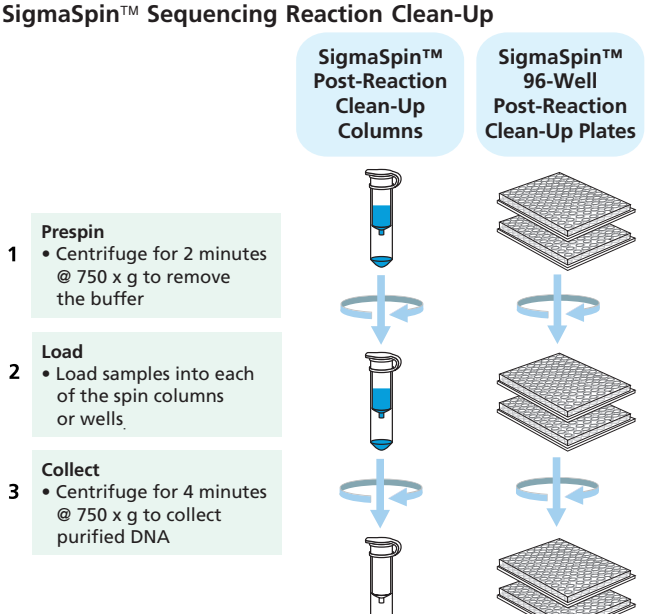
# Sequencing

(Continuation of)

**SigmaSpin™ Sequencing Reaction Clean-Up**




SigmaSpin™ Post-Reaction Clean-Up technology comes in two convenient forms, 96-well plates and single spin columns. Each format comes ready for immediate use.

| | | |
|---|---|---|
| S 5059 | **SigmaSpin™ Post-Reaction Clean-Up Columns** | 50 each<br>70 each |
| S 4309 | **SigmaSpin™ 2 Post-Reaction Clean-Up Plates** | 2 each |
| S 4434 | **SigmaSpin™ 10 Post-Reaction Clean-Up Plates** | 10 each |
| S 4559 | **SigmaSpin™ 50 Post-Reaction Clean-Up Plates** | 25 each<br>2 × 25 each |

# Cloning

## Directional Cloning System

### Director Universal Cloning System

**RDC-1** 1 kit

-20°C

WET ICE

The Director Universal Cloning System provides a simple, rapid and universal method to directionally clone PCR products into a vector cleaved with 5′ overhang-producing restriction endonucleases. Directionality is achieved by pairing directionally designed PCR primers (e.g., containing restriction sites) with any appropriately digested plasmid. The kit contains an optimized nucleotide triphosphate mix, containing dATPαS and dGTPαS, that is used for the PCR step. After PCR, the cohesive 5′ termini of the amplicon are generated by Exonuclease III digestion instead of being generated by traditional restriction enzyme digestions. The dA/GTPαS that was incorporated into the amplicon during PCR protects it from over-digestion by Exonuclease III. The nucleotide mix in the kit is specially formulated so that the amplicon terminates at a statistically determined array of 3′ dA/GαS sites. PCR primers are designed such that the 5′ termini compliment the 5′ overhangs of the predigested plasmid. The simple three step procedure (PCR, Exonuclease III digestion and rapid ligation/transformation) can be completed in one day. The typical cloning efficiency using this method is greater than 80%.

**Features and Benefits**

• Universal - PCR amplicon can be cloned into any expression vector
• High Cloning Efficiency - Typically >80%
• High Expression Efficiency - Typically >66%
• High Fidelity - Long and accurate, hotstart enzyme generates amplicons up to 20 kb with fidelity up to 6.5x greater than standard *Taq* DNA polymerase
• Fast - Simple three-step procedure allows completion in less than one day

sufficient for 25 PCR reactions

**Components:**

10x AccuTaq™ LA DNA Polymerase Buffer, 250 µl
Control PCR Template, 1ng/µl, 10 µl
Control RDC primer-R (with 5′ phosphorylation), 25 µl
Control RDC primer-F (with 5′ phosphorylation), 25 µl
Exo-Deoxynucleotide Mix (20x), 62.5 µl
ExoNuclease III, 100 units/µl, 25 µl
JumpStart™ REDAccuTaq™ LA DNA Polymerase, 1 unit/µl, 62.5 µl
Molecular biology grade water, 500 µl

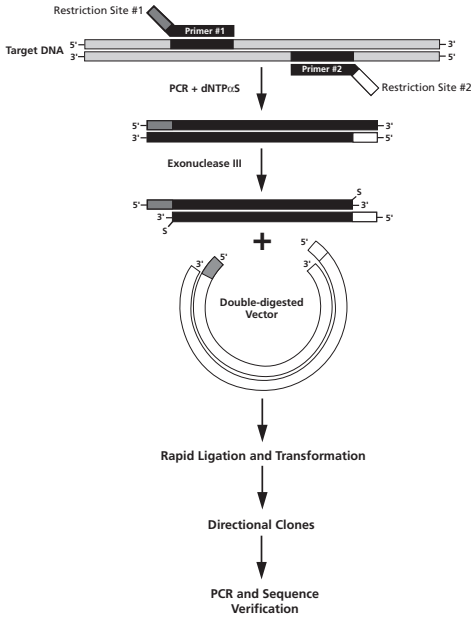


R: 36/37/38 S: 26-36

# Cloning

## Directional Cloning System

Outline of Procedures for Director™ Universal Cloning Using ExoClone™ Technology




### Quick-Link™ DNA Ligation Kit

**LIG-2** 1 kit
–20°C
DRY ICE

Quick-Link has been optimized for efficient blunt and cohesive ligations performed at room temperature with a short incubation, replacing the previous methods requiring 16 °C and long incubations. It comes with pre-made buffers, depending on the buffer conditions of the DNA, for fast and easy set up times.

**Features and Benefits**

- Fast 5 minute ligation.
- Perform at room temperature (does not require any cooling device).
- High ligation efficiency as detected by number of transformed colonies bearing a ligation product.
- Optimized for blunt- and sticky- ends ligation of restriction endonuclease digested inserts as well as PCR products.
- Bacterial transformation can be performed directly with the reaction mixture.
- Suitable for cloning into plasmids as well as phages, addition of linker (adapter), recircularization of linear DNA and concatamers formation.

1 kit sufficient for 50 ligation reactions

**Components:**

T4 DNA ligase,
2× Ligation buffer A,
5× Ligation buffer B,

**References**

1. Lehman, I.R., DNA ligase: structure mechanism and function. *Science* **186**, 790 (1974)
2. Rossi, R., Functional characterization of the T4 DNA ligase: a new insight into the mechanism of action *Nucl. Acids Res.* **25**, 2106 (1997)
3. Hayashi, K., et al., Regulation of inter- and intramolecular ligation with T4 DNA ligase in the presence of polyethylene glycol *Nucl. Acids Res.* **14**, 7617-7631 (1986)

## Reagents for Cloning

### GenElute™ PCR Clean-Up Kit

**NA1020** **sufficient for 70 purifications** 1 kit
RT

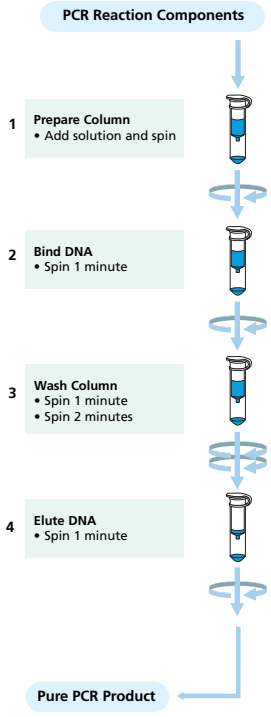
The GenElute PCR Clean-Up Kit is designed for rapid purification of single-stranded or double-stranded PCR amplification products (100 bp to 10 kb) from other components in the reactions, such as excess primers, nucleotides, DNA polymerase, oil and salts (Fig. 1). This kit combines the advantages of silica binding with a convenient spin column format, eliminating the need for expensive resins or toxic organic compounds such as phenol and chloroform.

DNA is bound on a silica membrane within the spin column. The bound DNA is washed and the clean, concentrated DNA is eluted in the buffer of choice. Each column can purify up to 100 µl or 10 µg of PCR amplified DNA and recover up to 95% of PCR products between 100 bp and 10 kb. More than 99% of the primers and most primer-dimers (<40 bp) are removed. Purified DNA can be used in enzymatic reactions, conventional or automated sequencing (Fig. 2), cloning and microarray analysis.

**Features and Benefits**

- Purifies up to 100 µl or 10 µg of PCR amplified DNA in 8 minutes
- Recovers up to 95% of PCR products between 100 bp and 10 kb
- Removes over 99% of primers and other components
- Eliminates the need to remove mineral oil by organic extraction
- 40% more purification preps supplied than market leader

R: 11-22-36/37/38-67 S: 7-16-24/25-26-36

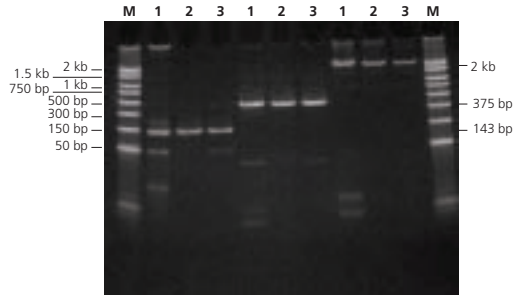


# Cloning

## Reagents for Cloning

(Continuation of)

### GenElute™ PCR Clean-Up Kit




**Comparison of PCR product recovery and primer removal.**

**Figure 1.** Three separate PCR products were purified with the GenElute™ PCR Clean-Up Kit and a comparable kit from Supplier Q. Products were 143 bp from corn leaf, 375 bp from pBR322, 2 kb from human blood. Samples were analyzed on a 20% TBE acrylamide gel and visualized by staining with SYBR® Green II
**Lanes 1:** Unpurified Reaction
**Lanes 2:** GenElute™ PCR Clean-Up Kit
**Lanes 3:** Supplier Q

**Purified PCR products are suitable for Automated Sequencing**

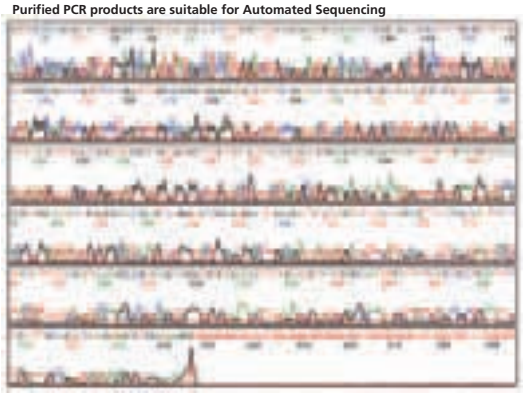


**Figure 2.** Sequence was resolved on an ABI 3100 from a purified, 645 bp corn leaf PCR product. The PCR product was purified with the GenElute™ PCR Clean-Up Kit. The DNA extraction and PCR were performed using Sigma's Extract-N-Amp™ Plant PCR Kit. The sequence was obtained by using ABI BigDye™ terminator chemistry and the same primers as the original PCR.

### BL21 Competent Cell Uni-packs

◆◆ NEW

The *E. coli* BL21 strain is widely known as the strain of choice for expression of target proteins in bacterial systems. It lacks both *lon* and *ompT* proteases, which promote recombinant protein stability. Sigma's Uni-Pack BL21 cells are chemically competent cells at an efficiency of ≥$10^6$ cfu/µg of pUC18 DNA. Included in these kits are 5 mL of SOC and 50 µl of 0.2 µg/ml pUC18 control plasmid. All strains are provided in ready-to-use 50 µl aliquots.

Additionally, strains designated as DE3 carry a copy of the T7 RNA polymerase gene on their chromosome driven by the *lacUV5* promoter. Therefore, when expressing a target gene under a T7 promoter based system, the BL21(DE3) strains offer a source of T7 RNA polymerase with simple IPTG induction.

For researchers who need tighter control over induction, hosts carrying the pLysS or pLysE plasmids are available. Both encode the T7 lysozyme gene, which is a natural inhibitor of T7 RNA polymerase. This enzyme will reduce background levels of polymerase activity in uninduced cells. The pLysS host produces low amounts of the T7 lysozyme while the pLysE containing strain provides more stringent control over transcription with much higher amounts of the enzyme.

**B 8808** **BL21 Competent Cell Uni-packs** 11 reactions
-70°C
DRY ICE
Standard BL21 strain that can be used with any promoter

**B 8683** **BL21 Competent Cell Uni-packs (DE3)** 11 reactions
-70°C
DRY ICE
Standard BL21(DE3) strain that carries a copy of the T7 RNA polymerase gene on their chromosome driven by the *lacUV5* promoter. Therefore, when expressing a target gene under a T7 promoter based system, the BL21(DE3) strains offer a source of T7 RNA polymerase with simple IPTG induction.

**B 8933** **BL21 Competent Cell Uni-packs (DE3)pLysS** 11 reactions
-70°C
DRY ICE
For researchers who need control over induction. Hosts carrying the pLysS plasmids encode the T7 lysozyme gene, which is a natural inhibitor of T7 RNA polymerase. This enzyme will reduce background levels of polymerase activity in uninduced cells. The pLysS host produces low amounts of the T7 lysozyme.
S: 23-24/25

**B 9058** **BL21 Competent Cell Uni-packs (DE3)pLysE** 11 reactions
-70°C
DRY ICE
For researchers who need tighter control over induction. Hosts carrying the pLysE plasmids encode the T7 lysozyme gene, which is a natural inhibitor of T7 RNA polymerase. This enzyme will reduce background levels of polymerase activity in uninduced cells. The pLysE containing strain provides more stringent control over transcription with much higher amounts of the enzyme.

# Cloning

## Culture Media

| Recommended for maintenance and propagation of *E. coli* and plasmid growth | | |
|---|---|---|
| | **Broth** | **Agar** |
| For increased yield of plasmid DNA | T 0918, Terrific Broth (Modified)<br>T 9179, Terrific Broth (Modified) EZMix | n/a |
| High salt concentration | L 3522, Luria Broth | L 3147, Luria Agar |
| Medium salt concentration | L 3022, LB Broth<br>L 7658, LB Broth EZMix | L 2897, LB Agar<br>L 7533, LB Agar EZMix |
| Low salt concentration | L 3397, Luria Broth Base (Miller's Modification) | L 3272, Luria Agar Base (Miller's Modification) |
| **Recommended for maintenance and propagation of *E. coli* and M13 bacteriophage** | | |
| | Y 2377, 2X YT Microbial Medium<br>Y 2627, 2X YT Microbial Medium EZMix | |
| **Recommended for maintenance and propagation of recombinant lambda phage** | | |
| | N 3518, NZM Broth<br>N 3643, NZCYM Broth<br>N 6905, NZCYM Broth EZMix | |
| **Recommended for propagation of competent *E. coli* and maximizing transformation efficiency** | | |
| | S 1797, SOC Medium<br>H 8032, Hanahan's Broth (SOB Medium) | |
| **Recommended for general bacteriological use** | | |
| | Y 1625, Yeast Extract<br>Y 1626, Yeast Extract EZMix | |
| **Recommended for propagation of yeast** | | |
| | **Broth** | **Agar** |
| | Y 1375, YPD Broth | Y 1500, YPD Agar |
| **Incomplete media (Addition of a carbon source required)** | | |
| Contains amino acids | Y 1250, Yeast Nitrogen Base | |
| Without amino acids | Y 0626, Yeast Nitrogen Base | |
| Without amino acids and ammonium sulfate | Y 1251, Yeast Nitrogen Base | |
| **Supplements for Yeast Nitrogen Base (Y 0626) lacking specific amino acids** | | |
| Without histidine | Y 1751, Yeast Synthetic Drop-Out Medium Supplement | |
| Without leucine | Y 1376, Yeast Synthetic Drop-Out Medium Supplement | |
| Without tryptophan | Y 1876, Yeast Synthetic Drop-Out Medium Supplement | |
| Without leucine and tryptophan | Y 0750, Yeast Synthetic Drop-Out Medium Supplement | |
| Without uracil | Y 1501, Yeast Synthetic Drop-Out Medium Supplement | |
| Without histidine, tryptophan and uracil | Y 2001, Yeast Synthetic Drop-Out Medium Supplement | |

### LB Agar

**L 2897** **powder** 250 g
RT 35 g per liter 1 kg

**Preparation instructions**

1. Suspend 35 g in 1 L of distilled water.
2. Heat to boiling while stirring to dissolve all ingredients completely.
3. Autoclave for 15 minutes at 121 °C.

To prepare Lennox L Agar: Add 1 g glucose and proceed with preparation instructions as above.

To prepare the medium of Enquist and Sternberg: Aseptically add 10 ml sterile 1 M magnesium sulfate after autoclaving.

**Components:**

Tryptone (pancreatic digest of casein), 10 g/L
Yeast extract, 5 g/L
NaCl, 5 g/L
Agar, 15 g/L

# Cloning

## Culture Media

### LB Agar EZMix™ Powder

**L 7533** RT — The EZMix powders provide the advantage of being granulated and dust-free. Therefore, because there is no dust hazard, safer and more accurate measurements can be taken. In addition, the EZmix powders dissolve faster and more completely than standard media. For more routine formulations, packet sizes have been pre-measured for added convenience.
35.6 g per liter
Available in preweighed 500 ml packages or large quantity bottles.
**Preparation instructions**
1. Suspend 35.6 g in 1 L of distilled water.
2. Heat to boiling while stirring to dissolve all ingredients completely.
3. Autoclave for 15 minutes at 121 °C.
To prepare Lennox L Agar: Add 1 g glucose and proceed with preparation instructions as above.
To prepare the medium of Enquist and Sternberg: Aseptically add 10 ml of sterile 1 M magnesium sulfate after autoclaving. The growth characteristics are the same as LB agar powder.
**Components:**
Tryptone (pancreatic digest of casein), 10 g/L
Yeast extract, 5 g/L
NaCl, 5 g/L
Agar, 15 g/L
Inert binder (EZMix only), 0.6 g/L

6 × 500 mL
1 kg

### LB Agar

**L 7025** RT — **tablet**
For convenient preparation of small quantities of medium without weighing.
1.68 g per tablet
Dissolve the tablet in 50 ml of water. The finished medium will contain 10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl, 15 g/L agar and 1.6 g/L inert tableting aids.
LB agar tablets have same high quality formulation as LB agar powder with the added advantage of being in tablet form, eliminating the need for weighing and handling. The growth characteristics are the same as LB agar powder.
contains 1.6 g/L inert tableting aids

100 tablets
500 tablets

### LB Broth

**L 3022** RT — **powder**
20 g per liter
**Preparation instructions**
1. Suspend 20 g in 1 L of distilled water.
2. Autoclave for 15 minutes at 121 °C.
To prepare Lennox L Broth: Add 1 g glucose and proceed with preparation instructions as above.
To prepare the medium of Enquist and Sternberg: Aseptically add 10 ml of sterile 1 M magnesium sulfate after autoclaving.
**Components:**
Tryptone (pancreatic digest of casein), 10 g/L
Yeast extract, 5 g/L
NaCl, 5 g/L

250 g
1 kg
6 × 1 kg

### LB Broth EZMix™ Powder

**L 7658** RT — The EZMix powders provide the advantage of being granulated and dust-free. Therefore, because there is no dust hazard, safer and more accurate measurements can be taken. In addition, the EZmix powders dissolve faster and more completely than standard media. For more routine formulations, packet sizes have been pre-measured for added convenience.
20.6 g per liter
Convenient package sizes of 500 ml and 5 liters.
**Preparation instructions**
1. Suspend 20.6 g in 1 L of distilled water.
2. Autoclave for 15 minutes at 121 °C.
To prepare Lennox L Broth: Add 1 g glucose and proceed with preparation instructions as above.
To prepare the medium of Enquist and Sternberg: Aseptically add 10 ml of sterile 1M magnesium sulfate after autoclaving. The growth characteristics are the same as LB broth.
**Components:**
Enzymatic casein digest, 10 g/L
Yeast extract, 5 g/L
NaCl, 5 g/L
Inert binder (EZMix only), 0.6 g/L

6 × 500 mL
2 × 5 L
1 kg

### LB Broth

**L 7275** RT — **tablet**
For convenient preparation of small quantities of medium without weighing.
1.1 g per tablet
Dissolve the tablet in 50 ml of water. The finished medium will contain 10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl, and 2 g/L inert tableting aids.
LB broth tablets have same high quality formulation as LB Broth (Lennox L broth) with the added advantage of being in tablet form, eliminating the need for weighing and handling. The growth characteristics are the same as LB broth.

100 tablets
500 tablets

### Luria Agar

**L 3147** RT — (Miller's LB agar)
For maintenance and propagation of *Escherichia coli*.
40 g per liter
**Preparation instructions**
1. Suspend 40 g in 1L of distilled water.
2. Heat to boiling while stirring to dissolve.
3. Autoclave for 15 minutes at 121 °C.
4. Cool to 50°C prior to dispensing into sterile petri dishes.
To prepare the medium of Luria and Burrows: Add 1 g glucose to medium and proceed with preparation instructions above.
To prepare the medium of Luria, Adams and Ting (also known as LC agar): Aseptically add 25 ml of sterile 0.1 M calcium chloride after autoclaving.
**Components:**
Tryptone (pancreatic digest of casein), 10 g/L
Yeast extract, 5 g/L
NaCl, 10 g/L
Agar, 15 g/L
**References**
1. Luria, S.E., and Burrous, J.W., Hybridization between *Escherichia coli* and *Shigella*. *J. Bacteriol.* **74**, 461-476 (1955)
2. Luria, S.E., et al., Transduction of lactose-utilizing ability among strains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. *Virology* **12**, 348-390 (1960)
3. Miller, J.H., *Experiments in Molecular Genetics* , Cold Spring Harbor, NY (1972), 433
4. *Difco Manual* 11th ed., Sparks, MD (1998), 239

250 g
1 kg

# Cloning

## Culture Media

### Luria Agar Base (Miller's Modification)

**L 3272** RT — For maintenance and propagation of *Escherichia coli*. 30.5 g per liter — 250 g, 1 kg

**Preparation instructions**
1. Suspend 30.5 g in 1 L of distilled water.
2. Heat to boiling while stirring to dissolve.
3. Autoclave for 15 minutes at 121 °C.
4. Cool to 50°C prior to dispensing into petri dishes.

**Components:**
Tryptone (pancreatic digest of casein), 10 g/L
Yeast extract, 5 g/L
NaCl, 0.5 g/L
Agar, 15 g/L

**References**
1. Miller, J.H., *Experiments in Molecular Genetics* , Cold Spring Harbor, NY (1972), 433
2. *Difco Manual* 11th ed., Sparks, MD (1998), 271

### Luria Broth

**L 3522** RT — (Miller's LB broth) For maintenance and propagation of *Escherichia coli*. 25 g per liter — 250 g, 1 kg

**Preparation instructions**
1. Suspend 25 g in 1 L of distilled water.
2. Autoclave for 15 minutes at 121 °C.

To prepare the medium of Luria, Adams and Ting (also known as LC broth): Aseptically add 25 ml of sterile 0.1 M calcium chloride after autoclaving.

**Components:**
Tryptone (pancreatic digest of casein), 10 g/L
Yeast extract, 5 g/L
NaCl, 10 g/L

**References**
1. Luria, S.E., and Burrous, J.W., Hybridization between *Escherichia coli* and *Shigella*. *J. Bacteriol.* **74**, 461-476 (1955)
2. Luria, S.E., et al., Transduction of lactose-utilizing ability among strains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. *Virology* **12**, 348-390 (1960)
3. Miller, J.H., *Experiments in Molecular Genetics* , Cold Spring Harbor, NY (1972), 433
4. *Difco Manual* 11th ed., Sparks, MD (1998), 241

### Luria Broth (Miller's Modification)

**L 3397** RT — For maintenance and propagation of *Escherichia coli*. 15.5 g per liter — 250 g, 1 kg

**Preparation instructions**
1. Suspend 15.5 g in 1 L of distilled water.
2. Autoclave for 15 minutes at 121°C.

**Components:**
Tryptone (pancreatic digest of casein), 10 g/L
Yeast extract, 5 g/L
NaCl, 0.5 g/L

**References**
1. Miller, J.H., *Experiments in Molecular Genetics* , Cold Spring Harbor, NY (1972), 433
2. *Difco Manual* 11th ed., Sparks, MD (1998), 272

### SOC Medium

**S 1797** 2-8°C — Used primarily for growing competent *Escherichia coli* and for maximizing transformation efficiency. 0.2 µm filtered — 10 × 5 mL, 100 mL

**Components:**
Tryptone (pancreatic digest of casein), 2% (w/v)
Yeast extract, 0.5% (w/v)
NaCl, 8.6 mM
KCl, 2.5 mM
$MgSO_4$, 20 mM
Glucose, 20 mM

**References**
Sambrook, J., et al., *Molecular Cloning: A Laboratory Manual* 2nd ed., Plainview, NY (1989), 1.76-1.81 & A.2

### Terrific Broth, modified

**T 0918** RT — **powder** 47.6 g per liter — 250 g, 1 kg

**Preparation instructions**
1. Suspend 47.6 g and 8 ml glycerol in 1 L of distilled water.
2. Autoclave for 15 minutes at 121 °C.

**Components:**
Tryptone (pancreatic digest of casein), 12 g/L
Yeast extract, 24 g/L
$K_2HPO_4$, 9.4 g/L
$KH_2PO_4$, 2.2 g/L

### Terrific Broth, Modified EZMix™ Powder

**T 9179** RT — The EZMix powders provide the advantage of being granulated and dust-free. Therefore, because there is no dust hazard, safer and more accurate measurements can be taken. In addition, the EZmix powders dissolve faster and more completely than standard media. For more routine formulations, packet sizes have been pre-measured for added convenience. Recommended concentration: 48.2g per liter Package sizes of 500 ml and 5 liters. — 6 × 500 mL, 2 × 5 L, 1 kg

**Preparation Instructions**
1. Suspend 48.2 g and 8 ml glycerol in 1 L of distilled water.
2. Autoclave for 15 minutes at 121 °C.

The growth characteristics are the same as Terrific broth.

**Components:**
Tryptone (pancreatic digest of casein), 12 g/L
Yeast extract, 24 g/L
$K_2HPO_4$, 9.4 g/L
$KH_2PO_4$, 2.2 g/L
Inert binder (EZMix only), 0.6 g/L

# Gene Expression Analysis

### Arrayer Calibration Solution

**C 2110** 2-8°C — Ready-to-use solution for calibration of arrayers prior to printing microarrays. Useful in determining that each pin of the arrayer is printing spots of equal size and uniform morphology. For use with split-pin and pin-ring arrayers. — 10 mL

DNase, RNase. . . . . free