

- ☐ 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)

TX-TL Day 2: Stage 1 and 2 Growth

- ☐ Stage 1 miniculture (do 2x):
 - ☐ Mix & warm for 30 min 2 minicultures:
 - ☐ 4mL 2xYT
 - ☐ 270 µL phosphate solution
 - ☐ 4 µL chloramphenicol
 - ☐ 5 colonies to each miniculture → 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 µL chloramphenicol
 - ☐ At 8hr mark, inoculate w/50 µL Stage 1 culture (use 50uL of each if both grow well, otherwise choose darker stage 2).
 - ☐ Split into 2x50 mL → Shake @37°C ~7-8 hr

Date: _____

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 μ L 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

☐ 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 OD595

Date: _____

Lysate Protein Concentration: