

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 (250 μ L O/N in 25 mL media);

One of these will be Induced, the other Uninduced control

OR one 24mL culture in a flask (240 μ L O/N in 24 mL media)

Grow in shaker at 37C until OD₆₀₀ = .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.