ExAssist Interference-Resistant Helper Phage

with XLOLR Strain

Instruction Manual

Catalog #211203

Revision C.0

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211203-12

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MATERIALS PROVIDED

Materials provided	Quantity
XLOLR Strain	0.5-ml bacterial glycerol stock
XL1-Blue MRF′ Strain	0.5-ml bacterial glycerol stock
ExAssist interference-resistant helper phage ($\sim 1.0 \times 10^{10}$ pfu/ml)[a-c]	1 ml

[a] Retiter after 1 month. Store at –80°C.

[b] Supercoiled, single-strand size is 7.3 kb [comigrates with ~5 kb of double-stranded linear DNA on a 1% (w/v) agarose gel].

[c] We recommend VCSM13 Interference-Resistant Helper Phage and R408 Interference-Resistant Helper Phage for single-stranded rescue. ExAssist interference-resistant helper phage has α-complementing β-galactosidase sequences which may interfere with sequencing or site-directed mutagenesis where oligonucleotide primers hybridize to β-galactosidase sequences (e.g., M13–20 primer).

STORAGE CONDITIONS

Helper Phage: –80°C
Bacterial Glycerol Stocks: –80°C

Revision C.0

INTRODUCTION

The ExAssist interference-resistant helper phage with XLOLR strain is designed to allow efficient excision of the pBK-CMV phagemid from the ZAP Express vector, while eliminating problems associated with helper phage co-infection. The ExAssist helper phage contains an amber mutation that prevents replication of the phage genome in a nonsuppressing *Escherichia coli* strain such as XLOLR cells. This allows only the excised phagemid to replicate in the host, removing the possibility of productive co-infection from the ExAssist helper phage.

BACTERIAL HOST STRAINS

Host Strain Genotypes

Host strain	Genotype
XLOLR strain[a]	*Δ(mcrA)183 Δ(mcrCB-hsdSMR-mrr)173 endA1 thi-1 recA1 gyrA96 relA1 lac* [F′ *proAB lacI*^{q}Z*ΔM15* Tn*10* (Tet^{r})] Su^{-} (nonsuppressing) λ^{r} (lambda resistant)
XL1-Blue MRF′ strain	*Δ(mcrA)183 Δ(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* [F′ *proAB lacI*^{q}Z*ΔM15* Tn*10* (Tet^{r})]

[a] Use the XLOLR strain for excision only.

Recommended Media

Host strain	Agar plates and liquid medium for bacterial streak and glycerol stock	Liquid medium for bacterial cultures prior to phage attachment	Agar plates and top agar for plaque formation	Agar plates for excision protocol
XLOLR strain	LB-tetracycline[a]	LB broth with supplements[a-c]	—	LB-kanamycin[a]
XL1-Blue MRF′ strain	LB-tetracycline[a]	LB broth with supplements[a-c]	NZY[a]	—

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

[b] LB broth with 0.2% (w/v) maltose and 10 mM $MgSO_4$.

[c] Maltose and magnesium supplements are required for optimal lambda phage receptor expression on the surface of the XL1-Blue MRF′ host cell. The media supplements are not required for helper phage infection, but are included in both protocols for simplified media preparation.

Establishing an Agar Plate Bacterial Stock

The bacterial host strains are shipped as bacterial glycerol stocks. On arrival, prepare the following plates from the bacterial glycerol stocks.

Note *Do not allow the contents of the vial to thaw. The vials should be stored immediately at –20° or –80°C, but most strains remain viable longer if stored at –80°C. It is best to avoid repeated thawing of the host strains in order to maintain extended viability.*

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 containing the appropriate antibiotic (see *Recommended Media*), if one is necessary.

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.

Preparing a –80°C Bacterial Glycerol Stock

1. In a sterile 50-ml conical tube, inoculate 10 ml of LB broth with the appropriate antibiotic (see *Recommended Media*) with one colony from the plate. Grow the cells to late log phase.

2. Add 4.5 ml of a sterile glycerol-liquid medium solution (prepared by mixing 5 ml of glycerol + 5 ml of the 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.

Growth of Cells for Phage Infection

Bacterial cultures for phage infection should be started from a fresh plate using a single colony and should be grown overnight with vigorous shaking at 30°C in 50 ml of LB broth supplemented with 0.2% (w/v) maltose and 10 mM $MgSO_4$. (Do not use tetracycline in the presence of magnesium.) The lower temperature ensures that the cells will not overgrow. The cells should be spun at 1000 × *g* for 10 minutes then gently resuspended in 25 ml of 10 mM $MgSO_4$. Before use, dilute cells to an OD_{600} of 1.0 with 10 mM $MgSO_4$. Bacterial cells prepared in this manner can be used for all phage manipulations described within the manual. Highest efficiencies are obtained from freshly prepared cells.

HELPER PHAGE

Storing the Helper Phage

The ExAssist helper phage is supplied in 7% dimethylsulfoxide (DMSO) and should be stored at –80°C. The helper phage may be stored for short periods of time at –20°C or 4°C. It is important to titer the helper phage prior to each use. Expect titers of approximately 10^{10} pfu/ml. If the titer drops over time, prepare a fresh high-titer stock of the helper phage as outlined in *Amplifying the Helper Phage*.

Titering the Helper Phage

1. Transfer a colony of XL1-Blue MRF´ cells into 10 ml of LB broth with supplements in a 50-ml conical tube. Incubate the conical tube with shaking at 37°C until growth reaches an OD_{600} of 1.0.

2. Dilute the ExAssist helper phage (10^{-4}–10^{-7}) in SM buffer (See *Preparation of Media and Reagents*) and combine 1 µl of each dilution with 200 µl of XL1-Blue MRF´ cells (OD_{600} = 1.0).

3. Incubate the helper phage and the XL1-Blue MRF´ cells for 15 minutes at 37°C to allow the phage to attach to the cells.

4. Add 3 ml of NZY top agar, melted and cooled to ~48°C, and plate immediately onto dry, prewarmed NZY agar plates. Allow the plates to set for 10 minutes.

5. Invert the plates and incubate overnight at 37°C.

   Note *ExAssist plaques will have a cloudier appearance than lambda phage plaques.*

6. To determine the titer [in plaque-forming units per milliliter (pfu/ml)], use the following formula:

$$\left[\frac{\text{Number of plaques (pfu)} \times \text{dilution factor}}{\text{Volume plated (µl)}}\right] \times 1000\ \mu\text{l / ml}$$

   where the volume plated (in microliters) refers to the volume of the helper phage solution added to the cells.

Amplifying the Helper Phage

1. Transfer a colony of XL1-Blue MRF´ cells into 10 ml of LB broth with supplements in a 50-ml conical tube. Incubate the conical tube with shaking at 37°C until growth reaches an OD_{600} of 0.3.

   Note *An OD_{600} of 0.3 corresponds to 2.5×10^8 cells/ml.*

2. Add the ExAssist helper phage at a multiplicity of infection (MOI) of 10:1 or 1:1 (phage-to-cells ratio).

3. Incubate the conical tube at 37°C for 15 minutes to allow the phage to attach to the cells.

4. Incubate the conical tube with shaking at 37°C for 8 hours.

5. Heat the conical tube at 65°C for 15 minutes.

6. Spin down the cell debris and transfer the supernatant to a fresh conical tube.

7. The titer of the supernatant should be between 7.5×10^{10} and 1.0×10^{12} pfu/ml.

8. Add dimethylsulfoxide (DMSO) to a final concentration of 7% (v/v) and store at –80°C.

9. For further details about helper phage titering or amplification, please see *Titering the Helper Phage* or Reference 1.

In Vivo Excision Protocol Using the ExAssist Interference-Resistant Helper Phage with XLOLR Strain

Single-Clone Excision Protocol

Day 1

1. Core the plaque of interest from the agar plate and transfer the plaque to a sterile microcentrifuge tube containing 500 µl of SM buffer and 20 µl of chloroform. Vortex the microcentrifuge tube to release the phage particles into the SM buffer. Incubate the microcentrifuge tube for 1–2 hours at room temperature or overnight at 4°C. (This phage stock is stable for up to 6 months at 4°C.)

2. Grow separate 50-ml overnight cultures of XL1-Blue MRF´ and XLOLR cells in LB broth with supplements at 30°C.

Day 2

3. Gently spin down the XL1-Blue MRF´ and XLOLR cells (1000 × *g*). Resuspend each of the cell pellets in 25 ml of 10 mM $MgSO_4$. Measure the OD_{600} of the cell suspensions, then adjust the concentration of the cells to an OD_{600} of 1.0 (8×10^8 cells/ml) in 10 mM $MgSO_4$.

4. Combine the following components in a 14-ml BD Falcon polypropylene round-bottom tube:

   200 µl of XL1-Blue MRF´ cells at an OD_{600} of 1.0
   250 µl of phage stock (containing $>1 \times 10^5$ phage particles)
   1 µl of the ExAssist helper phage ($>1 \times 10^6$ pfu/µl)

   Note *Briefly spin the lambda phage stock to ensure that the chloroform is separated completely before removing the aliquot used in the excision reaction.*

5. Incubate the BD Falcon polypropylene tube at 37°C for 15 minutes to allow the phage to attach to the cells.

6. Add 3 ml of LB broth with supplements and incubate the BD Falcon polypropylene tube for 2.5–3 hours at 37°C with shaking. Because clonal representation is not relevant, single-clone excision reactions can be safely performed overnight.

   Note *The turbidity of the media is not indicative of the success of the excision.*

7. Heat the BD Falcon polypropylene tube at 65–70°C for 20 minutes to lyse the lambda phage particles and the cells. Spin the tube at 1000 × *g* for 15 minutes to pellet the cell debris.

8. Decant the supernatant into a sterile 14-ml BD Falcon polypropylene round-bottom tube. This stock contains the excised pBK-CMV phagemid packaged as filamentous phage particles. (This stock may be stored at 4°C for 1–2 months.)

9. To plate the excised phagemids, add 200 µl of freshly grown XLOLR cells from step 3 (OD_{600} = 1.0) to two 1.5-ml microcentrifuge tubes. Add 100 µl of the phage supernatant (from step 8 above) to one microcentrifuge tube and 10 µl of the phage supernatant to the other microcentrifuge tube.

10. Incubate the microcentrifuge tubes at 37°C for 15 minutes.

11. Plate 200 µl of the cell mixture from each microcentrifuge tube on LB-kanamycin agar plates (50 µg/ml) and incubate the plates overnight at 37°C.

Due to the high-efficiency of the excision process, it may be necessary to titrate the supernatant to achieve single-colony isolation.

Colonies appearing on the plate contain the pBK-CMV double-stranded phagemid with the cloned DNA insert. Helper phage will not grow, since helper phage is unable to replicate in the Su^- (nonsuppressing) XLOLR strain and does not contain kanamycin-resistance genes. XLOLR cells are also resistant to lambda phage infection, thus preventing lambda phage contamination after excision.

To maintain the pBK-CMV phagemid, streak the colony on a new LB-kanamycin agar plate. For long-term storage, prepare a bacterial glycerol stock and store at –80°C.

VCSM13 helper phage is recommended for the single-stranded rescue procedure. The single-stranded rescue procedure can be found in the Agilent *pBluescript II Phagemid Vectors Instruction Manual*.

Mass Excision Protocol

Day 1

1. Grow separate 50-ml overnight cultures of XL1-Blue MRF´ and XLOLR cells in LB broth with supplements at 30°C.

Day 2

2. Gently spin down the XL1-Blue MRF´ and XLOLR cells (1000 × *g*). Resuspend each of the cell pellets in 25 ml of 10 mM $MgSO_4$. Measure the OD_{600} of the cell suspensions, then adjust the concentration of the cells to an OD_{600} of 1.0 (8×10^8 cells/ml) in 10 mM $MgSO_4$.

3. In a 50-ml conical tube, combine a portion of the amplified lambda bacteriophage library with XL1-Blue MRF´ cells at a MOI of 1:10 lambda phage-to-cell ratio. Excise 10- to 100-fold more lambda phage than the size of the primary library to ensure statistical representation of the excised clones. Add ExAssist helper phage at a 10:1 helper phage-to-cells ratio to ensure that every cell is co-infected with lambda phage and helper phage.

   For example, use

   10^7 pfu of the lambda phage (i.e., 10- to 100-fold above the primary library size)
   10^8 XL1-Blue MRF´ cells (1:10 lambda phage-to-cell ratio, noting that an OD_{600} of 1.0 corresponds to 8×10^8 cells/ml)
   10^9 pfu of ExAssist helper phage (10:1 helper phage-to-cells ratio)

   Note *Briefly spin the lambda phage stock to ensure that the chloroform is separated completely before removing the aliquot used in the excision reaction.*

4. Incubate the conical tube at 37°C for 15 minutes to allow the phage to attach to the cells.

5. Add 20 ml of LB broth with supplements and incubate the conical tube for 2.5–3 hours at 37°C with shaking.

   Notes *Incubation times for mass excision in excess of 3 hours may alter the clonal representation.*

   The turbidity of the media is not indicative of the success of the excision.

6. Heat the conical tube at 65–70°C for 20 minutes to lyse the lambda phage particles and the cells.

7. Spin the conical tube at 1000 × *g* for 10 minutes to pellet the cell debris and then decant the supernatant into a sterile conical tube.

8. To titer the excised phagemids, combine 1 µl of this supernatant with 200 µl of XLOLR cells from step 2 in a 1.5-ml microcentrifuge tube.

9. Incubate the microcentrifuge tube at 37°C for 15 minutes.

10. Plate 100 µl of the cell mixture onto LB-kanamycin agar plates (50 µg/ml) and incubate the plates overnight at 37°C.

    Note *It may be necessary to further dilute the cell mixture to achieve single-colony isolation.*

At this stage, colonies may be selected for plasmid preps, or the cell mixture may be plated directly onto filters for colony screening.

TROUBLESHOOTING

Observation	Suggestions
The number of colonies is too low	The molar ratios of lambda phage to cells to helper phage is critical. Verify the ZAP Express phage titer. It may be necessary to make a high-titer stock of the lambda phage and to repeat the excision procedure
	Increase the excision time to increase the number of colonies
	The excision products should be plated on kanamycin plates, since the pBK-CMV phagemid is kanamycin resistant
	The lambda phage stock aliquot used for in vivo excision cannot contain chloroform, as chloroform lyses the *E. coli* cells. Briefly spin the lambda phage stock to ensure that the chloroform is separated completely before removing the aliquot

PREPARATION OF MEDIA AND REAGENTS

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

LB Broth

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 pH to 7.0 with 5 N NaOH
Autoclave

LB Broth with Supplements

Prepare 1 liter of LB broth
Autoclave
Add the following filter-sterilized supplements prior to use
10 ml of 1 M $MgSO_4$
3 ml of a 2 M maltose solution or 10 ml of 20% (w/v) maltose

LB–Kanamycin Agar (per Liter)

Prepare 1 liter of LB agar
Autoclave
Cool to 55°C
Add 5 ml of 10-mg/ml, filter-sterilized kanamycin
Pour into petri dishes (~25 ml/100-mm plate)

LB–Tetracycline Broth (per Liter)

Prepare 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)

Prepare 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

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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

NZY Top Agar (per Liter)

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

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 (~25 ml/100-mm plate or ~80 ml/150-mm plate)

REFERENCES

1. Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

ENDNOTES

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MSDS INFORMATION

Material Safety Data Sheets (MSDSs) are provided online at *http://www.genomics.agilent.com*. MSDS documents are not included with product shipments.