



PROTOCOL

pStable Ind Vector Induction Protocol

For use with Ansa Clonal products

Ansa's pStable Inducible (Ind) vectors are used when sequences are challenging for *E. coli* to propagate. Inducible vectors minimize transgene expression to a very low copy number. Copy number can be further suppressed with glucose down to ~ 1 copy per cell. In the absence of glucose, copy number can be induced by arabinose to 50-100 copies per cell.

This protocol describes two induction methods:

- Method 1: Auto-induction (suited for high yield miniprep)
- Method 2: Two Step Induction (suited for high yield miniprep and creating a glycerol stock)

Materials Required (for either method):

- Sterile LB Broth (Teknova; PN: L8000)
- Sterile 20% Arabinose Solution (Teknova; PN: A2010)
- Sterile 20% Glucose Solution (Teknova; PN: G0520)
- Sterile LB Agar plates containing antibiotic and optional 0.2% glucose
- Competent cloning strain (i.e. DH5- α or DH10- β)

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Method 1: Auto-induction

1. Transform Ansa DNA sample(s) into a competent strain, i.e., DH5- α or DH10- β , using heat shock or electroporation. For DNA constructs > 20kb, electroporation is recommended.
2. Plate transformed cells onto LB Agar plates formulated with an antibiotic (12.5 μ g/mL), e.g., Kanamycin, Ampicillin or Chloramphenicol, and incubation overnight at 37°C.
3. Formulate the **auto-induction media** by combining (in order) LB Broth with 0.1% glucose, 0.02% arabinose and antibiotic (12.5 μ g/mL).
Note: Antibiotic concentration may require optimization.
4. In a 96 deep well plate, inoculate 1.2mL of auto-induction media with picked colonies and incubate at 37°C until point of saturation (18-20 hours).
5. Centrifuge the deep well plate at 4000 RCF for 10 minutes to pellet the cells and proceed to miniprep.

Method 2: Two Step induction

1. Transform Ansa DNA sample(s) into a competent *E. coli* strain, i.e., DH5- α or DH10- β , using heat shock or electroporation. For DNA constructs > 20kb, electroporation is recommended.
2. Plate transformed cells onto LB Agar plates formulated with an antibiotic (12.5 μ g/mL), e.g., Kanamycin, Ampicillin or Chloramphenicol, and incubation overnight at 37°C.
3. Formulate the **low copy media** by combining (in order) LB Broth with 0.2% of glucose and antibiotic (12.5 μ g/mL).
4. In a 96 deep well plate, inoculate 1.2mL of low copy media with picked colonies and incubate colonies at 37°C until point of saturation (18-20 hours).
5. To make the glycerol stocks, remove 300 μ L of the liquid culture and mix well with 300 μ L of 50% sterilized glycerol, and store in -80°C until needed.
6. Continue to culture by adding 300 μ L of fresh LB Agar with 0.04% of arabinose. The final concentration of arabinose is ~0.01% in 1.2 mL of media.
7. Incubate at 37°C for 5 hours.
8. Centrifuge the deep well plate at 4000 RCF for 10 minutes to pellet the cells and proceed to miniprep.

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