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pIND Vector Protocol

For use with Ansa Clonal products

The pIND vector is utilized by Ansa when sequences are challenging for E.Coli to propagate. The vector minimizes transgene expression to a very low copy number. The copy number can be further suppressed by glucose to ~ 1 copy. In absence of glucose, the copy number can be induced by arabinose to 50-100 copies/cell.

This protocol contains two ways in which to induce the pIND plasmid:

- Method 1 – autoinduction - 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; CAT No: L8000; shelf life 780 days)
- Sterile 20% Arabinose Solution (Teknova; CAT No: A2010; shelf life 730 days)
- Sterile 20% Glucose Solution (Teknova; CAT No: G0520; shelf life 1095 days)
- Sterile LB Agar plates containing the appropriate antibiotic for your IND Vector and optional 0.2% glucose if the sequence is highly complex or toxic
- Competent E. Coli cloning strain (i.e. DH5-α or DH10-β)
- Sterile Antibiotic (appropriate antibiotic for your IND Vector)

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Method 1 – Autoinduction

1. Transform your Ansa DNA sample into your chosen competent E.Coli cloning strain (standard techniques, i.e. heat shock or electroporation work well). For DNA constructs that are over 20kb, we recommend transformation by electroporation.
2. Plate transformation mix onto LB Agar plates with the appropriate antibiotic for your IND Vector.
    a. We find overnight growth at 37 C is sufficient for viable colonies to be visible.
3. Make the **auto-induction media** by combining (in order) LB Broth with 0.1% glucose and 0.02% arabinose. Add the appropriate antibiotic for your IND Vector last (we recommend 25 µg/mL for both Kanamycin and Chloramphenicol and 50 ug/mL liquid culture and 100 ug/mL in agar plates for Ampicillin, however this may require optimization). Use aseptic technique.
4. The next day, pick colonies of pIND transformed cells into 1.2mL of auto-induction media made in Step 3.
    a. Grow colonies until point of saturation, we recommend 18-24 hours.
5. Spin down cells and miniprep using your method of choice.

Method 2 – 2 step induction

1. Transform your Ansa DNA sample into your chosen competent E.Coli cloning strain (standard techniques i.e. heat shock or electroporation work well). For DNA constructs that are over 20kb, we recommend transformation by electroporation.
2. Plate transformation mix onto LB Agar plates.
    a. We find overnight growth at 37 C is sufficient for viable colonies to be visible.
3. Make the **low copy media** for pIND by combining (in order) LB Broth with 0.2% of glucose and appropriate antibiotic for your IND Vector (we recommend 25 µg/mL for both Kanamycin and Chloramphenicol and 50 ug/mL liquid culture and 100 ug/mL in agar plates for Ampicillin, however this may require optimization). Use aseptic technique.
4. The next day, pick colonies from the transformed plates in Step 2 into 1.2 mL of the low copy media made in step 3 and incubate at 37 C for 18 hours.
5. To make the glycerol stocks, remove 300 µl of the liquid culture and mix well with 300 µl of 50% sterilized glycerol, and store in -80 deg C until needed.
6. To continue the culture and induce the pIND plasmid, add 300 µl of fresh LB with 0.04% of arabinose to the culture. Final concentration of arabinose is ~0.01% in 1.2 mL of culture.
7. Culture at 37 deg C for 5 hours before harvesting with miniprep method of your choice.