

CopyControl™ Induction Solution

Cat. No. CCIS125

The CopyControl™ Induction Solution is used to induce CopyControl clones and clones retrofitted with the EZ::TN™ <orV/KAN-2> Transposon, grown in TransforMax™ EPI300™ *E. coli*, from single copy number to high copy number.

The Induction Solution induces expression of a mutant *trfA* gene contained in the TransforMax EPI300 cells. Expression of *trfA* gene results in initiation of replication from the *orV* high copy origin of replication and subsequent amplification of the CopyControl clones and clones containing the EZ::TN <orV/KAN-2> Transposon to high copy number.

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.

Product Specifications

Storage Temperature: 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 1000X concentrate in sterile water. 25 ml are provided.

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

Related Products: The following products are also available:

- CopyControl™ BAC Cloning Kits
- CopyControl™ PCR Cloning Kits
- CopyControl™ Fosmid Library Production Kit
- EZ::TN™ <orV/KAN-2> Insertion Kit
- TransforMax™ EPI300™ *E. coli*

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Induction of CopyControl Fosmid, CopyControl PCR Clones and Fosmids Retrofitted with an EZ::TN <oriV/KAN-2> Transposon to High Copy Number

CopyControl Fosmid clones, CopyControl PCR clones and low copy number fosmid clones retrofitted with the EZ::TN <oriV/KAN-2> Transposon and 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.

Important: The Growth Media for amplifying CopyControl Fosmid clones and CopyControl PCR clones and low copy number fosmid clones retrofitted with an EZ::TN <oriV/KAN-2> Transposon are different. Be sure to use the appropriate Growth Media for the clones that you are amplifying.

Growth Media for CopyControl Fosmid Clones and CopyControl PCR Clones

LB + chloramphenicol (12.5 µg/ml)

Growth Media for Fosmid Clones Retrofitted with an EZ::TN <oriV/KAN-2> Transposon

LB + chloramphenicol* (12.5 µg/ml) + kanamycin (50 µg/ml)

* or other selectable marker present on the cloning vector backbone

1. Add 5 ml of the appropriate **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	500 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.

Induction of CopyControl BAC Clones and BAC Clones Retrofitted with an EZ::TN <ori/KAN-2> Transposon to High Copy Number

CopyControl BAC clones and low copy number BAC clones retrofitted with an EZ::TN <ori/KAN-2> Transposon and grown in TransforMax EPI300 cells can be amplified to 10-20 copies per cell. Generally, 1 ml of an induced culture will provide a sufficient amount of BAC DNA for most applications including sequencing and fingerprinting. Procedures for amplifying CopyControl BAC clones and low copy number BAC clones retrofitted with an EZ::TN <ori/KAN-2> Transposon in 1.5 ml tubes and in deep-well, 96 well plates are provided. The induction volumes can be scaled up as required by the user.

Important: The Growth Media for amplifying CopyControl BAC clones and low copy number BAC clones retrofitted with an EZ::TN <ori/KAN-2> Transposon are different. Be sure to use the appropriate Growth Media for the type of BAC clones that you are amplifying.

Growth Media for CopyControl BAC Clones

LB + chloramphenicol (12.5 µg/ml)

Growth Media for BAC Clones Retrofitted with an EZ::TN <ori/KAN-2> Transposon

LB + chloramphenicol* (12.5 µg/ml) + kanamycin (50 µg/ml)

* or other selectable marker present on the cloning vector backbone

Amplification of BAC clones in 1.5 ml tubes

1. Dispense 1 ml of the appropriate **Growth Media** into 1.5 ml tubes. Inoculate each tube with an isolated single BAC clone from an overnight plate.
2. Incubate the cultures at 37°C overnight **without** shaking.
3. Following overnight incubation, mix each tube and then aspirate off 800 µl of culture medium from each and discard.
4. Add 800 µl of fresh **Growth Media** into each tube containing the remaining 200 µl of the overnight culture. Mix by vortexing.
5. Incubate the tubes for 30 minutes at 37°C with shaking at 250 rpm. After 30 minutes, the O.D₆₀₀ will be at 0.4 - 0.6.
6. Thaw the CopyControl Induction Solution and mix thoroughly. Add 1 µl of 1000X CopyControl Induction Solution (to a 1X final concentration) to each tube. Incubate each for 2 hour at 37°C with **vigorous** shaking. 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).
7. Isolate DNA from the induced culture by your method of choice.

Amplification of CopyControl BAC clones in deep-well (2 ml) 96 well plates

1. Dispense 1 ml of the appropriate **Growth Media** into each well of a deep-well plate. Inoculate each well with an isolated single BAC clone from an overnight plate.
2. Seal the plate with porous microtiter plate sealer, incubate at 37°C overnight **without** shaking.
3. Following overnight incubation, mix the cultures by shaking and then aspirate off 800 µl of culture medium from each well and discard.
4. Add 800 µl of fresh **Growth Media** into each well containing the remaining 200 µl of the overnight culture. Mix by shaking or vortexing.
5. Incubate the plate for 30 minutes at 37°C with shaking at 250 rpm. After 30 minutes, the O.D₆₀₀ will be at 0.4 - 0.6.
6. Thaw the CopyControl Induction Solution and mix thoroughly. Add 1 µl of 1000X CopyControl Induction Solution (to a 1X final concentration) to each well. Incubate each for 2 hour at 37°C with **vigorous** shaking. Aeration is critical!
7. Isolate DNA from the induced culture by your method of choice.