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Simplifying Genomics

CopyControl™ Induction Solution

Cat. No. CCIS125

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1. Introduction

The CopyControl™ Induction Solution is used to induce CopyControl clones developed in the CopyControl pCC1 and pCC2 vectors, grown in TransforMax™ EPI300™ *E. coli*, from single-copy number to high-copy number.

The Induction Solution induces expression of a mutant *trf*A gene contained in the TransforMax EPI300 cells. Expression of *trf*A gene results in initiation of replication from the *ori*V high-copy origin of replication and subsequent amplification of the CopyControl clones and clones developed in the CopyControl pCC1 and pCC2 vectors.

The CopyControl Induction Solution can be added to CopyControl clones growing in culture or to agar media prior to plating of CopyControl clones transformed into TransforMax EPI300 cells.

2. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle. Mix thoroughly after thawing.

Size and Formulation: CopyControl Induction Solution is supplied as a 1,000X concentrate in sterile water. 25 ml are provided.

Quality Control: CopyControl Induction Solution is function-tested to induce CopyControl fosmid clones from single-copy number to high-copy number at a 1X final concentration.

3. Induction of CopyControl Fosmid High-Copy Number

CopyControl Fosmid clones grown in TransforMax EPI300 cells can be amplified to 10-50 copies per cell. The induction process can be done in any culture volume desired depending on the needs of the user. Generally, a 1-ml induced culture will provide a sufficient amount of DNA for most applications including sequencing and fingerprinting. Here we provide the procedure for amplifying the clones in 1 ml, 5 ml, and 50 ml cultures.

Growth Media for CopyControl Fosmid Clones
LB + chloramphenicol (12.5 µg/ml)

1. Add 5 ml of the Growth Media to 15-ml tubes for each fosmid or PCR clone that will be induced to high-copy number.
2. Individually inoculate the media with a small portion of the desired fosmid or PCR clones grown on an overnight plate.
3. Grow the cultures overnight at 37°C with shaking. These cultures will be used as inocula for the copy number amplification procedure.
4. From the table below, combine the appropriate volumes of fresh Growth Media, the overnight culture and the CopyControl Induction Solution for the desired volume of induction culture. Aeration of the induction cultures is critical. Therefore, to maximize the surface area of the culture solution in the tube, perform the induction in the largest volume tubes that reasonably meets your needs and resources. For example, induce clones to high-copy number in 1 ml of culture, using 1.5-ml tubes or larger, 5 ml cultures in 15-ml tubes and 50 ml cultures in 125-ml flasks.

Total volume of clone induction culture	Volume of fresh LB + chloramphenicol (12.5 µg/ml)	Volume of overnight 5 ml culture	Volume of 1000X CopyControl Induction Solution†
1 ml	800 µl	200 µl	1 µl
5 ml	4.5 ml	500 µl	5 µl
50 ml	45 ml	5 ml	50 µl

† Mix thoroughly after thawing.

5. Vigorously shake the tubes at 37°C for 5 hours. Aeration is critical! Shake the tubes in a manner that will maximize aeration of the cultures (for example 1.5-ml tubes can be taped horizontally to the shaking table).
6. Centrifuge the cells and purify the DNA by your standard lab methods.