



Protein Expression Competent Cell Pack

Instruction Manual

Catalog #230246

Revision B

Research Use Only. Not for Use in Diagnostic Procedures.

230246-12



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Protein Expression Competent Cell Pack

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Protein Expression Competent Cell Pack

MATERIALS PROVIDED

Materials provided	Tube color	Quantity	Efficiency (cfu/ μ g of pUC18 DNA) ^a
BL21(DE3) competent cells ^b	Green	1 $\times$ 0.2 ml	$\geq 1 \times 10^6$
BL21-Gold(DE3) competent cells ^c	Red-orange	2 $\times$ 0.1 ml	$\geq 1 \times 10^8$
BL21-CodonPlus (DE3)-RIPL competent cells ^d	Purple	2 $\times$ 0.1 ml	$\geq 1 \times 10^6$
BL21-Gold(DE3)pLysS competent cells ^e	Clear	2 $\times$ 0.1 ml	$\geq 1 \times 10^8$
pUC18 control plasmid (0.1 ng/ μ l in TE buffer ^f)	—	4 $\times$ 10 μ l	—
β -Mercaptoethanol (1.42 M)	—	25 μ l	—
XL10-Gold β -mercaptoethanol mix	—	50 μ l	—

^a These competent cell efficiencies are guaranteed when cells are used according to the specifications outlined in this instruction manual.

^b Larger quantities of this competent cell strain are available: catalog #200131 (5 $\times$ 0.2 ml).

^c Larger quantities of this competent cell strain are available: catalog #230132 (10 $\times$ 0.1 ml).

^d Larger quantities of this competent cell strain are available: catalog #230280 (10 $\times$ 0.1 ml).

^e Larger quantities of this competent cell strain are available: catalog #230134 (10 $\times$ 0.1 ml).

^f See *Preparation of Media and Reagents*.

STORAGE CONDITIONS

Store cells immediately at -80°C . Do not place the cells in liquid nitrogen.

pUC18 Control Plasmid: -80°C

β -Mercaptoethanol Mixes: -80°C

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INTRODUCTION

The Protein Expression Competent Cell Pack is designed to allow you to empirically determine which strain is best suited to express your protein without the cost associated with purchasing large quantities of any one strain. Our BL21(DE3) competent cells are all-purpose strains for high-level protein expression and easy induction in T7 expression systems.¹ The cell pack includes four BL21(DE3) strains possessing different genotypic features to resolve potential protein expression obstacles such as expressing toxic or heterologous proteins in *Escherichia coli*.

Host Strains and Genotypes

Host strain ^a	Genotype	Antibiotic Resistance
BL21 (DE3) strain	<i>E. coli</i> B F ⁻ <i>dcm ompT hsdS</i> (r _B ⁻ m _B ⁻) <i>gal λ</i> (DE3)	—
BL21-Gold(DE3) strain	<i>E. coli</i> B F ⁻ <i>ompT hsdS</i> (r _B ⁻ m _B ⁻) <i>dcm</i> ⁺ Tet ^r <i>gal λ</i> (DE3) <i>endA Hte</i>	Tet ^r
BL21-CodonPlus (DE3)-RIPL strain	<i>E. coli</i> B F ⁻ <i>ompT hsdS</i> (r _B ⁻ m _B ⁻) <i>dcm</i> ⁺ Tet ^r <i>gal λ</i> (DE3) <i>endA Hte</i> [<i>argU proL Cam</i> ^r] [<i>argU ileY leuW Strep/Spec</i> ^r]	Tet ^r Cam ^r Strep/Spec ^r
BL21-Gold(DE3)pLysS strain ^b	<i>E. coli</i> B F ⁻ <i>ompT hsdS</i> (r _B ⁻ m _B ⁻) <i>dcm</i> ⁺ Tet ^r <i>gal λ</i> (DE3) <i>endA Hte</i> [pLysS Cam ^r]	Tet ^r Cam ^r

^a These strains, derivatives of *E. coli* B, are general protein expression strains that lack both the Lon protease and the OmpT protease, which can degrade proteins during purification.² The Dcm methylase, naturally lacking in *E. coli* B, is inserted into the genomes.

Competent Cell Strain Features

The BL21(DE3) competent cells and variants are all-purpose strains for high-level protein expression and easy induction. These expression strains are ideal for performing protein expression studies that utilize the T7 RNA polymerase promoter to direct high-level expression (e.g., expression from Affinity pCAL vectors and pET vectors). Derived from *E. coli* B, these expression strains naturally lack the Lon protease, which can degrade recombinant proteins. In addition, these strains are engineered to be deficient for a second protease, the OmpT protein.

The BL21-Gold(DE3) competent cells* and BL21-Gold(DE3)pLysS competent cells* are improved versions of Agilent's BL21(DE3) competent cells. These BL21-Gold-derived expression strains incorporate major improvements over the original BL21 strain.³ The BL21-Gold strains feature the Hte phenotype present in Agilent's highest efficiency competent cell strain, XL10-Gold.⁴ The presence of the Hte phenotype increases the transformation efficiency of the BL21-Gold cells to $\geq 1 \times 10^8$ cfu/ μ g of pUC18 DNA. In addition, the gene that encodes endonuclease I (*endA*), which rapidly degrades plasmid DNA isolated by most miniprep procedures, is inactivated. These two improvements, increased transformation efficiency and production of high-quality miniprep DNA, allow direct cloning of many protein expression constructs.

* U.S. Patent No. 6,706,525.

Additionally, the BL21-Gold(DE3)pLysS competent cells provide tighter control of protein expression for expression of toxic proteins. To reduce basal activity of T7 RNA polymerase in the uninduced state, the strain carries a low-copy-number pACYC-based plasmid that carries an expression cassette from which the T7 lysozyme gene is expressed at low levels. T7 lysozyme binds to T7 RNA polymerase and inhibits transcription by this enzyme. On IPTG induction, overproduction of the T7 RNA polymerase renders low-level inhibition by T7 lysozyme virtually ineffective.

The BL21-CodonPlus (DE3)-RIPL competent cells* are derived from Agilent's high-performance BL21-Gold competent cells. These cells enable efficient high-level expression of heterologous proteins in *E. coli*, which is frequently limited by the rarity of certain tRNAs that are abundant in the organisms from which the heterologous proteins are derived.^{II} Forced high-level expression of heterologous proteins can deplete the pool of rare tRNAs and stall translation. The BL21-CodonPlus (DE3)-RIPL strain is engineered to contain extra copies of genes that encode the tRNAs that most frequently limit translation of heterologous proteins in *E. coli*. Availability of tRNAs allows high-level expression of many heterologous recombinant genes that are poorly expressed in conventional BL21 strains.

The BL21-CodonPlus (DE3)-RIPL cells contain extra copies of the *argU*, *ileY*, *leuW*, and *proL* tRNA genes. This strain rescues expression of heterologous proteins from organisms that have either AT- or GC-rich genomes. BL21-CodonPlus(DE3)-RIPL cells specifically contain a ColE1-compatible, pACYC-based plasmid containing extra copies of the *argU* and *proL* tRNA genes and a ColE1- and pACYC-compatible pSC101-based plasmid containing extra copies of the *argU*, *ileY*, and *leuW* tRNA genes.

* U.S. Patent No. 6,706,525.

^{II} A complete compilation of codon usage of the sequences in the GenBank® database is available at <http://www.kazusa.or.jp/codon/>.

Considerations

Protein Toxicity

Proteins expressed from the T7 promoter can often be toxic to *E. coli* host cells. If using a BL21(DE3) strain as the primary host strain for expression, some caution should be exercised because even low-level expression can result in accumulation of a toxic gene product. If the gene to be expressed is suspected of being toxic to *E. coli*, we recommend transforming non-DE3 cells with the gene of interest and then inducing expression with CE6 bacteriophage. This procedure provides the tightest control of expression for genes under the control of the T7 promoter. When using IPTG induction, uninduced expression levels are reduced when using the BL21-Gold (DE3)pLysS host strain, compared to the BL21-Gold (DE3) host strain.

Composition of Miniprep DNA Prepared from Transformed BL21-CodonPlus (DE3)-RIPL Cells

Miniprep DNA obtained following transformation of the BL21-CodonPlus (DE3)-RIPL competent cells with the expression plasmid of choice will be a mixture containing both the expression plasmid and the tRNA-encoding plasmids. The BL21-CodonPlus(DE3)-RIPL strain contains a ColE1-compatible pACYC-based plasmid that confers chloramphenicol resistance. This plasmid is ~3.5 kb and will be observed when analyzing miniprep results by gel electrophoresis. The strain also contains a ColE1- and pACYC-compatible pSC101-based plasmid that confers streptomycin resistance. Although the 4.7 kb pSC101-based plasmid is maintained at relatively low copy number, the plasmid may be observed when analyzing miniprep DNA by gel electrophoresis.

Confirming the Presence of the tRNA-Encoding Plasmids in BL21-CodonPlus (DE3)-RIPL Cells Before Induction

The presence of the pACYC-based plasmid can be confirmed by verifying the chloramphenicol-resistance phenotype using LB-agar medium supplemented with 34 µg/ml chloramphenicol. Before inducing expression of the protein of interest, we recommend growing the overnight culture in the presence of the expression plasmid-specific antibiotic and 50 µg/ml chloramphenicol to assure the presence of the pACYC-based plasmids at the time of induction. When analyzing a cell extract by gel electrophoresis, chloramphenicol acetyl transferase, the protein that provides chloramphenicol resistance, will be observed at ~25,660 Da.

For the BL21-CodonPlus(DE3)-RIPL strain, the presence of the pSC101-based plasmid can be confirmed by the streptomycin- or spectinomycin-resistance phenotype. If phenotypic verification is desired, we recommend using LB-agar medium supplemented with 75 µg/ml streptomycin. It is not, however, necessary to add streptomycin to the growth medium prior to induction in order to maintain the pSC101-based plasmid. The plasmid contains a functional partitioning locus that ensures proper plasmid segregation, preventing plasmid loss even in the absence of a selective agent. When analyzing cell extracts by gel electrophoresis followed by Coomassie® staining, the 37.5-kD protein that confers streptomycin resistance is not typically detected.

TRANSFORMATION GUIDELINES

Important *For optimal transformation efficiency, please read the guidelines outlined in the following sections before proceeding with the Transformation Protocol.*

Storage Conditions

The competent cells are very sensitive to slight variations in temperature. Storing the competent cells at the bottom of a -80°C freezer directly from the dry ice shipping container is required in order to prevent a loss of transformation efficiency. Transferring tubes from one freezer to another may also result in a loss of efficiency. The transformation efficiency of the competent cells is guaranteed when the competent cells are used according to the specifications outlined in this instruction manual.

Use of 14-ml BD Falcon Polypropylene Round-Bottom Tubes

It is important to use 14-ml BD Falcon polypropylene round-bottom tubes (BD Biosciences Catalog #352059) for the *Transformation Protocol* because the critical incubation period during the heat pulse is optimized specifically for the thickness and shape of these tubes.

Aliquoting Cells

Store the competent cells on ice at all times while aliquoting. It is essential to place the 14-ml polypropylene tubes on ice before the competent cells are thawed and to aliquot the competent cells directly into the prechilled 14-ml polypropylene tubes. It is also important to use 100 μl of competent cells/transformation. Using an inadequate volume of competent cells results in lower transformation efficiencies.

Quantity of DNA Added

Greatest efficiencies (i.e., transformants/microgram of DNA) are observed when adding 1 μl of DNA at a concentration of 0.1 ng/ μl per 100 μl of competent cells. Although the overall transformation efficiency may be lower, a greater number of colonies will be obtained when transforming up to 50 ng.

Length of the Heat Pulse

For BL21(DE3) competent cells: Optimal transformation efficiencies are observed when transformation reactions are heat-pulsed for 45–50 seconds. Transformation efficiencies decrease sharply when heat-pulsed for <45 seconds or for >60 seconds.

For BL21-Gold(DE3), BL21-Gold(DE3)pLysS, and BL21-CodonPlus (DE3)-RIPL competent cells: Optimal transformation efficiencies are observed when transformation reactions are heat-pulsed for 20–25 seconds. Transformation efficiencies decrease sharply when the duration of the heat pulse is <20 seconds or >25 seconds.

Use of β -Mercaptoethanol

β -Mercaptoethanol has been shown to increase transformation efficiency. For optimal efficiency, use the recommended amount of β -mercaptoethanol provided in this kit. (Using an alternative source of β -ME may reduce transformation efficiency.)

TRANSFORMATION PROTOCOL

1. Thaw the competent cells on ice. Preheat SOC medium (see *Preparation of Media and Reagents*) in a 42°C water bath for use in step 8.

Note Store the competent cells **on ice at all times** while aliquoting. It is essential that the 14-ml BD Falcon polypropylene round-bottom tubes are placed on ice before the competent cells are thawed and that 100 μ l of competent cells are aliquoted directly into each **prechilled** polypropylene tube. If applicable, pipet the remaining competent cells into 100- μ l aliquots and freeze the aliquots at -80°C . Do not pass the frozen competent cells through more than one freeze-thaw cycle.

2. Gently mix the competent cells. Aliquot 100 μ l of the competent cells into the appropriate number of prechilled 14-ml BD Falcon polypropylene round-bottom tubes. Prepare an additional 100- μ l aliquot of cells for use as a transformation control.
3. When transforming **BL21(DE3) competent cells**, add 1.7 μ l of the β -mercaptoethanol (β -ME; 1.42 M) provided with this kit or a fresh 1:10 dilution of a 14.2 M stock solution of β -ME (diluted in distilled water) to each polypropylene tube containing the competent cells, for a final concentration of 25 mM β -ME. Swirl the contents of the tubes gently. Incubate the cells on ice for 10 minutes, swirling gently every 2 minutes.

When transforming **BL21-CodonPlus (DE3)-RIPL competent cells**, add 2.0 μ l of the 1:10 dilution of the XL10-Gold β -mercaptoethanol mix to each polypropylene tube containing the competent cells. Swirl the contents of the tubes gently. Incubate the cells on ice for 10 minutes, swirling gently every 2 minutes.

Note The *BL21-Gold(DE3)* and *BL21-Gold(DE3)pLysS* competent cells do not require the addition of β -mercaptoethanol.

4. Add 1–50 ng of expression plasmid DNA to each transformation reaction and swirl gently. For the control transformation reaction, add 1 μ l of the pUC18 control plasmid to a separate 100- μ l aliquot of the competent cells and swirl gently.
5. Incubate the reactions on ice for 30 minutes.

6. **For BL21(DE3) competent cells:** Heat-pulse each transformation reaction in a 42°C water bath for 45 seconds

For BL21-Gold(DE3), BL21-Gold(DE3)pLysS, and BL21-CodonPlus (DE3)-RIPL competent cells: Heat-pulse each transformation reaction in a 42°C water bath for 20 seconds.

The duration of the heat pulse is critical for optimal transformation efficiencies.

7. Incubate the reactions on ice for 2 minutes.
8. Add 0.9 ml of preheated (42°C) SOC medium to each transformation reaction and incubate the reactions at 37°C for 1 hour with shaking at 225–250 rpm.
9. Using a sterile spreader, spread ≤ 200 μ l of the cells transformed with the experimental DNA onto LB agar[§] plates that contain the appropriate antibiotic.

For the pUC18 control plasmid, plate the volume of the control transformation reaction listed in Table IV, according to the BL21-CodonPlus strain used, on an LB–ampicillin agar plate.[§]

Notes *The cells may be concentrated by centrifuging at 200 $\times$ g for 3–5 minutes at 4°C if desired. Resuspend the pellet in 200 μ l of SOC broth.*

If plating ≤ 100 μ l of the transformation reaction, first place a 100- μ l pool of SOC medium onto the plate. Pipet the cells from the transformation reaction into the pool of SOC and then spread the mixture. If plating >100 μ l, spread the cell suspension onto the plates directly.

When spreading the cells, tilt and tap the spreader to remove the last drop of cells.

10. Transformants will appear as colonies following overnight incubation at 37°C. See the table below for the results expected for the pUC18 control transformation.

Transformation Summary for the pUC18 Control Plasmid

Host strain	Plating quantity (μ l)	Expected colony number (cfu)	Efficiency (cfu/ μ g of pUC18 DNA)
BL21(DE3) competent cells	200	>20	$\geq 1 \times 10^6$
BL21-Gold(DE3) competent cells	5	>50	$\geq 1 \times 10^8$
BL21-CodonPlus (DE3)-RIPL competent cells	200	>20	$\geq 1 \times 10^6$
BL21-Gold(DE3)pLysS competent cells	5	>50	$\geq 1 \times 10^8$

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

INDUCTION OF TARGET PROTEIN USING IPTG

The following induction protocol is a general guide for expression of genes under the control of IPTG-inducible promoters on an analytical scale (1 ml of induced culture). Most commonly, this protocol is used to analyze protein expression of individual transformants when using BL21(DE3) host strains in combination with plasmids containing T7 promoter constructs (e.g. pET vectors). Expression cassettes under the control of the *trp/lac* hybrid promoter, *tac*, can be also induced using this protocol. In the case of *tac* promoter constructs, non-DE3 lysogen strains can be employed as hosts.

Note *The transformation procedure described above will produce varying numbers of colonies depending on the transformation efficiency obtained for the expression plasmid. It is prudent to test more than one colony as colony-to-colony variations in protein expression are possible.*

1. Inoculate 1-ml aliquots of LB broth (see *Preparation of Media and Reagents*) containing the antibiotic required to maintain the expression plasmid with single colonies from the transformation. Shake at 220–250 rpm at 37°C overnight.

Note *For the BL21-CodonPlus (DE3)-RIPL and BL21-Gold(DE3)pLysS host strains, the overnight culture must contain chloramphenicol at a final concentration of 50 µg/ml to maintain the pACYC-based plasmid, in addition to the antibiotic required to maintain the expression plasmid. (If using the BL21-CodonPlus(DE3)-RIPL strain, the additional pSC101-based plasmid is maintained without antibiotic selection. It is **not** necessary to add streptomycin to growth medium.)*

2. The next morning, pipet 50 µl of each culture into fresh 1-ml aliquots of LB broth containing no selection antibiotics. Incubate these cultures with shaking at 220–250 rpm at 37°C for 2 hours.
3. Pipet 100 µl of each of the cultures into clean microcentrifuge tubes and place the tubes on ice until needed for gel analysis. These will serve as the non-induced control samples.
4. To the rest of the culture in each tube add IPTG to a final concentration of 1 mM. Incubate with shaking at 220–250 rpm at 37°C for 2 hours.

Note *These values for IPTG concentration and induction time are starting values only and may require optimization depending on the gene expressed.*

5. After the induction period, place the cultures on ice.

6. Pipet 20 μ l of each of the induced cultures into clean microcentrifuge tubes. Add 20 μ l of 2 $\times$ SDS gel sample buffer (see *Preparation of Media and Reagents*) to each microcentrifuge tube.
7. Mix the non-induced samples held on ice to resuspend the cells. Pipet 20 μ l from each tube into a clean microcentrifuge tube. Add 20 μ l of 2 $\times$ SDS gel sample buffer to each of the 20- μ l aliquots of cells.
8. Heat all tubes to 95°C for 5 minutes. Load the associated non-induced and induced samples in adjacent lanes for analysis by SDS-PAGE. Stain the protein gel with Coomassie Brilliant Blue stain.

Note *When analyzing a cell extract by gel electrophoresis, chloramphenicol acetyl transferase, the protein that provides chloramphenicol resistance, will be observed at ~25,660 Da.*

TROUBLESHOOTING

Observation	Suggestion
Plasmid instability	Inserts containing repeated sequence(s) or secondary structure may undergo rearrangement if established in the BL21-Gold series of competent cells as they are <i>recA</i> ⁺ . To minimize this possibility, establish the insert in a recombination-deficient host strain, such as Agilent's SURE competent cells (<i>recB</i> ⁻ and <i>recJ</i> ⁻) or XL1-Blue competent cells (<i>recA</i> ⁻), prior to expression in BL21-Gold cells.
Problems associated with induction time	The optimal induction time depends on the physicochemical characteristics of the protein and toxicity of the protein to <i>E. coli</i> . In certain cases, accumulation of target protein may kill cells at saturation while allowing normal growth in logarithmically growing cultures, while in other cases target protein may continue to accumulate in cells well beyond the recommended 3-hour induction period. To determine the optimal induction period, a time course may be carried out during which a small portion of the culture is analyzed by SDS-PAGE at various times following induction.
Inclusion bodies	If the protein is insoluble, it may be necessary to alter one or more of the growth conditions (e.g., induce expression at 30°C, decrease the IPTG concentration, alter the induction time, alter the timing of induction, and/or increase aeration).
Clone toxicity	More tightly controlled induction may be achieved by infecting BL21-Gold cells with bacteriophage CE6 than by IPTG-induction of BL21-Gold (DE3) cells. When using IPTG induction, uninduced expression levels are reduced when using the BL21-Gold (DE3)pLysS host strain, compared to the BL21-Gold (DE3) host strain.
Proteolytic degradation	If protein degradation occurs, which results in bands of reduced molecular weight, inclusion of various commercially available protease inhibitors in the cell lysis buffer can be highly effective in reducing this degradation.
Gel analysis of miniprep restriction digestion results in multiple bands	The expression plasmid(s) harbored in the BL21-CodonPlus (DE3)-RIPL and BL21-Gold(DE3)pLysS strains may be detected in miniprep DNA. A pACYC-based plasmid (3.5 kb) is present in the BL21-CodonPlus (DE3)-RIPL and BL21-Gold(DE3)pLysS strains. An additional 4.7 kb pSC101-based plasmid is present in the BL21-CodonPlus(DE3)-RIPL strain. Insert cloning and verification should be performed in a general cloning strain (e. g., XL1-Blue competent cells) prior to expression in BL21 strains harboring the expression plasmid(s).
Gel analysis of cell extract from BL21-CodonPlus (DE3)-RIPL and BL21-Gold(DE3)pLysS host strains result in an extra protein band at ~25,660 Da	The chloramphenicol acetyl transferase expressed from the pACYC-based plasmids present in the BL21-CodonPlus (DE3)-RIPL and BL21-Gold(DE3)pLysS host strains is typically detected in protein gel analysis of cell extracts.

PREPARATION OF MEDIA AND REAGENTS

LB Broth (per Liter) 10 g of NaCl 10 g of tryptone 5 g of yeast extract Add dH₂O 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 Agar (per Liter) 10 g of NaCl 10 g of tryptone 5 g of yeast extract 20 g of agar Add deionized H₂O to a final volume of 1 liter Adjust pH to 7.0 with 5 N NaOH Autoclave Cool to 55°C, and then add antibiotic, if required Pour into petri dishes (~25 ml/100-mm plate)
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)	SOC Medium (per 100 ml) Note <i>This medium should be prepared immediately before use</i> 1 ml of a 2 M filter-sterilized glucose solution or 2 ml of 20% (w/v) glucose SOB medium to a final volume of 100 ml Filter sterilize
SOB Medium (per Liter) 20.0 g of tryptone 5.0 g of yeast extract 0.5 g of NaCl Add deionized H₂O to a final volume of 1 liter Autoclave Add 10 ml of filter-sterilized 1 M MgCl₂ and 10 ml of filter-sterilized 1 M MgSO₄ prior to use	2× SDS gel sample buffer 100 mM Tris-HCl (pH 6.5) 4% SDS (electrophoresis grade) 0.2% bromophenol blue 20% glycerol Note <i>Add dithiothreitol to a final concentration in the 2× buffer of 200 mM prior to use. This sample buffer is useful for denaturing, discontinuous acrylamide gel systems only.</i>
TE Buffer 10 mM Tris-HCl (pH 7.5) 1 mM EDTA	

REFERENCES

1. Weiner, M. P., Anderson, C., Jerpseth, B., Wells, S., Johnson-Browne, B. *et al.* (1994) *Strategies* 7(2):41–43.
2. Grodberg, J. and Dunn, J. J. (1988) *J Bacteriol* 170(3):1245-53.
3. Jerpseth, M., Jerpseth, B., Briester, L. and Greener, A. (1998) *Strategies* 11(1):3-4.
4. Jerpseth, B., Callahan, M. and Greener, A. (1997) *Strategies* 10(2):37–38.

ENDNOTES

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