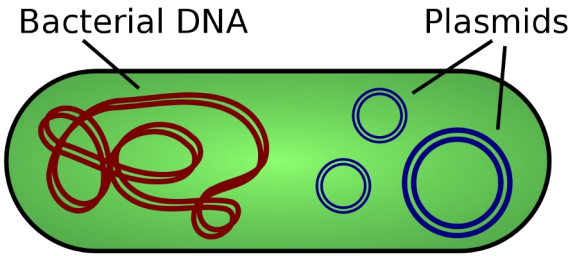
Laboratory 5

Objectives

- To isolate plasmid DNA
  - recombinant plasmid pcDNA6-Amy2 CDS-myc-His
  - parental plasmid pcDNA6/myc-His
- To determine the concentration and purity of purified plasmids
- To analyse plasmids by restriction enzyme digestion
  - Does the plasmid contain the expected Amy2 insert?

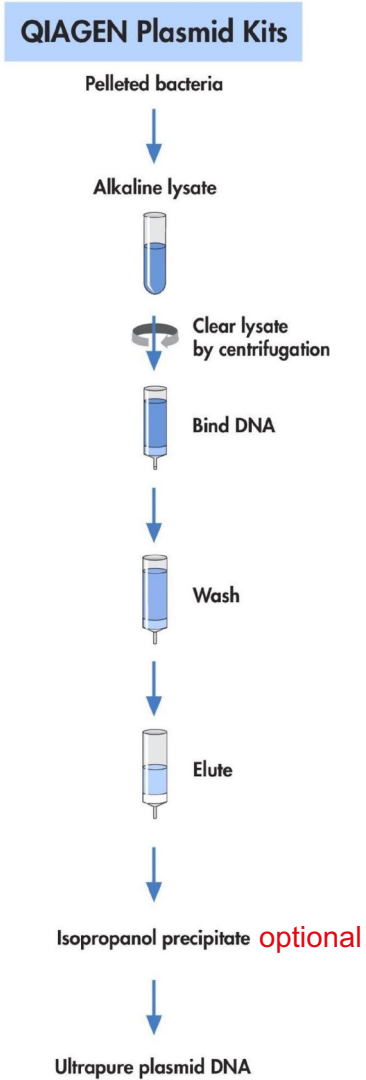
Isolation of plasmid DNA

- Plasmids are prepared from bacterial cultures grown in the presence of an antibiotic (here: Ampicillin).
- Bacterial overnight cultures from two clones (=single colonies)
  - pcDNA6-Amy2 CDS-myc-His (recombinant plasmid)
  - pcDNA6/myc-His (parental plasmid)



Miniprep procedure

- The miniprep procedure is based on the **alkaline lysis** method.
- Steps involved:
  1. Resuspension of bacterial pellet
  2. Alkaline lysis in the presence SDS
  3. Neutralization and clearing of the lysate
  4. Adsorption of DNA to the QIAprep silica membrane
  5. Washing and elution of plasmid DNA



Safety reminders

- Be careful not to contaminate your pipettes or benches with the bacterial cultures.

- Buffers P2, N3, and PB contain irritants. Wear gloves and safety goggles when handling these buffers.

1. Resuspension of bacterial pellets

- Resuspend pellet in buffer P1 (contains RNase A) by vortexing
- No cell clumps should be visible after resuspension of the pellet

2. Alkaline lysis in the presence of SDS

- Lyse cells in buffer P2. Mix by inverting the tube 4-6x. Do not vortex! (To prevent contamination of plasmid DNA with chromosomal DNA)
- SDS solubilizes the phospholipid and protein components of the cell membrane, leading to lysis and release of the cell contents
- Alkaline conditions denature the chromosomal and plasmid DNA, as well as proteins.

3. Neutralization and clearing

- Neutral pH allows renaturation of plasmid DNA.
- Chromosomal DNA, proteins and cell wall debris are precipitated and removed by centrifugation.

4. Adsorption of DNA to the silica membrane

- Plasmid DNA selectively binds to the silica membrane in high salt buffer.
- RNA, residual proteins and other metabolites are found in the flow-through.

5. Washing and elution of plasmid DNA

- Washing steps to remove endonucleases and salts.
- Elution of plasmid DNA in EB buffer (10mM Tris-HCl pH 7.5).

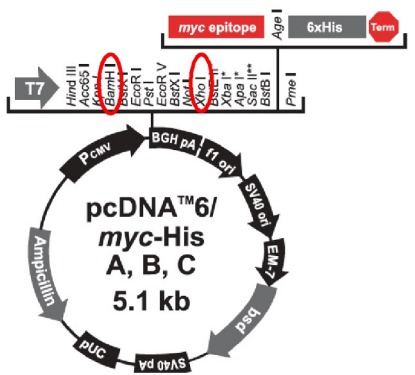
Analysis of plasmid DNA

- NanoDrop to measure concentration + purity
- Agarose gel electrophoresis to visualize restriction fragments

NanoDrop: DNA Yield and Purity

- **Yield** depends on plasmid copy number per cell, growth of the bacterial culture, and elution volume.
- **Concentration**
  - 1.0 A260 unit of dsDNA = 50 µg / ml
- **DNA purity**
  - A260 / A280 ratio should be around 1.8 for pure DNA sample
- Why is this ratio lower than for RNA?

Restriction digest



- Plasmids
  - pAmy2-His(pcDNA6/*myc*-His + Amy2 insert)
  - pcDNA6/*myc*-His
- Restriction enzymes
  - *Bam*HI and *Xho*I (enzymes used to clone the *Amy2* cDNA)
  - *Eco*RI (enzyme that cuts within the *Amy2* cDNA)
- Benchling
  - Simulate restriction digest
  - Create virtual gel

Analysis of restriction fragments by agarose gel electrophoresis

- Circular plasmids migrate differently than linear forms
- DNA ladder to estimate the fragment size
- For your ELN: add the size of fragments next to the gel (ladder and restriction fragments)

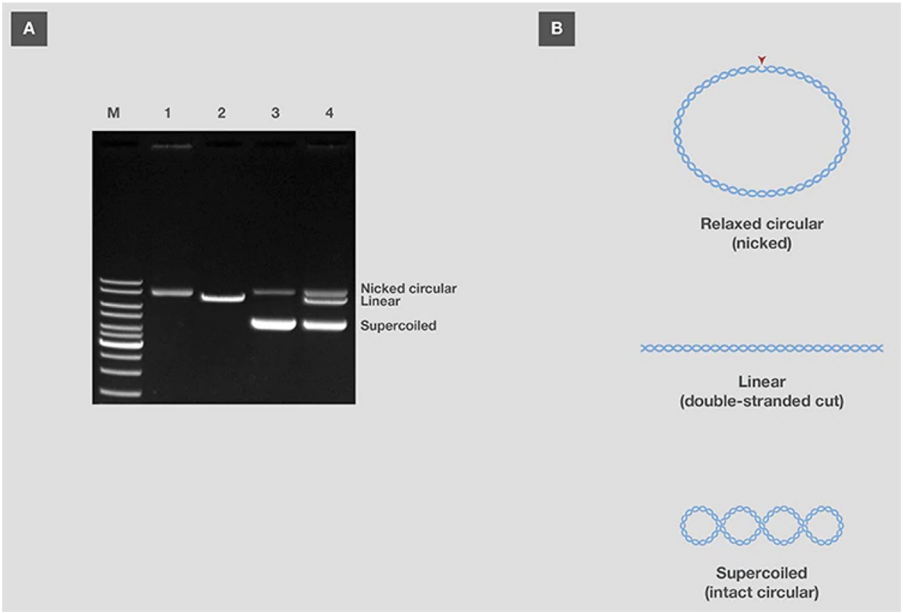


Figure 1. Electrophoretic migration of the same DNA in various conformations
Source : https://www.thermofisher.com/ch/en/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/na-electrophoresis-education/na-electrophoresis-considerations.html