

Lab: DNA Ligation/Bacterial Transformation
Teacher Prep!

Day 1: DNA Ligation

- Just have reagents ready – students will prepare sample solution and three controls. No additional prep is necessary.

Day 2: Innoculation and plate preparation

- Solutions to make:
 - o Add 200µl DMF (dimethyl formamide) into the microcentrifuge tube containing the X-gal powder.
 - o Add 200µl of sterile water into the microcentrifuge tube containing IPTG powder.
 - o Transformation Solution Buffer (TS buffer) – Add 35ml of ice cold sterile water into a 50ml polypropylene tube containing powdered TS buffer. Mix the contents thoroughly, and keep the unused solution in the freezer (along with unused ampicillin and unused white cells).
- Resuspend the plasmid DNA samples with 200µl of sterile water. Dissolve the plasmid DNA by mixing the solution up and down with a clean pipet tip. Keep the tubes in the refrigerator or freezer for future use.
- Sterilize the bacterial media – add 100ml distilled water into a clean 500ml beaker containing 2.5g powder LB media. Mix thoroughly and autoclave. Store in refrigerator.

Day 3: Transformation

- Have incubator/hot water bath on hand (42 deg C)

