

Trouble shooting

Excessive chromosomal DNA can result from poor lysis or from high culture density. Dilute heavy cultures to an OD600 of 4.0 to 6.0.

Do not vortex sample at lysis step. Gentle inversion of sample is sufficient.

Do not shorten the incubation times or chromosomal DNA may not pellet.

If your centrifuge generates less than 20,000 x g, extend the spin time to achieve approximately the same g x time value.

At step 7, be certain to spin out the remaining wash buffer as directed.

In step 8, allow the elution buffer to incubate on the matrix for at least 2 minutes before recovering the eluant.

DNA loss often occurs at the precipitation step. The pellet is barely visible due to the small amount of DNA present. Orient the microfuge tubes in the rotor in a consistent manner so that the relative position of the pellet is known. Take care when pipetting supernatants to not dislodge the pellet.

Very low yield from a clone that has previously produced higher yields, is often due to recombination resulting in insert loss. Restriction digestion and electrophoresis should be used to confirm the insert size. Since BAC's have a fixed copy number, recombination will result in a smaller insert and a lower yield.

DNA isolated from some E.coli strains (e.g. HB101 and derivatives) does not sequence well. Our recommendation is to use DH10B host strains.

Related Products

Product	Cat#	Pack size
Chemical competent cells TZ101 α ; 10 x 0.1mL	M3434.0010	10 transformations
Chemical competent cells TZ101 α ; 30 x 0.1mL	M3434.0030	30 transformations
Chemical competent cells for large / toxic plasmids; TZ102 α , 10 x 0.1mL	M3435.0010	10 transformations

Genaxxon bioscience Bacterial Artificial Chromosome Purification Kit

Product	Cat#	Package size
Big BAC DNA Isolation Kit	S5302.0550	5 x 250mL culture volume
96 well plate BAC DNA Isolation Kit	S5302.0196	1 x 96 well plate
96 well plate BAC DNA Isolation Kit	S5302.0496	4 x 96 well plate

Purification protocol using the PSI Clone Big BAC Kit

Introduction: The PSI CLONE Big BAC DNA isolation kit accommodates culture volumes of 25 to 250mL. Any volume within this range may be processed by scaling the reagents to the culture media at a 1:10 ratio. The BAC DNA is captured in batch mode using a highly efficient, hydrophilic non-silica anion exchanger. The BAC DNA/resin complex is poured into a fritted column for washing and recovery of the DNA. The gravity-based wash steps remove RNA and protein contaminants. Elution is easily achieved in a small volume of elution buffer by inserting the column into a 50mL centrifuge tube and centrifuging at low g-force. Typical yields are 5 to 10 micrograms pure BAC DNA from 25 to 30mL of culture.

The PSI CLONE Big BAC DNA isolation protocol is easily adjusted to process any volume from 25 to 250mL of culture. A scaling factor 1:10 is used for all reagents except the Elution Buffer. It is recommended that volumes be processed in units of 25 or 50mL. (50mL is the largest volume of culture that can be processed in readily available glassware or plasticware e. g. plastic screwtop centrifuge tubes. Aliquots of 250mL can be processed in one lot only by using appropriately sized glassware, centrifuge tubes and elution columns and since these are not easily available, we recommend a maximum of 50mL batches). If culture volumes greater than 50mL are processed, the fritted column tubes may be washed out and reused.

Material required but not supplied:

Ice bath

High speed centrifuge (Sorvall RC5 or equivalent)

50mL conical centrifuge tubes

Kit components

Resuspension Buffer (Buffer 1)	25mL
Lysis Buffer (Buffer 2)	25 mL
Neutralisation Buffer (Buffer 3)	25 mL
BAC DNA Binding Resin (Buffer 4)	25 mL
Wash Buffer (Buffer 5)	80 mL
Elution Buffer (Buffer 6)	10 mL
RNAse A	5 mg (2 x 2.5 mg vials)
Elution columns	5

All components except the combined Resuspension Buffer and RNAse may be stored at room temperature. After adding RNAse to Resuspension Buffer, store the combination at 4°C.

Scaling Factors

This Big BAC DNA isolation protocol may be easily adjusted to process any volume from 25 – 250mL of culture. A scaling factor of 1:10 is used for all reagents except the Elution Buffer. An elution volume of 750µL is suitable for all processing volumes when the columns supplied are used. It is recommended that volumes be processed in units of 25 or 50mL.

Although 250mL can be processed in one lot using appropriately sized glassware, centrifuge tubes and elution columns, 50mL is the largest volume of culture that can be processed in readily available glass or plastic ware (typically plastic screwtop centrifuge tubes). If culture volumes < 50mL are processed the fritted column tubes may be washed out and reused.

The protocol on the following page uses a 30mL culture as an example. For other culture volumes, substitute the appropriate reagent volume (i.e. for 50mL cultures use 5mL of reagents).

Protocol for 25 – 250mL culture media using Big BAC DNA Kit

This protocol is designed to isolate template quality BAC DNA from cultures of 30mL LB medium (containing 12µL/mL chloramphenicol) with a culture density as measured by OD600 between 4.0 and 6.0. Typical yields are between 5 to 7µg DNA per prep.

1. Remove 1mL Resuspension Buffer and add it to the RNAse A tube to dissolve the RNAse A, add this back to the Resuspension buffer and mix. Store any unused portion of this combination at 4°C.
2. Add 3mL resuspension buffer to the cell pellet and resuspend by gentle pipetting.
3. Add 3mL lysis buffer and mix by GENTLE (!) inversion. Allow the lysis to continue for 20 minutes at room temperature.
4. Add 3mL neutralization buffer and mix by GENTLE (!) inversion until a thick white precipitate forms and then incubate on ice for 20 minutes.
5. Centrifuge the precipitate at 25,000 x g (using an SS-34 rotor or equivalent) for 20 minutes to clarify the lysate. Pour off the lysate into a clean tube; if the lysate is not clear, GENTLY (!) invert it to mix and centrifuge again.
6. Add 3mL BAC binding resin to the clarified lysate and invert several times. Incubate at room temperature for 10 minutes (inverting every 2 minutes to mix). Pour the solution into the fritted column barrel provided and allow it to drain by gravity.
7. Add 3 x 3mL wash buffer allowing it to drain by gravity, then place the column in an empty 50 ml conical tube (Falcon Tube or equivalent) and remove the excess wash buffer by centrifugation at 750 x g for 2 minutes in a bench top centrifuge. Discard the wash.
8. Add 750 microliters elution buffer to the column and incubate it for 2 minutes. Centrifuge as above using a clean centrifuge tube.
9. Precipitate the DNA by adding 1 volume isopropanol (not included) and mixing. Centrifuge at 20,000 x g for 30 minutes. Remove supernatant. Wash the DNA by adding 200 microliters 70% ethanol and mixing. Centrifuge at 20,000 x g for 5 minutes. Remove the excess ethanol and allow the pellet to air dry for about 5 minutes.
10. Dissolve the pellet in a suitable volume of TE or other low salt buffer of your choice.