Transpack Packaging Extract

For Lambda Transgenic Shuttle Vector Recovery

INSTRUCTION MANUAL

Catalog #200220 (400 packaging reactions), #200221 (100 packaging reactions), and #200223 (50 packaging reactions)
Revision A

For In Vitro Use Only
200220-12

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TRANSPACK PACKAGING EXTRACT

For Lambda Transgenic Shuttle Vector Recovery

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MATERIALS PROVIDED

Materials provided	Catalog number		
	#200220	**#200221**	**#200223**
Transpack packaging extract	400 packaging reactions[a]	100 packaging reactions[a]	50 packaging reactions[a]
Escherichia coli strain SCS-8 host strain	1 ml of bacterial glycerol stock	1 ml of bacterial glycerol stock	1 ml of bacterial glycerol stock
Control genomic DNA from Big Blue transgenic rodents	20 µl	20 µl	20 µl

[a] One set of Transpack packaging extract consists of five red tubes, each tube containing ~10 µl of Transpack packaging extract, and one blue tube containing ~70 µl of Transpack packaging extract. One set of Transpack packaging extract is enough for five total packaging reactions.

STORAGE CONDITIONS

All Components: –80°C

Note *Do not thaw the Transpack packaging extract prior to use!*

ADDITIONAL MATERIALS REQUIRED

Preparing the SCS-8 Plating Cultures

LB–tetracycline agar plates[§]
NZY broth[§]
Maltose [20% (w/v)] and $MgSO_4$ (1 M) solution, Stratagene Catalog #722000[§]
$MgSO_4$ (10 mM) solution, sterile[§]

Performing the Packaging Reaction

Wide-bore pipet tips
SM buffer[§]
Chloroform (optional)

Titering the Packaged DNA Samples

NZY agar plates[§] (two 100-mm plates/packaged DNA sample)
NZY top agarose[§] (3–4 ml/100-mm plate)
Disposable 5-ml tubes, sterile

[§] See *Preparation of Media and Reagents*.

Revision A

INTRODUCTION

The use of lambda packaging extracts is necessary with the Big Blue transgenic rodent mutagenesis assay system, an in vivo assay for genetic toxicity in mice and rats. The Big Blue transgenic rodent mutagenesis assay system employs a recoverable transgenic lambda phage shuttle vector containing a bacterial target gene. The assay is performed by isolating genomic DNA from rodent tissues and recovering the lambda shuttle vector from the genomic DNA using Transpack packaging extract.* Transpack packaging extract rescues the shuttle vector from the genomic DNA and packages the vector into viable lambda phage particles, which can then infect *Escherichia coli* to form plaques on a bacterial lawn. The plaques are evaluated for mutations in the target gene using a variety of methods.[1,2,3,4]. Transpack packaging extract is free of all known restriction systems that have been shown to reduce rescue efficiency and is deficient of β-galactosidase activity, which can affect mutant detection.[2,5,6,7,8]

Optimal rescue efficiencies are obtained when high-molecular-weight genomic DNA is used, with full-length chromosomal DNA being the theoretical ideal. Good results have been obtained with the RecoverEase DNA isolation kit.‡

Note *We recommend reading the entire instruction manual before attempting the experimental protocols.*

* U.S. Patent No. 5,188,957.

‡ [Stratagene Catalog #720202 (30 reactions) and #720203 (15 reactions)]

PREPARING THE SCS-8 PLATING CULTURES

Note *The packaging reaction (see* Performing the Packaging Reaction*) may be performed while the liquid cell cultures of the host strain are growing. We recommend using the SCS-8 host strain provided in order to achieve the best plating results for genomic DNA with the Transpack packaging extract; however,* E. coli *host strains other than the SCS-8 host strain have been used with success.*

1. Using a sterile inoculating loop, streak splinters of solid ice from the bacterial glycerol stock containing the SCS-8 host strain onto an LB–tetracycline agar plate.[§]

2. Incubate the bacterial streak plate overnight in a stationary 37°C air incubator. (Bacterial streak plates can be stored at 4°C for up to one week.)

3. In a sterile 50-ml conical tube, inoculate 20 ml of NZY broth,[§] supplemented with 250 µl of a 20% (w/v) maltose and 1 M $MgSO_4$ solution,[§] with several colonies from the bacterial streak plate. The SCS-8 cells cultured in NZY broth supplemented with the maltose–$MgSO_4$ solution is referred to as the SCS-8 liquid culture.

   Note *Do not use tetracycline for the preparation of the SCS-8 liquid culture.*

4. Incubate the SCS-8 liquid culture at 37°C for 4–6 hours with shaking at 250–300 rpm.

5. Centrifuge the conical tube at ~1000 × *g* for 10 minutes to pellet the bacterial cells.

6. Discard the supernatant and gently resuspend the pellet in 10 ml of sterile 10 mM $MgSO_4$ (see *Preparation of Media and Reagents*).

   Note *The bacterial cells may be stored at 4°C in 10 mM $MgSO_4$ but should be used within 48 hours.*

7. Dilute the SCS-8 bacterial cell suspension to an OD_{600} of 0.5 with sterile 10 mM $MgSO_4$. The prepared suspension of SCS-8 cells at an OD_{600} of 0.5 is referred to as the SCS-8 plating culture.

   Note *We recommend using the SCS-8 plating culture within 1–2 hours after dilution.*

[§] See *Preparation of media and Reagents*.

PERFORMING THE PACKAGING REACTION

Notes *Transpack packaging extract is extremely temperature sensitive and cannot be refrozen once thawed. In addition, contaminants in the genomic DNA such as residual phenol, chloroform, and ethanol have been shown to inhibit packaging.*

One Transpack set consists of 5 red tubes and one blue tube, which is enough for 5 packaging reactions. Since Transpack cannot be refrozen once thawed, it is most cost effective to package in sets of 5 reactions at a time.

Genomic DNA of high molecular weight (> 100 kb fragments) gives the best results in the Transpack packaging reaction. Actions which shear the DNA should be minimized (ex. pipetting, vortexing, shaking, diluting, etc…). To pipet the genomic DNA as directed in step 3, below, use a wide-bore pipet tip. Placing the wide-bore pipet tip to the bottom of the microcentrifuge tube containing the sample and carefully twisting the tip against the bottom of the tube may be necessary.

1. Remove five red tubes of Transpack packaging extract from a –80°C freezer and quickly place each red tube on **dry ice** until ready for use.

2. Quickly thaw one red tube of Transpack packaging extract between your fingers or hands until the contents of the tube are approximately three-quarters thawed. Leave the remaining tubes on dry ice until ready for further use.

   Note *Immediately before thawing each red tube, a 1–2 second pulse in a microcentrifuge may be required to collect the frozen contents from the tube sides and cap.*

3. Using a wide-bore pipet tip, transfer ≥8 µl of the genomic DNA sample to the red tube containing the Transpack packaging extract. Mix the packaging reaction by pipetting the reaction up and down three times with the wide-bore pipet tip and place the tube in a 30°C water bath. (If necessary, pulse the tube in a microcentrifuge to force the contents to the bottom of the tube before placing the tube in the water bath.)

4. Repeat steps 2 and 3 to package the remaining samples in the set.

   Note *A genomic DNA solution containing high molecular weight fragments that is very concentrated will be very viscous (i.e. it should be very difficult to pipet even with a wide-bore pipet tip). Starting with a very viscous genomic DNA sample, 8 µl of DNA per 10 µl of packaging extract is within the optimal DNA concentration range for the packaging reaction. If the genomic DNA sample is slightly less viscous, it is possible to use as much as 12 µl of DNA per 10 µl of packaging extract.*

5. Incubate each packaging reaction at 30°C for 90 minutes.

6. Remove one blue tube of Transpack packaging extract from a –80°C freezer for every set of five packaging reactions performed and quickly place the blue tube on **dry ice** until ready for use.

7. Thaw the blue tube of Transpack packaging extract by holding the tube between your fingers or hands until the contents of the blue tube are **completely thawed**. Quickly transfer 12 µl of Transpack packaging extract from the blue tube to one of the red tubes containing a packaging reaction. Mix the packaging reaction by pipetting the reaction up and down three to five times with the wide-bore pipet tip and return the tube to the 30°C water bath.

   Note *For very viscous DNA preparations only (e.g., DNA prepared using RecoverEase), packaging efficiencies can be improved using a **standard-bore** yellow tip for the **second** addition of Transpack, followed by vigorous pipetting to break down the viscosity before returning the tube to 30°C.*

8. Repeat step 7 for the remaining samples.

9. Incubate each packaging reaction at 30°C for an additional 90 minutes.

10. Dilute each completed packaging reaction by adding sterile SM buffer (see *Preparation of Media and Reagents*) to a final volume of 1 ml, then vortex at maximum speed for 10 seconds and place on ice. Multiple reactions for a particular DNA sample may be pooled at this time. The completed packaging reaction, diluted with SM buffer, is referred to as the packaged DNA sample.

Note *If the entire packaged DNA sample will not be plated on the same day it was packaged, add 50 µl of chloroform per ml of packaged DNA sample, gently vortex and store at 4°C for up to 2 weeks.*

PACKAGING THE CONTROL GENOMIC DNA

Use the following procedure to test the efficiency of Transpack packaging extract with the provided control genomic DNA from Big Blue transgenic rodents. Treat the genomic DNA control sample as one in a set of five packaging reactions.

1. Incubate the control genomic DNA at 30°C for 10 minutes.

2. Using a wide-bore pipet tip, gently pipet the DNA up and down 3–4 times to resuspend the DNA.

3. Use 8 µl of control genomic DNA from Big Blue transgenic rodents in one red tube. Proceed with steps 2–10 in *Performing the Packaging Reaction*.

4. Proceed to *Titering the Packaged DNA Samples*.

   Note *Rescue efficiencies should be ≥100,000 pfu/packaging reaction.*

TITERING THE PACKAGED DNA SAMPLES

Note *The protocol outlined below is one method that may be used to titer a packaged DNA sample. Although this protocol proves successful, the researcher is not limited to use of this method alone and may choose any alternate method that accurately estimates the number of plaques recovered per packaging reaction.*

1. Vortex each packaged DNA sample briefly.

2. For each packaged DNA sample to be titered, transfer 200 µl of prepared SCS-8 plating culture to a sterile 5-ml test tube *in duplicate*.

3. *Carefully* transfer 1 µl of each packaged DNA sample to each of the duplicate test tubes that contain the 200-µl aliquots of SCS-8 plating culture.

   Note *Dip only the tip of a standard (yellow) pipet tip into the packaged DNA sample, and avoid transferring any of the excess liquid that may be present on the outside of the pipet tip. Change the pipet tip between duplicate transfers of each packaged DNA sample.*

4. Incubate each test tube containing the packaged DNA sample and SCS-8 plating culture at 37°C for 15 minutes to allow phage to adsorb to host cells.

5. Add 3–4 ml of molten NZY top agarose[§] cooled to 50°C to each tube.

6. Quickly but gently pour the contents of each tube over a separate 100-mm NZY agar plate[§] and agitate the plate to ensure a smooth and even distribution before the top agarose hardens.

7. Invert and incubate each agar plate overnight in a stationary 37°C air incubator.

8. Count the number of phage plaques on each agar plate and determine the mean.

In general, the packaged DNA sample is not homogeneous and titering the reaction yields only a rough estimate of the number of phage plaques per packaging reaction. In practice, the actual titer of a packaged DNA sample is usually ~30% higher than the values obtained with the above method (probably due to phage trapped in the DNA and downward settling of the DNA). Therefore, multiplying the number of plaques per microliter by 1.3 generally gives a more reliable estimation of phage titer.

Example *If the number of phage plaques on the first NZY agar plate is 80 pfu and the number of phage plaques on the second NZY agar plate is 89 pfu, then the mean of both plates is 84.5 pfu. Determine the actual number of plaques per microliter of packaged DNA sample by multiplying the mean by 1.3 as follows:*

$$\frac{84.5\ pfu}{\mu l} \times 1.3 = \frac{109.85\ pfu}{\mu l}$$

Next, calculate the actual number of plaques per packaging reaction as follows:

$$\frac{109.85\ pfu}{\mu l} \times \frac{1000\ \mu l}{packaging\ reaction} = \frac{109{,}850\ pfu}{packaging\ reaction}$$

Note *If screening for mutations in the* lacI *gene using plaque color, please refer to the* Big Blue Transgenic Rodent Mutagenesis Assay System Instruction Manual *(*Color Screening Assay*) for details on plating and screening packaged DNA of Big Blue transgenic rodents.*

[§] See *Preparation of Media and Reagents*.

TROUBLESHOOTING

Observation	Suggestion(s)
The SCS-8 liquid culture appears clear during the growth phase	The SCS-8 liquid culture may possibly be contaminated with phage. Prepare and grow a fresh SCS-8 liquid culture.
An absence of plaques but presence of a bacterial lawn on the NZY titer plates	The packaging reaction is unsuccessful or is omitted from the SCS-8 cell–phage mixture prior to plating. Contact Stratagene Technical Services Department (see *Ordering Information and Technical Services* on the inside front cover of this instruction manual) or the appropriate distributor (see *Stratagene Distributors*).
Appearance of a spotted, grainy, or irregular bacterial lawn on the NZY titer plate	Ensure that chloroform or contaminating bacteria are removed from packaged DNA sample. Spin the packaged DNA sample in a microcentrifuge at high speed for 30 seconds to separate the chloroform phase from the reaction or use sterile technique to prevent the introduction of contaminating bacteria.
An absence of bacterial lawns on the NZY titer plates	To ensure NZY top agarose is at the optimal temperature, allow the NZY top agarose to cool to 48°–50°C.
	Check that the SCS-8 plating culture is added to the packaged DNA sample prior to plating.
A low number of plaques present on the NZY titer plates	Use a greater amount of the genomic DNA sample for the packaging reaction or increase the concentration of the genomic DNA sample.
	Ensure the genomic DNA is not degraded or sheared. If more tissue is available, prepare additional genomic DNA samples. Use of the RecoverEase DNA isolation kit [Stratagene Catalog #720202 (30 reactions) and #720203 (15 reactions)] is highly recommended.
	Ensure that contaminating ethanol and/or salts are removed from the genomic DNA sample by reprecipitating the genomic DNA using 100% (v/v) ethanol and 200 mM NaCl; ensure that all liquid is removed following precipitation.

PREPARATION OF MEDIA AND REAGENTS

Maltose [20% (w/v)] and $MgSO_4$ (1 M) Solution (per 100 ml)

20.0 g of maltose
24.6 g of $MgSO_4 \cdot 7H_2O$
Add dH_2O to a final volume of 100 ml
Filter sterilize
Store at 4°C for up to 6 months

SM Buffer (per Liter)

5.8 g of NaCl
2.0 g of $MgSO_4 \cdot 7H_2O$
50.0 ml of 1 M Tris-HCl (pH 7.5)
5.0 ml of 2% (w/v) gelatin
Add deionized H_2O to a final volume of 1 liter
Adjust the pH to 7.5
Autoclave for 30 minutes
Store at room temperature for up to 1 year

$MgSO_4$ (10 mM) (per Liter)

2.46 g of $MgSO_4 \cdot 7H_2O$
Add dH_2O to a final volume of 1 liter
Autoclave for 30 minutes
Store at room temperature for up to 1 year

NZY Broth (per Liter)

5.0 g of NaCl
2.0 g of $MgSO_4 \cdot 7H_2O$
5.0 g of yeast extract
10.0 g of casein peptone
Add 800 ml of dH_2O
Adjust the pH to 7.0 with 1 N NaOH
Add dH_2O to a final volume of 1 liter
Autoclave for 30 minutes

NZY Top Agarose (per Liter)

5.0 g of NaCl
2.0 g of $MgSO_4 \cdot 7H_2O$
5.0 g of yeast extract
10.0 g of casein peptone
Add 800 ml of dH_2O
Adjust the pH to 7.0 with 1 N NaOH
Add 7.0 g agarose
Add dH_2O to a final volume of 1 liter
Stir on a hot plate until the agarose dissolves
Pour into 100-ml bottles
Autoclave for 30 minutes
Store at room temperature for up to 1 year

Note *Alternatively, the top agarose component of the Big Blue media (Stratagene Catalog #720300) can be prepared according to instructions on the certificate of analysis, poured into 100-ml bottles, and autoclaved.*

***Prior to use**, melt the prepared NZY top agarose completely in microwave and cool the NZY top agarose to 50°C*

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NZY Agar Plates (per Liter)

5.0 g of NaCl
2.0 g of $MgSO_4 \cdot 7H_2O$
5.0 g of yeast extract
10.0 g of casein peptone
Add 15 g of agar
Add 800 ml of dH_2O
Adjust the pH to 7.0 with 1 N NaOH
Add dH_2O to a final volume of 1 liter
Autoclave for 30 minutes
Mix well and allow to cool to 55°C
Pour into sterile petri dishes (25–50 ml/ 100-mm plate)
Store plates at 4°C

Note *Alternatively, the bottom agar component of the Big Blue media (Stratagene Catalog #720300) can be prepared according to instructions on the certificate of analysis and poured into sterile petri dishes.*

LB–Tetracycline Agar Plates (per Liter)

10 g of NaCl
10 g of Bacto-tryptone
5 g of yeast extract
20 g of agar
Add 800 ml of dH_2O
Adjust pH to 7.0 with 1 N NaOH
Add dH_2O to a final volume of 1 liter
Autoclave for 30 minutes
Mix well and allow to cool to 55°C
Add 12.5 mg of tetracycline (dissolved in 10 ml of 50% ethanol and filter-sterilized)
Pour into sterile petri dishes (25–50 ml/ 100-mm plate)
Store plates at 4°C protected from light (tetracycline is light-sensitive)

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MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.

STRATAGENE

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For Lambda Transgenic Shuttle Vector Recovery

Catalog #200220 (400 packaging reactions), #200221 (100 packaging reactions), and #200223 (50 packaging reactions)

QUICK-REFERENCE PROTOCOLS

Preparing the SCS-8 Plating Cultures

- Using a sterile inoculating loop, streak splinters of the bacterial glycerol stock onto an LB–tetracycline agar plate
- Incubate the plate overnight in a stationary 37° air incubator
- In a sterile 50-ml conical tube, inoculate 20 ml of NZY broth, supplemented with maltose–$MgSO_4$ solution, with several colonies from the bacterial streak plate
- Incubate the SCS-8 liquid culture at 37°C for 4–6 hours with shaking at 250–300 rpm
- Centrifuge the conical tube at ~1000 × *g* for 10 minutes
- Gently resuspend the pellet in 10 ml of sterile 10 mM $MgSO_4$
- Dilute the SCS-8 bacterial cell suspension to an OD_{600} of 0.5 with sterile 10 mM $MgSO_4$

Performing the Packaging Reaction

Note *Perform packaging reactions while the bacterial cell cultures are growing (see* Preparing the SCS-8 Plating Cultures*)*

- Remove the required number of red tubes from a –80°C freezer and place each tube on dry ice
- Initiate each packaging reaction using a single red tube of Transpack packaging extract
  - Hold a red tube between your fingers or hands until the contents are approximately three-quarters thawed
  - Use a wide-bore pipet tip, transfer ≥8 µl of the genomic DNA sample to the red tube, mix by pipetting up and down three times, and place at 30°C
  - Repeat until each genomic DNA sample has been transferred to a red tube and placed at 30°C
  - Incubate all packaging reactions at 30°C for 90 minutes

- Remove one blue tube from a –80°C freezer for every five packaging reactions performed and place the tube on dry ice
- Complete each packaging reaction using a 12-µl aliquot from the blue tube
  - Hold the blue tube between your fingers or hands until the contents are completely thawed
  - Use a wide-bore pipet tip, quickly transfer 12 µl from the blue tube to one of the red tubes, mix by pipetting up and down three to five times, and place at 30°C
  - Repeat until each packaging reaction has received a 12-µl aliquot from the blue tube and placed at 30°C
  - Incubate at 30°C for an additional 90 minutes
- Add sterile SM buffer to a final volume of 1 ml to dilute each completed packaging reaction and vortex on medium speed for 3–5 seconds
- If the entire packaged DNA sample will not be plated on the same day it was packaged, add 50 µl of chloroform per ml of packaged DNA sample, gently vortex and store at 4°C for up to 2 weeks

Titering the Packaged DNA Samples

- Prepare, melt, and cool the NZY top agarose to 50°C in a water bath
- Transfer 200 µl of SCS-8 plating culture to a sterile 5-ml test tube *in duplicate*
- Transfer 1 µl of each packaged DNA sample to each of the duplicate test tubes that contain the 200-µl aliquots of SCS-8 plating culture
- Incubate the tubes at 37°C for 15 minutes
- Add 3–4 ml molten top agarose (cooled to 50°C) to each tube
- Quickly spread the contents of each tube onto a 100-mm NZY plate
- Invert and incubate each agar plate at 37°C overnight
- Count the number of plaques on each plate to estimate titer