Inoculum for Maxi Growth of Membrane Proteins

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Materials/Reagents/Equipment	Vendor
MDAG Plates—look at Studier's protocol	
MDG Media-- look at Studier's protocol	
Antibiotics (AB)	
Luria Broth	Add 2 mM MgS04, 0.2% glucose
ZYM or ZYP Media	
1 M IPTG	

Purpose: To make sure that there is no expression of the target prior to induction on either the plates or the starter media.

Procedure

Day 1

1. Take plasmid and transform background cells using Z competent cells or Chiron treated competent cells. Plate onto MDAG plates/antibiotics (AB) and incubate overnight at 37˚C.
2. Note date and type of plate used for transformation.

Day 2

1. In the middle of the afternoon, take several colonies from the plate and inoculate necessary MDG media/AB (make sure you add 100 μg/ml methionine if using B834 (DE3) since it is a met auxotroph) in a flask. Shake overnight at 37˚C. This starter is stable for 3-4 weeks at 4˚C.

Day 3

1. Dilute the starter 1:100 into LB with desired antibiotics. Grow at 37˚C at 210 rpm until OD_{600} = 0.8-1.0. Record time and OD at induction. Cool media down to 20˚C and then induce @ 0.3 mM IPTG. Grow overnight at 20˚C at 160 rpm. Record time and OD.
2. Spin cell paste in Evolution centrifuge. Decant. Freeze cell paste at -80˚C.