PhageMaker® System

Description

PhageMaker System	6 extracts	69307-3
	11 extracts	69307-4

The PhageMaker system is optimized for maximum packaging efficiencies of cDNA and genomic DNA inserts ligated with any bacteriophage λ vector. Using a test insert with Novagen's λ vector arms, efficiencies >10^7 pfu/µg arms are typically obtained, >2×10^8 pfu/µg with Control DNA. This single-tube system is extremely easy to use; simply add up to 5µg of ligated vector plus insert to the extract and incubate at room temperature for 2 hours. PhageMaker provides three important advantages for in vitro packaging. First, the negligible background of <10 pfu per extract is orders of magnitude lower than convential two-extract systems because the endogenous λ DNA is unpackagable due to a deletion of the *cos* sites (1, 2). Second, the extract is devoid of *Eco*K and other restriction systems (*mcrA, mcrBC, mrr*) that recognize methylated DNA (3). Therefore, PhageMaker is especially recommended for constructing highly representative cDNA and genomic DNA libraries in which the insert DNA is methylated. Third, the one-tube configuration allows the use of multiple aliquots from the same tube, thus providing a significant cost savings over other systems.

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Components

- 6 or 11 PhageMaker Extracts in 50µl aliquots
- Ligated PhageMaker Control DNA
- Test host strain LE392

Storage: Store all components at -70°C.

Packaging Reaction

1. Allow the PhageMaker extract to thaw on ice The volume of extract is 50µl. The extract tube can be centrifuged briefly at 4°C if necessary to collect the contents at the bottom of the tube. The extract can be subdivided into several prechilled tubes for testing several samples at once. Scale down the amounts of ligation reaction and SM buffer added in the following steps in proportion to the amount of extract used.
2. Add up to 10µl of ligation reaction per 50µl extract. Mix gently by stirring with the pipet tip (do not vortex!). There is a linear relationship between amount of DNA added and number of plaque forming units (pfu) for amounts of DNA between 50ng and 5µg. A vial of ligated lambda control DNA is provided with the system. To test the packaging efficiency independently, add 0.5µg of the control DNA to the 50µl extract. More consistent results have been observed when the control DNA is diluted 1:5 with TE to a final concentration of 0.1µg/µl. 5µl of the diluted DNA can then be used in a packaging reaction.
3. Incubate the mixture at 22°C for 2hr.
4. Stop the reaction by adding SM to 0.5ml (*e.g.* 440µl SM if 10µl DNA was added to 50µl of extract). If the packaging reaction is to be stored for more than 24hr, also add 25µl chloroform and invert to mix.
5. The packaged phage may be stored for a week at 4°C, although we recommend amplifying primary libraries within 48hr to avoid possible decreases in titer. If the library is not to be amplified, allow the chloroform to settle out (or spin briefly), remove the aqueous phase to a fresh tube, add fresh DMSO to a final concentration of 7% (for 0.5ml this is 35µl) and store the phage at -70°C.

Titering Phage

1. Grow the appropriate host strain in LB medium supplemented with 0.2% maltose and 10mM $MgSO_4$ at 37°C to an OD_{600} of 1.0. Note that the Control DNA packaging reaction must be plated on a *supF* host (*e.g.* the provided LE392 strain).

2. Store the host cells at 4°C until needed (up to 72hr).
3. Make a series of dilutions of the phage in SM buffer. As a general guideline, an appropriate dilution for recombinant phage in typical packaging reactions is 1/1000 to 1/10,000. When the positive control DNA is used, dilutions should be made to $1/10^5$. Make a 1/100 dilution first by adding 10µl packaging reaction to 990µl SM and then make further dilutions from there.
4. In a sterile tube with at least a 4ml capacity, mix 100µl of the host cells with 100µl of the appropriate phage dilution. Add the host cells first to avoid cross-contamination of the phage between samples.
5. Incubate the host/phage mixture at 37°C for 20 min to allow the phage to adsorb to the host (shaking is not necessary).
6. Add 3ml of molten top agarose (no warmer than 47°C) to the tube containing the host and phage. Vortex briefly and immediately pour the contents of the tube onto an LB plate. Spread the top agarose evenly by gently swirling the plate.
7. Allow the plate to sit undisturbed on a level surface for a few minutes until the top agarose hardens, then incubate the plate inverted at 37°C overnight.
8. Count the plaques and calculate the phage titer. The phage titer (pfu/ml) is the number of plaques on a plate times the dilution factor times ten (to account for the fact that 0.1ml of dilution was plated). For example: if there were 200 plaques on a plate made from a 1/1000 dilution, there are $200 \times 1000 \times 10 = 2 \times 10^6$ pfu/ml of packaging reaction. If 0.5µg of arms were packaged in a 50µl reaction that was brought to 0.5ml (1µg arms/ml), the titer is 2×10^6 pfu/µg arms. The positive control DNA should produce more than 200 plaques when packaged as above and plated at 10^{-5}, which corresponds to a titer of 2×10^8 pfu/ml.

Note: Plaque formation can be delayed by using top agarose at too high a concentration (>0.8%; sometimes caused by pipetting from the bottom of an insufficiently mixed bottle).

Recipes

LB
Per liter:
10g tryptone
5g yeast extract
10g NaCl
adjust pH to 7.5 with 1N NaOH
Autoclave
(for plates, add 15g/l agar)

Top agarose
Per 100ml:
1g bacto-tryptone
0.5g NaCl
0.6g agarose
Autoclave

SM
Per liter:
5.8g NaCl
2g $MgSO_4 \cdot 7H_2O$
50ml 1M Tris-HCl pH 7.5
5ml 2% gelatin
Autoclave

Troubleshooting

Possible Problems/causes	Controls/Solutions
Insufficient cDNA insert: Low cloning efficiency	Monitor packaging with Control DNA and arms + Control Insert ligation. Be sure to use LE392 (*supF*) for plating packaged Control DNA and ER1647 for λSCREEN™-1 ligations. Check cDNA recovery by gel analysis. Do not freeze ligations prior to packaging. Do not exceed 10µl ligation in 50µl packaging reaction.
Bad (old) plating cells: Low cloning efficiency	Regrow fresh plating cells and replate Control DNA and arms + insert packaging reactions. Be sure to use LE392 (*supF*) when plating Control DNA reactions. Be sure to add supplemental Mg^{2+} and maltose to LB media during plating cell growth. Take inoculum for ER1647 plating cells from a fresh streak prepared on LB agar containing tetracycline.

References

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2. Rosenberg, S.M. (1987) *Meth. Enzymol.* **53**, 95-103.
3. Gunther, E.J., Murray, N.E. and Glazer, P.M. (1993) *Nucleic. Acids. Res.* **21**, 3903-3904.