

- Prepare 6 x 0.75 L+200mL of 2xYT:
 - Prepare 3.2 L 2xYT
 - 3x 46.5 g 2xYT in 1.4 L water
 - OR (Alternative Measurements)
 - 2x 62g 2xYT in 2 L water +
 - 1x 31 g 2xYT in 1 L water +
 - 1x: 6.2g 2xYT in 200 mL water
 - Autoclave (with plate mix below)
- Prepare phosphate solution
 - 300 mL dibasic phosphate solution
 - 165 mL monobasic phosphate sol.
 - Sterile filter
- Prepare a 2xYT+P.P+Chlor plate (can be replaced by Chlor/LB plate)
 - Mix:
 - 1.24g 2xYT
 - 0.44g Bacto-Agar
 - water to 40 mL
 - stir bar
 - Autoclave (with 2xYT above)
 - Let cool to ~50°C, stirring, then add:
 - 2.5 mL phosphate solution
 - 60 µL chloramphenicol stock
 - Mix, pour, let cool 1 hr.
- Prepare 2L S30 buffer B (2x for a total of 4L if doing dialysis):
 - Mix:
 - 10.88g Mg-glutamate
 - 24.4g K-glutamate
 - stir bar
 - water to 1.9 L
 - Stir until dissolved
 - Stir, pH to 8.2 w/ 2M Tris (& acetic acid if necessary)
 - Water to 2 L
 - Autoclave
 - Store at 4C

TX-TL Day 1: Plates

- Streak 2 plates with *E. coli* glycerol stock (Multiple plates recommended – choose the healthiest later)
- 37°C incubator (~19 hr)
- Check DTT at -80°C (need 2x1mL @ 1M)

Date: _____

TX-TL Day 2: Stage 1 and 2 Growth

- Stage 1 miniculture (do 2x):
 - Mix & warm for 30 min 2 minicultures:
 - 4mL 2xYT
 - 270 μ L phosphate solution
 - 4 μ L chloramphenicol
 - 5 colonies to each miniculture \rightarrow shake @ 37°C ~8 hr (choose colonies from the healthiest plate).
- Stage 2 miniculture:
 - At 7.5 hr mark, mix & warm for 30 min:
 - 100 mL 2xYT
 - 6.6 mL phosphate solution
 - 100 μ L chloramphenicol
 - At 8hr mark, inoculate w/50 μ L Stage 1 culture (use 50uL of each if both grow well, otherwise choose darker stage 2).
 - Split into 2x50 mL \rightarrow Shake @37°C ~7-8 hr

Time into incubator: _____

Time into stage 1: _____

OD after stage 1: _____

Time into stage 2: _____

TX-TL Day 3: Stage 3 Growth

- Add 93 mL phosphate solution to 1.4 L 2xYT → add water to 1.5L.
- Add 0.75 L 2xYT+P to 6x4L flasks → At 7.5 hr mark, warm in shaker for 30 min.
- Add 7.5 mL stage 2 culture
- Grow @ 220 rpm, 37°C until OD ~2-4: checking OD periodically (~3-4 hr).
 - To avoid overgrowth, **check OD every 20-30 minutes.** Remove from shaker at OD ~3. Lower ODs yield less extract and higher ODs (above 4) may result in weaker extract.
- While growing:
 - Pre-chill floor centrifuge to 4°C
 - Weigh 6 empty 50 mL Falcons → chill on ice.
 - Thaw 2mL 1M DTT
 - Chill S30B buffer @4°C
- Culture → 6x1 L centrifuge bottles → balance within 1g.
- Spin 12 min @ 5000g, 4°C
- While spinning, add 2 mL 1M DTT to 2L S30B buffer → Mix → to ice
- 2x:
 - Decant supernatant → blot dry
 - 150 mL S30B buffer to each bottle
 - Shake until no more clumps
 - Spin 12 min @ 5000g, 4°C
- Decant supernatant → blot dry
- 40 mL S30B buffer to each bottle → transfer to pre-weighed falcon tubes
- Spin 8 min @ 2000g, 4°C
- Decant off sup. -> Spin 2 min @ 2000 g, 4°C
- Remove residual supernatant by pipetting
- Optional: Flash freeze pellets, store @ -80°C

Date: _____

OD into stage 3: _____

ODs in stage 3: _____

OD at spin: _____

Pellet weights: _____

TX-TL Day 4: French Press, Run-off, and Dialysis

- Put French Press pieces in cold room
- Weight pellets → add 1.4mL S30B/1g pellet
- Vortex to homogenize
- French press once
 - Move platform all the way down
 - Add handle to cylinder cap
 - Glycerol parts
 - Cylinder upside-down, insert rod
 - Fill with TX-TL, push piston up until just enough head to fit cylinder cap
 - Cylinder cap on (should squirt a bit)
 - Flip cylinder to press, lock in place
 - Selector to “high”, keep pressure at 640, tap handle lightly to adjust
 - Cleanup: Wash parts with water, EtOH, then milliQ water.
- Immediately add 3 µL DTT/1mL lysate, mix
- Spin 30 min @30,000g, in centrifuge tubes, 4°C
- Collect lysate → Spin 30 min @30,000g, 4°C
- Pipette supernatant into 50mL Falcons
- Run Off Reaction
 - Incubate 60 min @37°C, aerating (with lid slightly loose)
- Spin 15 min @15,000g, 4°C to clarify (repeat once if there is lots of sediment).
- Immediately decant+pipette supernatant → ice
- Dialysis [in cold room]: # 10k MWCO Dialysis Cassettes = Extract Volume / 3 rounded up.
- 2 ml of 1 M DTT to 2 L of S30B. Mix and split into two sterile 1 L beakers with sterile magnetic stirrer into each beaker; keep at 4 °C.
- Load cassettes with 3 ml of extract.
- After loading suck out excess air with syringe.
- Dialyze in 1L of S30B stirring, at 4 °C for 45 min.
- Move cassettes to a new L of S30B buffer and dialyze for another 45 min.
- Aliquot → flash freeze in LN2 → Store @-80°C (save a little for Bradford)

mL Extract Collected:

mL extract after all spins:

mL Extract After Dialysis:

TX-TL Bradford Assay

Date: _____

 Bradford assay: Thaw BSA standard (2mg/mL) from -80°C. 1 mg/mL BSA standard: 25 μ L BSA stock 25 μ L water 0.1 mg/mL BSA standard 10 μ L BSA stock 90 μ L water 1/20x sample: 2 μ L sample 38 μ L water Add 800 μ L water each to 7 cuvettes Prepare cuvettes: (0 mg/mL) nothing (1 mg/mL) 10 μ L 0.1 mg/mL standard (2 mg/mL) 20 μ L 0.1 mg/mL standard (4 mg/mL) 4 μ L 1 mg/mL standard (6 mg/mL) 6 μ L 1 mg/mL 2 μ L 1:20x sample 4 μ L 1:20x sample Add 200 μ L Bradford dye to each → pipette to mix Incubate at RT 5 min (<60 min) Measure OD595Lysate Protein Concentration: