

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 and use 5 mL of diluted culture per prep.

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.

Lack of lysate flow through column: Be sure to include Step 7 (Dilution). The PSI Pressure Tip (included in Kit) may be used to initiate lysate flow. Attach the Pressure Tip to a 3 mL Luer Lock syringe, pull back on the plunger, apply tip straight onto the column and depress the plunger to expel the lysate at a flow rate of 1-2 drops/sec. Do not release the plunger until loading is complete.

At Step 10, be certain to spin out the remaining wash buffer as directed. In Step 11, 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 (approx. 1 microgram). 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 (< 50 ng/prep) 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 0.1 ml	M3434.0010	10 transformations
Chemical competent cells for large / toxic plasmids; TZ102 α , 2 x 0.5 ml	M3435.0010	10 transformations

Genaxxon bioscience Bacterial Artificial Chromosome Purification Kit

Product	Cat#	Package size
Mini BAC DNA Isolation Kit	S5302.0255	25 x 5 ml Preps
Big BAC DNA Isolation Kit	S5302.0550	5 x 250 ml 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 **Mini BAC DNA** isolation kit facilitate the isolation of high quality BAC DNA. Sufficient DNA is obtained to allow for at least one sequencing reaction as well as other biochemical characterisations (e.g. restriction digest). Typical yields between 0.6 and 1.0 μ g are observed from an overnight 3 – 5 ml culture (We recommend growing cultures for 20—24 hours in Terrific Broth with 20 micrograms/ml chloramphenicol. This should result in an OD(600) of 4.0—6.0).

Bacterial Artificial Chromosomes are vectors with an origin of replication from the F factor episome. This allows the stable cloning of large DNA fragments (> 150 kb). The F factor origin is a stringent replicon and allows for 1-2 copies of each BAC molecule/cell. The low copy number of BAC clones presents a problem for users in limiting the potential yield to 100-200 ng/mL. Current protocols require large culture volumes (50-200 mL) to obtain microgram quantities of BAC DNA in sufficient purity for molecular biology experiments. These large volume protocols for BAC DNA isolation generally involve enzymatic steps or organic extractions and many are unique to individual laboratories.

The PSI Clone Mini BAC DNA Kit utilizes a simple spin column protocol free of organics to yield high purity BAC DNA.

Material required but not supplied:

- Microcentrifuge • sterile test tubes and 1.5 mL microcentrifuge tubes • Ice bath
- Isopropanol • 70% Ethanol

Kit components

Resuspension Buffer (1)	13 mL	RNase A	1 vial
Lysis Buffer (2)	13 mL	BAC DNA columns	25
Neutralisation Buffer (3)	13 mL	Tube retainers	4
Equilibration Buffer (4)	15 mL	PSI Pressure Tip*	1
Wash Buffer (5)	50 mL	Instruction Manual	1
Elution Buffer (6)	10 mL		

* included in the BAC column bag

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.

Recommended sequencing conditions

Successful sequence was obtained by using the ABI PRISM BigDye Terminator Cycle sequencing Ready Reaction Kit as follows:

Sequencing Mixture		PCR conditions (PE 9600)			
Template	200-400 ng	11.0 µL	Step 1.	98°C	5 min.
Primer M13 ¹	3.2 pmol	1.0 µL	Step 2.	98°C	30 sec.
Reaction Mix		8.0 µL	Step 3.	55°C ²	20 sec.
Total		20.0 µL	Step 4.	60°C	4 min.
			Step 5.	Cycle to Step 2	30 X
			Step 6.	60°C	5 min.
			Step 7.	4°C	- - -

¹ M13 forward primer – GTA AAA CGA CGG CCA GT (Tm=53) sequence is present in pBeloBAC11.

² The annealing temperature may vary for primers with higher Tm.

Sequencing products were cleaned using CentriSep (Cat-No. S5300) and dried in a Savant Speed-Vac. It is important to add the entire sequencing reaction onto the gel to achieve adequate signal intensity. Resuspend the reaction in a minimal volume (e.g. 1 µL) and load the entire amount in the well.

Protocol for 3 – 5 ml culture media using Mini BAC DNA Kit

This protocol is designed to isolate template quality BAC DNA from cultures of 5 ml LB medium (containing 20 µg/ml chloramphenicol) with a culture density as measured by OD600 between 4.0 and 6.0. Typical yields are between 0.6 to 1.0 µg DNA per prep.

We recommend growing cultures for 20-24 hours.

1. Add 0.5 mL Resuspension Buffer (1) to the RNase A tube. Mix gently. Transfer 0.25 mL of this RNase A solution to remaining Resuspension Buffer (1). Mix well
2. Centrifuge a 3-5 mL overnight culture. Pour off the culture broth.
3. Resuspend the cell pellet by gently pipetting in 0.5 mL Resuspension Buffer (1) containing RNase A. Transfer to a sterile 1.5 mL micro-centrifuge tube.
4. Add 0.5 mL Lysis Buffer (2) and mix by GENTLE inversion (NOTE: lysate may not be clear). Incubate at room temperature for 10 min.
5. Add 0.5 mL Neutralization Buffer (3) and mix by GENTLE (!) inversion until a thick white precipitate forms. Chill in an ice bath for 10 minutes.
6. Centrifuge (20,000 x g) for 10 minutes, collect the supernatant (approx. 1.4 ml) and transfer to a sterile test tube (e. g. 15 ml Falcon tube or equivalent).
7. **Dilute the cleared lysate with 1.0 ml sterile water.** Mix by pipetting.
8. Add 0.5 ml equilibration buffer to the BAC column, vortex, and allow the column to drain by gravity.
9. Apply the dilute lysate (approx. 2.4 ml; it will take two applications) to the column and drain by gravity. The "PSI pressure tip" may be used to initiate the flow of lysate.
10. Wash BAC column with 2 x 1.0 ml Wash Buffer (5) and drain by gravity. Centrifuge at 750 x g for 2 minutes to dry the column.
11. Add 50 to 100 microliters of Elution Buffer (6) to the top of the resin and incubate 2 minutes at room temperature. Centrifuge at 750 x g for 2 minutes to collect the eluent.
12. Precipitate the BAC DNA by adding 1 volume of isopropyl alcohol (not included) and mixing. Centrifuge for 30 minutes at 20,000 x g to pellet the DNA. Remove the supernatant. Wash the pellet with 200 microliters of 70% ethanol (not included). Centrifuge again at 20,000 x g for 5 minutes. Remove the excess ethanol and air dry.
13. Resuspend your pure BAC DNA in 20 microliters TE or other low salt buffer of your choice.