Mini-Induction in a T7 polymerase-containing strain

DAY I
Streak cells for colonies

DAY II
Inoculate 3mL O/N culture from colony
Grow at 37C

DAY III

Back-dilute 1:100 in fresh media, NO IPTG yet
- Start two 25mL cultures in flasks (250mL O/N in 25 mL media);
  - One of these will be Induced, the other Uninduced control
- OR one 24mL culture in a flask (240mL O/N in 24 mL media)

Grow in shaker at 37C until OD_{600} = .3 - .5 (preferably around .4; this is mid- to end-log)
- This will take 60 – 90 minutes (check at 90 min; it could take up to 2.5 hrs depending on the strain.)

IF going from a single 20mL culture, split it to make an Induced and Uninduced culture
- Important: pay attn to vol of Induced culture; vol of Uninduced ctrl is less important

Add IPTG to the Induced culture, to 1mM concentration
- From a 1 M solution, this is a 1:1000 dilution
- Use IPTG aliquots, not stock

Grow at 37C in shaker for 1-3 hours (4 hrs for FtsZ)

Take two 1mL samples from both cultures (4 samples total) every hour
- Sample 1: OD; expect $OD_{Induced} < OD_{Uninduced}$.
- Sample 2: Spin down
  - Pull off supernatant using a pipet—be careful not to disturb the pellet
  - Freeze pellet in -80C until needed

Pellets from both cultures need to be frozen at -80C so they can be compared on a protein gel.