

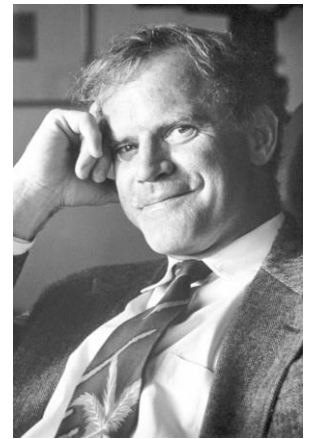
Lab on cell-free synthetic biology

Week 3

Outline

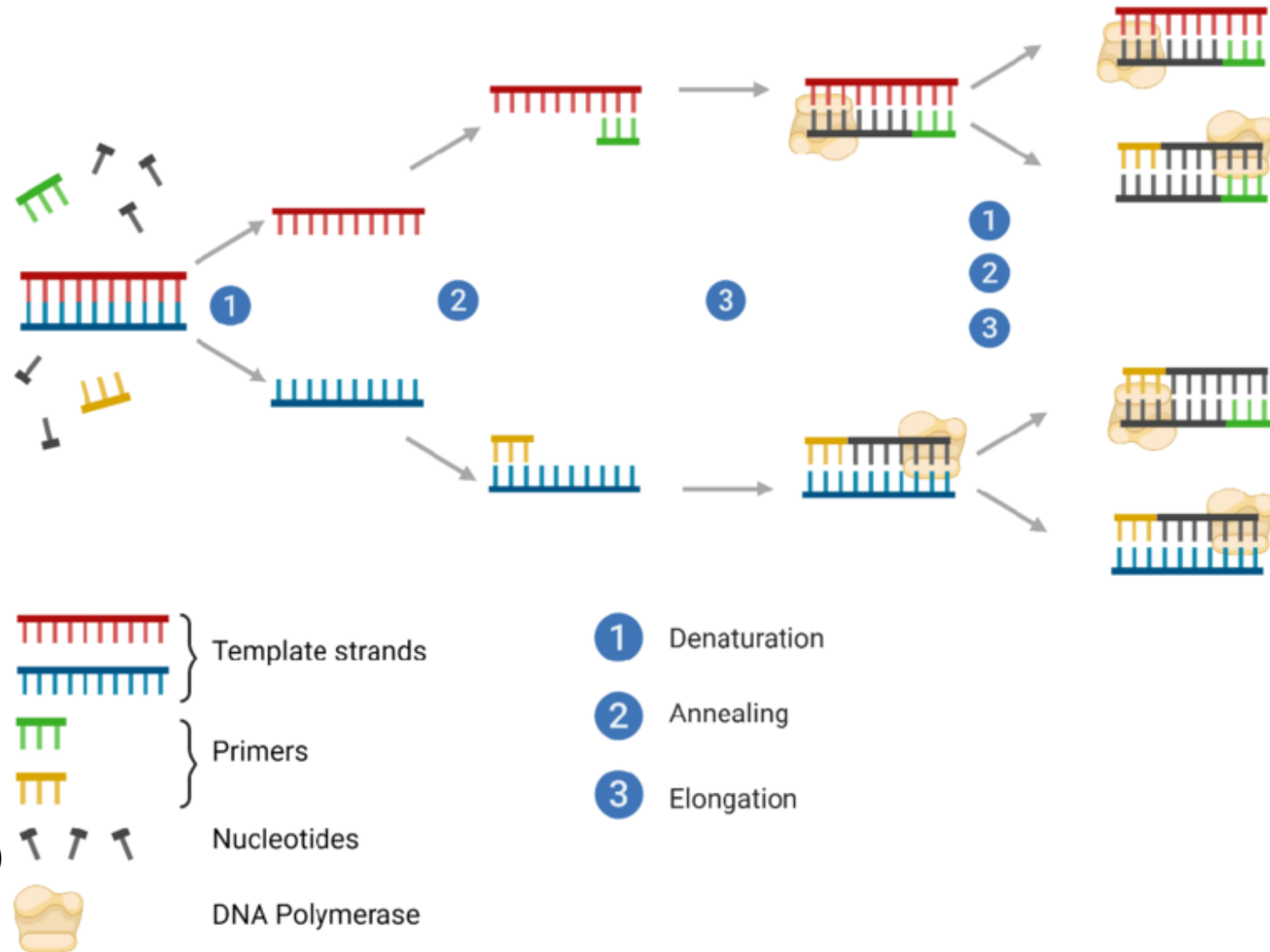
- Colony PCR + DNA electrophoresis
- Protein expression in the cell
- Protein expression in the cell-free system

Polymerase chain reaction



- Invented by Kary B. Mullis (The Nobel Prize in Chemistry in 1993)
- “Mullis recalled that, while driving in the vicinity of his country home in Mendocino County (with his girlfriend, who also was a chemist at Cetus), he had the idea to use **a pair of primers to bracket the desired DNA sequence and to copy it using DNA polymerase.**”

What is required in PCR?



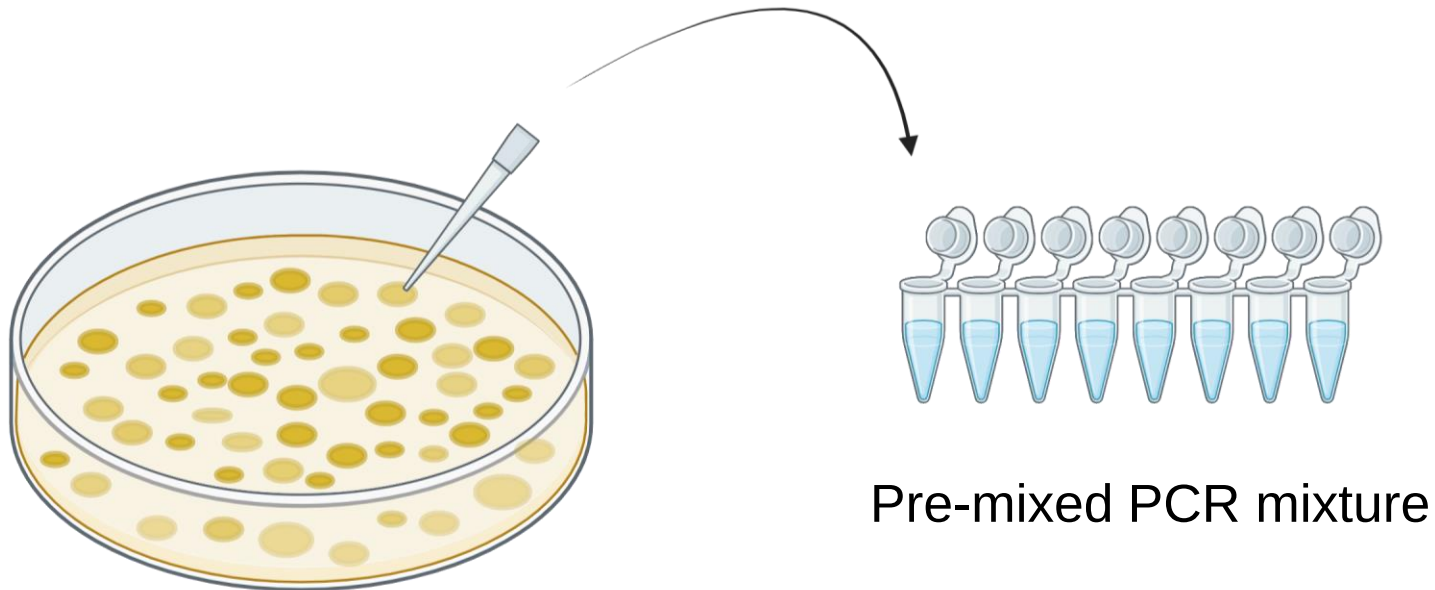
Mg^{2+}

DMSO (Dimethyl sulfoxide)



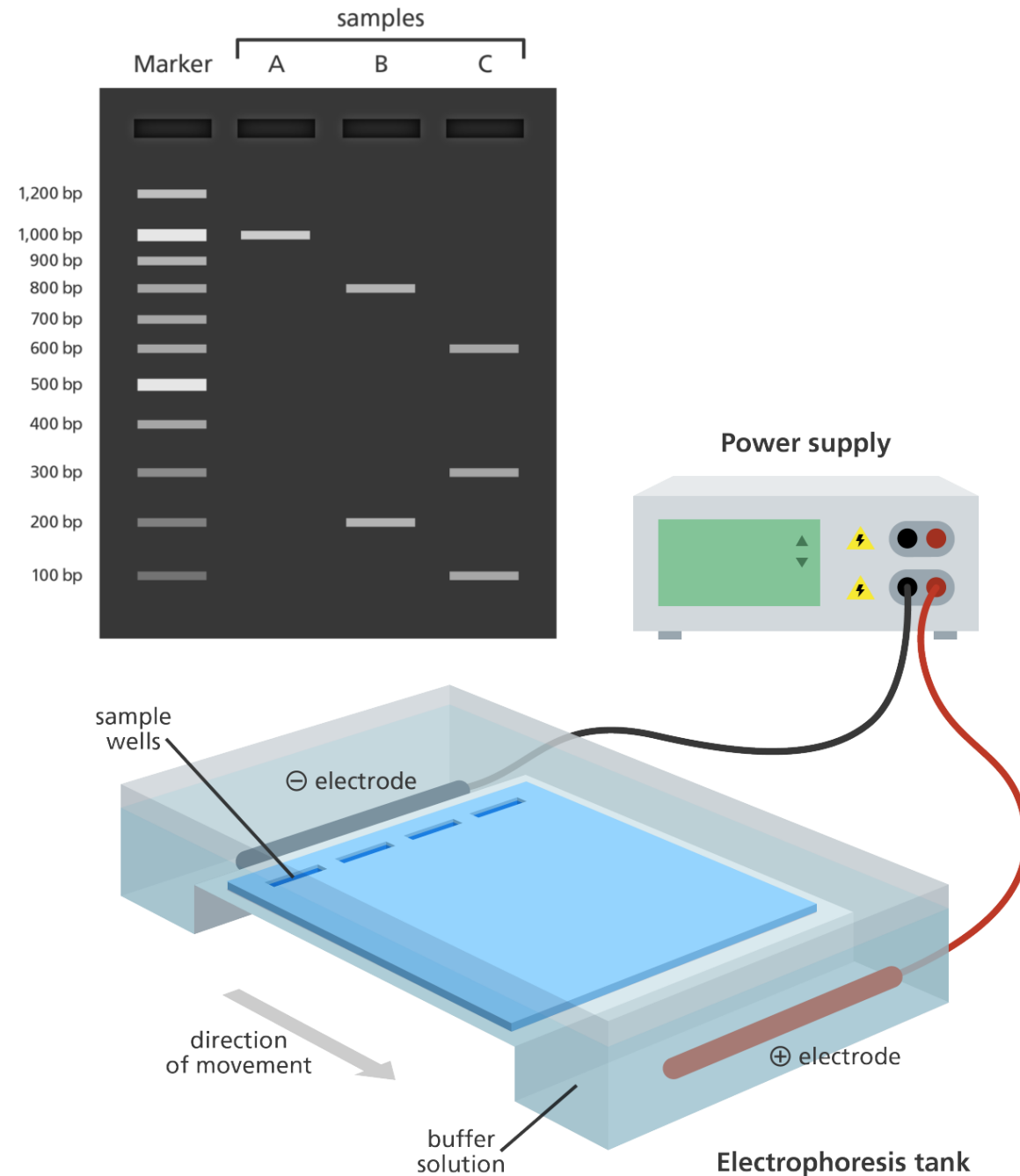
Colony PCR

- Colony PCR is a convenient method for determining the presence or absence of insert DNA in plasmid constructs.



Gel electrophoresis

- To separate different sizes of charged molecules (DNA, RNA, proteins, etc.)
- Agarose gel percentage depends on the DNA size
- DNA gel stain (ethidium bromide, SYBR safe, etc.)
- Marker
- Loading dye (mixed with the DNA samples for visualizing the migration)
- Check the electrode!!!



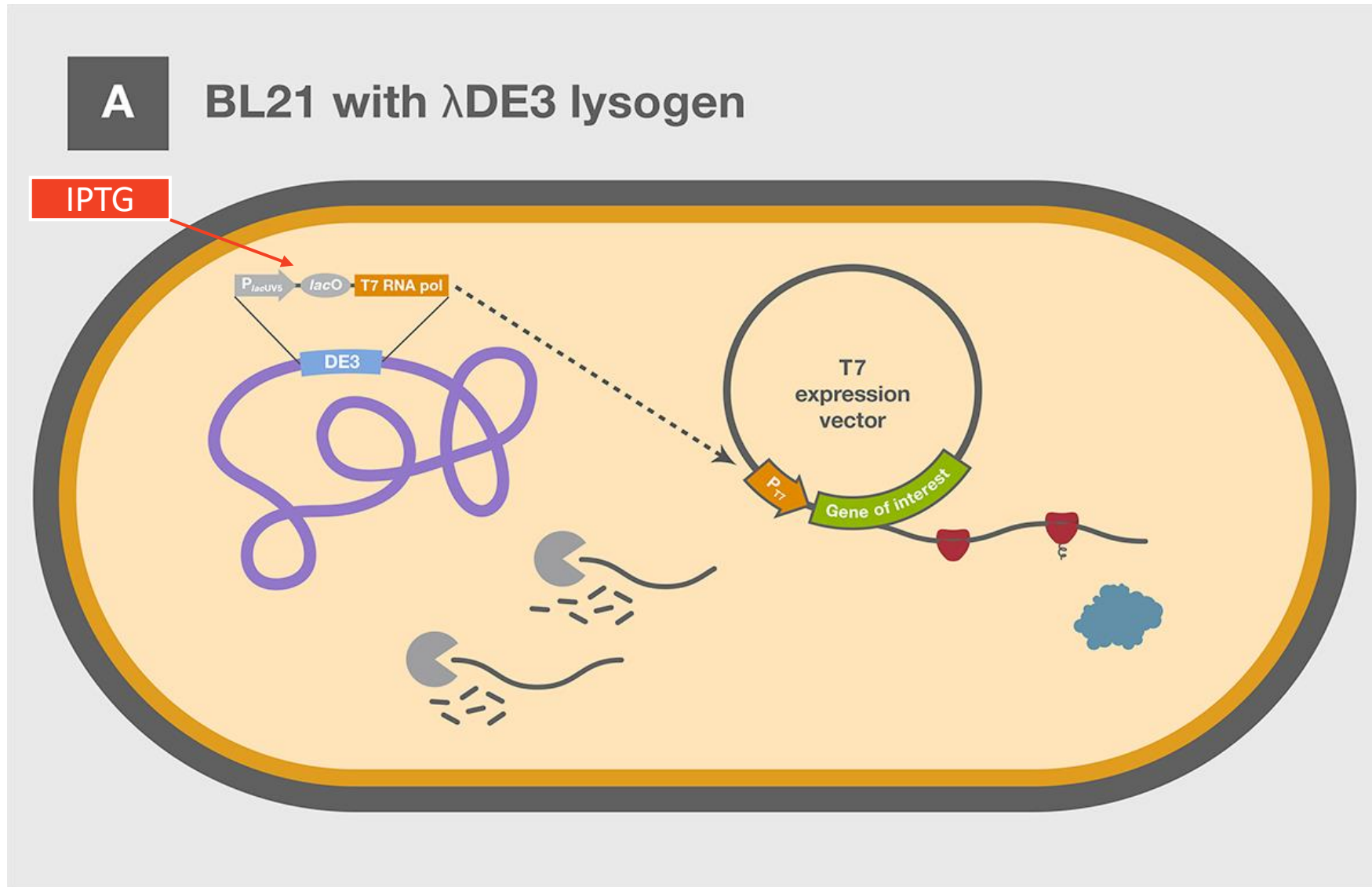
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Protein expression in *E. coli* BL21(DE3)

- Deficient in a cytoplasmic protease, Lon, and an outer membrane protease, OmpT.
- The BL21 strain is engineered to contain a λ DE3 lysogen to express the T7 RNA polymerase under the control of *lacUV5* promoter
- The *lacUV5* promoter is positively regulated by **IPTG** (isopropylthio- β -galactoside)
- Genes to be expressed can be cloned downstream of the T7 RNA polymerase promoter on the vector and induced from DE3 via IPTG

Protein expression in *E. coli* BL21(DE3)



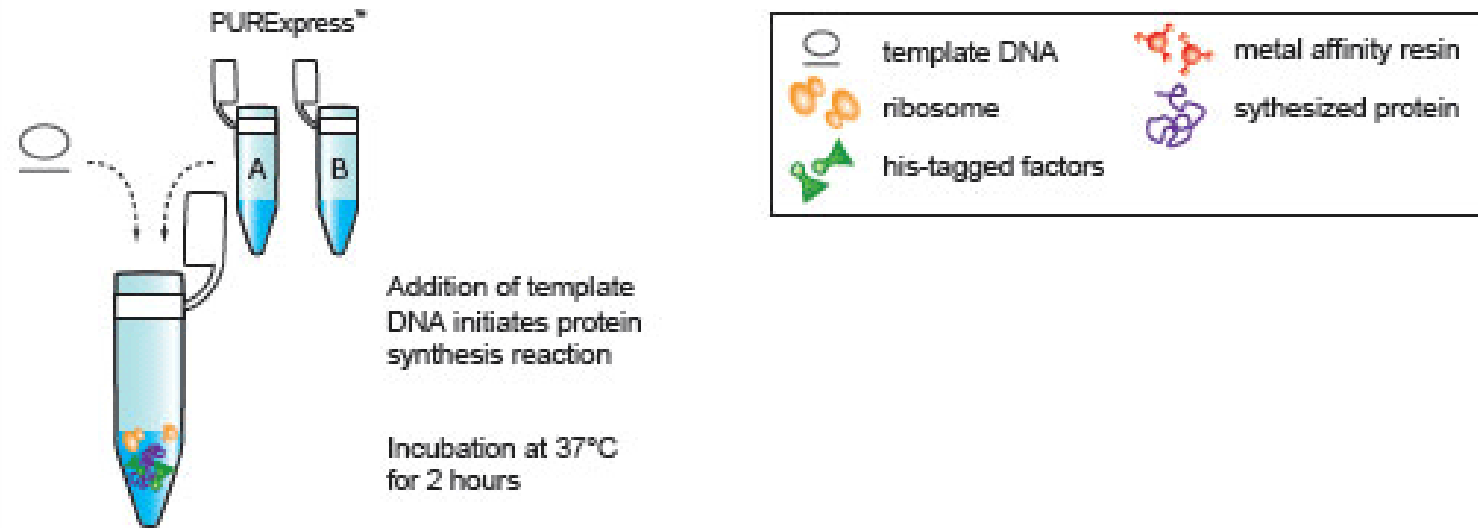
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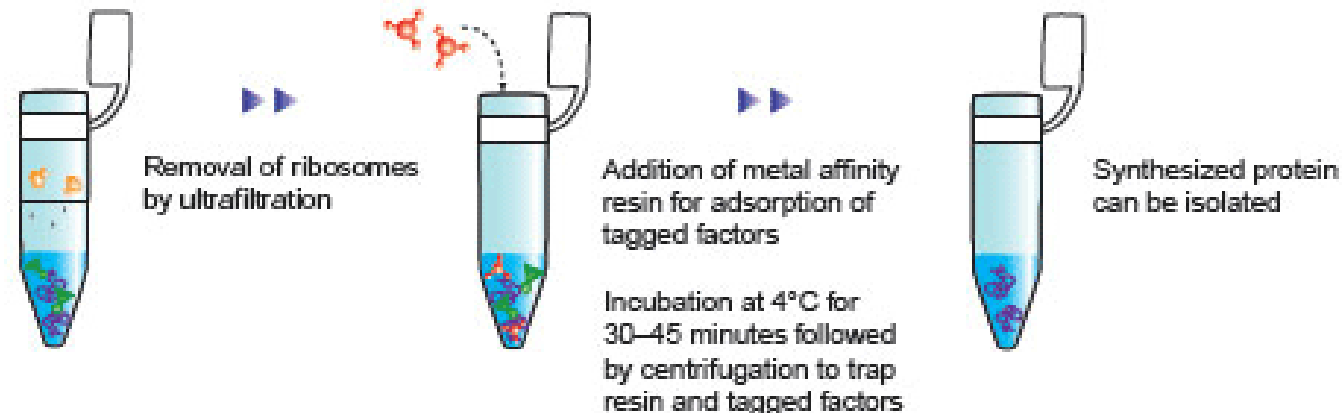
PURE reaction (commercial)

DNA
↓
RNA
↓
Protein

Protein synthesis using PURExpress



Reverse purification using affinity chromatography (reagents not included)



Next week

- Protein purification
- SDS-PAGE

References

- Jia B, Jeon CO. High-throughput recombinant protein expression in *Escherichia coli*: current status and future perspectives. Open Biol. 2016 Aug;6(8):160196.