

PLASMID PREPARATION FOR IUE SURGERY

DAY 1 - Bacterial Transformation:

- Before beginning this procedure, make sure you have the following available per plasmid:
 - 250µl LB medium: *use 25 capsules per liter of water, autoclave (liquid cycle for 20 min) to dissolve. (MP Biomedicals, cat# 3002-021)*
 - 1 LB agar plate with ampicillin: *use 20 tablets per liter of water, autoclave (liquid cycle for 20 min) to dissolve. (Sigma, cat# L7025-100TAB)*
 - *Once dissolved, allow solution to cool so that you can handle it, and add ampicillin:*
 - *Using a 100mg/ml stock of Ampicillin (HyClone/Fisher, cat# SV30071.01), add 1µl Amp for every 1ml LB agar medium (final concentration = 100µg/ml). Ampicillin plates don't last forever – usually a few months – so if your bacteria grow in a lawn (not separate colonies), this may be why.*
 - *Pour plates before the agar reaches room temperature (otherwise it will solidify!). Each plate should have a ~5mm layer of agar.*
 - *Once agar is solid, invert plates and leave to dry overnight. Store at 4°C.*

- 1.) Thaw competent DH5α bacterial cells on ice, 5-10 min.
 - *DH5α cells are kept in the -80°C freezer, and should be thawed just long enough so that liquid can be drawn up. Repeated freeze-thaw cycles can destroy competent cells.*
 - *Turn on water bath, temperature set to 42°C.*
- 2.) Add 30µl bacteria to bacterial tubes, keep on ice.
- 3.) Add 8-15µg of your desired plasmid to the bacterial tubes, tap to mix.
 - *The amount of DNA added is approximate, any number in this range should be enough. For example, if your stock plasmid is 5.0µg/µl, use 2µl, if it is lower, use more. Do not vortex the tubes with competent cells, this can shear genomic DNA.*
- 4.) Incubate DH5α cells + plasmid DNA on ice for 20 minutes.
 - *Allowable range is 10-30 minutes, adjust time if necessary.*
 - *At this time, remove LB-agar plates (with appropriate antibiotic) from cold storage, and leave upside down at room temperature to warm up.*
- 5.) Heat shock the bacteria at 42°C for 1 min, remove immediately and place tubes on ice.
- 6.) Add 600µl LB medium to tubes.
 - *To prevent contamination of LB stock and bacterial solution, perform this step near a flame, under a hood if possible.*
 - *Do not add antibiotic at this stage!*

- 7.) Incubate in shaker (~250rpm) at 37°C for 45min-1hr.
 - *At the end of this time, make spreaders out of long-tipped glass Pasteur pipets (bend in flame).*
- 8.) Remove 50µl from broth mixture and pipet onto agar plate and spread evenly. When dry, turn upside down and incubate overnight at 37°C.

DAY 2 - Growing Cultures for Maxi Prep:

- 1.) In the morning, remove plates from incubator.
- 2.) Prepare bacterial tubes with 2 ml LB medium and 2µl Ampicillin.
 - *Perform this step near a flame.*
 - *Using a 100mg/ml stock of ampicillin, add 1µl Amp for every 1ml LB medium (final concentration = 100µg/ml).*
- 3.) Using a pipet tip, gently scrape a colony from the plate and eject tip into the bacterial tube.
 - *Make sure you only touch one colony – we want exact clones only!*
 - *Select a colony that is medium size for the plate and isolated.*
- 4.) Incubate in shaker (~250rpm) at 37°C for 4-8 hrs.
 - *During this time, autoclave 500ml flasks and centrifuge bottles (250 or 500ml size, depending on the centrifuge being used at the beginning of Day 3). DO NOT CLOSE THE CAP ON THE PLASTIC CENTRIFUGE BOTTLES WHILE AUTOCLAVING. This would result in a pressure change inside the bottle, deform it, and would render it unusable.*
- 5.) Add 250ml LB medium and 250µl Ampicillin to 500ml flasks. Pour the broth from the cultured bacterial tubes into corresponding flasks of fresh medium, and incubate in the shaker overnight at 37°C.

DAY 3 – Maxi Prep:

- 1.) In the morning, remove flasks from the shaker.
 - *If the culture grew successfully, your medium should be cloudy.*
- 2.) Start the protocol for Plasmid DNA Purification found in the booklet of the QIAfilter Maxi Kit at STEP 3 with the following changes.
 - *Use the directions for “QIAfilter Maxi,” 250ml. Amounts of reagents to be used should be printed in red.*
 - *Step 3.) Harvest the bacterial cells by centrifugation at **4500 rpm** for 15 min at 4°C.*

- Step 7.) *Instead of incubating, **centrifuge** lysate after P3 at **7000 rpm for 10 min.***
- *Perform Step 8.) while you wait. When centrifugation and equilibration is done, pour supernatant into QIAfilter Cartridge and proceed with Step 9.)*
- Step 13.) *Precipitate DNA with 10.5ml room-temperature isopropanol and centrifuge at **3400 rpm for 30 min.***
- Step 14.) *Wash DNA pellet with 5ml room-temperature 70% ethanol and centrifuge at **3400 rpm for 1 hour.***
- Step 15.) *Carefully pour out ethanol, inverting the tube only once to prevent loss of pellet. After pouring out the liquid, keep tube inverted for 5 minutes to allow pellet to air-dry.*
- Step 16.) *Wash DNA pellet with room-temperature 95% ethanol and centrifuge at **3400 rpm for 30 min.***
- Step 17.) *Perform drying procedure as indicated in Step 15.), then dissolve in 100-150µl TE buffer. This amount should result in a relatively high final concentration, which is good for surgery prep.*

3.) Quantify using the NanoDrop. Remember to use TE Buffer as your blank.

YOUR PLASMIDS ARE READY TO USE FOR IUE SURGERY PREP!