**CAVAN plasmid miniprep:**

This procedure is based on the alkaline lysis procedure developed by Birnboim and Doly (Nucleic Acids Research 7:1513, 1979).

Procedure:

• Inoculate 1 colony in 3 ml LB+amp; O/N 37°C shaking

• Put cell suspension in 2 mL tube

• Spin down at 14000 RPM for 30s

• Resuspend with **300μl Resuspension solution** (vortex)

• Add **300 μl Lysis solution** and invert 3X (do NOT vortex)

• Incubate up to 5 minutes (not longer!)

• Add **300μl Neutralization** solution and invert 3X (do NOT vortex)

• Incubate up to 5 minutes

• Spin down for 10 min at 14000 RPM at 4°C

• Pipet the supernatant in a new epp

• Add 750μl of Isopropanol (precipitation)

• Vortex and incubate 2 min at RT

• Spin down at 4°C for 15 min (14000 RPM)

• Discard supernatant

• Wash pellet with 300 μl of 70% EtOH

• Spin down at 4°C for 1 min at 14000RPM

• Discard supernatant

• Repeat washing step

• Dry pellet with speedvac for 30 min

• Add 50 μl of TE-buffer

The procedure takes advantage of the fact that plasmids are relatively small supercoiled DNA molecules and bacterial chromosomal DNA is much larger and less supercoiled. This difference in topology allows for selective precipitation of the chromosomal DNA and cellular proteins from plasmids and RNA molecules. The cells are lysed under alkaline conditions, which denatures both nucleic acids and proteins, and when the solution is neutralized by the addition of Potassium Acetate, chromosomal DNA and proteins precipitate because it is impossible for them to renature correctly (they are so large). Plasmids renature correctly and stay in solution, effectively separating them from chromosomal DNA and proteins.

Solutions:

Resuspension solution: 50 mM Tris-HCl (121,14 g/mol), 10 mM EDTA (292,248 g/mol), 100 µg/mL RNase A, pH 8.0

Stock solution:

1L: 6,057 g Tris + 3,722 g EDTA.2H2O ; adjust to pH 8,0 (HCl)

500 mL: 3,0285 g Tris + 1,861 g EDTA.2H2O ; adjust to pH 8,0 (HCl)

Working solution:

Add 0,6 ml of RNase (20mg/ml) to 100ml of resuspension solution store at 4°C

When the cells are lysed in the next step, the RNase will catalyze hydrolysis of all RNA molecules into nucleotides, but the DNA will not be affected.

Lysis solution: 1% SDS; 0,2 M NaOH (39,99711 g/mol)

1L: 8,0 g NaOH pellets in 900 ml MQ-water; autoclave; add 100 ml of SDS 10%

500 mL: 4,0 g NaOH pellets in 450 ml MQ-water; autoclave; add 50 ml of SDS 10%

SDS is an ionic detergent which disrupts cell membranes and destabilizes all hydrophobic interactions holding various macromolecules in their native conformation. The high pH of the 0.2 M NaOH also denatures macromolecules by changing the condition of ionizable groups (ionizing certain groups and deionizing others). The clearing you see is because the cells are lysing. The viscosity of the solution is increased by the increase in concentration of macromolecules in solution (a result of the cell lysis).

Neutralisation solution: 3.0 M Potassium Acetate (KCH3COO; 98,14 g/mol), pH 5.5

1L: 294,45 g KAc in 500 ml H2O; pH to 5,5 with acetic acid (CH3COOH); H2O to 1L

500 mL: 147,225 g KAc in 250 ml H2O; pH to 5,5 with acetic acid, H2O to 500 mL

This is really the key step in the alkaline lysis procedure. The low pH of the Potassium acetate solution neutralizes the NaOH and when the pH returns to near-neutrality then the macromolecules renature. The proteins and large DNA molecules do not renature correctly however. They form hydrophobic, ionic and hydrogen bonds with each other non-specifically because the correct conformation of the molecule was not maintained during denaturation. The plasmid DNA molecules, however, never really fully denatured because they are small circular molecules, which are supercoiled. Even though the hydrogen bonds between base pairs were broken by the high pH, they reform correctly when the pH is lowered. The large DNA molecules (chromosomal DNA) and proteins form precipitates because they bind to each other in a large aggregate but the plasmids don't precipitate because they renature correctly and don't become part of the large multi-molecule aggregates. Thus plasmid DNA remains in solution while proteins and other DNA molecules precipitate.

TE-buffer (1X): 1mM EDTA (292,248 g/mol), 10 mM Tris (121,14 g/mol); HCl pH 8.0

1L (10X): 3,722 g EDTA .2H2O ; 12,114 g Tris

100 mL (10X): 0,3722 g EDTA .2H2O ; 1,2114 g Tris

TE buffer is commonly used to redissolve DNA because it contains EDTA. The EDTA will chelate magnesium ions, which are a cofactor for most nucleases (enzymes which degrade nucleic acids). If your DNA prep becomes contaminated with a nuclease (like the ones produced by the cells in your skin) then the nuclease will be inactivated by the fact that the magnesium cofactor is unavailable in the solution (because it is chelated by the EDTA).