Gigapack® III Gold Packaging Extract, Gigapack® III Plus Packaging Extract, and Gigapack® III XL Packaging Extract

INSTRUCTION MANUAL

Catalog #200201 (Gigapack III Gold-4), #200202 (Gigapack III Gold-7), and #200203 (Gigapack III Gold-11)
Catalog #200204 (Gigapack III Plus-4), #200205 (Gigapack III Plus-7), and #200206 (Gigapack III Plus-11)
Catalog #200207 (Gigapack III XL-4), #200208 (Gigapack III XL-7), and #200209 (Gigapack III XL-11)
Revision #053003c

For In Vitro Use Only

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Gigapack® III Gold Packaging Extract, Gigapack® III Plus Packaging Extract, and Gigapack® III XL Packaging Extract

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Gigapack® III Gold Packaging Extract, Gigapack® III Plus Packaging Extract, and Gigapack® III XL Packaging Extract

MATERIALS PROVIDED

Materials provided	Quantity		
	Catalog #200201	**Catalog #200202**	**Catalog #200203**
Gigapack® III Gold-4 packaging extract (blue tube)[a]	4 × 25 µl	—	—
Gigapack® III Gold-7 packaging extract (blue tube)[a]	—	7 × 25 µl	—
Gigapack® III Gold-11 packaging extract (blue tube)[a]	—	—	11 × 25 µl
λcI857 *Sam*7 wild-type lambda control DNA[b]	1.05 µg	1.05 µg	1.05 µg
VCS257 host strain[c]	1 ml	1 ml	1 ml
	Catalog #200204	**Catalog #200205**	**Catalog #200206**
Gigapack® III Plus-4 packaging extract (blue tube)[a]	4 × 25 µl	—	—
Gigapack® III Plus-7 packaging extract (blue tube)[a]	—	7 × 25 µl	—
Gigapack® III Plus-11 packaging extract (blue tube)[a]	—	—	11 × 25 µl
λcI857 *Sam*7 wild-type lambda control DNA[b]	1.05 µg	1.05 µg	1.05 µg
VCS257 host strain[c]	1 ml	1 ml	1 ml
	Catalog #200207	**Catalog #200208**	**Catalog #200209**
Gigapack® III XL-4 packaging extract (red-orange tube)[a]	4 × 25 µl	—	—
Gigapack® III XL-7 packaging extract (red-orange tube)[a]	—	7 × 25 µl	—
Gigapack® III XL-11 packaging extract (red-orange tube)[a]	—	—	11 × 25 µl
λcI857 *Sam*7 wild-type lambda control DNA[b]	1.05 µg	1.05 µg	1.05 µg
VCS257 host strain[c]	1 ml	1 ml	1 ml

[a] Gigapack III packaging extract is very sensitive to slight variations in temperature. Storing the packaging extracts at the bottom of a –80°C freezer directly from the dry ice shipping container is required in order to prevent a loss of packaging efficiency. Transferring tubes from one freezer to another may also result in a loss of efficiency. **Do not allow the packaging extracts to thaw!** Do not store the packaging extracts in liquid nitrogen as the tubes may explode.

[b] The λcI857 *Sam*7 wild-type lambda control DNA is shipped frozen and should be stored at –80°C immediately on receipt.

[c] The VCS257 host strain, included for plating the λcI857 *Sam*7 positive control, is shipped as a frozen bacterial glycerol stock (see *Preparation of Host Strains* for additional storage instructions) and should also be stored at –80°C immediately on receipt. This control host strain is a derivative of DP50 *supF* and should be used only when plating the packaged test DNA. The control DNA used with Gigapack III Gold packaging extract requires a *supF* mutation in the bacterial host to plate efficiently.

STORAGE CONDITIONS

Packaging Extracts: –80°C (Do not allow the packaging extracts thaw! Do not store the packaging extracts in liquid nitrogen as the tubes may explode.)

Lambda Control DNA: –80°C (Store at 4°C after thawing.)

Bacterial Glycerol Stock: –80°C

Revision #053003c

INTRODUCTION

Packaging extracts are used to package recombinant lambda phage with high efficiency. The single-tube format of Gigapack® III packaging extract simplifies the packaging procedure and increases the efficiency and representation of libraries constructed from highly methylated DNA. Each packaging extract is restriction minus ($HsdR^-$ $McrA^-$ $McrBC^-$ $McrF^-$ Mrr^-) to optimize packaging efficiency and library representation. When used in conjunction with restriction-deficient plating cultures, Gigapack III packaging extract improves the quality of DNA libraries constructed from methylated DNA.[1,2,3,4].

Because choice of an *in vitro* packaging extract designed to achieve specific experimental goals is important, Stratagene offers three different packaging extracts: Gigapack III Gold packaging extract, Gigapack III Plus packaging extract, and Gigapack III XL packaging extract. Gigapack III Gold packaging extract produces the highest packaging efficiency commercially available (2×10^9 pfu/µg) and is designed for use in cDNA and genomic library construction. Gigapack III Plus packaging extract is an economical alternative to Gigapack III Gold packaging extract when the highest packaging efficiency is not required. Gigapack III XL packaging extract preferentially packages large inserts (i.e., 47–51-kb recombinants), which eliminates the need for time-consuming size fractionation steps and the loss associated with sizing columns or sucrose gradients. This packaging extract is designed for use in genomic lambda and cosmid library construction.

Optimal packaging efficiencies are obtained with lambda DNAs that are concatemeric. Ligations should be carried out at DNA concentrations of 0.2 µg/µl or greater, which favors concatemers and not circular DNA molecules that only contain one *cos* site. DNA to be packaged should be relatively free from contaminants. *Polyethylene glycol (PEG), which is contained in some ligase buffers, can inhibit packaging.* The volume of DNA added to each extract should be between 1 and 4 µl. To obtain the highest packaging efficiency [i.e., the number of plaque-forming units per microgram (pfu/µg) of DNA], package 1 µl of the ligation reaction and never more than 4 µl. Increased volume (i.e., >4 µl) yields more plaque-forming units per packaging reaction, but fewer plaque-forming units per microgram of DNA.

DNA that is digested with restriction enzymes and religated packages less efficiently (by a factor of 10–100) than uncut lambda DNA. For example, uncut wild-type lambda DNA packages with efficiencies exceeding 1×10^9 pfu/µg of vector when using a Gigapack III packaging extract. However, predigested arms, when ligated to a test insert, yield ~$5 \times 10^6 - 1 \times 10^7$ recombinant plaques/µg of vector.

PREPARATION OF HOST STRAINS

Note *The appendix is included as a convenient reference for preparing host strains other than the VCS257 control host strain provided with the packaging extracts (see* Appendix: Host Strain Preparation).

The host strain is shipped as a bacterial glycerol stock. For the appropriate media, please refer to the following table:

Host strain	Agar plates for bacterial streak[a]	Medium for bacterial glycerol stock[a]	Medium for bacterial cultures for titering phage (final concentration)[a]
VCS257 strain	LB[b]	LB[b]	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$

[a] NZY medium (see *Preparation of Media and Reagents*) may be substituted for LB medium in all cases.

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

On arrival, prepare the following from the bacterial glycerol stock using the appropriate media as indicated in the previous table:

1. Revive the stored cells by scraping off splinters of solid ice with a sterile wire loop.

2. Streak the splinters onto an LB agar plate.

3. Incubate the plate overnight at 37°C.

4. Seal the plate with Parafilm® laboratory film and store the plate at 4°C for up to 1 week.

5. Restreak the cells onto a fresh plate every week.

Preparation of a –80°C Bacterial Glycerol Stock

1. In a sterile 50-ml conical tube, inoculate 10 ml of appropriate liquid medium containing the appropriate antibiotic, if one is necessary, with one colony from the plate. Grow the cells to an OD_{600} between ~1.0 and 2.0.

2. Add 4.5 ml of a sterile glycerol–liquid medium solution (5 ml of glycerol + 5 ml of appropriate medium) to the bacterial culture from step 1. Mix well.

3. Aliquot into sterile centrifuge tubes (1 ml/tube).

This preparation may be stored at –20°C for 1–2 years or at –80°C for more than 2 years.

Preparation of Host Bacteria

Note *Prepare an overnight culture of the VCS257 strain (see the table in* Preparation of Host Strains*) prior to performing the protocol for the positive wild-type lambda DNA control (see* Positive Wild-Type Lambda DNA Control for the Gigapack III Packaging Extract*).*

1. Streak the bacterial glycerol stock onto the appropriate agar plates (see *Appendix: Host Strain Preparation*). Incubate the plates overnight at 37°C.

   Note *Do not add antibiotic to the medium in the following step. The antibiotic will bind to the bacterial cell wall and will inhibit the ability of the phage to infect the cell.*

2. Inoculate an appropriate medium, supplemented with 10 mM $MgSO_4$ and 0.2% (w/v) maltose (see *Appendix: Host Strain Preparation*), with a single colony.

3. Grow at 37°C, shaking for 4–6 hours (do not grow past an OD_{600} of 1.0). Alternatively, grow overnight at 30°C, shaking at 200 rpm.

   Note *The lower temperature keeps the bacteria from overgrowing, thus reducing the number of nonviable cells. Phage can adhere to nonviable cells resulting in a decreased titer.*

4. Pellet the bacteria at 500 × *g* for 10 minutes.

5. Gently resuspend the cells in half the original volume with sterile 10 mM $MgSO_4$.

6. Dilute the cells to an OD_{600} of 0.5 with sterile 10 mM $MgSO_4$.

   Note *The bacteria should be used immediately following dilution.*

PACKAGING PROTOCOL

Note *Polyethylene glycol, which is contained in some ligase buffers, can inhibit packaging.*

1. Remove the appropriate number of packaging extracts from a –80°C freezer and place the extracts on dry ice.

2. Quickly thaw the packaging extract by holding the tube between your fingers until the contents of the tube just begins to thaw.

3. Add the experimental DNA **immediately** (1–4 µl containing 0.1–1.0 µg of ligated DNA) to the packaging extract.

4. Stir the tube with a pipet tip to mix well. **Gentle** pipetting is allowable provided that air bubbles are not introduced.

5. Spin the tube quickly (for 3–5 seconds), if desired, to ensure that all contents are at the bottom of the tube.

6. Incubate the tube at room temperature (22°C) for 2 hours.

7. Add 500 µl of SM buffer (see *Preparation of Media and Reagents*) to the tube.

8. Add 20 µl of chloroform and mix the contents of the tube gently.

9. Spin the tube briefly to sediment the debris.

10. The supernatant containing the phage is ready for titering. The supernatant may be stored at 4°C for up to 1 month.

Positive Wild-Type Lambda DNA Control for the Gigapack III Packaging Extract

Use the following procedure to test the efficiency of the Gigapack III packaging extract with the λ*c*I857 *Sam*7 wild-type lambda control DNA.

1. Thaw the frozen wild-type lambda control DNA on ice and gently mix the control after thawing.

2. Using 1 µl of the wild-type lambda control DNA (~0.2 µg), proceed with steps 1–10 in the *Packaging Protocol*.

   Note *Because of the high titer achieved with the wild-type lambda control DNA, Stratagene recommends stopping the control packaging reaction with 1 ml of SM buffer. This should make the plaques easier to count.*

3. Prepare two consecutive 10^{-2} dilutions of the packaging reaction from step 10 in the *Packaging Protocol* in SM buffer. (The final dilution is 10^{-4}.)

4. Add 10 µl of the 10^{-4} dilution to 200 µl of the VCS257 host strain. (This strain is recommended for plating the wild-type lambda control DNA only.) Incubate at 37°C for 15 minutes. Add 3 ml of LB top agar (see *Preparation of Media and Reagents*), melted and cooled to ~48°C, and quickly pour the dilution onto dry, prewarmed LB agar plates.

5. Incubate the plates for at least 12 hours at 37°C.

6. Count the plaques. Approximately 400 plaques should be obtained on the 10^{-4} dilution plate when the reaction is stopped with 1 ml of SM buffer.

TITERING THE PACKAGING REACTION

Note *For titering cosmid libraries, see* Titering the Cosmid Packaging Reaction.

1. Streak the bacterial glycerol stock onto the appropriate agar plates (see *Appendix: Host Strain Preparation*). Incubate the plates overnight at 37°C.

   Note *Do not add antibiotic to the medium in the following step. The antibiotic will bind to the bacterial cell wall and will inhibit the ability of the phage to infect the cell.*

2. Inoculate an appropriate medium, supplemented with 10 mM $MgSO_4$ and 0.2% (w/v) maltose (see *Appendix: Host Strain Preparation*), with a single colony.

3. Grow at 37°C, shaking for 4–6 hours (do not grow past an OD_{600} of 1.0). Alternatively, grow overnight at 30°C, shaking at 200 rpm.

   Note *The lower temperature keeps the bacteria from overgrowing, thus reducing the number of nonviable cells. Phage can adhere to nonviable cells resulting in a decreased titer.*

4. Pellet the bacteria at 500 × *g* for 10 minutes.

5. Gently resuspend the cells in half the original volume with sterile 10 mM $MgSO_4$.

6. Dilute the cells to an OD_{600} of 0.5 with sterile 10 mM $MgSO_4$.

   Note *The bacteria should be used immediately following dilution.*

7. Prepare dilutions of the final packaged reaction in SM buffer. Add 1 µl of the final packaged reaction to 200 µl of host cells diluted in 10 mM $MgSO_4$ to an OD_{600} of 0.5. If desired, also add 1 µl of a 1:10 dilution of the packaged reaction in SM buffer to 200 µl of host cells.

8. Incubate the phage and the bacteria at 37°C for 15 minutes to allow the phage to attach to the cells.

9. Add 3 ml of LB top agar (48°C) and plate immediately on prewarmed LB agar plates.

10. Incubate the plates at 37°C for 12 hours.

11. Count the plaques and determine the titer in plaque-forming units per milliliter (pfu/ml).

TITERING THE COSMID PACKAGING REACTION

1. Streak the bacterial glycerol stock onto the appropriate agar plates (see *Appendix: Host Strain Preparation*). Incubate the plates overnight at 37°C.

   Note *Do not add antibiotic to the medium in the following step. The antibiotic will bind to the bacterial cell wall and will inhibit the ability of the phage to infect the cell.*

2. Inoculate an appropriate medium, supplemented with 10 mM $MgSO_4$ and 0.2% (w/v) maltose (see *Appendix: Host Strain Preparation*), with a single colony.

3. Grow at 37°C, shaking for 4–6 hours (do not grow past an OD_{600} of 1.0). Alternatively, grow overnight at 30°C, shaking at 200 rpm.

   Note *The lower temperature keeps the bacteria from overgrowing, thus reducing the number of nonviable cells. Phage can adhere to nonviable cells resulting in a decreased titer.*

4. Pellet the bacteria at 500 × *g* for 10 minutes.

5. Gently resuspend the cells in half the original volume with sterile 10 mM $MgSO_4$.

6. Dilute the cells to an OD_{600} of 0.5 with sterile 10 mM $MgSO_4$.

   Note *The bacteria should be used immediately following dilution.*

7. Prepare a 1:10 and a 1:50 dilution of the cosmid packaging reaction in SM buffer.

8. Mix 25 µl of each dilution with 25 µl of the appropriate bacterial cells at an OD_{600} of 0.5 in a microcentrifuge tube and incubate the tube at room temperature for 30 minutes.

9. Add 200 µl of LB broth to each sample and incubate for 1 hour at 37°C, shaking the tube gently once every 15 minutes. This incubation will allow time for expression of the antibiotic resistance.

10. Spin the microcentrifuge tube for 1 minute and resuspend the pellet in 50 µl of fresh LB broth.

11. Using a sterile spreader, plate the cells on LB agar plates with the required amount of the appropriate antibiotic. Incubate the plates overnight at 37°C.

APPENDIX: HOST STRAIN PREPARATION

Host strain	Agar plate for bacterial streak	Medium for glycerol stock	Medium for bacterial cultures for titering phage (final concentration)[a]
AG-1 strain	LB	LB	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
BB4 strain	LB–tetracycline[b]	LB–tetracycline[b]	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
C600 strain	LB	LB	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
JM101 strain[c]	M9[d]	M9[b]	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
JM109 strain[c]	M9	M9	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
JM110 strain[d]	M9	M9	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
LE392 strain	LB	LB	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
NM514 strain	LB	LB	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
NM522 strain[c]	M9	M9	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
NM554 strain	LB	LB	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
P2392 strain	LB	LB	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
SCS110 strain[d]	M9	M9	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
SURE® strain[e]	LB–tetracycline	LB–tetracycline	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
VCS257 strain	LB	LB	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
XL1-Blue strain	LB–tetracycline	LB–tetracycline	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
XL1-Blue MR strain	LB	LB	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
XL1-Blue MRF′ strain	LB–tetracycline	LB–tetracycline	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
XL1-Blue MRF′ Kan strain	LB–kanamycin	LB–kanamycin	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
XL1-Blue MRA strain	LB	LB	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
XL1-Blue MRA (P2) strain	LB	LB	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
Y1088 strain	LB–ampicillin[b]	LB–ampicillin[b]	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
Y1089r$^-$ strain	LB–ampicillin	LB–ampicillin	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$
Y1090r$^-$ strain	LB–ampicillin	LB–ampicillin	LB with 0.2% (w/v) maltose–10 mM $MgSO_4$

[a] NZY medium (see *Preparation of Media and Reagents*) may be substituted for LB medium in all cases.

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

[c] Will grow two times slower on M9 medium.

[d] The amino acids—threonine, leucine, valine, and isoleucine—must be added to the M9 medium and plated at a concentration of 50 mg/liter for proper growth. Filter-sterilize the amino acids with the glucose and add as instructed.

[e] When growing lambda phage for plaque formation, incubate plates at 39°C.

TROUBLESHOOTING

Observation	Suggestion(s)
Packaging efficiency is too low	Gigapack III packaging extract is very sensitive to slight variations in temperature; therefore, store the packaging extracts at the bottom of a –80°C freezer and avoid transferring tubes from one freezer to another
	Do not allow the packaging extracts to thaw
	Avoid use of ligase buffers containing PEG, which can inhibit packaging
	Ligate at DNA concentrations of 0.2 µg/µl or greater and package between 1 and 4 µl of the ligation reaction
	Never package >4 µl of the ligation reaction, which causes dilution of the proteins contained within the packaging extract
Neither a bacterial lawn nor plaques is observed on the plate	Be sure to spin down the chloroform completely prior to removing an aliquot for titering

PREPARATION OF MEDIA AND REAGENTS

Note *All media must be autoclaved before use.*

LB Broth (per Liter)

10 g of NaCl
10 g of tryptone
5 g of yeast extract
Add deionized H_2O to a final volume of 1 liter
Adjust to pH 7.0 with 5 N NaOH
Autoclave

LB Agar (per Liter)

10 g of NaCl
10 g of tryptone
5 g of yeast extract
20 g of agar
Add deionized H_2O to a final volume of 1 liter
Adjust pH to 7.0 with 5 N NaOH
Autoclave
Pour into petri dishes (~25 ml/100-mm plate)

LB–Ampicillin Broth (per Liter)

1 liter of LB broth, autoclaved
Cool to 55°C
Add 10 ml of 10-mg/ml filter-sterilized ampicillin

LB–Ampicillin Agar (per Liter)

1 liter of LB agar, autoclaved
Cool to 55°C
Add 10 ml of 10-mg/ml filter-sterilized ampicillin
Pour into petri dishes (~25 ml/100-mm plate)

LB–Kanamycin Broth (per Liter)

1 liter of LB broth
Autoclave
Cool to 55°C
Add 50 mg of filter-sterilized kanamycin

LB–Kanamycin Agar (per Liter)

1 liter of LB agar
Autoclave
Cool to 55°C
Add 50 mg of filter-sterilized kanamycin
Pour into petri dishes (~25 ml/100-mm plate)

LB–Tetracycline Broth (per Liter)

1 liter of LB broth
Autoclave
Cool to 55°C
Add 12.5 mg of filter-sterilized tetracycline
Store broth in a dark, cool place as tetracycline is light-sensitive

LB–Tetracycline Agar (per Liter)

1 liter of LB agar
Autoclave
Cool to 55°C
Add 12.5 mg of filter-sterilized tetracycline
Pour into petri dishes (~25 ml/100-mm plate)
Store plates in a dark, cool place or cover plates with foil if left out at room temperature for extended time periods as tetracycline is light-sensitive

LB Top Agar (per Liter)

1 liter of LB broth
Add 0.7% (w/v) agarose
Autoclave

NZY Top Agar (per Liter)

1 liter of NZY broth
Add 0.7% (w/v) agarose
Autoclave

M9 Medium (per Liter)

6.0 g of dibasic sodium phosphate (Na_2HPO_4)
3.0 g of monobasic potassium phosphate (KH_2PO_4)
1.0 g of ammonium chloride (NH_4Cl)
0.5 g of NaCl
Add deionized H_2O to a final volume of 1 liter
Autoclave
While autoclaving, prepare the following solution:

1.0 ml of 1 M $MgSO_4$
2.0 g of glucose
0.1 ml of 1 M $CaCl_2$
1.0 ml of 1 M thiamine-HCl
Add deionized H_2O to a final volume of 10 ml and filter sterilize

Add the above solution to the cooled M9 media

NZY Agar (per Liter)

5 g of NaCl
2 g of $MgSO_4 \cdot 7H_2O$
5 g of yeast extract
10 g of NZ amine (casein hydrolysate)
15 g of agar
Add deionized H_2O to a final volume of 1 liter
Adjust the pH to 7.5 with NaOH
Autoclave
Pour into petri dishes (~80 ml/150-mm plate)

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
Autoclave

NZY Broth (per Liter)

5 g of NaCl
2 g of $MgSO_4 \cdot 7H_2O$
5 g of yeast extract
10 g of NZ amine (casein hydrolysate)
Add deionized H_2O to a final volume of 1 liter
Adjust the pH to 7.5 with NaOH
Autoclave

REFERENCES

1. Kretz, P. L., Reid, C. H., Greener, A. and Short, J. M. (1989) *Nucleic Acids Res* 17(13):5409.
2. Kretz, P. L. and Short, J. M. (1989) *Strategies in Molecular Biology* 2(2):25-26.
3. Kohler, S. W., Provost, G. S., Kretz, P. L., Dycaico, M. J., Sorge, J. A. *et al.* (1990) *Nucleic Acids Res* 18(10):3007-13.
4. Kretz, P. L., Kohler, S. W. and Short, J. M. (1991) *J Bacteriol* 173(15):4707-16.

ENDNOTES