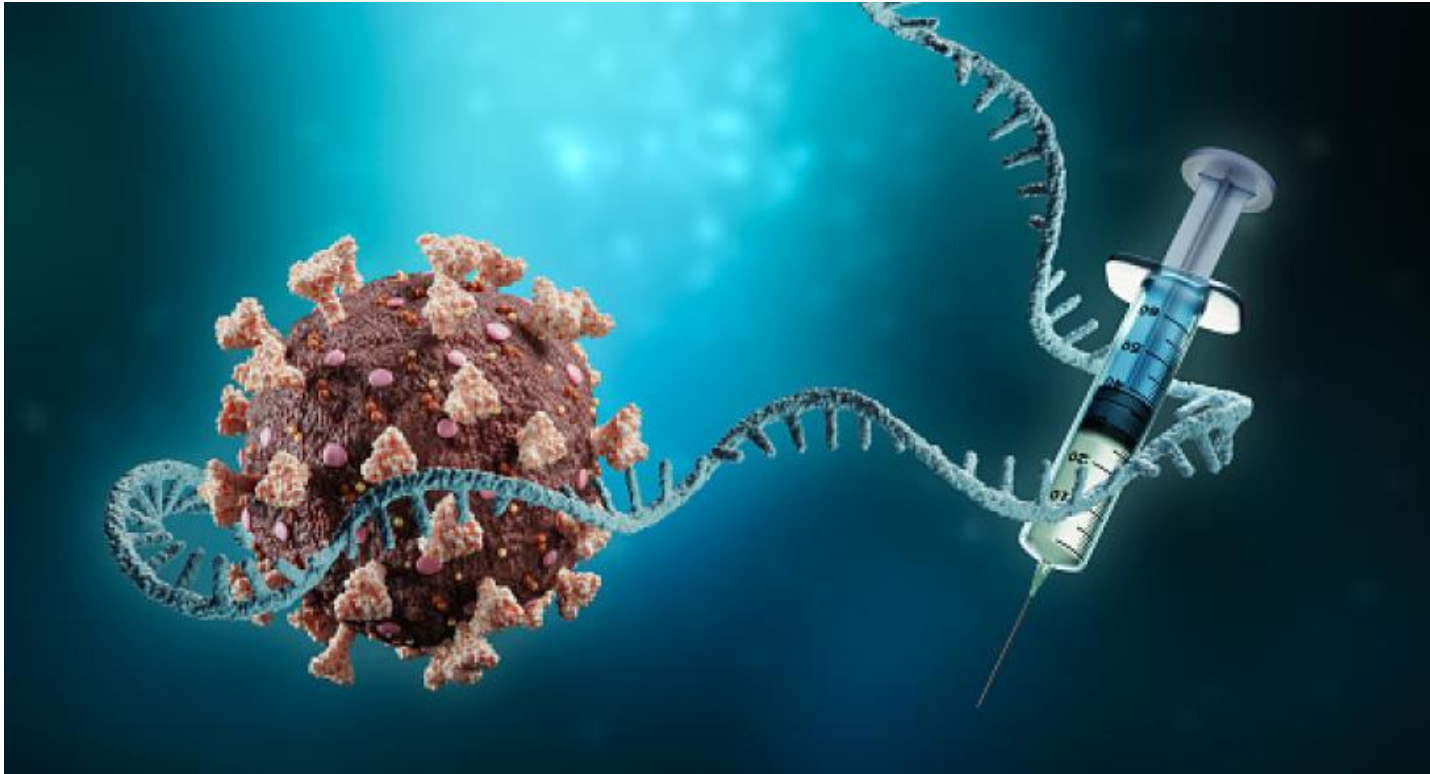


# mRNA Vaccine Production: Biorisk Considerations

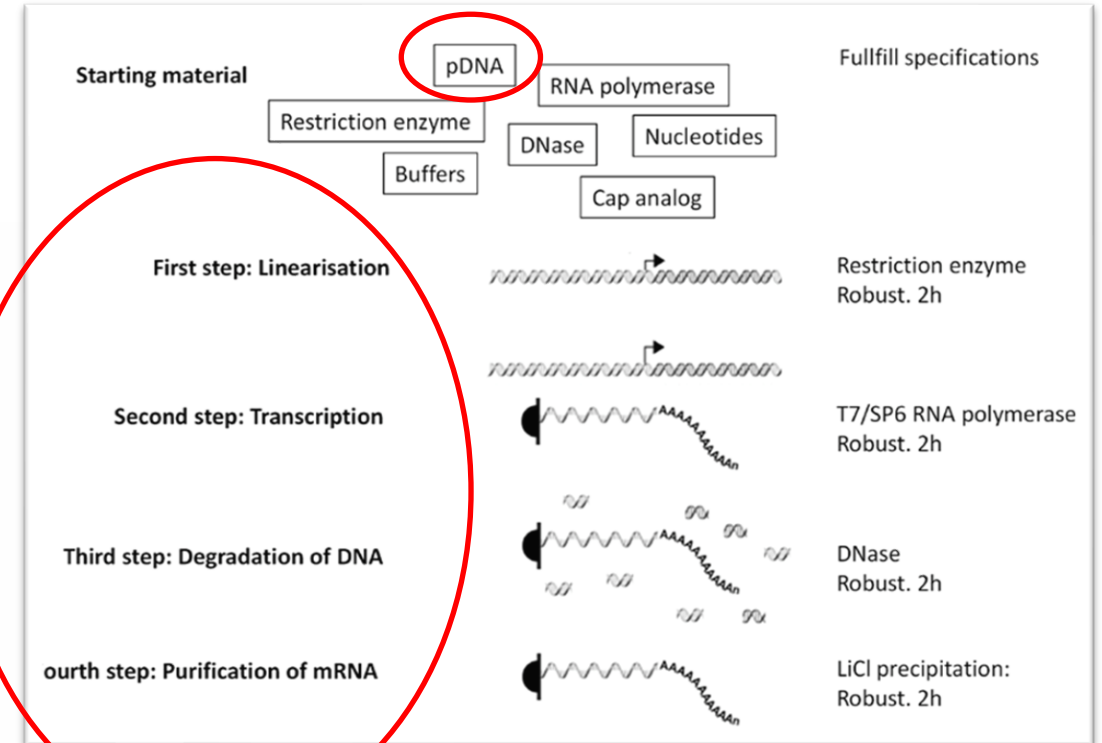
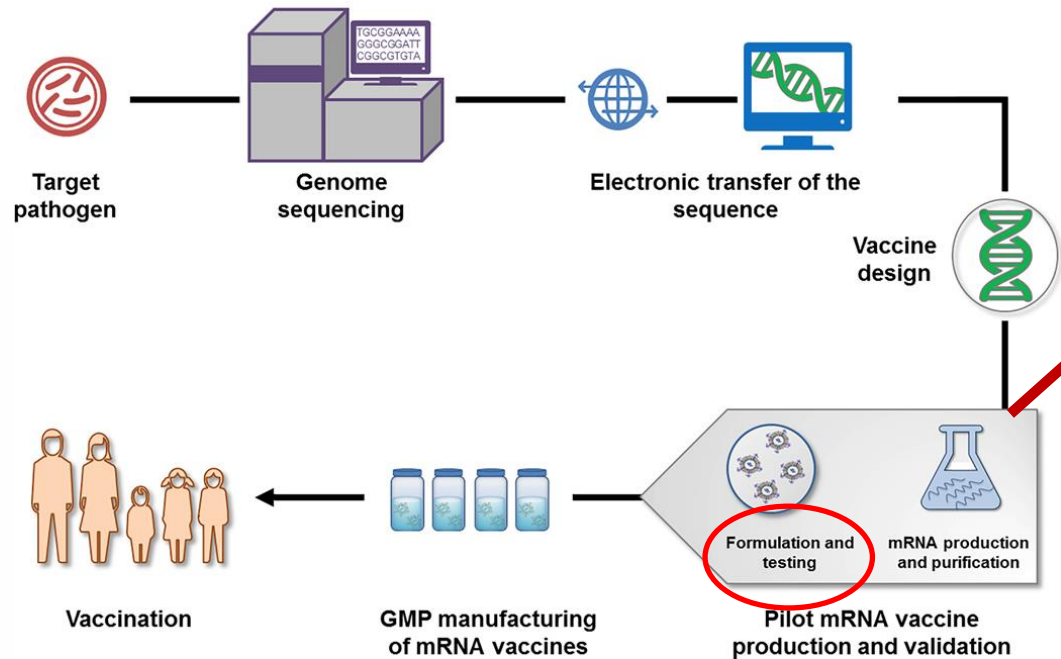


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# Overview



# Production of plasmid DNA

Biological risk has to be considered: **this phase is subject to legislation on contained use**

- Recombinant (plasmid carrying) *E. coli*, grown in culture flasks
  - Host strain K12 derived?
  - Non-transferable plasmid?
  - Risk enhancing insert?
  - Larger volumes for production purposes?
- Standard operation, presumably at risk level 1



# Lab work: Testing and QC of mRNA

- QC tests on the process and the production environment
- QC tests on the final product (mRNA)
  - “potency test” to analyze the activity of the mRNA:
    - Electroporation of mRNA in target cells; visualization
  - Immuno monitoring on the patients’ own cells
    - Cells may have to be expanded, e.g. by transduction by viral vectors

Biosafety aspects: Not the mRNA molecules, but the target cells may pose a biohazard

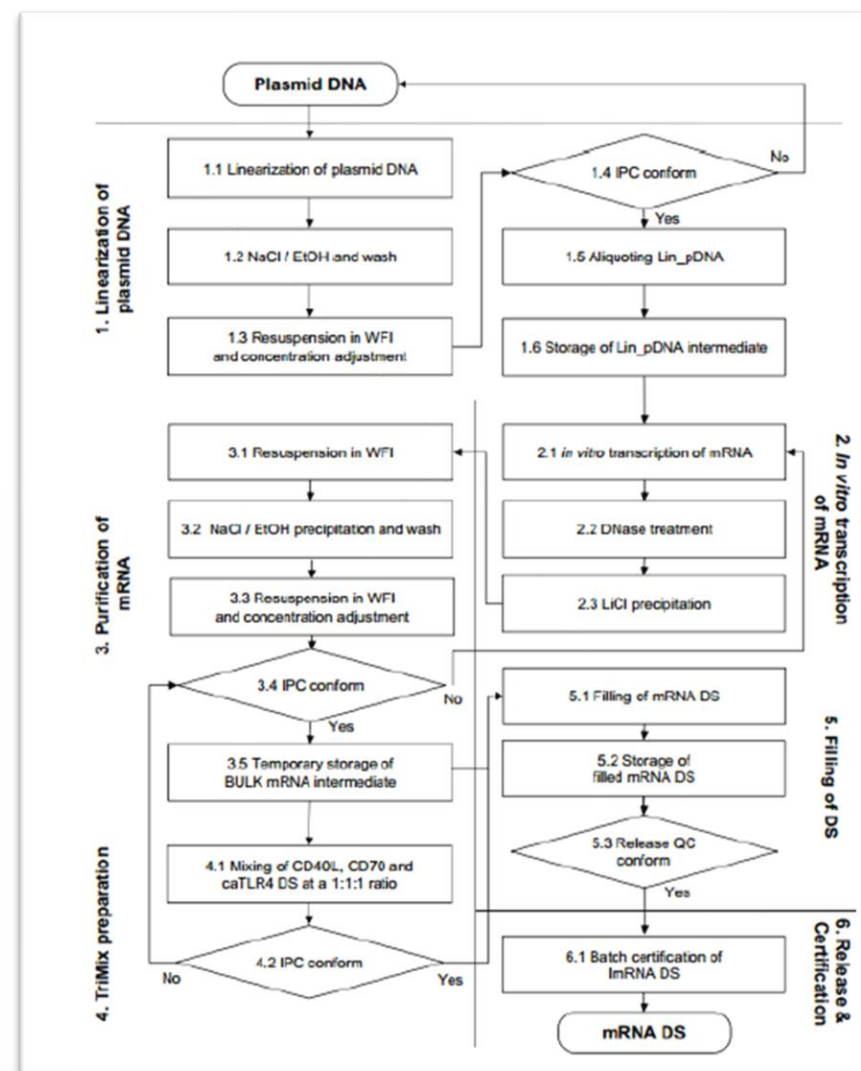
- Potential (unknown) presence of pathogens in human cells:  
*subject to legislation on workers’ protection against biological agents*
- Genetic modifications, viral transduction of target cells:  
*subject to contained use legislation*



# Handling of “naked” nucleic acids

pDNA → mRNA using a purely *in vitro*, enzymatic procedure, in the absence of living organisms

- Is this an activity subject to the “contained use” legislation ?
- Is there any biohazard associated with this activity ?





# Handling of “naked” nucleic acids

## Is this an activity subject to the “contained use” legislation ?

- European directive 2009/41/EG: only about “organisms”  
“naked” DNA or RNA is not an organism;  
directive does not apply
- Belgium: same position
- In other countries, different interpretations possible, e.g.:
  - UK: DNA/RNA is not an organism, hence no GMO, except if the complete sequence of a virus is involved.
  - NL: use of “naked” DNA/RNA is within the scope of the regulation, because can be incorporated in human cells if injected, creating GMO cells as a result.



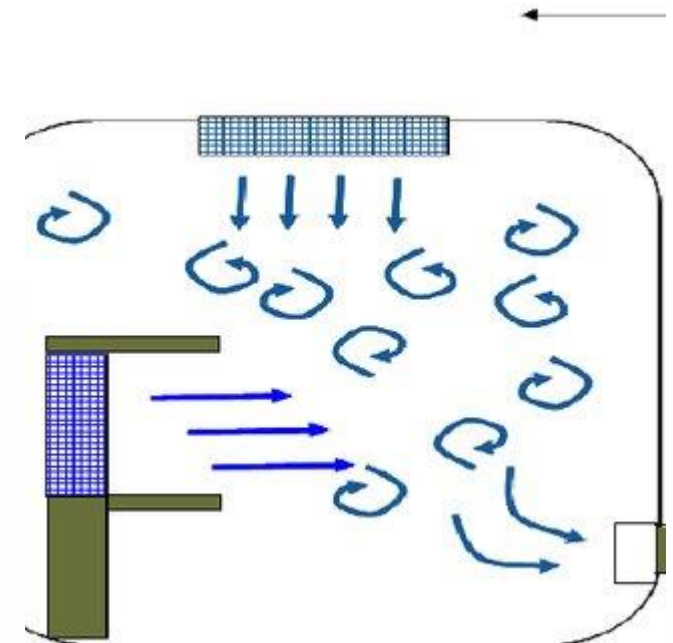
# Handling of “naked” nucleic acids

## Is there any biorisk associated with nucleic acids?

Enzymatic production in high grade cleanroom

- Airstream away from the subject
- Air currents could float towards the operator
- “PPE” is meant to prevent releasing particles

Consequence: operator may be exposed



# Handling of “naked” nucleic acids

## Is there any biorisk associated with nucleic acids?

Basic question: is uptake and unwanted integration of DNA/RNA in cells / genome possible?

Basic answer: Naked DNA/RNA is actually shown to be able to enter cells and be expressed.

- If at all, then at a very low frequency
- But some sequences may promote recombination
- Viral DNA/RNA: potential interaction with other viruses
- Specific sequences may promote uptake by bacteria
- Usual suspects: toxins, oncogenes, ...



# Handling of “naked” nucleic acids

## Is there any biorisk associated with nucleic acids?

General considerations:

Increased risk when handling DNA or RNA that codes for:

- highly biologically active molecules,
- full length viral genomes (cloned or isolated directly from a virus)
- active biological modifiers such as siRNA.

Risks can be over-estimated:

- Effective means needed by which DNA/RNA can gain entry into a cell and cause harm.
- For RNA, further factors complicate risk assessment:
  - Stability of RNA molecule
  - ubiquitous nature of RNase in the environment

# mRNA Vaccine Production: Biorisk Considerations



**Thank you for your attention**

... and thank you, colleagues @ Perseus !

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