Bacteria Transformation
Colony Pick-up
Miniprep
Restriction Enzyme Digestion
What are these steps for?

- Insert
- Vector
- Ligation Reaction
- Desired Construct
What are these steps for?

vector

Ligation Reaction

Desired construct

Ligation by-product

insert
Bacteria transformation

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.
Selection of successful transformant

kanamycin
Bacteria transformation select DNA constructs

Growth

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Restriction Enzyme Digest

Growth

Restriction Digest
Two bands

One band
Bacteria Transformation/plating

- Use chemically treated cells (MnCl$_2$, dimethyl sulfoxide)
- On ice 30 min followed by heat-shock
- Incubate with liquid medium without antibiotics
- Plate on selection plate
Alkaline lysis method of plasmid purification

- Denature protein and chromosomal DNA with SDS (strong detergent) and NaOH (solution 2)
- Neutralize with potassium acetate and centrifuge (solution 3)
- SDS makes precipitate with potassium
- Protein and chromosomal DNA will precipitate
- Plasmid DNA is compact and remain on supernatant.
- Remove remaining salt with isopropanol precipitation