Synthetic Genes Protocol: Handling BACs

BACs (Bacterial Artificial Chromosomes) are synthetic vectors derived from *E. coli* F factor plasmids. These plasmids contain a very low copy origin of replication which replicates at 1–2 copies per cell. These vectors are particularly useful because of their ability to carry large fragments of DNA (up to 100s of kb in size) and are less prone to instability and recombination than high copy number plasmids.

IDT and SGI-DNA Custom Gene Synthesis

IDT and SGI-DNA offer custom gene synthesis, with ordering provided through the IDT website. MiniGene™ Synthetic Genes can be ordered in lengths from 50–500 bp, and Genes from 501–2 Mb. All Genes and MiniGene Synthetic Genes are constructed using high quality IDT oligonucleotides. Prior to shipping, all Genes and MiniGene Synthetic Genes are sequence verified. The sequencing method is determined by IDT and SGI-DNA as part of the manufacturing process.

Genes over 5000 bp in length will be provided in BAC vectors, as will some large genes under that limit. For more information, contact genes@idtdna.com

Handling BACs

Handling large plasmids requires special care particularly when their size is larger than 30 kb. In this size range, avoid vortexing, triturating with a small bore pipet tip, and other physical manipulations that might result in shearing of the DNA.

Resuspending Your BAC

You will recieve 1 µg of BAC vector, containing your sequence. Centrifuge the tube for 3–5 sec at a minimum of 3000 x g to pellet the material to the bottom of the tube.

1. Dilute BAC in ddH_2O to a concentration of 0.1–10 ng/µL. It is essential that there is a very low salt concentration in the plasmid suspension to prevent arcing during transformations.
2. If the BAC stocks are resuspended in TE buffer, make sure to dilute at least 100X with water, or remove salts with a size exclusion column or other method.

Growth

BACs contain a chloramphenicol resistance gene and can be selectively grown in LB medium containing 12.5 µg/mL of chloramphenicol.

Storing BAC

1. Long term storage of BACs should be in TE pH 7.5–8.0 and at –20°C. Under these conditions, BACs can be stored for periods of up to several years and still retain viability.
2. For the most stable storage, glycerol stocks should be used [1].

Transformation

The best method for transforming BACs is electroporation. The following protocol a published example for transforming BACs using common lab reagents and electroporation equipment [2]. Several commercial kits are also available for subcloning BACs.

Transformation (cont.)

Required Materials

1. Electrocompetent *E. Coli*, compatible with BACs we suggest: *BAC-Optimized Replicator™ v2.0 & 10G BAC-Optimized Electrocompetent Cells (Lucigen)*.
2. BAC in ddH_2O to a concentration of 0.1–10 ng/µL (See Above: *Resuspending Your BAC*)
3. 1 mm electroporation cuvettes
4. SOC media

Procedure

1. Wash and UV sterilize 1 mm electroporation cuvettes, place on ice and prepare cultures tubes with 0.5ml SOC.
2. Thaw cells on wet ice and aliquot 25–30mL to the cuvettes.
3. Add 1 µL BAC plasmid to the tube and mix gently by stirring with a pipette tip.
4. Electroporate using settings of 100 Ohms, 2.4KV, 25mFa.

Purification

The following are important considerations for growing an purifying BAC-containing *E. coli* cultures [3].

- Due to their low copy number, approximately 10X more culture must be purified to achieve the same mass of BAC as is typical with a high copy vector.
- Small scale preps of BACs can be obtained from as little as 5 mL of culture using standard alkaline lyses techniques and typically yield 200-400 ng of material [4].
- Larger cultures, of up to 500 mL can yield micrograms of material.
- BACs can be induced with arabinose to increase their copy number. The efficiency of induction is dependent on the strain of *E. coli* used, the size of the insert, and the sequence composition of the inserted DNA. In general, small inserts will result in larger copy number. An increase from 1 copy per cell to 20–40 copies per cell is common with inserts that are smaller than 20 kb.
    - Note that some sequences will be toxic to *E. coli* upon arabinose induction and may result in poor or reduced yields.
    - Grow cultures to an A_{600} of 0.2–0.3 in LB in a shaking incubator at 37°C .
    - Add L-arabinose to a final concentration of 0.01%
    - Return the culture to the shaking incubator for 3–5 hrs
- Several commercial BAC purification kits are available.
    - Millipore 96-well Plasmid and BAC Preparation Kit.
    - PureLink® HiPure BAC Buffer Kit.
    - Nucleobond BAC 100.

References

1. Koenig GL. (2003) Viability of and Plasmid Retention in Frozen Recombinant Escherichia coli over Time: a Ten-Year Prospective Study Appl Environ Microbiol. 69(11): 6605–6609.
2. Sheng Y., Mancino V., and Birren B. (1995) Transformation of Escherichia coli with large DNA molecules by electroporation. Nucleic Acids Res. 23(11): 1990–1996.
3. Wild J., Hraddecna Z., and Szybalski W. (2002) Conditionally amplifiable BACs: switching from single-copy to high-copy vectors and genomic clones. Genome Research. 12(9):1434-44.
4. Sambrook J., and Russell D.W., editors. (2001) Molecular Cloning: A Laboratory Manual. 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.