2021 |
| CHMP adoption of conclusions: | 20 May 2021 |

EMA-Document from May 2021

*„This report covers the following post-authorisation commitment undertaken by the MAH: REC7: The MAH should provide the resilts of the studies performed to enhance the robustness of the DNase digestion step in the active substance manufacturing process."*

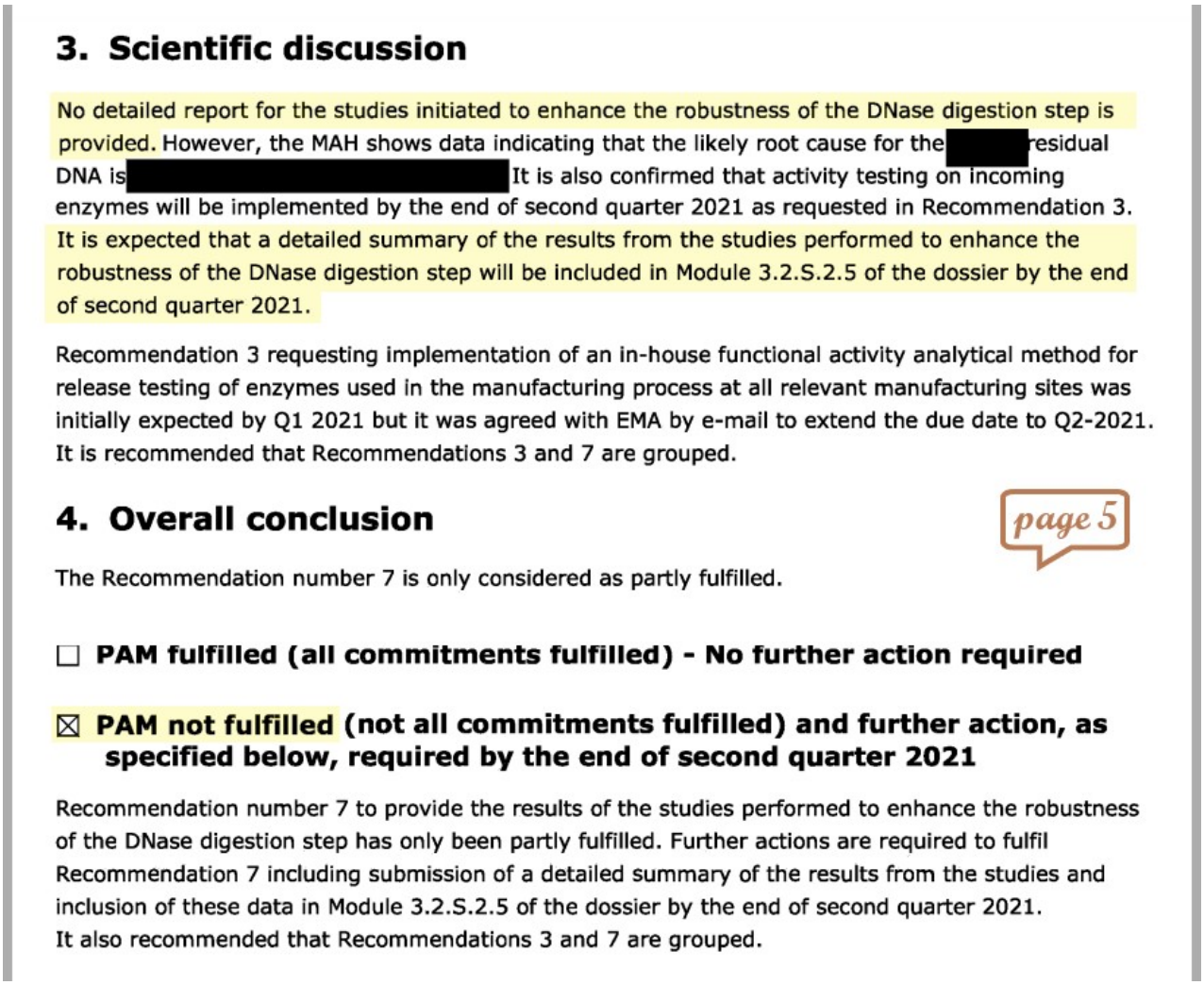
In this excerpt of the EMA document, „MAH" refers to the Marketing Authorization Holder, which is the entity holding the market authorization (BioNTech) for the medicinal product Comirnaty.

The passage states that the MAH has taken on a post-authorization commitment to provide the results of studies conducted to improve the robustness of the DNase digestion step in the active substance manufacturing process.

To clarify this:

- As described in section 3.5.2., **DNase** is an enzyme that degrades DNA.
- The **manufacturing process of the active substance** refers to the process by which the actual active substance of the drug is produced.
- The **DNase digestion step** is a specific step in the manufacturing process where DNase is used to digest and remove DNA (see chapter 3.5.2. Purification of synthetic mRNA – Process 2).
- The **robustness** of this step refers to how stable and reliable this step is in the manufacturing process, and how well it functions in different situations.

The EMA requires the MAH to provide the results of studies conducted to ensure that this specific step in the manufacturing process of the active substance is reliable and stable. This is to ensure the quality and safety of the medicinal product, especially with regard to potential impurities from DNA residues.



## 3. Scientific discussion

No detailed report for the studies initiated to enhance the robustness of the DNase digestion step is provided. However, the MAH shows data indicating that the likely root cause for the [redacted] residual DNA is [redacted] It is also confirmed that activity testing on incoming enzymes will be implemented by the end of second quarter 2021 as requested in Recommendation 3. It is expected that a detailed summary of the results from the studies performed to enhance the robustness of the DNase digestion step will be included in Module 3.2.S.2.5 of the dossier by the end of second quarter 2021.

Recommendation 3 requesting implementation of an in-house functional activity analytical method for release testing of enzymes used in the manufacturing process at all relevant manufacturing sites was initially expected by Q1 2021 but it was agreed with EMA by e-mail to extend the due date to Q2-2021. It is recommended that Recommendations 3 and 7 are grouped.

## 4. Overall conclusion

page 5

The Recommendation number 7 is only considered as partly fulfilled.

☐ **PAM fulfilled (all commitments fulfilled) - No further action required**

☒ **PAM not fulfilled (not all commitments fulfilled) and further action, as specified below, required by the end of second quarter 2021**

Recommendation number 7 to provide the results of the studies performed to enhance the robustness of the DNase digestion step has only been partly fulfilled. Further actions are required to fulfil Recommendation 7 including submission of a detailed summary of the results from the studies and inclusion of these data in Module 3.2.S.2.5 of the dossier by the end of second quarter 2021.
It also recommended that Recommendations 3 and 7 are grouped.

EMA-Document from May 2021

*„No detailed report for the studies initiated to enhance the robustness oft he DNase digestions step is provided."*

*„It is expected that a detailed summary of the results from the studies performed to enhance the robustness of the DNase digestion step will be included in Module 3.2S.2.5 of the dossier by the end of second quarter 2021."*

### *„PAM not fulfilled"*

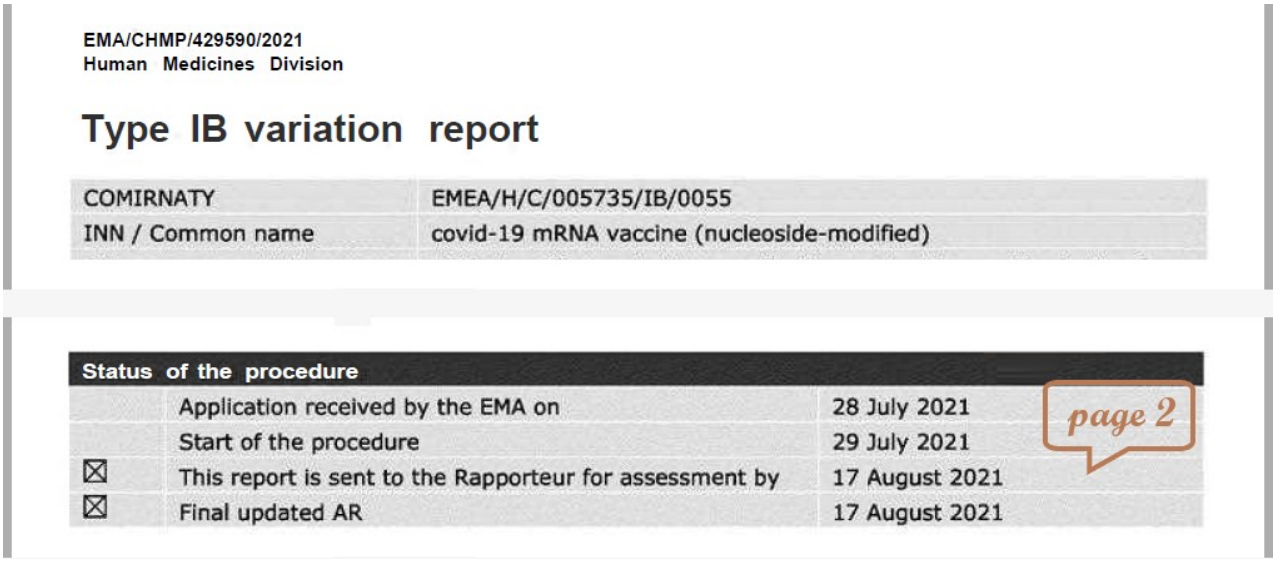
This section of the EMA document indicates that the MAH did not provide detailed reports on the studies conducted to improve the robustness of the DNase digestion step.

„PAM" stands for „Post-Authorization Measure", which means that a measure must be taken after a drug has been authorized. In this case, „PAM not fulfilled" means that the measure that the MAH (BioNTech) had to take with regard to reporting on the studies to improve DNase digestion was not fulfilled.

The passage states that the MAH is expected to submit a detailed summary of the results from the studies conducted to improve the robustness of the DNase digestion step by the end of the second quarter of 2021 in module 3.2.S.2.5 of the dossier.

### EMA document from August 2021

The discussion on „Improving the robustness of the DNase digestion step" continues.



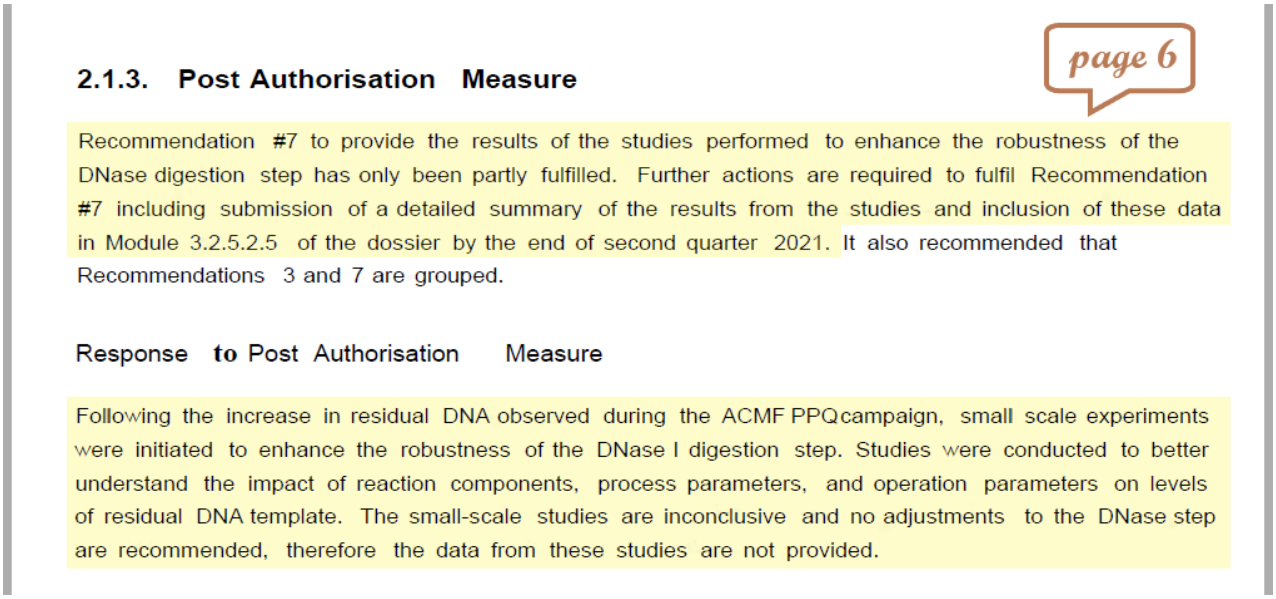
EMA/CHMP/429590/2021
Human Medicines Division

**Type IB variation report**

| | |
|---|---|
| COMIRNATY | EMEA/H/C/005735/IB/0055 |
| INN / Common name | covid-19 mRNA vaccine (nucleoside-modified) |

| Status of the procedure | | |
|---|---|---|
| | Application received by the EMA on | 28 July 2021 |
| | Start of the procedure | 29 July 2021 |
| ☒ | This report is sent to the Rapporteur for assessment by | 17 August 2021 |
| ☒ | Final updated AR | 17 August 2021 |

page 2



page 6

**2.1.3. Post Authorisation Measure**

Recommendation #7 to provide the results of the studies performed to enhance the robustness of the DNase digestion step has only been partly fulfilled. Further actions are required to fulfil Recommendation #7 including submission of a detailed summary of the results from the studies and inclusion of these data in Module 3.2.5.2.5 of the dossier by the end of second quarter 2021. It also recommended that Recommendations 3 and 7 are grouped.

**Response to Post Authorisation Measure**

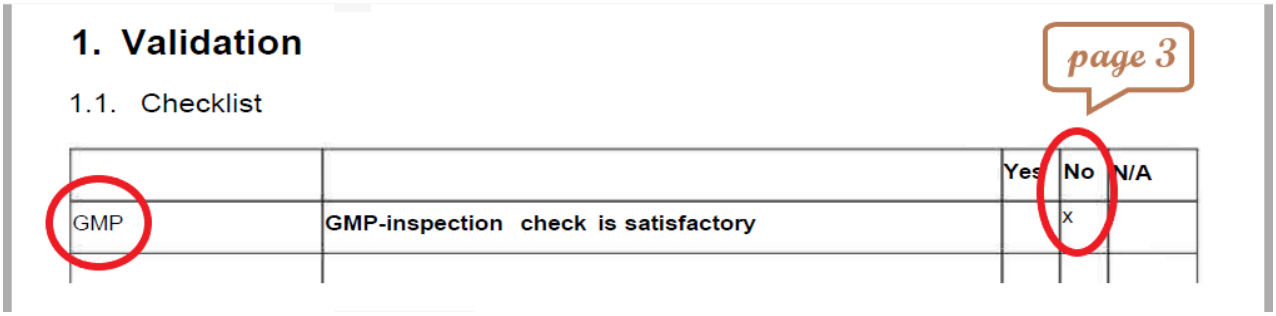
Following the increase in residual DNA observed during the ACMF PPQcampaign, small scale experiments were initiated to enhance the robustness of the DNase I digestion step. Studies were conducted to better understand the impact of reaction components, process parameters, and operation parameters on levels of residual DNA template. The small-scale studies are inconclusive and no adjustments to the DNase step are recommended, therefore the data from these studies are not provided.

EMA-Document from August 2021

*Recommendation #7 to provide the results of the studies performed to enhance the robustness of the DNase digestion step has only been partly fulfilled. Further actions are required to fulfil Recommendation #7 including submission of a detailed summary of the results from the studies and inclusion of these data in Module 3.2.5.2.5 of the dossier by the end of second quarter 2021.*

Response to Post Authorisation Measure:
*Following the increase in residual DNA observed during the ACMF PPQcampaign, small scale experiments were initiated to enhance the robustness of the DNase I digestion step. Studies were conducted to better understand the impact of reaction components, process parameters, and operation parameters on levels of residual DNA template. The small-scale studies are inconclusive and no adjustments to the DNase step are recommended, therefore the data from these studies are not provided.*

The text states that recommendation no. 7 (REC7) regarding the submission of study results to improve the robustness of the DNase digestion step has only been partially fulfilled. It states that further action is required. BioNTech's response to this recommendation includes small-scale trials to improve the robustness of the DNase I digestion step. These trials should help to better understand the effects of various factors on the amount of residual DNA. However, it is concluded that the studies conducted are inconclusive and therefore no adjustments to the DNase step are recommended. As a result, the data from these studies are not provided.



1. Validation

page 3

1.1. Checklist

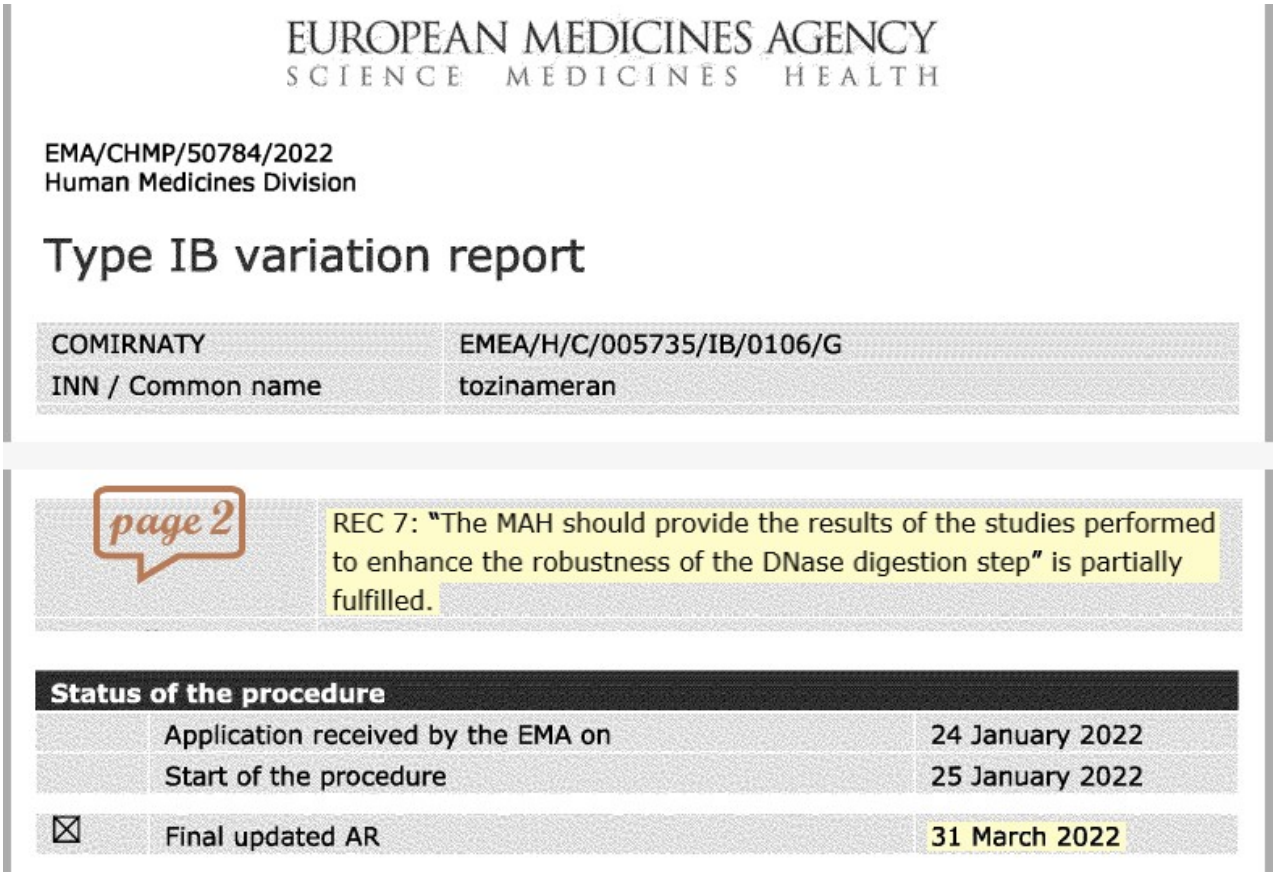
| | | Yes | No | N/A |
|---|---|---|---|---|
| GMP | GMP-inspection check is satisfactory | | X | |

EMA-Document from August 2021

In the EMA report on page 3, the „**GMP Inspection check**" was rated as „**No**." This assessment indicates that during an inspection of Good Manufacturing Practice (GMP), deficiencies or inadequacies regarding compliance with certain requirements or standards were identified. At that time, the product Cormirnaty was NOT compliant with GMP standards.

### EMA document from March 2022

In the EMA document from March 2022, we find the following regarding the status „REC 7":



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

EMA/CHMP/50784/2022
Human Medicines Division

Type IB variation report

| | |
|---|---|
| COMIRNATY | EMEA/H/C/005735/IB/0106/G |
| INN / Common name | tozinameran |

page 2

REC 7: "The MAH should provide the results of the studies performed to enhance the robustness of the DNase digestion step" is partially fulfilled.

| Status of the procedure | | |
|---|---|---|
| | Application received by the EMA on | 24 January 2022 |
| | Start of the procedure | 25 January 2022 |
| ☒ | Final updated AR | 31 March 2022 |

EMA-Document from March 2022

*„REC 7: „The MAH should provide the results of the studies performed to enhance the robustness of the DNase digestion step" is partially fulfilled."*

| Recommendation | Status |
|---|---|
| 7. The MAH should provide the results of the studies performed to enhance the robustness of the DNase digestion step in the active substance manufacturing process. | **Partly fulfilled**<br><br>REC/027 ongoing, CHMP conclusion 20/05/2021: Further actions are required to fulfil Recommendation 7 including submission of a detailed summary of the results from the studies and inclusion of these data in Module 3.2.S.2.5 of the dossier by the end of second quarter 2021. It also recommended that Recommendations 3 and 7 are grouped.<br><br>VAR IB-55: No results are provided, since the Applicant considers the small-scale study to be inconclusive and no adjustment to the DNase digestion step is recommended. This is not found acceptable, and data should be provided to support that no change is needed. In addition, the correlation of DNase I activity and levels of Residual DNA template as measured by the in-house methods should be sufficiently evaluated.<br><br>VAR IB-106-G: In order to complete REC #7, data from the two additional sites Mainz/Rentschler and Marburg should be provided. Section 3.2.S.2.5 of the dossier should be updated with data on residual DNA levels from all three sites. In addition, section 3.2.S.2.2 should be updated with information on the enhanced process control. If possible, the minor adjustments should be reflected in the related acceptance criteria. |

page 16

EMA-Document from March 2022

*„REC/027 ongoing, CHMP conclusion 20/05/2021: Further actions are required to fulifil Recommendation 7 including submission of a detailed summary of the results from the studies and inclusion of these data in Module 3.2.S.2.5 of the dossier by the end of second quarter 2021."*

*„VAR IB-55: No results are provided, since the Applicant considers the small-scale study to be inconclusive and no adjustment to the DNase digestion step is recommended. This is not found acceptable, and data should be provided to support that no change is needed. In addition, the correlation of DNase I activity and levels of Residual DNA template as measured by the in-house methods should be sufficiently evaluated."*

*„VAR IB-106-G: In order to complete REC #7, data from the two additional sites Mainz/Rentschler and Marburg should be provided. Section 3.2.S.2.5 of the dossier should be updated with data on residual DNA levels from all three sites. In addition, section 3.2.S.2.5 should be updated with information on the enhanced process control. If possible, the minor adjustments*

*should be reflected in the related acceptance criteria.“*

This section of the EMA document refers to the review of the fulfilment of certain obligations (Recommendation, REC) of the Marketing Authorization Holder (MAH) in connection with the manufacture of the medicinal product „tozinameran“.

„Tozinameran“ is the active ingredient name for the COVID-19 vaccine from Pfizer-BioNTech. The vaccine is marketed under the brand name „Comirnaty“. „Tozinameran“ is the international non-proprietary name (INN) for the active ingredient established by the World Health Organization (WHO). The term is used to identify the specific active ingredient of the vaccine independently of brand names.

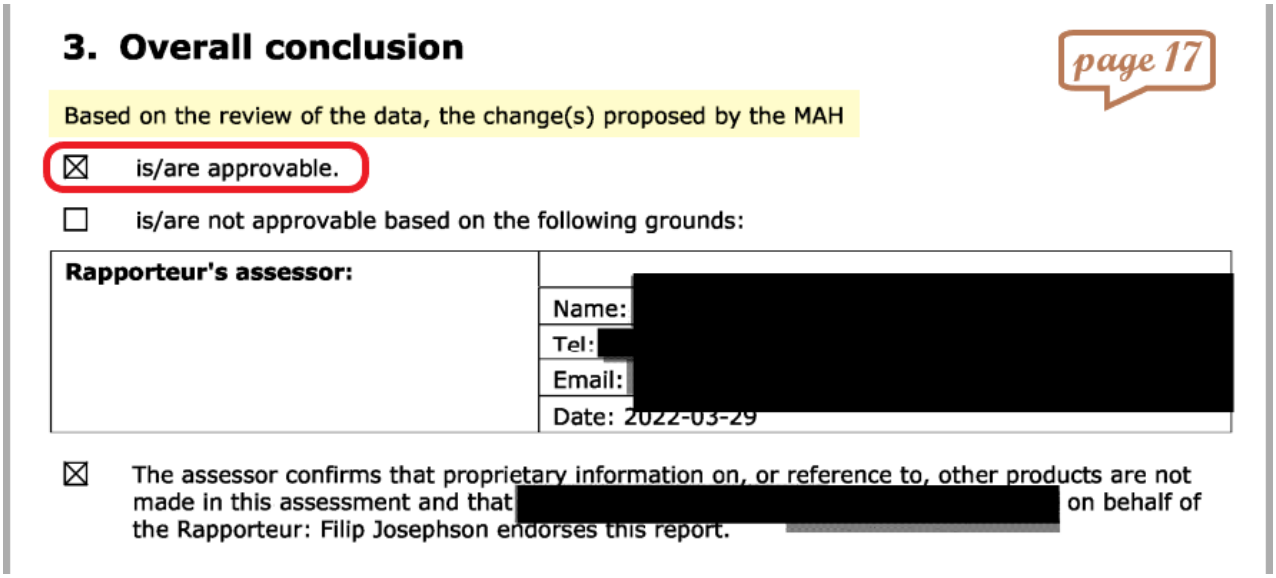
**Partially fulfilled**: It is noted that the commitment to present the results of the studies performed to improve the robustness of the DNase digestion step has been partially fulfilled. However, it is noted that further actions are required to fully comply with Recommendation 7 (REC 7).

Special considerations regarding each variable (VAR):

- **VAR IB-55:** No results are provided as the applicant considers the study inconclusive and does not recommend adjusting the DNase digestion step. However, this is not accepted, and data are requested to demonstrate that no changes are necessary. Furthermore, the correlation between DNase I activity and residual DNA as measured by the company’s own methods should be adequately evaluated.
- **VAR IB-106-G:** To complete recommendation 7 (REC7), data from the two additional sites Mainz/Rentschler and Marburg should be provided. Section 3.2.S.2.5 of the dossier should be updated with data on residual DNA content from all three sites. Additionally, Section 3.2.S.2.5 should be updated with information on enhanced process control. If possible, minor adjustments should be reflected in the corresponding acceptance criteria.

Overall, the official EMA document shows that in March 2022 – over a year after the mass use of the BioNTech vaccine – this point has not been clearly clarified.

Despite the „**Partly Fulfilled**“ finding, the EMA considers the variations proposed by the MAH (BioNTech) to be „**is/are approvable**“:



**3. Overall conclusion**

page 17

Based on the review of the data, the change(s) proposed by the MAH

☒ is/are approvable.

☐ is/are not approvable based on the following grounds:

| **Rapporteur's assessor:** | Name: [redacted]<br>Tel: [redacted]<br>Email: [redacted]<br>Date: 2022-03-29 |
|---|---|

☒ The assessor confirms that proprietary information on, or reference to, other products are not made in this assessment and that [redacted] on behalf of the Rapporteur: Filip Josephson endorses this report.

EMA-Document from March 2022

To better understand the dispute between EMA and Pfizer regarding the robustness of DNase I, the article from ThermoFisher SCIENTIFIC provides further insights. It points out that it can be challenging to completely remove all DNA contaminations.

# 6.2. The Paul Ehrlich Institute (PEI)

### 6.2.1. What is the PEI and what is its role?

The Paul Ehrlich Institute (PEI) is a federal authority in Germany, operating under the Federal Ministry of Health. It is named after the German medical researcher Paul Ehrlich and is located in Langen, Hessen. The PEI plays a central role in drug safety and vaccine approval in Germany. Here are some of the main tasks of the PEI:

**Approval:** The PEI is responsible for the approval of medicines and vaccines. It checks the quality, efficacy and safety of new medicines and vaccines before they are approved for the market.

**Monitoring:** The PEI continuously monitors the safety and quality of medicinal products and vaccines that are already on the market. It ensures that the products are manufactured in accordance with the applicable standards and meet the requirements for safety, purity and efficacy. It collects and evaluates reports on side effects and adverse events in order to identify potential risks at an early stage and ta-

ke appropriate measures to protect public health.

**Research and development:** The PEI also conducts research in the field of drug safety and vaccine development. It works closely with other national and international authorities as well as with academic institutions and industry to gain new insights and promote innovative approaches to improve health.

### 6.2.2. Questions to the Paul-Ehrlich-Institut (PEI)

#### 27.09.2023 – Letter from ÄFI to PEI

ÄFI = Ärztinnen und Ärzte für individuelle Impfentscheidung e.V
(Physicians for Individual Vaccination Decisions e.V)

Due to the study by Kevin McKernan and considering the responsibility of the PEI for the monitoring and quality assurance of vaccines in Germany, „Ärztinnen und Ärzte für individuelle Impfentscheidung e.V" (Physicians for Individual Vaccination Decisions) formulate important questions regarding the topic of „DNA contamination in the mRNA vaccines from Pfizer/BioNTech and Moderna" in a letter to the PEI on September 27, 2023.

**Question 1:** Are the safety assessments of vaccines conducted or initiated by the PEI designed to detect, classify, and quantify such foreign substances (DNA-containing fragments/plasmids)?

**Question 2:** If yes: What were the findings of the PEI? Has the PEI obtained knowledge indicating significant DNA contamination in the mentioned mRNA vaccines?

**Question 3:** If no: After the above-mentioned results became known in spring 2023, did the PEI take precautions in the safety testing protocol to rule out contamination for the batches of both vaccine manufacturers used in Germany?

#### 19.10.2023 – PEI response to ÄFI

In the response from the PEI, the following is stated:

*„The Paul-Ehrlich-Institut is an official control laboratory in the European OMCL*

*network and has tested most batches of the COVID-19 vaccine product Comirnaty (in all indications and concentrations) approved by the EU Commission in accordance with OMCL guidelines and approval specifications and, where applicable, granted state batch release for Germany."*

*„For parameters such as the residual DNA content in the vaccine, which are **only tested experimentally by the manufacturer**, the OMCL checks the manufacturer's test results to see whether the limits specified in the authorization have been met in each batch."*

The batch release tests to be carried out by the OMCL for the COVID-19 vaccines are summarized here in Table 2.

*„The batches tested and released by the Paul Ehrlich Institute did not show any objections. Therefore, no further safety measures were necessary. Batches that do not meet all criteria are not approved for the German market."*

**In summary, the response from the Paul Ehrlich Institute confirms that there is residual DNA content, and the experimental verification of vaccine batches is carried out exclusively by the manufacturer.**

The discussion gains momentum, leading to a large number of further inquiries from healthcare professionals on the topic. In response, the Paul Ehrlich Institute (PEI) publishes a letter with information for healthcare professionals on December 22, 2023.

### 22.12.2023 – Letter from the PEI with information for healthcare professionals

From this letter, the key statements of the PEI are analyzed. To improve clarity, the statements are divided into separate sections.

**Section I**
*„Many of the data and investigations circulating in the public regarding alleged contaminations of COVID-19 mRNA vaccines are based on methodological deficiencies. Additionally, there is the issue of potentially improper storage of the examined vaccine doses."*

The PEI thereby implies **incompetence** on the part of the scientists who dared to

examine the vaccine for residual DNA content. These scientists include, among others:

**Dr. Kevin McKernan** led the research and development team at the Human Genome Project. He founded Medicinal Genomics and co-founded Agencourt Bioscience Corporation. He is a renowned genomicist and has developed groundbreaking sequencing technologies.

**Dr. David J. Speicher** is an Assistant Professor of Biology and Health Sciences at Redeemer University in Hamilton, Ontario. He holds a Ph.D. in virology and has extensive experience in detecting infectious diseases using molecular biology techniques. His research focuses on detecting infectious diseases using saliva biomarkers, and he has been a director of a COVID-19 testing facility.

**Dr. Phillip Buckhaults**, a professor at the University of South Carolina, is an experienced molecular biologist and cancer geneticist with extensive expertise in utilizing next-generation sequencing technologies for global gene expression analysis and detecting gene mutations. He is also a proponent of vaccination.

**Dr. Jessica Rose** is a Canadian researcher with a bachelor's degree in applied mathematics and a master's degree in immunology from Memorial University of Newfoundland. She also holds a PhD in computational biology from Bar-Ilan University and two post-doctoral degrees: one in molecular biology from the Hebrew University of Jerusalem and one in biochemistry from the Technion Institute of Technology.

**Prof. Dr. König** studied biology, chemistry and medicine, habilitated in „Medical Microbiology and Infection Immunology" and is an external professor at the Faculty of Medicine at Otto von Guericke University in Magdeburg.

... and so on. The list is extensive.

**Section II**

*„Experimental determinations, e.g. of residual DNA by third parties in vaccine doses available on the market, must fulfill the following criteria in order to provide scientifically valid results:"*

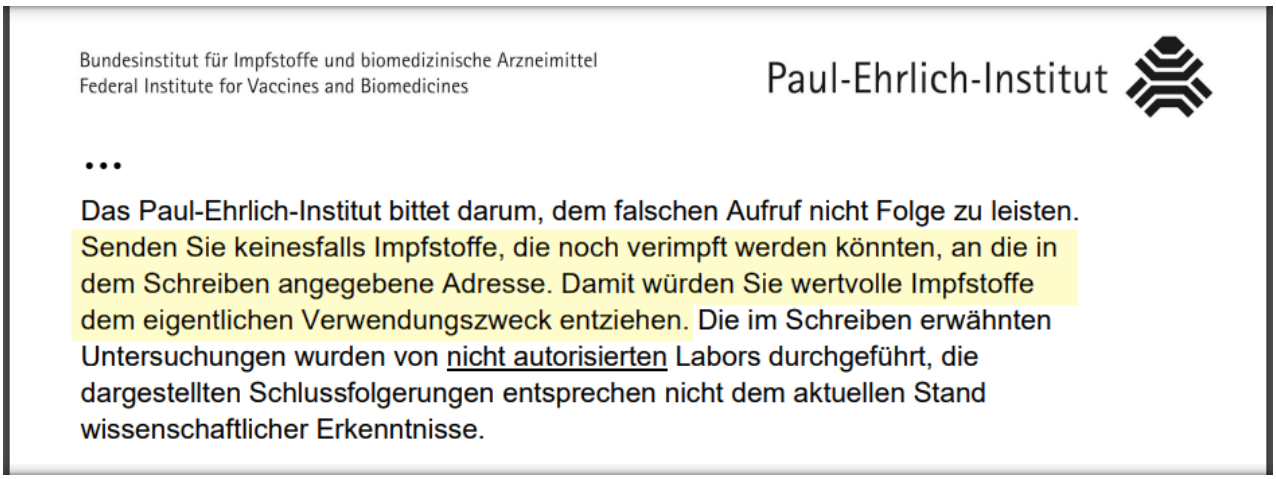
*„(i) They may not be carried out on samples taken from expired (expiration date exceeded) vaccine vials or from opened or improperly stored vaccine vials."*

**a) Access to vaccine doses**

It should be noted that vaccines are usually distributed by public health authorities

to medical institutions, general practitioners, specialists, pharmacies and vaccination centers. This means that these institutions have a kind of monopoly on access to and distribution of these products.

In this context, the PEI's appeal to „doctors and pharmacists" dated December 5, 2023 listed below is noteworthy.



Bundesinstitut für Impfstoffe und biomedizinische Arzneimittel
Federal Institute for Vaccines and Biomedicines

Paul-Ehrlich-Institut

...

Das Paul-Ehrlich-Institut bittet darum, dem falschen Aufruf nicht Folge zu leisten. Senden Sie keinesfalls Impfstoffe, die noch verimpft werden könnten, an die in dem Schreiben angegebene Adresse. Damit würden Sie wertvolle Impfstoffe dem eigentlichen Verwendungszweck entziehen. Die im Schreiben erwähnten Untersuchungen wurden von nicht autorisierten Labors durchgeführt, die dargestellten Schlussfolgerungen entsprechen nicht dem aktuellen Stand wissenschaftlicher Erkenntnisse.

*„Do not send any vaccines that could still be administered to the address provided in the letter. By doing so, you would deprive valuable vaccines of their intended use."*

At the same time, millions of valuable vaccine doses are being discarded. Just five days earlier, on December 1, 2023, ZEIT ONLINE headlined, „Now the panic buying is taking its revenge". The article states:

*„Just this year, 95 million doses of COVID vaccine expired in central warehouses and had to be destroyed... This amounts to about a third of the 458 million doses delivered to Germany so far... And the number of expired vaccine doses is expected to continue rising, as the Health Ministry reports that another* ***56 million vaccine doses are currently stored in federal central warehouses****."*

**This raises questions.** Why is the provision of individual doses for testing prevented when there is an obvious oversupply of vaccine doses? What concerns does the Paul Ehrlich Institute (PEI) have in this regard? These additional tests could potentially refute the results of the independent studies and laboratories. Why doesn't the PEI test itself? To what extent does this reaction by the PEI help to build trust?

### b) Klaus Cichutek on „Regulatory requirements for vaccine quality"

In a paper entitled „Accelerated development of COVID-19 vaccines: Technology Platforms, Benefits and Associated Risks", Klaus Cichutek, who was President of the Paul Ehrlich Institute from 2009 until the end of 2023, along with others, authored and endorsed the following statements:

**Vaccine Quality-Related Regulatory Requirements**
As for common vaccines, the establishment of a fully quality-assured manufacturing process is of fundamental importance for COVID-19 vaccine development. This requires detailed process-specific developments and specifications as well as the implementation of suitable control measures including in-process controls. **The entire production of vaccines must meet the requirements of „Good Manufacturing Practice" (GMP)** … A certain degree of flexibility has been applied for COVID-19 vaccines as regards the manufacturing range of these process performance qualification lots or by taking into account the respective data for similar products of the same platform technology….
**These tests also include controls and the minimization of any possible contamination.** …
**Approved vaccines are also subject to batch testing by the Paul-Ehrlich-Institut or another official control laboratory from the European OMCL network.** This ensures that release tests of particular importance for quality assurance, such as potency, must undergo an additional experimental check by a qualified public test laboratory before the official batch release for the market.

**Requirements for Clinical Trials**
Nevertheless, after approval, further studies on the effectiveness („effectiveness in everyday use") and safety of the vaccines will also be carried out by the federal authorities in order to ensure that the vaccines retain their positive benefit–risk profile after approval in their intended usage in broad sections of the population.

The re-examination of the benefit-risk assessment of the medicinal product under investigation includes, for example:

- individual reports of expected serious adverse side effects with an unexpected outcome,
- a clinically relevant increase in the frequency of expected serious adverse side effects,
- Events connected to the conduct of the study or the development of the investigated medicinal product that could potentially affect the safety of the

persons concerned.

How do these statements contradict the aforementioned request in the PEI letter dated December 05, 2023?

**c) Could the residual DNA content increase in an expired and sealed mRNA vaccine vial?**

The half-life of DNA is 521 years, meaning that half of the DNA molecules in a sample decay after this time. Even as DNA breaks down and fragments into smaller pieces, the total amount of DNA in a sample remains unchanged. Qubit fluorometry and qPCR measure DNA concentration per unit volume, but the total amount of DNA in the sample remains the same even as it breaks into smaller fragments. Therefore, the residual DNA content in an mRNA vaccine vial does not increase even after a short period of 2 years, even under suboptimal storage conditions.

**Section III**

*„(ii) The methodology used to determine the amount of residual DNA must be proven to be appropriate and reproducible – in particular, test interferences due to the presence of lipid nanoparticles in the vaccine vials should have been excluded (which cannot be guaranteed when testing on the final vaccine vial).“*

The study by Speicher et al. states the following: *„Vaccine in these three vials was diluted 10-fold serially to assess LNP inhibition in qPCR (Figure 5). We observed the expected ~3.3 Cq response after the 1:10 dilution (1:10, 1:100, 1:1000) suggesting that there is some LNP inhibition that could impact the quantitation of DNA at these dilutions (Figure 6). Therefore, the data from the 1:10 dilutions were used for further analysis. This dilution, as well as the fact that some of the doses were designed to be diluted before use, was accounted for in our calculations.“*

This indicates that measures were taken to account for potential test interferences due to the presence of lipid nanoparticles in the vaccine vials.

**Section IV**

*„(iii) The method used must have been validated to provide reliable and verifiable*

*results."*

*„In the frequently cited preprint publications by McKernan et al. (April 2023) and Speicher et al. (October 2023), there is insufficient information provided regarding whether the mentioned conditions were met, as well as details on the traceability of the chosen methodology. Method validation is essential to ensure that the methodology used yields reliable and reproducible results at all times and regardless of the operator, and that the method is suitable for its intended purpose. The aforementioned conditions for obtaining scientifically sound measurement results in residual DNA determination are adhered to by the manufacturers."*

Dr. Kevin McKernan discusses this topic in an interview with Prof. Dr. Paul Cullen and Prof. Dr. Henrieke Stahl:

*„Our methods are in the public domain and have been reproduced by independent laboratories. However, the PEI does not carry out its own tests for DNA on the vaccine, but leaves this testing to the manufacturers. The manufacturers, in turn, have not, as far as we know, provided any detailed insight into their testing procedures; only an EMA document disclosed by a cyberattack provides some insight into the testing methods. If the manufacturers or the PEI were to publish their own methods and self-collected data transparently, we would be willing to make comparisons and possibly learn from each other."*

*„Up to this point, our methods are not only the only publicly available measurement methods, but also the best validated. This is because they were immediately subjected to various „stress tests", such as a gradient PCR, determination of the lower detection limits, an analysis of the PCR primer concentrations and a PCR inhibition analysis. In addition, the amplified DNA fragments were also sequenced by a third party using a gold standard, the so-called Sanger method. Standard sequencing using the Illumina and Oxford Nanopore method was also carried out in several independent laboratories."*

*„As can be seen from the above-mentioned „Rolling Review" document, the EMA has accepted qPCR for DNA measurement and fluorometry and spectrometry in the ultraviolet light spectrum for RNA measurement. This procedure contradicts the Moderna patent US10.077.439, which was filed by company boss Stéphane Bancel. Here it is shown that qPCR underestimates the amount of DNA because it cannot detect fragments with a length of less than about 100 base pairs."*

## 6.3. Brief summary

- The European Medicines Agency (EMA) has been aware of the problem of „manufacturing-related residual DNA impurities" since the beginning. As early as February 2021, it specifically requested additional data from the manufacturer to clarify the robustness (i.e. reliability) of the DNase digestion step (see REC7), as this is used to remove the DNA.
- In March 2022, the EMA noted (VAR IB-55) that the applicant did not submit results as it questioned the conclusiveness of the small scale study and did not recommend an adjustment to the DNase digestion step. This position was not accepted by the EMA and the applicant was requested to provide data demonstrating that no change was required. Furthermore, it was emphasized that the correlation between the DNase digestion step and the residual DNA content should be sufficiently evaluated. Despite these concerns, formal approval was granted by the EMA.
- The Paul-Ehrlich-Institut (PEI) confirms the existence of residual DNA in the vaccine and the fact that the experimental testing of the vaccine batches is carried out exclusively by the manufacturer.
- The PEI questions the measurement results reported by independent laboratories regarding the residual DNA in the vaccine. The authority is questioning the measurement methods used and the quality of the vaccine samples used.
- The Paul-Ehrlich-Institut (PEI) has sent a „letter to doctors and pharmacists" asking them not to send vaccine doses to independent laboratories for further testing. This appeal comes at a time when there is a surplus of vaccine doses.
- Although the experimental data of McKernan et al. and Speicher et al. are based on established procedures that meet current scientific standards and are also used by Pfizer/BioNTech and Moderna, the PEI makes no effort to clarify the dispute through further independent experimental investigations involving all parties.

# 7. Are further investigations necessary?

The statements in Chapter 6 give the impression that neither the manufacturers of mRNA vaccines nor the responsible regulatory authorities are interested in further professional engagement with the findings from Chapter 5.

The main arguments of the regulatory authorities were summarized by McKernan:

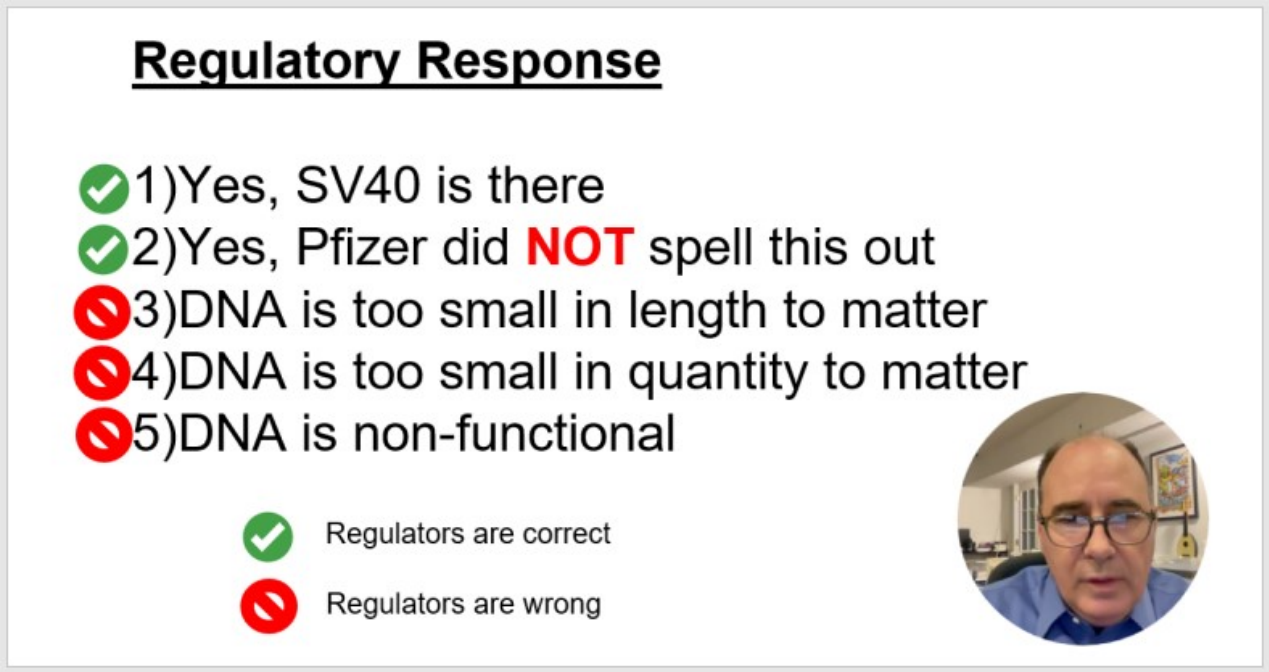



Fig. 52: Regulatory Response [Powerpoint-recording Massachusetts-Slides, Slide 14]

It is important to investigate whether the authorities' conclusions that the DNA is too short, the amount of DNA is too small or the DNA is not functional are clear and undisputed. It is important to critically scrutinize these conclusions and to examine the arguments in more detail to determine whether they are indeed correct or whether further investigations are required.



## 7.1. Official statements regarding residual DNA

Two of Moderna's patents should be highlighted here.

**Patent US 10077439 B2 „Removal of DNA fragments in mRNA production process"**

This patent from Moderna (2014), authored by Stéphane Bancel (CEO of Moderna) and others, explicitly states that residual DNA in drug products could trigger activation of the innate immune response and potentially cause oncogenic effects in certain patient populations.

**[0003]** The **DNA template** used in the mRNA manufacturing process **must be removed** to ensure the efficacy of therapeutics and safety, **because residual DNA in drug products may induce activation of the innate response and has the potential to be oncogenic in patient populations**. Regulatory guidelines may also require the quantification, control, and removal of the DNA template in RNA products. Currently available or reported methods do not address this deficiency.

**Patent US 10898574 B2 „Delivery and formulation of engineered nucleic acids"**

Another patent from Moderna (2021) clearly states the following:
„There are multiple problems with prior methodologies of delivering pharmaceutical compositions in order to achieve effective protein expression both for therapeutics and bioprocessing applications. **For example, introduced DNA can integrate into host cell genomic DNA at some frequency, resulting in alterations and/or damage to the host cell genomic DNA. Alternatively, the heterologous deoxyribonucleic acid (DNA) introduced into a cell can be inherited by daughter cells (whether or not the heterologous DNA has integrated into the chromosome) or by offspring.**"

**The „FDA Guidance on Prophylactic DNA Vaccines: Analysis and Recommendations" from 2009 states:**

„DNA is considered a contaminant of „conventional" (non-DNA) vaccines. Manufacturers typically minimize or remove extraneous DNA during the production process to minimize human exposure to potentially injurious material. Not surprisingly, concerns that plasmid DNA might integrate into the host genome, increasing the likelihood of malignant transformation, genomic instability, or cell growth dysregulation were raised when DNA vaccines were first proposed for clinical use."

## 7.2. Further studies on the topic

### 7.2.1. Issues associated with residual cell-substrate DNA in viral vaccines

This **study**, entitled „Issues associated with residual cell-substrate DNA in viral vaccines", points out that the presence of some residual cellular DNA derived from the production cell substrate in viral vaccines is unavoidable. Whether this DNA poses a safety risk is unknown. DNA has two biological activities that need to be considered. Firstly, DNA can be oncogenic; secondly, DNA can be infectious.

### 7.2.2. High spontaneous integration rates of end-modified linear DNAs upon mammalian cell transfection

In this **study** entitled „High spontaneous integration rates of end-modified linear DNAs upon mammalian cell transfection", the frequency of integration of genetic material into the genome was investigated after cells were treated with different forms of DNA. It was found that about 10 to 20% of the cells were stably transfected after treatment with different forms of linear DNA. In other words, a considerable percentage of the originally treated cells took up the genetic material. Integration of linear DNAs occurred with similar frequency despite altered DNA ends. These results suggest that the cells have mechanisms to eliminate non-natural DNA ends and subsequently rescue the DNA by integration.
The study suggests that non-integrating gene therapy methods may need to be further developed before they can be used more widely.

### 7.2.3. Double-stranded DNA induces a prothrombotic phenotype in the vascular endothelium

This **study**, entitled „Double-stranded DNA induces a prothrombotic phenotype in the vascular endothelium", investigates the effects of double-stranded DNA (dsDNA) on the inner wall of blood vessels, also known as the vascular endothelium. The researchers treated human endothelial cells in the laboratory with synthetic dsDNA. They found that this led to an increased production of molecules that activate the blood coagulation system. This in turn led to accelerated blood clotting in vitro. An injection of dsDNA into mice (in vivo) also accelerated the formation of blood clots in the blood vessels, which led to a shortened time to complete vascular occlusion with cessation of blood flow. This study shows that double-stranded DNA (dsDNA), both of viral origin and endogenous (naturally present in the body) origin, induces direct prothrombotic effects in the vascular endothelium.

# 7.3. SV40 Sequences

The SV40 components play a significant role in gene therapy. The promoter and the enhancer are two key elements of SV40 DNA used in gene therapy to enhance the expression of foreign genes in target cells.

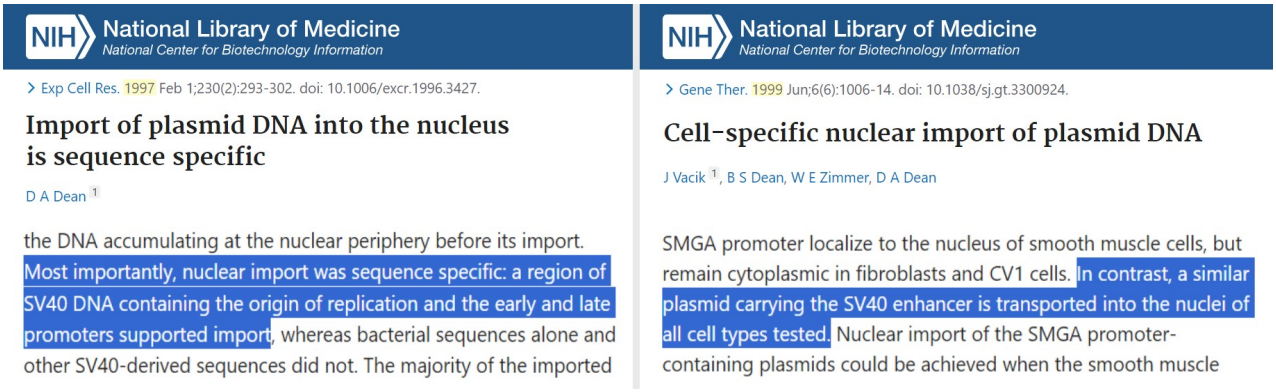
The promoter is a specific DNA sequence that initiates the transcription of a neighboring gene. In SV40, the promoter comprises both the origin of replication and the early and late promoter regions. This structure enables the promoter to efficiently regulate the expression of foreign genes.

The enhancer is a regulatory DNA sequence that can amplify the activity of a promoter, regardless of orientation or distance relative to the promoter. The SV40 enhancer is known for its strong enhancer activity and is often used in gene therapy vectors to increase the expression of introduced genes.

A key aspect of SV40 components in gene therapy is their ability to support import into the nucleus. The SV40 enhancer can use specific signaling pathways to pass through the nuclear pores and efficiently enter the nucleus. In this context, the following studies are worth mentioning:

- **SV40-based gene therapy vectors: turning an adversary into a friend**
- **Effect of a DNA Nuclear Targeting Sequence on Gene Transfer and Expression of Plasmids in the Intact Vasculature**
- **Nuclear Targeting of Plasmids and Protein-DNA Complexes**

As early as 1997, a **study** found that specific SV40 sequences are important for the transport of molecules into the cell nucleus. In a later **study** in 1999, it was discovered that the SV40 enhancer successfully transports the plasmid into the nuclei of various cell types. These research results show how certain SV40 sequences can control transport into the cell nucleus.



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> Exp Cell Res. 1997 Feb 1;230(2):293-302. doi: 10.1006/excr.1996.3427.

**Import of plasmid DNA into the nucleus is sequence specific**

D A Dean [1]

the DNA accumulating at the nuclear periphery before its import. Most importantly, nuclear import was sequence specific: a region of SV40 DNA containing the origin of replication and the early and late promoters supported import, whereas bacterial sequences alone and other SV40-derived sequences did not. The majority of the imported

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> Gene Ther. 1999 Jun;6(6):1006-14. doi: 10.1038/sj.gt.3300924.

**Cell-specific nuclear import of plasmid DNA**

J Vacik [1], B S Dean, W E Zimmer, D A Dean

SMGA promoter localize to the nucleus of smooth muscle cells, but remain cytoplasmic in fibroblasts and CV1 cells. In contrast, a similar plasmid carrying the SV40 enhancer is transported into the nuclei of all cell types tested. Nuclear import of the SMGA promoter-containing plasmids could be achieved when the smooth muscle

1997: *„Most importantly, nuclear import was sequence specific: a region of SV40 DNA containing the origin of replication and the early and late promoters supported import, whereas bacterial sequences alone and other SV40-derived sequences did not."*

1999: *„In contrast, a similar plasmid carrying the SV40 enhancer is transported into the nuclei of all cell types tested."*

## 7.4. SV40-Promoter and the p53-Tumor-Suppressor Gene

Kevin McKernan mentioned in a presentation [Massachusetts slides, slide 23] a study on the interaction between the SV40 promoter and p53.

p53 is a key protein in tumor suppression, often referred to as the „guardian of the genome". It regulates the cell cycle, DNA repair, apoptosis (programmed cell death), and senescence (cell aging). Mutations in the p53 gene are frequently found in many types of cancer and lead to loss of function and disrupted regulation of cell growth and division.

A **study** found that p53 interacts with a specific region of the SV40 viral genome known as the „early promoter". The statement in the study reads: „The p53 protein binds to the SV40 early promoter."

Hypothetically, this could have various effects:

**p53 loss of function:** If the SV40 early promoter binds to p53 and impairs its normal function, this could lead to a loss of function of p53. p53 is an important tumor suppressor that normally protects the cell from uncontrolled growth. A loss of function of p53 could therefore lead to an increased risk of cancer or other undesirable

cellular changes.

**Disruption of the p53-mediated signaling cascade:** p53 regulates the expression of many genes involved in cell cycle regulation, apoptosis (programmed cell death) and DNA repair. If the SV40 early promoter binds to p53 and interferes with its normal transcriptional activity, this could lead to disruption of these important cellular processes, potentially resulting in genetic instability or other cellular problems.

**Interference with other p53 interactions:** p53 interacts with many other proteins and regulatory elements in the nucleus to carry out its functions. If SV40 DNA sequences enter the nucleus and interact with p53, they could interfere with other important p53 interactions, leading to far-reaching effects on cellular physiology.

These are hypothetical scenarios and therefore further experimental studies would be required to understand the actual effects of SV40-p53 interaction.

McKernan also refers to this in his presentation [Massachusetts slides, slide 23]. He said that the SV40-p53 interaction needs to be investigated for genotoxicity and that there are currently no studies on genotoxicity. It needs to be analyzed and evaluated whether there are integration risks or oncogenic risks.

## 7.5. Residual DNA and the lipid nanoparticles

In the study, Speicher et al. found that **the plasmid DNA is probably packaged in lipid nanoparticles**. The function of lipid nanoparticles is to protect their contents from degradation by enzymes in the body. The lipid nanoparticles would provide protection for the enclosed DNA and allow it to cross the cell membrane and enter the cells. Once the DNA has entered the cell, the SV40 enhancer, if part of the DNA sequence, could help to transport the DNA sequence into the cell nucleus. This would increase the risk of integration of the plasmid DNA into the genome.

Dr. Phillip Buckhaults describes this with the following analogy:

**20 Greek soldiers wandering around outside the walls of Troy are not a big deal. 20 Greek soldiers packed inside a large wooden horse are a different matter.**

The conclusion drawn by Speicher et al. in their study is as follows:

*„The FDA and WHO guidelines did not consider packaging of DNA in lipid nanoparticles, likely resulting in longer DNA persistence as well as increased transfection efficiency (successfull transport of genetic material into a target cell). Furthermore, the guidelines did not consider cumulative dosing with LNP-based modRNA."*

*„Our findings extend existing concerns about vaccine safety and call into question the relevance of guidelines conceived before the introduction of efficient transfection using LNPs (Lipid nanoparticles). With several obvious limitations, we urge that our work is replicated under forensic conditions and that guidelines be revised to account for highly efficient DNA transfection and cumulative dosing."*

# 7.6. Possible integration into the DNA

### 7.6.1. A further study

The following **study** first examined the „Presence of viral spike protein and vaccinal spike protein in the blood serum of patients with long-COVID syndrome". Of the 81 long-COVID patients analyzed, fragments of the vaccine spike protein were found in 2 patients, two months after vaccination. The authors of the study asked themselves whether a permanent production of spike proteins could be based on DNA changes. To clarify this question, they conducted a supplementary study.

In the supplementary **study**, blood samples from patients with long-COVID syndrome were tested for the „presence of spike protein sequences of the BNT162b2 vaccine in the DNA". To do this, they used specific DNA tests to detect the genetic code of the COVID vaccine in the cell genomes of the study participants. The results indicated the presence of spike protein sequences similar to those of the vaccine.

In the discussion, attention was drawn to the possibility that the results could indicate potential integration of the vaccine spike protein into the patients' DNA. Connections were also made to other studies showing similar results. However, the study also acknowledges its own limitations, such as the need for further investigations to confirm vaccine integration and to rule out potential sources of error. Overall, the authors recommend further studies with larger samples and control groups

to assess the specificity and prevalence of vaccine integration in long-COVID patients.

### 7.6.2. Smaller DNA fragments

To ensure patient safety with regard to DNA contamination of host cells, the Food and Drug Administration (FDA) has published industry guidelines for the manufacturing of cell and gene therapies:

„*Since some cell substrates also harbor tumorigenic genetic sequences or retroviral sequences that may be capable of transmitting infection, we recommend that you take steps to minimize the biological activity of any residual DNA associated with your viral preparation. This can be accomplished by reducing the size of the DNA to below the size of a functional gene and by decreasing the amount of residual DNA. We recommend that you* ***limit the amount of residual DNA*** *for continuous non-tumorigenic cells to* ***less than 10 ng/dose and the DNA size to below approximately 200 base pairs***.

*If you are using cells that are tumor-derived or have tumorigenic phenotypes or other characteristics that may give rise to special concerns, the limitation of specific residual DNA quantities may be needed to assure product safety…"*

Although FDA guidelines prescribe the limitation of residual DNA in vaccines, this does not necessarily mean that DNA fragments smaller than 200 base pairs (bp) have no impact or play no role.

Even **small DNA fragments can theoretically be incorporated into the genome** if they enter the cells and are recognized by the cellular mechanisms. Whether these fragments can play a functional role in the genome depends on various factors, including their sequence, their position in the genome and the availability of repair mechanisms. Therefore, even very small DNA fragments, including those under 200 base pairs, could be integrated into the genome and have biological effects.

Kevin McKernan writes in his substack:

Some guidelines focus exclusively on DNA fragments larger than 200 base pairs. However, the assumption that fragments below 200 base pairs are not relevant is

based on flawed logic. This assumption is based on the assumption that DNA in this size range is not able to encode an open reading frame (ORF), i.e. a specific section of DNA or RNA that contains information for translation into a protein. Should this nevertheless be possible, it is assumed that the resulting peptide would be so short (66 amino acids) that it is of no significance, or that the short DNA is rapidly degraded.

„A few reasons this argument is flawed.
1) The regulations didn't anticipate this DNA being inside nuclease resistant LNPs, therefore short length doesn't imply rapid degradation.
2) Even small DNA fragments can be functional. The 72bp SV40 Enhancer is one such element that is known to recruit transcription factors and mobilize the DNA to the nucleus. David Deans work covers this.
3) Even small fragments, if integrated can knock a human gene out of frame and cause problems."

Kevin McKernan emphasized in a presentation that the amount of DNA in nanograms is not the only decisive factor, but also the molarity, i.e. the number of DNA molecules in a certain amount of solution (dose). „A 200-base fragment is like a shotgun pellet that enters your genome and has a much higher propensity to integrate and damage your genome."

### 7.6.3. Larger DNA fragments

The presence of larger DNA fragments is another issue that should be investigated. McKernan explains this situation in an interview:

„*Sutton et.al. have shown that hybrids, i.e. pairs of DNA and RNA, can block the enzyme DNase, which is responsible for the degradation of DNA. Such hybrids could also be present in mRNA vaccines. The N1-methyl-pseudouridine in the modified RNA of the vaccine could cause it to interact with the DNA and bind to it. Sequencing of the plasmid DNA was less effective in the region of the spike gene, suggesting that DNA:RNA hybrids may interfere with the sequencing reaction. It is likely that such hybrids block enzymatic reactions, as DNase normally targets double-stranded DNA (i.e. DNA:DNA hybrids). I have described this problem in detail in my substack.*"

### 7.6.4. Dr. Phillip Buckhaults

In his hearing at the Senate of South Carolina in September 2023, Buckhaults stated:

*„We do this in the lab all the time, we take pieces of DNA, we mix them up with a lipid complex like Pfizer vaccine is in. We pour it onto cells and a lot of it gets into the cells and a lot of it gets into the DNA of those cells and it becomes a permanent fixture of the cells. It's not just a temporary thing, it is in that cell and all of its progyny from now and forever more.* ***So that's why I'm kind of alarmed about this DNA being in the vaccine, it's different from RNA because it can be permanent. This is a real Hazard for genome modification of Long Live semantic cells like stem cells*** *and it could cause theoretically this is all a theoretical concern but it's pretty reasonable based on molecular biology that it could case a sustained autoimmune tact toward that tissue. It's also a very real theoretical risk of future cancer in some people depending on where in the genome this foreign piece of DNA lands, it can interrupt a tumor suppressor or activate an endogen."*

*„DNA is a longlived information storage device. What you were born with you're going to die with and pass on to your kids DNA lasts for hundreds of thousands of years. And it can last for Generations if you and get pass it on to your kids right so alterations to the DNA they stick around RNA by its nature is temporary it doesn't last and that feature of RNA was part of the sales pitch for the vaccine."* ...

*„The pseudo Uridin was supposed to make the RNA last a little bit longer but still it's a transient phenomenon we're talking hours to days. And then proteins once proteins are made they also don't last forever they last for hours to days.* ***But something that makes its way into DNA has the potential to last for a very long time maybe a lifetime.***"

*„In my view somebody should go about sequencing DNA samples from stem cells of people who are vaccinated and find out if this theoretical risk has happened or not."*

### 7.6.5. Dr. Jürgen O. Kirchner

When asked by The Epoch Times „In your opinion, what is the risk of being injected

with foreign DNA?", Kirchner replies:

„*That's a very important question. First of all, what does DNA do? When the DNA is introduced into the cells, it can migrate into the cell nucleus and integrate itself into the human DNA, where it can either switch off genes, switch on genes – including switching on cancer genes. And if these are pieces of DNA that are too large to enter the cell nucleus – there are also so-called bacterial plasmids that were also found in the vaccines – then this plasmid can already show activity outside the cell nucleus, i.e. it is read. And the fatal thing is that the plasmid found in the vaccines contains the spike gene and a gene for antibiotic resistance. ... This needs to be scientifically investigated.*"

### 7.6.6. Preliminary indications of DNA integration

At the International Crisis Summit-5 (ICS-5) on February 23, 2024 in Washington DC, Kevin McKernan presented the latest research on possible DNA integration. The whole presentation can be seen under this link.

From minute 9:50 onwards, the results of the tests on OVCAR3 and MCF7 cells treated with the vaccine are presented.

**OVCAR3 cells:** This cell line is derived from ovarian carcinoma, which is a malignant tumor of the ovaries. **MCF7 cells:** This cell line is derived from a breast cancer (mammary tumor). Both cell lines are established models for studying cancer biology, testing drugs and developing therapies.

Kevin McKernan reported that DNA was detected in the cell cultures treated in this manner. „*We can see the DNA there. It's a very high copy number... It's about a 3000-fold coverage of the entire vaccine genome.*"

In simple terms, this means that the genetic information of the vaccine has entered the cells. The researchers were able to visualize the DNA inside the cells and found that it was present in very high numbers.

The statement „approximately 3000-fold coverage of the entire vaccine genome" refers to how often the DNA sequences of the vaccine were found in the cells. A „3000-fold coverage" means that the DNA sequences were present on average

about 3000 times in the cells examined. This indicates that a large amount of the vaccine's genetic information has entered the cells.

Kevin McKernan continued: „*We have also found two genome integrations. These have not yet been replicated, like all of our qPCR data, so it's still very early days, but we have fusions of DNA between the spike sequence and chromosome 12 and between the spike sequence and chromosome 9. This needs to be confirmed with long-read sequencers, which will allow us to cover the entire integration event.... But we shouldn't see anything like this... So we need to do a little more validation, but it's still a little worrying.*"

More information on this here.

## 7.7. Calls for further investigations

WDR also reported on the topic of DNA contamination in mRNA vaccines on January 18, 2024:


Here is a summary of the statements.

Dr. Thomas Voshaar, pulmonary physician at the Moers Clinic, answered the following questions for WDR:

***„Do you agree that it is time for a re-evaluation of these mRNA vaccines?"***

*„Yes, absolutely. There are two things that have come to light. One is that DNA may be present in very small quantities due to the manufacturing process. That is a WHO specification. You have to question why this is reasonable and why it is allowed. Because they say there is no relevant risk and that's why I'm reassured at the moment. But we don't know that. There are no studies at all on how little is harmless and at what point it might be dangerous."*

***„But why not? Why don't these studies exist?"***

*„Yes, that really is a question. Of course, this new vaccine was developed with this new technology under relatively high pressure, time pressure, and of course this is always emphasized positively, and that's why some things may have fallen by the wayside. But once again, there was a consensus, including at the WHO, that it was acceptable that certain amounts of DNA could be present due to the manufacturing process. But it remains the case that it is a contaminant. We would rather not have it in there. And now there is also the fact that some laboratories have found that certain batches contain more and larger pieces of DNA than would actually be permitted."*

***„That means, what are you specifically demanding?"***

*„One definitely needs to hold the Paul-Ehrlich-Institut (PEI) accountable once again. The Paul-Ehrlich-Institut primarily relies on reviewing the protocols provided by the manufacturer. However, this cannot be left solely to the manufacturer; instead, other laboratories must be commissioned to thoroughly investigate the matter."*

***„We have requested a statement from BioNTech regarding DNA contaminations, but have NOT received any responses."***

# 7.8. Brief summary

- Studies show that the presence of plasmid DNA originating from the production process of mRNA vaccines is unavoidable..
- The presence of plasmid DNA in the vaccine is not without concern. Whether

these DNA remnants pose a safety risk needs to be specifically investigated.
- Laboratory investigations demonstrate the potential for integration of linearized DNA sequences into mammalian cells, as well as the potentially critical effects of dsDNA on the functionality of human endothelial cells.
- Studies suggest that residual DNA may potentially be encapsulated within lipid nanoparticles. This could enable it to traverse the cell membrane and enter cells.
- The presence of SV40 components such as promoter and enhancer carries the potential risk of transporting DNA fragments into the cell nucleus and could also impair the proper functionality of the p53 tumor suppressor gene.
- Globally, more and more scientists are highlighting these potential hazards and emphasizing the need for additional careful examinations.

# 8. Epilogue

The mRNA technology is undoubtedly an impressive testament to human intelligence. At the same time, it underscores the extreme complexity not only of the individual technological production steps but also of the interactions of these preparations with the human body. Like any highly complex technology platform, it has its pros and cons.

The debate over DNA contamination in mRNA vaccines, as summarized in this paper, underscores the urgent need for further investigation into the reliability of the technological production process, the standardization of measurement methods and quality assurance thresholds, as well as the assessment of the long-term effects of such therapeutics.

For products designed for future global use, diligence and thoroughness should be paramount.

**And questions remain:**

Why are mRNA vaccine manufacturers silent on this issue?

Why aren't the authorities taking action to refute or gain more clarity on the results and concerns raised by independent labs and scientists through their own manufacturer-independent experimental investigations?

Let us recall the poignant words of former Chancellor Mrs. Merkel at the beginning of the COVID-19 pandemic:

**„We are a community where every life and every person matters."**

. . .

**Acknowledgments**

I would like to express my sincere gratitude to the numerous scientists, researchers, and dedicated individuals who continuously contribute to exploring various aspects of this complex issue through their studies, articles, and presentations. Their dedication and efforts are invaluable and significantly contribute to the advancement of our understanding.

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Sources (as of 15.03.2024)

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Bacteria, BioNTech, Chromatography, Comirnaty, Covid-19, David Speicher, Diafiltration, DNA contamination, DNA fragments, DNA integration, DNase I, EMA, FDA, Fluorescence assay, Foreign DNA, Health, Kevin McKernan, Lipid nanoparticle, LNP, Moderna, mRNA Vaccine, Oxford Nanopore technology, Pandemic, PEI, Pfizer, Plasmid, Plasmid DNA, Process 2, qPCR, Residual DNA, SARS-CoV-2, Spike protein, SV40, Tangential flow filtration, Tozinameran, Transfection efficiency, UF/DF, Ultrafiltration, UV spectroscopy, Vaccine production, WHO