Manual

MaxPlax Lambda Packaging Extracts

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BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC

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1. Introduction

MaxPlax Lambda Packaging Extracts are a convenient, high-efficiency transduction system designed for in vitro lambda packaging reactions. MaxPlax Lambda Packaging Extracts are supplied as predispensed single-tube reactions that have been optimised for packaging of methylated and unmethylated DNA. The packaging extracts routinely yield packaging efficiencies of >1 × 10^9 pfu/µg of Ligated Lambda Control DNA. The extracts can be used in the construction of representative cDNA libraries and genomic cloning of highly modified (methylated) DNA into λ-phage or cosmid vectors.

Traditional packaging extracts are derived from two complementary lysogenic *E. coli* strains, BHB2690 and BHB2688, as described by Hohn (1979).[1] The MaxPlax extracts utilise a new packaging strain, NM759*, reported by Gunther, Murray and Glazer (1993).[2] This strain, which replaces strain BHB2690 in the preparation of the sonication extract, is a restriction- free K12-derived strain deficient in the production of λ-phage capsid protein D. When combined with the complementary freeze-thaw extract from strain BHB2688**,[1] deficient in the production of λ-phage capsid protein E, an extremely high-efficiency of packaging for λ DNA is obtained. Moreover, the ability to package λ DNA bearing the mammalian methylation pattern is greatly enhanced, as evidenced by the high efficiency of λ-vector rescue from transgenic mouse DNA.[2] The lack of restriction activity has been shown to be crucial for the high efficiency rescue of lambda shuttle vectors from transgenic mouse DNA.[2,3]

*NM759: [W3110 recA56, Δ(mcrA) e14, Δ(mrr-hsd-mcr), (λimm434, clts, b2, red3, Dam15, Sam7)/λ]

**BHB2688: [N205 recA–, (λimm434 clts, b2, red3, Eam4, Sam7)/λ]

2. Product designations and kit components

Product	Kit size	Catalog number	Reagent description	Part numbers	Volume
MaxPlax Lambda Packaging Extracts	5 extracts	MP5105	MaxPlax Lambda Packaging Extract	SS000437-D	5 × 60 µL
			LE392MP Control Plating Strain Glycerol Stock	SS001000-D	250 µL
			Ligated Lambda Control DNA (0.02 µg/µL)	SS000602-D	50 µL
MaxPlax Lambda Packaging Extracts	10 extracts	MP5110	MaxPlax Lambda Packaging Extract	SS000437-D	10 × 60 µL
			LE392MP Control Plating Strain Glycerol Stock	SS001000-D	250 µL
			Ligated Lambda Control DNA (0.02 µg/µL)	SS000602-D	50 µL
MaxPlax Lambda Packaging Extracts	20 extracts	MP5120	MaxPlax Lambda Packaging Extract	SS000437-D	20 × 60 µL
			LE392MP Control Plating Strain Glycerol Stock	SS001000-D	250 µL
			Ligated Lambda Control DNA (0.02 µg/µL)	SS000602-D	50 µL

Note: MaxPlax Lambda Packaging Extracts are supplied as freeze-thaw/sonicate extracts in unlabeled single tubes. The extracts, Ligated Lambda Control DNA, and LE392MP Control Plating Strain are packaged together in a CO_2-impermeable foil pouch.

Store the MaxPlax Lambda Packaging Extracts at –70 °C or below. Exposure to higher temperature will decrease packaging efficiencies.

***E. coli* strain LE392MP Genotype:**
[F– e14–(McrA–) Δ(*mcrC-mrr*) (Tet^R) *hsd*R514 *sup*E44 *sup*F58 *lac*Y1 or Δ(*lac*IZY)6 *gal*K2 *gal*T22 *met*B1 *trp*R55 λ–]

3. Product specifications

Storage: Store the LE392MP Control Plating Strain Glycerol Stock and the MaxPlax Lambda Packaging Extract at –70 °C. Exposure to higher temperatures will greatly compromise packaging extract efficiency. Avoid long-term storage of product in the presence of dry ice. Once removed from the foil package, avoid any exposure to dry ice. Store the Ligated Lambda Control DNA at –20 °C. After thawing, store the Control DNA at 4 °C.

Storage Buffers: MaxPlax Lambda Packaging Extracts are supplied as unlabeled single tubes of freeze-thaw/sonicate extracts. LE392MP Control Plating Strain is supplied as a glycerol stock. Ligated Lambda Control DNA is supplied in 1X Ligation Buffer.

Guaranteed Stability: MaxPlax Lambda Packaging Extracts are quality tested by packaging a ligation reaction containing a fosmid vector backbone and a 42 kb control insert DNA from the human X chromosome. MaxPlax Lambda Packaging Extracts are guaranteed to maintain a packaging efficiency of $>1.0 \times 10^7$ cfu/µg of control insert DNA, when stored as directed for 1 year from the date of purchase.

4. Example protocol

This protocol can be used for the positive control reaction as well as for experimental reactions. The positive control reactions must be plated on the control host bacterial strain (LE392MP) included with the MaxPlax Extracts. The proper bacterial plating strain for the experimental reactions will vary depending on the cloning vector used. See the vector manufacturer's recommendations for the proper strain and plating media requirements. Ligation reactions may be added directly to the packaging extracts. When doing so, it is important to: a) add a volume of 10 µL or less to the packaging reaction, and b) heat inactivate the ligase (that is, treatment at 65 °C for 15 minutes) as active DNA ligase will decrease packaging efficiencies.

Solutions

Phage Dilution Buffer

10 mM Tris-HCl (pH 8.3)
100 mM NaCl
10 mM $MgCl_2$

LB Broth (1 Liter)

10 g Bacto-tryptone
5 g Bacto-yeast extract
10 g NaCl
Adjust pH to 7.0 with NaOH

LB Plates

LB Broth with 1.5% (w/v) Bacto-agar

LB Top Agar

LB Broth with 0.7% (w/v) Bacto-agar

Plating bacteria preparation:

1. The day before performing the packaging reactions, inoculate 50 mL of supplemented (10 mM $MgSO_4$) LB broth with a single colony of the plating bacterial strain and shake overnight at 37 °C.
2. The day of the packaging reactions, inoculate 50 mL of supplemented (10 mM $MgSO_4$ + 0.2% maltose) LB broth with 5 mL of the overnight culture and shake at 37 °C to an OD_{600} = 0.8-1.0. Store the cells at 4 °C until needed; cells may be stored for up to 72 hours.

Plating bacteria preparation:

1. Thaw the appropriate number of packaging extracts at room temperature. For every two packaging reactions, thaw one extract then place on ice.
2. When thawed, immediately transfer half (25 µL) of each packaging extract to a second 1.5-mL tube and place on ice.
3. Add the substrate DNA (10 µL [0.2 µg] of the control DNA) to a tube containing 25 µL of extract. If performing an odd number of packaging reactions, the remaining 25 µL of extract can be refrozen at -70 °C.
4. Mix by pipetting several times; avoid the introduction of air bubbles. Return all of the contents to the bottom of the tube by brief centrifugation if necessary.
5. Incubate the reaction(s) at 30 °C for 90 minutes.
6. At the end of this incubation, add the additional 25 µL of thawed extract to each reaction tube at 30 °C (If performing two packaging reactions, thaw another tube of extract and add 25 µL to each tube.) and incubate the reaction(s) for an additional 90 minutes at 30 °C.
7. Add 500 µL of phage dilution buffer and mix by gentle vortexing.
8. Add 25 µL of chloroform and mix by gentle vortexing (store at 4 °C).
9. Assay the packaged phage by titering on the appropriate bacterial strain (LE392MP for the control).

Titering phage extracts:

1. Make serial dilutions of the packaged phage in phage dilution buffer. Use 10^{-5} and 10^{-6} dilutions for the control reactions.
   10^{-2} dilution is 10 µL of packaged phage particles into 990 µL of phage dilution buffer; vortex mix.
   10^{-4} dilution is 10 µL of 10-2 dilution into 990 µL phage dilution buffer; vortex mix.
   10^{-5} dilution is 100 µL of 10^{-4} dilution into 900 µL phage dilution buffer; vortex mix.
   10^{-6} dilution is 10 µL of 10^{-4} dilution into 990 µL phage dilution buffer; vortex mix.
2. Add 100 µL of the appropriate serial dilutions to 100 µL of prepared plating bacteria (use LE392MP for the control reactions) and incubate for 15 minutes at 37 °C.
3. Add 3.0 mL of melted supplemented (10 mM $MgSO_4$) LB top agar (cooled to ~48 °C). Vortex gently and pour onto pre-warmed (37 °C) LB plates. Allow the top agar to solidify and then incubate inverted overnight at 37 °C.
4. Count the plaques and determine the titer (pfu/mL) and packaging efficiency (See sample calculations).

Sample calculations:

If there were 110 plaques on a 10-6 dilution plate, then the titer, pfu/mL, (where pfu represents plaque forming units) of this reaction would be:

$$\frac{(\#\text{ of plaques})\,(\text{dilution factor})\,(1000\ \mu L/mL)}{(\text{volume of phage plated [}\mu L\text{]})} \quad \textbf{OR} \quad \frac{(110\ \text{pfu})\,(10^6)\,(1000\ \mu L/mL)}{(100\ \mu L)} = \mathbf{1.1 \times 10^9\ pfu/mL}$$

The packaging efficiency (pfu/µg DNA) of this reaction would be:

$$\frac{(\#\text{ of plaques})\,(\text{dilution factor})\,(\text{total reaction vol.})}{(\text{vol. of dilution plated})\,(\text{amount of DNA packaged})} \quad \textbf{OR} \quad \frac{(110\ \text{pfu})\,(10^6)\,(550\ \mu L)}{(100\ \mu L)\,(0.2\ \mu g)} = \mathbf{3 \times 10^9\ pfu/\mu g}$$

5. References

1. Hohn, E.G. (1979) *Methods Enzymol.* **68,** 299.
2. Gunther, E.G. *et al.,* (1993) *Nucl. Acids Res.* **21,** 3903.
3. Kohler, S.W. *et al.,* (1990) *Nucl. Acids Res.* **18,** 3007.

6. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

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