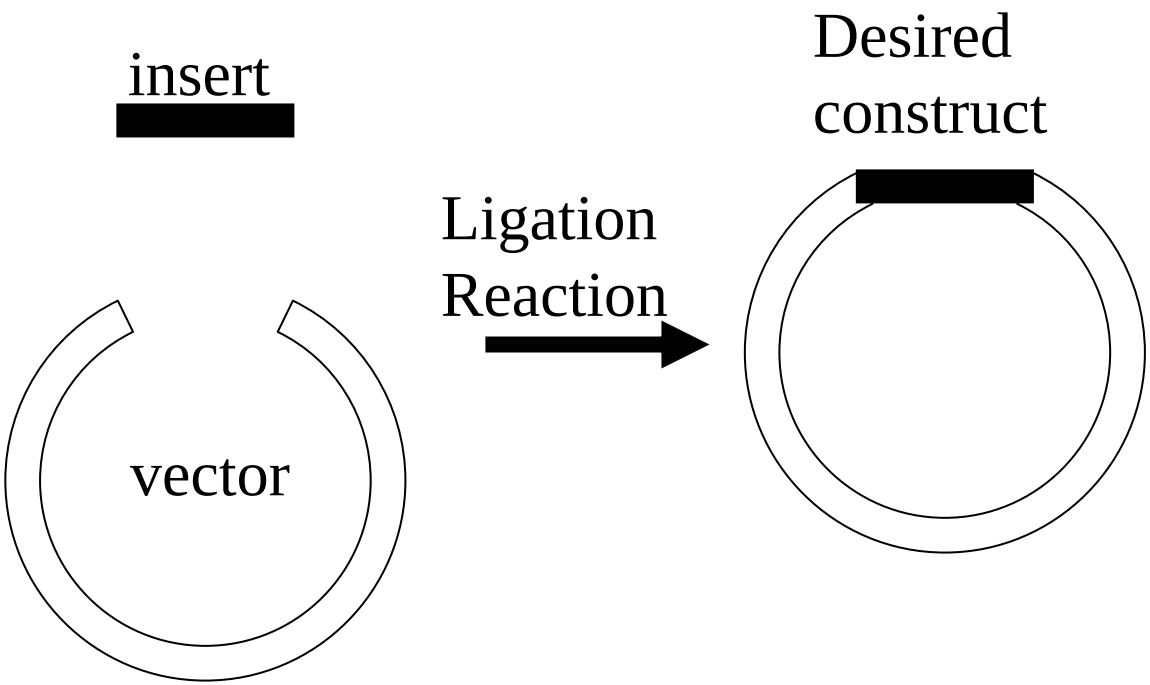
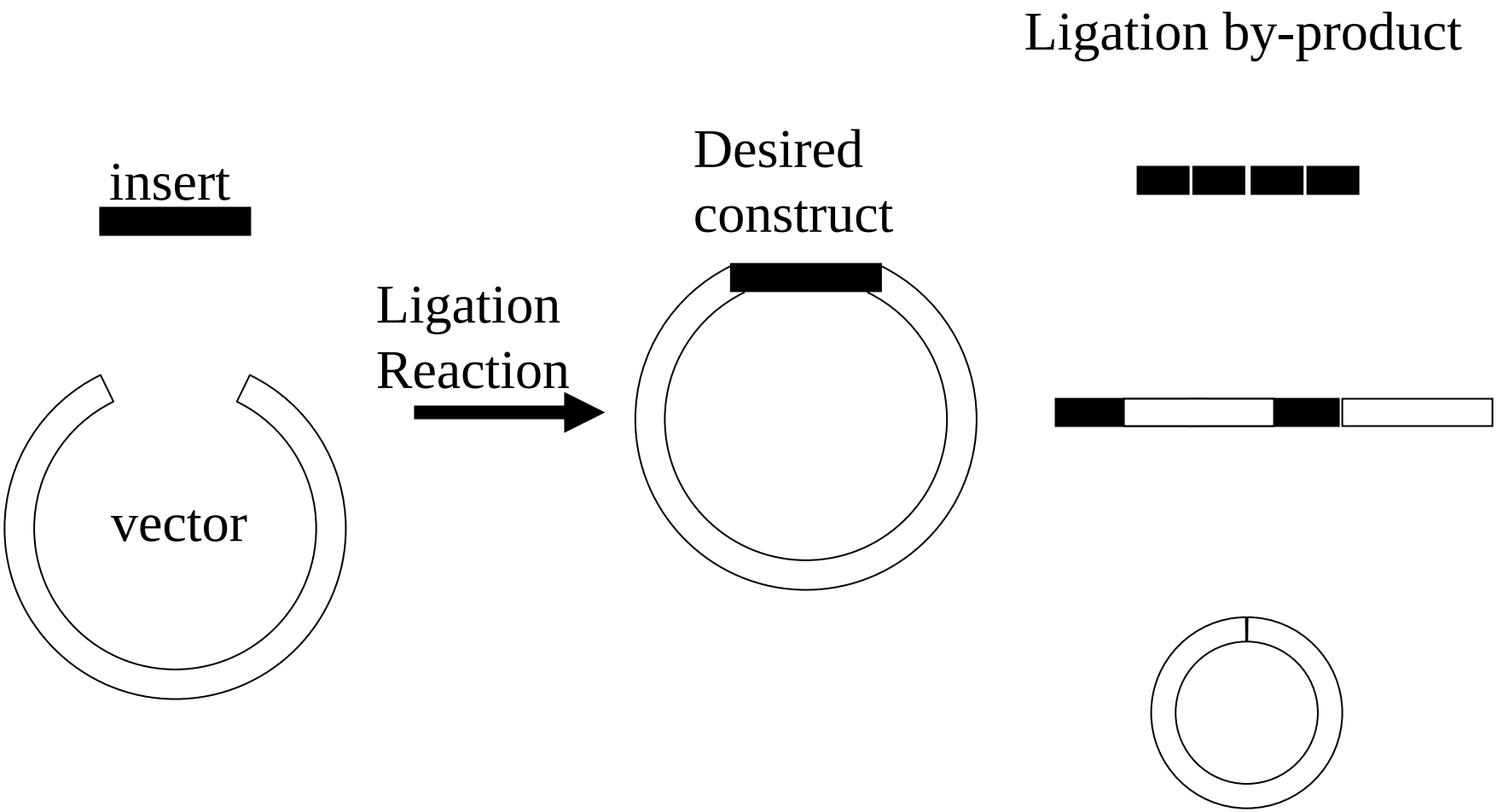
Bacteria Transformation
Colony Pick-up
Miniprep
Restriction Enzyme Digestion

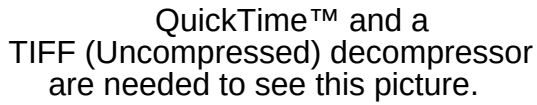
What are these steps for?



What are these steps for?



Bacteria transformation



Selection of successful transformant

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

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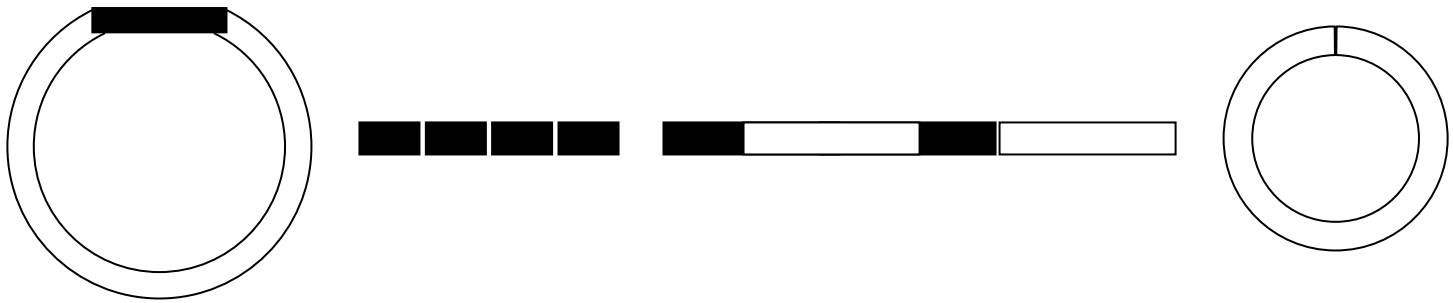
kanamycin

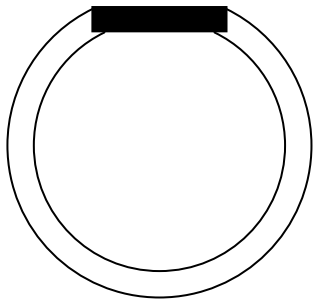
kanamycin

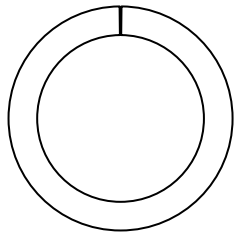
Bacteria transformation select DNA constructs



Growth	+	-	-	+

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