

Rapid Isolation of Plasmid DNA (MiniPrep)

General review/Cloning review (hopefully from one colony)

Alkaline lysis method

Growth of bacteria: Many minipreps are usually done concurrently.
Pellet and discard supernatant, and add GTE (glucose, TRIS, and EDTA)

Alkaline lysis

Add lysis buffer: SDS and NaOH: breaks apart membrane and genomic DNA.

Add potassium acetate: Will neutralize NaOH and make a flocculent made of genomic DNA and proteins.

Note: NaOH will denature DNA, but plasmid DNA loops remain linked. When KO-Ac is added, most DNA and proteins will clump, but plasmid DNA will re-anneal (as they are already linked) and as a result will stay in suspension. Diagram process below:

Alcohol ppt: Cold isopropanol, vortex and spin (plasmid will form pellet at bottom:

Technique Note: Microfuge tube is orientated so that the hinge is facing outward. After spinning, the pellet appears in the bottom of the tube, directly below the hinge (aids in locating.)

Tubes are opened and warmed briefly to drive off any residual alcohol.

RNAase treatment: Note: RNAases must be DNAase free. Incubate @ 37 °C for 1 hour.

Note gel results (specifically plasmid and insert)

Amount of plasmid visible is a function of copy rate. Low copy plasmids make about 20 plasmids/cell and high copy are 100-1000 plus copies.

Undigested RNA can be seen as smear at the bottom of the gel lane.

Alternative methods:

96 well plates: Same idea as above, but in 96 well plates (for replicates.)

Boiling miniprep: Lysozyme incubation, detergents and heat. Chromosomal DNA will pelletize as it stay attached to the cell membrane (so plasmid is in the supernatant.) This techniques results in lower quality DNA.

Commercial kits: We will use Qiagen minipreps. Strive to understand rational for each step.