

PREPARATION OF PACKAGING EXTRACTS—PROTOCOL II¹**Sonicated Extract from Induced BHB2690 (Prehead Donor)**

1. Make a subculture of the master stock of *E. coli* BHB2690. Verify its genotype, as described on page 258.
2. Read the OD₆₀₀ of a 100-ml overnight culture and inoculate 500 ml of NZM broth prewarmed to 32°C in a 2-liter flask with sufficient cells to give an initial OD₆₀₀ ≤ 0.1. Incubate with aeration at 32°C until an OD₆₀₀ = 0.3 is reached (2-3 hours).
It is important that the cultures be in the midlog phase of growth before induction.
3. Induce the lysogen by placing the flask into a water bath preheated to 45°C. Swirl the culture continuously for 15 minutes.
Alternatively, induce the culture by immersing the flask in a shaking water bath set at 65°C. As soon as the temperature inside the flask reaches 45°C, the culture should be transferred for 15 minutes to a water bath set at 45°C.
4. Incubate the induced cells at 38-39°C for 2-3 hours with vigorous aeration. Check for successful induction by adding a drop of chloroform to a small sample of culture; it should clear within a few minutes.
5. Recover the cells by centrifugation at 4000g for 10 minutes at 4°C.
6. Drain off as much liquid as possible. Remove any remaining medium with a pasteur pipette and Q-tips. Dry the walls of the centrifuge bottle with Kimwipes.
7. Add 3.6 ml of freshly prepared sonication buffer. Resuspend the pellet thoroughly and transfer the resulting homogeneous suspension to a small, clear plastic tube (Falcon no. 2054 or 2057).

Sonication buffer

20 mM Tris · Cl (pH 8.0)
1 mM EDTA
5 mM β-mercaptoethanol

8. Sonicate in short bursts (10 seconds) at maximum power using a microtip probe. The tube should be immersed in ice-water and the temperature of the sonication buffer should not be allowed to exceed 4°C. Allow the sample to cool for 20-30 seconds between each burst of sonication. Patience is critical!
Sonicate until the solution clears and its viscosity decreases.

¹Scalenghe et al. (1981); B. Hohn (unpubl.).

Note. The amount of sonication is critical, and the clearing and change in viscosity of the solution are not always readily apparent. When preparing these extracts for the first time, you should remove aliquots of the suspension after sonicating for various times. The aliquots are then processed and used in separate packaging reactions to determine the optimal sonication time.

9. Transfer the sonicated sample to a centrifuge tube and remove debris by centrifugation at 12,000*g* for 10 minutes at 4°C.
10. To the supernatant (3 ml) add an equal volume of cold sonication buffer and $\frac{1}{6}$ volume of freshly prepared packaging buffer. Dispense 15- μ l aliquots into precooled (4°C), 1.5-ml Eppendorf tubes. Immediately close the caps of the tubes, immerse them briefly in liquid nitrogen, and transfer them to -70°C for long-term storage.

Packaging buffer

- 6 mM Tris · Cl (pH 8.0)
- 50 mM spermidine
- 50 mM putrescine
- 20 mM MgCl₂
- 30 mM ATP (see note ii, page 262)
- 30 mM β -mercaptoethanol

Freeze/Thaw Lysate from Induced BHB2688 (Packaging Protein Donor)

1. Make a subculture of the master stock of *E. coli* BHB2688. Verify its genotype as described on page 258.
2. Read the OD₆₀₀ of a 100-ml overnight culture and inoculate 500 ml of NZM broth prewarmed to 32°C into each of three 2-liter flasks with sufficient cells to give an initial OD₆₀₀ ≤ 0.1. Incubate with aeration at 32°C until the OD₆₀₀ = 0.3 (2-3 hours).
3. Induce the lysogen by placing the flasks into a water bath preheated to 45°C. Swirl the flasks continuously for 15 minutes (see step 4, page 260).
4. Incubate the induced cells at 38-39°C for 2-3 hours with vigorous aeration. Check for successful induction by adding a drop of chloroform to a small sample of the culture; it should clear within a few minutes.
5. Recover the cells by centrifugation at 4000g for 10 minutes at 4°C.
6. Drain off as much liquid as possible. Remove any remaining medium with a pasteur pipette and Q-tips. Dry the walls of the centrifuge bottle with Kimwipes.
7. Resuspend the cells in a total of 3 ml of ice-cold sucrose solution. Distribute 0.5 ml of the suspension into each of six precooled (4°C) Eppendorf tubes. Add 25 μl of fresh, ice-cold lysozyme solution to each tube. Mix gently. Quickly close the cap of the tube and plunge it into liquid nitrogen.

Sucrose solution

10% sucrose
in 50 mM Tris · Cl (pH 8.0)

Lysozyme solution

2 mg/ml lysozyme
in 0.25 M Tris · Cl (pH 8.0)

8. Use forceps to remove the tubes from the liquid nitrogen. Thaw the extracts in ice. Add 25 μl of freshly prepared packaging buffer (see step 10, page 265) to each tube and mix.
9. Combine the thawed extracts in a centrifuge tube and centrifuge at 48,000g for 1 hour at 4°C.

10. Dispense 10 μ l of the supernatant into precooled (4°C) Eppendorf tubes. Immediately close the caps of the tubes and immerse them in liquid nitrogen. After all the aliquots have been frozen, remove the tubes from the liquid nitrogen and immediately transfer them to long-term storage at -70°C .

Note

The sonicated extract and the freeze/thaw extract can be combined at the time of preparation, if desired. Prepare the sonicated extract first, and freeze 15- μ l aliquots in open Eppendorf tubes arranged in a rack in liquid nitrogen. Add 10 μ l of freeze/thaw lysate directly into each tube. Close the cap of the tubes and immerse them in liquid nitrogen. Store at -70°C .

The major problem in preparing combined extracts is handling the frozen tubes and attempting to pipette 10 μ l of freeze/thaw lysate into a tube at -70°C . The efficiencies of packaging of extracts prepared by combining before or after freezing are similar.

PACKAGING IN VITRO—PROTOCOL II

1. Remove tubes from storage at -70°C and allow the packaging extracts to thaw on ice. The freeze/thaw lysate will thaw first. Transfer the freeze/thaw lysate to the still-frozen, sonicated extract.
2. Mix gently. When the combined extracts are almost totally thawed, add the DNA to be packaged (up to $1\ \mu\text{g}$ dissolved in $5\ \mu\text{l}$ of $10\ \text{mM}$ Tris \cdot Cl [pH 7.9] and $10\ \text{mM}$ MgCl_2). Mix with a sealed capillary pipette and incubate for 1 hour at room temperature.
3. Add 0.5 – $1\ \text{ml}$ of SM and a drop of chloroform and mix. Remove debris by centrifugation in an Eppendorf centrifuge for 30 seconds and measure the titer of the viable bacteriophage particles as described on page 64.

Notes

- i. Each batch of extracts should be tested with a standardized preparation of intact bacteriophage λ DNA.
- ii. These extracts exhibit a high degree of selectivity in the size of the DNA that is packaged (Sternberg et al. 1977). Recombinant DNAs that are 90% or 80% of wild-type bacteriophage λ in length are packaged with efficiencies 20-fold to 50-fold lower, respectively, than wild-type λ DNA.
- iii. The same packaging buffer may be used for packaging of both bacteriophage λ and cosmids.
- iv. See note to Packaging In Vitro—Protocol I, page 263.