TX-TL Prep

* Prepare 6 x 0.75 L+200mL of 2xYT:
  + Prepare 3.2 L 2xYT
    - 3x 46.5 g 2xY2 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

Date:

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 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

* Bradford assay:
  + Thaw BSA standard (2mg/mL) from -80°C.
  + 1 mg/mL BSA standard:
    - 25 μL BSA stock
    - 25 μL water
  + 0.1 mg/mL BSA standard
    - 10 μL BSA stock
    - 90 μL water
  + 1/20x sample:
    - 2 μL sample
    - 38 μL water
  + Add 800 μL water each to 7 cuvettes
  + Prepare cuvettes:
    - (0 mg/mL) nothing
    - (1 mg/mL) 10 μL 0.1 mg/mL standard
    - (2 mg/mL) 20 μL 0.1 mg/mL standard
    - (4 mg/mL) 4 μL 1 mg/mL standard
    - (6 mg/mL) 6 μL 1 mg/mL
    - 2 μL 1:20x sample
    - 4 μL 1:20x sample
  + Add 200 μL Bradford dye to each 🡪 pipette to mix
  + Incubate at RT 5 min (<60 min)
  + Measure OD595

Date:

Lysate Protein Concentration: