ABLE C Strain and ABLE K Strain Excision Protocol

Instruction Manual

Catalog #200305 (ABLE C strain and ABLE K strain)
#200306 (ABLE C strain)
#200307 (ABLE K strain)

Revision B

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MATERIALS PROVIDED

Host Strains and Genotypes

Host strain[a]	Reference	Genotype
ABLE C strain	1	*E. coli* C *lac*(LacZω⁻) [Kan^r McrA$^-$ McrCB$^-$ McrF$^-$ Mrr$^-$ HsdR(r_K^- m_K^-)] [F′ *proAB lacI*^{q}ZΔ*M15* Tn*10* (Tet^r)]
ABLE K strain	1	*E. coli* C *lac*(LacZω⁻) [Kan^r McrA$^-$ McrCB$^-$ McrF$^-$ Mrr$^-$ HsdR(r_K^- m_K^-)] [F′ *proAB lacI*^{q}ZΔ*M15* Tn*10* (Tet^r)]

[a] For host strain shipping and storage conditions, please see *Preparation of Host Cells*.

STORAGE CONDITIONS

Bacterial Glycerol Stocks: –80°C

ADDITIONAL MATERIALS REQUIRED FOR THE EXCISION PROTOCOL

f1 Helper Phage [ExAssist interference-resistant helper phage (>1 × 10^{10} pfu/ml)]
Host strains [SOLR strain and XL1-Blue strain (see table below for genotypes)]

Additional Host Strain Genotypes

Host strain	Genotype
SOLR strain [a,]	e14$^-$(McrA$^-$) Δ(*mcrCB-hsdSMR-mrr)171 sbcC recB recJ uvrC umuC*::Tn*5* (Kan^r) *lac gyrA96 relA1 thi-1 endA1* λ^R [F′ *proAB lacI*^{q}ZΔ*M15*] Su$^-$ (nonsuppressing)
XL1-Blue strain	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F′ *proAB lacI*^{q}ZΔ*M15* Tn*10* (Tet^r)]

[a] The SOLR strain should be used only for excision.

Revision B

INTRODUCTION

During cDNA library screening in plasmid systems, many clones cannot be propagated because their products are lethal to the host. Likewise, while toxic cDNA clones can be propagated in lambda systems, problems arise when attempting to subclone into plasmid systems, which require survival of the host. The ABLE C strain and the ABLE K strain[1] help solve these problems associated with toxic clones. The ABLE C strain and the ABLE K strain reduce the copy number of common cloning vectors by ~4- and 10-fold from XL1-BLue, respectively, enhancing the probability that a toxic clone will be propagated. Positive clones observed upon initial screening as lambda plaques can be excised or recloned into any convenient vector and can be introduced into the ABLE strains. Alternatively, plasmid libraries or excised phagemid libraries can be screened directly in these strains.

The ABLE strains and a standard high-copy-number host such as XL1-Blue can be screened simultaneously avoiding the need to repeat cloning operations or to investigate new host–vector systems. The ABLE strains are completely restriction minus and permit blue–white color selection. The ABLE strains propagate all ColE1-derived vectors (e.g., pBluescript II phagemids, pUC, pET, etc.) at lower copy numbers, thereby reducing the level of the cloned gene product.

PREPARATION OF HOST CELLS

The host strains have been sent as bacterial glycerol stocks. For the appropriate media and plates, please refer to the following table:

Host strain	Agar plates[a] for bacterial streak	Media[a] for bacterial glycerol stock	Media[a] for bacterial cultures for titering phage (final concentration)
ABLE C strain	LB–kanamycin[b]–tetracycline[c]	LB–kanamycin[b]–tetracycline[c]	LB (supplement not required)
ABLE K strain	LB–kanamycin[b]–tetracycline[c]	LB–kanamycin[b]–tetracycline[c]	LB (supplement not required)

[a] See *Preparation of Media and Reagents*.
[b] 50 µg/ml.
[c] 12.5 µg/ml.

On arrival, prepare the following from the bacterial glycerol stock:

Note *The host strains may thaw during shipment. The vials should be stored immediately at –20° or –80°C, but most strains remain viable longer if stored at –80°C. It is also 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 (see *Media and Reagents*) containing the appropriate antibiotic.

   Restreak the cells fresh each week.

Preparation of a –80°C Bacterial Glycerol Stock

1. In a sterile 50-ml conical tube, inoculate 10 ml of the appropriate liquid media with one or two colonies from the plate. Grow the cells to late log phase.

2. Add 4.5 ml of a sterile glycerol–liquid media solution (prepared by mixing 5 ml of glycerol + 5 ml of liquid media) 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.

In Vivo EXCISION PROTOCOL USING EXASSIST HELPER PHAGE

The ExAssist helper phage with SOLR strain is designed to allow the efficient excision of the pBluescript phagemid from Lambda ZAP vectors, while preventing 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 *E. coli* strain such as SOLR or ABLE cells. This allows only the excised phagemid to replicate in the host, removing the possibility of productive co-infection from the ExAssist helper phage. Since the ExAssist helper phage cannot replicate in the SOLR or ABLE strains, single-stranded rescue cannot be performed in these strains using this helper phage.

Note *The 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). The size of the ExAssist helper phage DNA single-strand is ~5 kb.*

Mass excision can be used to generate subtraction libraries and subtraction DNA probes. Converting the library to the phagemid form also allows screening of the phage library in eukaryotic cells by transformation of eukaryotic cells with supercoiled plasmid DNA.[2, 3]

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 (see *Media and Reagents*) 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 1 year at 4°C.)

2. Grow an overnight culture of XL1-Blue cells in LB broth supplemented with 0.2% (w/v) maltose–10 mM $MgSO_4$ at 30°C.

3. In addition, grow a 5-ml overnight culture of SOLR, ABLE C and ABLE K cells in LB broth containing 12.5 µg/ml of tetracycline and 50 µg/ml of kanamycin at 30°C.

Day 2

4. Make a 1/100 dilution of the cells using 0.5 ml of the overnight cultures and 50 ml of LB broth. Grow this culture at 37°C for 2–3 hours to mid log phase (OD_{600} = 0.2–0.5).

5. Gently spin down the XL1-Blue cells at 1500 × *g*. Resuspend the cells at an OD_{600} of 1.0 for single-clone excision and at an OD_{600} of 5.0 in 10 mM $MgSO_4$ for mass excision.

6. Allow the SOLR, ABLE C and ABLE K cells to grow to an OD_{600} of 0.5–1.0, while continuing with steps 7–11. Before the SOLR, ABLE C and ABLE K cells reach an $OD_{600} \geq 1$, remove the cells from the 37°C incubator to room temperature.

   Note *Use these cells on the same day.*

7. Combine the following components in a 50-ml conical tube:

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

   Note *When excising an entire library, ≥100-fold more of the amplified lambda phage should be excised than is found in the primary library to ensure statistical representation of the excised clones. The ExAssist helper phage should be added at a 1:1 phage-to-cell ratio.*

   For example, use:
   5 ml of OD_{600} = 5.0 cells
   10^{10} of the ExAssist helper phage
   10^9 pfu of the amplified library

8. Incubate the conical tube at 37°C for 15 minutes.

9. Add 3 ml of LB broth (25 ml of LB broth for mass excision) and incubate the conical tube for 2–2½ hours at 37°C with shaking. Incubation times for mass excision in excess of 3 hours may alter the clonal representation. Single-clone excision reactions can be safely performed overnight, since clonal representation is not relevant.

   Note *Cloudy growth may not always be seen.*

10. Heat the conical tube at 70°C for 15 minutes and then spin the tube again at 4000 × *g* for 15 minutes.

11. Decant the supernatant into a sterile conical tube. This stock contains the excised pBluescript phagemid packaged as filamentous phage particles. (The stock may be stored at 4°C for 1–2 months.)

12. To plate the excised phagemids, add 200 µl of freshly grown SOLR cells from step 6 (OD_{600} = 1.0) to two 1.5-ml microcentrifuge tubes. Add 100 µl of the phage supernatant (1 µl of the phage supernatant for mass excision) from step 11 above to one microcentrifuge tube and 10 µl of the phage supernatant to the other microcentrifuge tube.

13. Repeat step 12 using both the ABLE C and the ABLE K strains.

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

15. Plate 100 µl from each microcentrifuge tube on LB–ampicillin agar plates (50 µg/ml) (see *Media and Reagents*) 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 pBluescript double-stranded phagemid with the cloned DNA insert. Helper phage will not grow, since helper phage is unable to replicate in Su^- (nonsuppressing) SOLR, ABLE C and ABLE K strains. SOLR cells are also resistant to lambda phage infection, thus preventing lambda phage contamination after excision.

To maintain the pBluescript phagemid, streak the colony on a new LB-ampicillin agar plate. For long-term storage, prepare a bacterial glycerol stock and store at –80°C (see *Preparation of a –80°C Bacterial Glycerol Stock)*.

TROUBLESHOOTING

Observation	Suggestion
Low colony number	Excision efficiencies are directly related to the phage titer. If an excision is unsuccessful, it may be necessary to make a high-titer stock of the phage and to repeat the excision procedure)
	Incubate the plaque cores in SM buffer overnight to elute phage completely

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 H_2O to a final volume of 1 liter
Autoclave

LB Agar (per Liter)

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

LB Broth (per Liter)

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

LB–Ampicillin Agar (per Liter)

1 liter of LB agar
Autoclave
Cool to 55°C
Add 50 mg of filter-sterilized ampicillin
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–Tetracycline–Kanamycin Broth (per Liter)

1 liter of LB broth
Autoclave
Cool to 55°C
Add 12.5 mg of filter-sterilized tetracycline
Add 50 mg of filter-sterilized kanamycin
Store broth in a dark, cool place as tetracycline is light-sensitive

LB–Tetracycline–Kanamycin Agar (per Liter)

1 liter of LB agar
Autoclave
Cool to 55°C
Add 12.5 mg of filter-sterilized tetracycline
Add 50 mg of filter-sterilized kanamycin
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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MSDS INFORMATION

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