CELLS

DIvA CELLS

DIvA U2-OS cells (Stably expressing AsiSI-ER)
Puromycin resistant 1 µg/ml

Culture in :
DMEM Glutamax + glucose 4.5g/L –pyruvate (Invitrogen 61965)
+1mM sodium pyruvate (Invitrogen 11360)
+10% FBS
+penistreptomycin (Invitrogen 15140)

Please make sure to amplify and freeze some vials upon arrival as cells derive over time

AID-DIvA CELLS

AID DIvA U2-OS cell line (Stably expressing AsiSI-ER-AID)
G418 resistant 800µg/ml

Culture in :
DMEM Glutamax + glucose 4.5g/L - pyruvate (Invitrogen 61965)
+ 1mM sodium pyruvate
+ 10% FBS
+ penicillin/streptomycin

Please make sure to amplify and freeze some vials upon arrival as cells derive over time (induction and control get loose over time)

PLASMIDS

pBabe-AsiSI-ER

The *E. coli* Asi MET strain (which stably expresses the AsiSI methylase) **NEEDS** to be used to transform and amplify the plasmid encoding pBABE+AsiSI.

You will be provided 2 soft agar stabs and the plasmid:

- E coli Asi MET AsiSI (chloramphenicol Resistant).
- E coli Asi MET strain+ pBABE-AsiSI-ER, i.e.: transformed with the pBABE AsiSI ER (Chloramphenicol resistant and Ampicillin resistant).
- Plasmid encoding the pBABE AsiSI-ER (ampicillin resistant).

Bacteria are sent as a stab in soft agar (do not freeze upon reception, long term storage at Room temp). Take some bacteria from the stock and culture in 2 ml of LB media 2h without antibiotic with gentle shaking at 30°C. Then add ampicillin overnight in LB media (DO NOT ADD BOTH antibiotics as they have trouble to grow). You can then amplify bacteria in larger volume.

pAID-AsiSI-ER

The *E. coli* Asi MET/RecA strain (which stably expresses the AsiSI methylase) **NEEDS** to be used to transform and amplify the plasmid encoding the pAID-AsiSI.

You will be provided 2 soft agar stabs and the plasmid:

- E Coli Asi MET/RecA- strain (Chloramphenicol Resistant, Tetracyclin Resistant)
- E Coli AsiSI MET/RecA+AID Asi SI, i.e.: transformed with the pAID AsiSI ER (Kanamycin resistant, Chloramphenicol Resistant, Tetracyclin Resistant)
- Plasmid encoding the pAID-AsiSI-ER (kanamycin resistant).

Bacteria are sent as a stab in soft agar (do not freeze upon reception, long term storage at Room temp). Take some bacteria from the stock and culture in 2 ml of LB media 2h without antibiotic with gentle shaking at 30°C. Then add kanamycin overnight in LB media (DO NOT ADD ALL antibiotics as they have trouble to grow). You can then amplify bacteria in larger volume.