Custom cDNA Library in pcDNA™ 3.1

For isolating cDNAs, arraying cDNA, PCR of target sequences, and gene expression

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Contents

General Information ................................................................................................... iv

Introduction ............................................................................................................... 1
  Overview ................................................................................................................. 1

Methods ................................................................................................................... 3
  Using Custom cDNA Library .................................................................................. 3
  Transfection ............................................................................................................ 8
  Expression and Analysis ......................................................................................... 9
  Creating Stable Cell Lines ..................................................................................... 10

Appendix ................................................................................................................... 12
  Map of pcDNA™3.1 (+) .......................................................................................... 12
  Cloning Site of pcDNA™3.1 (+) ........................................................................... 13
  Recipes ..................................................................................................................... 14
  Additional Protocols .............................................................................................. 16
  Accessory Products ............................................................................................... 18
  Technical Support .................................................................................................. 19
  Purchaser Notification ............................................................................................ 20
  References ............................................................................................................... 21
General Information

Type of Libraries

This manual is supplied with the following Custom cDNA Libraries in pcDNA™3.1 (+):

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalog no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard library from tissue</td>
<td>11065-018</td>
</tr>
<tr>
<td>Standard library from RNA</td>
<td>11133-014</td>
</tr>
<tr>
<td>Microquantity library from tissue</td>
<td>11649-019</td>
</tr>
<tr>
<td>Microquantity library from RNA</td>
<td>11649-022</td>
</tr>
<tr>
<td>Full-length library</td>
<td>12028-015</td>
</tr>
<tr>
<td>Normalized library</td>
<td>11315-017</td>
</tr>
<tr>
<td>Full-length normalized library</td>
<td>12028-016</td>
</tr>
<tr>
<td>Amplified library</td>
<td>11499-019</td>
</tr>
</tbody>
</table>

Contents and Storage

Each Custom cDNA Library in pcDNA™3.1 (+) is supplied in 80% SOB medium, 20% (v/v) glycerol.

Store the library at –80°C.

Library Specification

The specifications for each type of Custom cDNA Library in pcDNA™3.1 (+) is described below.

For more details, refer to the Certificate of Analysis supplied with the library.

<table>
<thead>
<tr>
<th>Libraries</th>
<th>Features</th>
<th>Total Primary Clones</th>
<th>Ave. Insert Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>High representation</td>
<td>&gt;3 × 10⁶</td>
<td>&gt;1.2 kb</td>
</tr>
<tr>
<td>Microquantity</td>
<td>High representation from microquantity starting material</td>
<td>&gt;1 × 10⁶</td>
<td>&gt;1.2 kb</td>
</tr>
<tr>
<td>Full-Length</td>
<td>65–85% full-length clones</td>
<td>&gt;3 × 10⁶</td>
<td>&gt;1.6 kb</td>
</tr>
<tr>
<td>Normalized</td>
<td>10–100-fold reduction in abundant sequences</td>
<td>&gt;1 × 10⁶</td>
<td>&gt;1.2 kb</td>
</tr>
<tr>
<td>Amplified</td>
<td>1000-fold increase in the number of colony forming units (cfu)</td>
<td>&gt;1 × 10⁹</td>
<td>&gt;1.2 kb</td>
</tr>
</tbody>
</table>

Product Use

For research use only. Not intended for any human or animal diagnostic or therapeutic uses.
Introduction

Overview

Introduction

The Custom cDNA Library in pcDNA™3.1 (+) is suitable for isolating cDNAs, PCR of target sequences, cDNA sequencing, preparing cDNA arrays, and gene expression in eukaryotic cells. Each Custom cDNA Library is constructed using the SuperScript™ III reverse transcriptase to generate full-length and high-yield cDNA.

Library may be screened using the GeneTrapper® cDNA Positive Selection System, PCR or plate screening procedures, or by functional analysis using the eukaryotic CMV promoter.

pcDNA™3.1 (+)

Important features of pcDNA™3.1 (+) are listed below.

- The CMV promoter for efficient high-level expression of cloned cDNAs in eukaryotic cells
- T7 RNA polymerase promoter sites for in vitro transcription of RNA transcription in the sense orientation and sequencing through the insert
- The bovine growth hormone (BGH) polyadenylation signal for efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992)
- An f1 origin for single-strand DNA production to isolate desired clones using the GeneTrapper® cDNA Positive Selection System (page 7)
- SV40 early promoter and origin that allows efficient, high-level expression of the neomycin resistance gene and episomal replication in cells expressing SV40 large T antigen
- Neomycin resistance gene for selection of stable transfectants in mammalian cells (Southern and Berg, 1982)
- SV40 early polyadenylation signal for efficient transcription termination of mRNA
- Ampicillin resistance gene for selection of transformants in E. coli
- The pUC origin for high copy replication and maintenance of the plasmid in E. coli

For a map of pcDNA™3.1 (+), see page 12.
Preparing Custom Library

Custom cDNA Library in pcDNA™3.1 is prepared as follows:

- mRNA is isolated using two steps. First, total RNA is isolated from tissues or cells using the TRIzol® Reagent. Second, mRNA is isolated from total RNA using oligo (dT) in a filter syringe.
- First-strand cDNA is synthesized using SuperScript™ III Reverse Transcriptase
- Second-strand cDNA is synthesized using *E. coli* RNase H, *E. coli* DNA polymerase I, and *E. coli* DNA ligase
- cDNA is blunt-ended using T4 DNA Polymerase and digested with *Not I*
- cDNA is size-selected using column chromatography or agarose gel electrophoresis
- Size-selected cDNA is directionally cloned into the *Not I-EcoR V* region of the vector (*EcoR V* site is destroyed during cloning)
- Ligation mixture is transformed into competent ElectroMAX™ DH10B™ T1-Phage resistant *E. coli* and the number of primary recombinants is determined

If desired, the cDNA library can be amplified upon request once using a semi-solid procedure (Kriegler, 1990) to minimize representational biases (page 18).

Genotype of DH10B™ T1

The genotype of DH10B™ T1-Phage Resistant *E. coli* is: F’ mcrA Δ(mrr-hsdRMS-mcrBC)φ80lacZΔM15 ΔlacX74 deoR recA1 endA1 araD139 Δ(ara,leu)7697 galU galK rpsL nupG tonA (confers phage T1 and T5 resistance).
Methods

Using Custom cDNA Library

Introduction

Custom cDNA Library in pcDNA™3.1 (+) may be screened using the GeneTrapper® cDNA Positive Selection System, PCR or plate screening procedures, in vitro transcription analysis, or functional analysis using the eukaryotic CMV promoter.

General procedures for preparing DNA from the library and colony PCR are provided in this section. For detailed information on library screening refer to published references (Ausubel et al., 1994; Sambrook et al., 1989).

Materials Needed

- LB plate containing 100 μg/mL ampicillin

For preparing plasmid DNA

- Terrific Broth (page 14 for a recipe)
- Buffer I with RNase (page 14 for a recipe)
- Buffer II (page 15 for a recipe)
- 7.5 M ammonium acetate, chilled
- Isopropanol, chilled
- Phenol:Chloroform:Isoamyl alcohol (25:24:1)
- 70% ethanol

Plating the Library

To plate a library for screening or picking colonies:

1. Check the titer of the Custom cDNA Library on the Certificate of Analysis that is shipped with the library. If you wish to verify the library titer, see page 16.

2. Based on the library titer, dilute an aliquot of the library such that you can plate 500–1000 clones on a 75 mm plate or 2500–5000 clones on a 150 mm plate. Use LB plates containing 100 μg/mL ampicillin.

3. Incubate the plates at 37°C overnight.

4. Use the isolated colonies for colony PCR screening (page 6) or use a method of choice for screening.

Continued on next page
Using Custom cDNA Library, continued

Note
You may use a maxi plasmid DNA preparation method to isolate plasmid DNA. If you are using a maxi plasmid DNA preparation method, extract and precipitate the DNA as described, see page 5.

Preparing dsDNA from a Plasmid cDNA Library

1. Inoculate 100 mL Terrific Broth containing 100 μg/mL ampicillin with 2.5 × 10^6 cells from a primary library or 2.5 × 10^9 cells from an amplified library in a 500-mL flask.
2. Incubate the culture for 14–20 hours for an amplified library and 22–24 hours for a primary library at 30 °C with shaking at 275 rpm. Clones are prone to deletions when the culture is grown at >30°C.
3. Read the A_{590} of the culture. For accurate A_{590} determination, dilute the cells 1:10–1:20, such that the observed value is between 0.2–0.8.
4. Process ~500 OD_{590} units in two 50-mL centrifuge tubes.
5. Centrifuge the tubes at 4800 × g for 15 minutes at 4°C. Discard the supernatant. If desired the cell pellet can be stored at −80°C until ready for use.
6. Resuspend the cell pellets in a total volume of 10 mL Buffer I with RNase (cells must be <50 OD units/mL).
7. Add 10 mL of Buffer II to the resuspended cells. Invert the tubes to mix the cells and incubate for 5 minutes at room temperature. Do not exceed 5 minutes.
8. Add 10 mL cold 7.5 M ammonium acetate to the cell mixture. Invert the tubes to mix the cells and incubate for 10 minutes on ice.
9. Centrifuge the tubes at 3,000 × g for 15 minutes at 4°C. Pour the supernatant through cheesecloth or a clean, DNase-free, porous filter into a fresh 50-mL centrifuge tube. Avoid the white flocculant material.
10. Add an equal volume of cold isopropanol to the tube, mix well, and centrifuge the tubes at 3,000 × g for 15 minutes at 4°C. Discard the supernatant.
11. Resuspend the cell pellet in 1 mL Buffer I with RNase and transfer to a microcentrifuge tube.
12. Centrifuge the tubes at 14,000 × g for 1 minute at 4°C. Transfer the supernatant to a fresh microcentrifuge tube. Incubate the tube at 37°C for 10 minutes.
13. Incubate the tubes at 65 °C for 5 minutes. Split each sample into two equal parts (~500 μl each) in 1.5-mL microcentrifuge tubes and precipitate DNA (page 5).

Continued on next page
Extracting and Precipitating DNA

1. Add an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) to each sample from Step 13, previous page, and vortex the tubes for ~1 minute.

2. Centrifuge the tubes at 14,000 × g for 5 minutes at room temperature.

3. Avoiding the interface, transfer 450 mL of the upper (aqueous) phase to a fresh microcentrifuge tube.

4. Repeat the phenol:chloroform:isoamyl alcohol extraction at least twice. If an interface remains, repeat Steps 1–4 until the supernatant is clear.

5. Add an equal volume (~450 μl) of chilled isopropanol to each tube. Centrifuge the tubes at 14,000 × g for 15 minutes at 4°C. Discard the supernatant.

6. Add 500 μl 70 % ethanol to each tube. Centrifuge the tubes at 14,000 × g for 5 minutes at 4°C. Discard the supernatant.

7. Dry the pellet for 10 minutes at room temperature.

8. Completely dissolve the two pellets in 200 μl TE buffer. The plasmid DNA library concentration must be approximately 1 μg/μl.

9. Store the DNA at –20°C.

Continued on next page
Using Custom cDNA Library, continued

**Colony PCR Screening**

Use this PCR procedure to screen for the presence of specific cDNA or identify desired cDNA clones. The recommended primers for PCR and sequencing are shown on page 13.

1. Add 10 mL TE to each labeled, 0.5 mL microcentrifuge tube.

2. Pick individual colonies using a pipette tip and place the colonies directly into separate tubes containing TE. Pipet up and down to mix.

3. Incubate the tubes in a prewarmed thermal cycler at 99°C for 5 minutes.

4. Incubate the tubes on ice for 2 minutes.

5. Centrifuge briefly to collect the sample at the bottom of the tube. Place the tubes on ice.

6. Prepare the following reaction mix and add 40 mL of reaction mix to each tube.
   - 1X PCR Buffer (contains no MgCl2)
   - 0.2 mM dNTP mix
   - 0.5 μM primers
   - 2.4 mM MgCl2
   - 2.5 units Platinum® Taq DNA polymerase

7. Bring the volume to 50 μl with sterile water.

8. Perform PCR using the following program:

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>95°C</td>
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<tr>
<td>94°C</td>
<td>15 seconds</td>
<td></td>
</tr>
<tr>
<td>60°C</td>
<td>1 minute</td>
<td>40</td>
</tr>
<tr>
<td>72°C</td>
<td>1 minute</td>
<td></td>
</tr>
<tr>
<td>72°C</td>
<td>5 minutes</td>
<td>1</td>
</tr>
</tbody>
</table>

9. Transfer 10 μl of each reaction to a new tube containing 2 μl 10X gel loading buffer.

10. Electrophorese the samples on a 1.5% agarose gel and analyze your results.

   *Continued on next page*
Using Custom cDNA Library, continued

GeneTrapper® cDNA Positive Selection System

The GeneTrapper® cDNA Positive Selection System (see page 18 for ordering information) facilitates rapid isolation of cDNA clones from cDNA Library (representing $10^{12}$ DNA molecules) within 2–3 days.

Custom services for isolating cDNA clones from cDNA library using the GeneTrapper® method are available. For more details, visit www.lifetechnologies.com/evoquest or contact Technical Support (page 19).

Expression of Cloned cDNA

The CMV promoter in pcDNA™3.1 vector enables transient and stable expression (page 10) of cloned cDNAs in mammalian cells. Library and individual clones may be screened using a functional assay of choice. For transfection, see page 8.
Transfection

Introduction

This section provides general information for transfecting your clone into the mammalian cell line of choice. We recommend that you include a positive control vector and a mock transfection (negative control) in your experiments to evaluate your results.

Plasmid Preparation

Once you have isolated your desired clone, you must isolate plasmid DNA for transfection. Plasmid DNA for transfection into eukaryotic cells must be very clean and free from phenol and sodium chloride. Contaminants will kill the cells, and salt will interfere with lipid complexing, decreasing transfection efficiency. We recommend isolating plasmid DNA using the PureLink™ HQ Mini Plasmid DNA Purification Kit (cat. no. K2100-01).

Methods of Transfection

For established cell lines (e.g. HeLa), consult original references or the supplier of your cell line for the optimal method of transfection. We recommend that you follow exactly the protocol for your cell line. Pay particular attention to medium requirements, when to pass the cells, and at what dilution to split the cells. Further information is provided in Current Protocols in Molecular Biology (Ausubel et al., 1994).

Methods for transfection include calcium phosphate (Chen and Okayama, 1987; Wigler et al., 1977), lipid-mediated (Felgner et al., 1989; Felgner and Ringold, 1989) and electroporation (Chu et al., 1987; Shigekawa and Dower, 1988). Life Technologies offers a large selection of reagents for transfection including Lipofectamine™ 2000 (Catalog no. 11668-027) and the Calcium Phosphate Transfection Kit (Catalog no. K2780-01). Other transfection reagents are also available. For more information, refer to our Web site (www.lifetechnologies.com) or contact Technical Support (page 19).

Continued on next page
Expression and Analysis

**Introduction**

Expression of your gene of interest from the cDNA clone can be performed in either transiently transfected cells or stable cell lines (see page 10 for guidelines to create stable cell lines). You may use a functional assay or a Western blot analysis to detect your recombinant protein (see Analysis).

**Preparing Cell Lysates**

To detect your fusion protein by Western blot, prepare a cell lysate from transfected cells. A sample protocol is provided below. Other protocols are suitable.

1. Wash cell monolayers (~5 × 10⁵ to 1 × 10⁶ cells) once with phosphate-buffered saline (PBS) pH 7.4.
2. Scrape cells into 1 mL PBS and pellet the cells at 1500 × g for 5 minutes.
3. Resuspend in 50 μl Cell Lysis Buffer (see page 15 for a recipe). Other cell lysis buffers are suitable. Vortex.
4. Incubate cell suspension at 37°C for 10 minutes to lyse the cells. **Note:** You may prefer to lyse the cells at room temperature or on ice if degradation of your protein is a potential problem.
5. Centrifuge the cell lysate at 10,000 × g for 10 minutes at 4°C to pellet nuclei and transfer the supernatant to a fresh tube. Assay the lysate for protein concentration.
6. Add SDS-PAGE sample buffer (see page 15 for a recipe) to a final concentration of 1X and boil the sample for 5 minutes.
7. Load 20 μg of lysate onto an SDS-PAGE gel and electrophorese. Use the appropriate percentage of acrylamide to resolve your fusion protein.

**Analysis**

To facilitate separation and visualization of your recombinant fusion protein by polyacrylamide gel electrophoresis, a wide range of pre-cast NuPAGE® and Novex® Tris-Glycine polyacrylamide gels and electrophoresis apparatus are available from Life Technologies. To facilitate detection of antibodies by colorimetric or chemiluminescent methods, the ready-to-use WesternBreeze® Chromogenic Kits and WesternBreeze® Chemiluminescent Kits are available from Life Technologies. For more information, go to www.lifetechnologies.com or contact Technical Support (page 19).
Creating Stable Cell Lines

Introduction

The pcDNA™3.1 (+) vector contains the neomycin resistance genes to allow selection of stable cell lines using neomycin (Geneticin® Selective Antibiotic). If you wish to create stable cell lines, transflect your cDNA clone of interest into the mammalian cell line of choice and select for foci using Geneticin® Selective Antibiotic. General information and guidelines are provided, see pages 10–11.

To obtain stable transfectants, we recommend that you linearize your cDNA clone before transfection. While linearizing the vector may not improve the efficiency of transfection, it increases the chances that the vector does not integrate in a way that disrupts elements necessary for expression in mammalian cells. To linearize your construct, cut at a unique site that is not located within a critical element or within your gene of interest.

Geneticin® Selective Antibiotic

Geneticin® Selective Antibiotic blocks protein synthesis in mammalian cells by interfering with ribosomal function. It is an aminoglycoside, similar in structure to neomycin, gentamycin, and kanamycin. Expression in mammalian cells of the bacterial aminoglycoside phosphotransferase gene (APH), derived from Tn5, results in detoxification of Geneticin® Selective Antibiotic (Southern and Berg, 1982).

Continued on next page
Creating Stable Cell Lines, continued

Determining Antibiotic Sensitivity

To successfully generate a stable cell line expressing your protein of interest, you need to determine the minimum concentration of Geneticin® Selective Antibiotic required to kill your untransfected host cell line. Test a range of concentrations to ensure that you determine the minimum concentration necessary for your cell line.

1. Plate or split a confluent plate such that the cells are approximately 25% confluent. Prepare a set of 6–7 plates. Add the following concentrations of Geneticin® Selective Antibiotic (0, 50, 125, 250, 500, 750, and 1000 μg/mL Geneticin® Selective Antibiotic) to each plate.

2. Replenish the selective media every 3–4 days, and observe the percentage of surviving cells.

3. Count the number of viable cells at regular intervals to determine the appropriate concentration of antibiotic that prevents growth within 1–3 weeks after addition of the antibiotic.

Geneticin® Selection Guidelines

Once you have determined the appropriate Geneticin® Selective Antibiotic concentration to use for selection, you can generate a stable cell line expressing your cDNA clone. Geneticin® Selective Antibiotic is available separately from Life Technologies (see page 18 for ordering information). Use as follows:

1. Prepare Geneticin® Selective Antibiotic in a buffered solution (e.g. 100 mM HEPES, pH 7.3).

2. Use the predetermined concentration of Geneticin® Selective Antibiotic in complete medium.

3. Calculate concentration based on the amount of active drug.

4. Cells will divide once or twice in the presence of lethal doses of Geneticin® Selective Antibiotic, so the effects of the drug take several days to become apparent. Complete selection can take from 2–3 weeks of growth in selective medium.
Appendix

Map of pcDNA™ 3.1 (+)

The figure below shows the map and features of pcDNA™ 3.1 (+) vector. The complete sequence of pcDNA™ 3.1 (+) is available for downloading from our Web site (www.lifetechnologies.com) or by contacting Technical Support (page 19).

Comments for pcDNA3.1 (+) without insert
5428 nucleotides

CMV promoter: bases 232-819
T7 promoter/priming site: bases 863-882
pcDNA3.1/BGH reverse priming site: bases 1022-1039
BGH polyadenylation sequence: bases 1028-1252
f1 origin: bases 1298-1726
SV40 early promoter and origin: bases 1731-2074
Neomycin resistance gene (ORF): bases 2136-2930
SV40 early polyadenylation signal: bases 3104-3234
pUC origin: bases 3617-4287 (complementary strand)
Ampicillin resistance gene (bla): bases 4432-5428 (complementary strand)
ORF: bases 4432-5292 (complementary strand)
Ribosome binding site: bases 5300-5304 (complementary strand)
bla promoter (P3): bases 5327-5333 (complementary strand)

Continued on next page
Cloning Site of pcDNA™ 3.1 (+)

The cloning site for pcDNA™3.1 (+) is shown below. Restriction sites are labeled to indicate the cleavage site.

**Cloning Site of pcDNA™ 3.1 (+)**

689 CATTGAGGCT CAACTGGAGTT TGTTTTGGCA CCAAAATCAA CGGAAGTTTC CAAATATGTGC

749 TAACAAGCTCC GCCCATTTGA CGCAAATGGG CGGTAGGCCT GTAGGTGGG AGGTCTATAT

809 AAGCAGAGCT CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC

T7 promoter/primer binding site

Nhe I  |  Pme I  |  Apa II  |  Hind III  |  Asp718 I  |  Kpn I

BamHI  |  BstXI I  |  EcoRI I  |  Not I  |  Xho I

GGATCCACTA GTCCAGTGTT GTGGAATTCT GCAGAT | | | cDNA insert | GGC GGGGCTCGAG

929 GAATTCAGA GTCCAGTGGT GTGGAATTCT GCAGAT | | | | | pcDNA3.1/BGH reverse priming site

979 AGATCTAGAG GCCGTTTAA ACCCGCTGAT CAGGCTGAC TGGCCCTCT AGTTGCGAGC

1039 CATCTGTTGT TTGGGCTTCC CCCGTGCTTT TCTTGAACCT GGAAGGTGCC ACTCCACTG

1099 TCTTTTCCTA ATAAAATGAG GAAATGGCAT

enhancer region (3' end)
Recipes

**Terrific Broth**

1. Dissolve the following reagents in 800 mL of distilled water:
   - Tryptone 12 g
   - Yeast Extract 24 g
   - Glycerol 4 mL
2. Mix well and adjust the volume to 900 mL with distilled water.
3. Autoclave on liquid cycle for 20 minutes. Allow solution to cool to ~55°C.
4. Dissolve the following reagents in 80 mL of distilled water:
   - KH₂PO₄ (monobasic) 2.3 g
   - K₂HPO₄ (dibasic) 12.5 g
5. Mix well and adjust the volume to 100 mL with distilled water.
6. Autoclave on liquid cycle for 20 minutes. Allow solution to cool to ~55°C. Mix this solution with the solution prepared in Step 3.
7. After the media is cooled, add antibiotic to the desired concentration.
8. Store at +4°C.

**Buffer I with RNase**

15 mM Tris-HCl, pH 8.0
10 mM EDTA
100 μg/mL RNase A
1200 U/mL RNase T1

1. To prepare 1 liter Buffer I, mix the following:
   - 1 M Tris-HCl, pH 8.0 15 mL
   - 0.5 M EDTA 20 mL
   - Sterile water to 1000 mL
2. Mix well and store at room temperature.
3. Prepare 50 mL **fresh Buffer I with RNase** as follows:
   - Buffer I (Step 2) 50 mL
   - RNase T1 (1200 units/μl) 50 μl
   - RNase A (10 mg/mL) 500 μl
4. Mix well and store on 4°C until use. Use Buffer I with RNase as described on page 4.

*Continued on next page*
Recipes, continued

Buffer II

0.2 M NaOH
1% SDS

1. To prepare 50 mL Buffer II, mix the following:
   - 10% SDS  5 mL
   - 10 N NaOH 1 mL
   - Sterile water to 50 mL

2. Mix well and store at room temperature. Use Buffer II as described on page 4.

Cell Lysis Buffer

50 mM Tris, pH 7.8
150 mM NaCl
1% Nonidet P-40

1. This solution can be prepared from the following common stock solutions. For 100 mL, combine
   - 1 M Tris base 5 mL
   - 5 M NaCl 3 mL
   - Nonidet P-40 1 mL

2. Bring the volume up to 90 mL with deionized water and adjust the pH to 7.8 with HCl.

3. Bring the volume up to 100 mL. Store at room temperature.

To prevent proteolysis, you may add 1 mM PMSF, 1 μM leupeptin, or 0.1 μM aprotinin before use.

4X SDS-PAGE Sample Buffer

1. Combine the following reagents:
   - 0.5 M Tris-HCl, pH 6.8 5 mL
   - Glycerol (100%) 4 mL
   - β- mercaptoethanol 0.8 mL
   - Bromophenol Blue 0.04 g
   - SDS 0.8 g

2. Bring the volume to 10 mL with sterile water.

3. Aliquot and freeze at –20°C until needed.
Additional Protocols

Checking the Library Titer

The library titer is included in the Certificate of Analysis shipped with the library.

If desired, you may check the library titer using the protocol described below.

1. Thaw an aliquot (one tube) of the Custom cDNA Library in pcDNA™3.1 (+).
2. To 100 μl of the library aliquot, add 900 μl SOC medium and mix well. This is the 10⁻¹ dilution.
3. Prepare a series of dilutions with SOC medium as described below:
   For primary library: Prepare 10⁻², 10⁻³, and 10⁻⁴ dilutions of the library with SOC medium.
   For amplified library: Prepare 10⁻⁶, 10⁻⁻⁷, and 10⁻⁻⁸ dilutions of the library with SOC medium.
4. Plate 100 μl from each dilution on LB plates containing 100 μg/mL ampicillin.
5. Incubate the plates at 37°C overnight.
6. Count the number of colonies on each plate and calculate the titer as described.

Calculating the Titer

The library titer is calculated using the formula:

Titer (cfu/mL) = No. of colonies × dilution factor × 10

For example: The number of colonies on a 10⁻³ plate is 100, then the library titer is 100 × 10³ x10 = 1 × 10⁶ cfu/mL.

Continued on next page
Additional Protocols, continued

Verifying the Insert Size

You may verify the insert size by PCR or restriction enzyme analysis.

**PCR**

Perform colony PCR on 12–24 colonies as described on page 6 using primers described in page 13.

**Restriction Enzyme Analysis**

1. Plate the library to isolate individual colonies as described on page 3.
2. Prepare plasmid DNA from 12–24 colonies.
3. Prepare the following mix in each tube:
   
   - DNA sample: 0.5–2 µg
   - 10X REact® 3: 2 µl
   - Not I: 10–50 units
   - EcoR I: 10–50 units
   - Sterile water: to 20 µl
4. Incubate at 37°C for 1–2 hours.
5. Analyze insert size on a 1% agarose gel using appropriate DNA markers.

You can also use the CloneChecker™ System (page 18) to screen recombinant bacterial colonies for the presence of target plasmid DNA. If you are using the CloneChecker™ System, refer to the CloneChecker™ manual for details.
The table below lists additional products that may be used with Custom cDNA Library.

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Catalog no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneTrapper® cDNA Positive Selection System</td>
<td>5 cDNA captures</td>
<td>10356-020</td>
</tr>
<tr>
<td>Terrific Broth</td>
<td>500 g</td>
<td>22711-022</td>
</tr>
<tr>
<td>T7 Promoter Primer</td>
<td>327 pmoles</td>
<td>N560-02</td>
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<tr>
<td>2.5 mM dNTP Mix</td>
<td>1 mL</td>
<td>R725-01</td>
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<tr>
<td>Platinum® Taq DNA Polymerase</td>
<td>100 reactions</td>
<td>10966-018</td>
</tr>
<tr>
<td>Phenol:Chloroform:Isoamyl Alcohol, (25:24:1, v/v/v)</td>
<td>100 mL</td>
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<td>Lipofectamine™ 2000</td>
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<td>Geneticin® Selective Antibiotic</td>
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<tr>
<td>Ampicillin</td>
<td>200 mg</td>
<td>11593-019</td>
</tr>
<tr>
<td>PureLink™ HQ Mini Plasmid DNA Purification Kit</td>
<td>100 reactions</td>
<td>K2100-01</td>
</tr>
<tr>
<td>Not I</td>
<td>200 units</td>
<td>15441-025</td>
</tr>
<tr>
<td>EcoR I</td>
<td>5000 units</td>
<td>15202-013</td>
</tr>
<tr>
<td>REact® 3 Buffer</td>
<td>2 × 1 mL</td>
<td>16303-018</td>
</tr>
<tr>
<td>CloneChecker™ System</td>
<td>100 reactions</td>
<td>11666-013</td>
</tr>
<tr>
<td>Library Amplification Service</td>
<td>--</td>
<td>11499-019</td>
</tr>
<tr>
<td>Phosphate-Buffered Saline, pH 7.4</td>
<td>500 mL</td>
<td>10010-023</td>
</tr>
</tbody>
</table>
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