Accep**T**or™ Vector Kits

U.S. Patent No. 5,629,179 has been issued to Novagen® for the **A**ccep**T**or™ Vector method and kit for direct cloning of PCR products.

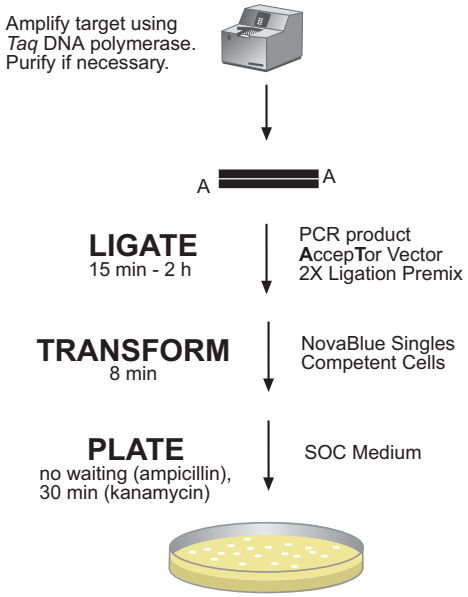
The pETBlue™ T7 expression system is covered under US Patent 5,693,489. For academic and non-profit laboratories, a non-distribution agreement accompanies the products. Commercial laboratories must obtain a research-use license from Brookhaven Science Associates prior to purchase of the products. **A**ccep**T**or™ products are sold for research use only.

About the System

Introductory pETBlue™-1 **A**ccep**T**or™ Vector Kit	1 kit (10 rxn)	70597-3
pETBlue™-1 **A**ccep**T**or™ Vector Kit	1 kit (20 rxn)	70598-3
	1 kit (40 rxn)	70598-4
pETBlue™-1 **A**ccep**T**or™ Vector	20 rxn	70599-3
	40 rxn	70599-4
Introductory pSTBlue-1 **A**ccep**T**or™ Vector Kit	1 kit (10 rxn)	70594-3
pSTBlue™-1 **A**ccep**T**or™ Vector Kit	1 kit (20 rxn)	70595-3
	1 kit (40 rxn)	70595-4
pSTBlue™-1 **A**ccep**T**or™ Vector	20 rxn	70596-3
	40 rxn	70596-4

Description

The **A**ccep**T**or™ Vector Kits are designed to simplify cloning of PCR products generated using non-proofreading thermostable DNA polymerases, such as *Taq*. These DNA polymerases leave single 3'-dA overhangs on their reaction products (1). The linearized **A**ccep**T**or™ vector DNA contains single 3'-dU overhangs and can be ligated to the PCR product without modification. Following transformation, the dU residues are replaced with dT residues as the plasmid replicates in the bacterial cell.



The kits contain Clonables™ 2X Ligation Premix, a unique ligation cocktail that includes ligase, buffer and cofactors to support rapid, efficient ligation. The kits also include NovaBlue Singles™ Competent Cells for simple, convenient transformation of the ligation. The combination of the **A**ccep**T**or™ vector and NovaBlue Singles allows screening based on blue/white colony color to distinguish those containing plasmids with inserts from those without inserts. The NovaBlue host strain is well suited for cloning and verification of constructs in the **A**ccep**T**or™ Vectors due to its high transformation efficiency, blue/white screening capability, and *rec*A *end*A mutations which result in high yields of excellent quality plasmid DNA.

The pETBlue™ -1 **A**ccep**T**or™ Vector Kit also includes Tuner™(DE3)pLacI Competent Cells. This strain carries a chromosomal copy of the T7 RNA polymerase gene and is designed for IPTG-inducible expression of target genes under the control of the T7*lac* promoter in the pETBlue™-1 vector. After plasmid DNA of clones is prepared from NovaBlue, it can be verified and then transformed into the Tuner™ (DE3)pLacI strain for expression in *E. coli*.

Components

Introductory **A**ccep**T**or™ Vector Kits

- 0.5 µg pETBlue™-1 or pSTBlue-1 **A**ccep**T**or™ Vector DNA, 50 ng/µl
- 10 µl Positive Control Insert, 15 ng/µl
- 1 × 55 µl Clonables™ 2X Ligation Premix
- 1.5 ml Nuclease-free Water
- 11 x 50 µl NovaBlue Singles™ Competent Cells
- 2 or 3 x 2 ml SOC Medium
- 10 µl Test Plasmid 0.2 ng/µl (amp^R)
- 0.2 ml Tuner™(DE3)pLacI Competent Cells*

*only with the pETBlue™-1 Kit

Accep**T**or™ **V**ector Kits

- 2 or 4 x 0.5 µg pETBlue™-1 or pSTBlue-1 **A**ccep**T**or™ Vector DNA, 50 ng/µl
- 10 µl Positive Control Insert, 15 ng/µl
- 2 or 4 x 55 µl Clonables 2X Ligation Premix
- 1.5 ml Nuclease-free Water
- 22 or 44 x 50 µl NovaBlue Singles Competent Cells
- 4,5,7, or 9 x 2 ml SOC Medium
- 10 µl Test Plasmid 0.2 ng/µl (ampR)
- 2 or 4 x 0.2 ml Tuner(DE3)pLacI Competent Cells*

* only with the pETBlue™-1 Kit

Accep**T**or™ **V**ector

- 2 or 4 x 0.5 µg pETBlue™-1 or pSTBlue-1 **A**ccep**T**or™ Vector DNA, 50 ng/µl
- 10 µl Positive Control Insert, 15 ng/µl

Storage

Store all components at –70°C.

Accep**T**or™ **V**ectors

The two available **A**ccep**T**or™ Vectors are summarized below. Vector maps and sequences are available at www.merck4biosciences.com

AccepTor™ Vector	Applications	Vector Advantages
pETBlue™-1	Protein expression: T7*lac*-driven, tightly controlled, high level expression in *E. coli*	No fusion tags Insert provides ATG start codon
pSTBlue™-1	Archiving, Subcloning, Sequencing, *In vitro* transcription	Dual opposed SP6/T7 promoters Amp or Kan selection Dual EcoRI sites flank insert

pETBlue™-1 **A**ccep**T**or™ Vector

The pETBlue™-1 vector enables high-level expression of target genes in *E. coli*. The expression of protein without N-terminal fusions tags is achieved by T7 RNA polymerase-driven transcription from a T7 promoter. The ribosome binding site in the vector is appropriately spaced 5' to the **A**ccep**T**or™ cloning site (EcoRV) to achieve translation initiation from an insert-provided ATG start. The sense primer for PCR amplification to produce the insert DNA must begin with an ATG start codon at its 5' end. Ligation with the **A**ccep**T**or™ Vector will give optimal spacing between the ribosome binding site and translation initiation site, providing for efficient protein synthesis in *E. coli*. There are no restrictions on the design of primers that specify the carboxyl terminus of the target sequence.

Sense primer: 5'-ATG $(XXX)_n$ -3'

pSTBlue-1 **A**ccep**T**or™ Vector

pSTBlue-1 is a general purpose vector with dual opposed T7 and SP6 promoters and both amp and kan resistance. It includes a convenient array of restriction sites flanking the cloning site which can be used for mapping and subcloning.

Preparation of the Insert

1. Amplify the target sequence via PCR using target-specific primers and a non-proofreading thermostable DNA polymerase, such as *Taq*. For expression with pETBlue™-1 include an ATG start codon as described above. Check a small amount of the PCR reaction on a gel to verify appropriately sized product.
2. If there are extraneous bands in the PCR reaction or if the template plasmid and the **A**ccep**T**or™ Vector have the same antibiotic resistence, the PCR product needs to be gel-purified prior to ligation. Use a standard method to elute the target band from the gel, e.g., SpinPrep™ Gel DNA Kit (Cat. No. 70852) or electroelution with D-Tube™ Dialyzers (Cat. No. 71507)
3. If the PCR is very clean (i.e., the gel shows a clear, distinct band of the desired size with no extraneous bands), gel purification is optional. Instead, use one of the following methods to remove polymerase activity.
   a) Purify the PCR product with SpinPrep PCR Clean-Up Kit (Cat. No. 70976) to remove polymerase, dNTPs, and primers.

      - or -

   b) Perform a chloroform extraction to inactivate Taq DNA polymerase. Add 1 volume chloroform:isoamyl alcohol (24:1) to the PCR reaction, vortex vigorously for 1 minute, centrifuge at 12,000 × g for 1 minute. Removal of dNTPs and small DNA is necessary if the PCR product is at a low concentration. If more than 2 µl would be required for the ligation, the PCR product should be concentrated and dNTPs removed by alcohol precipitation. Pellet Paint® Co-Precipitant (Cat. No. 69049) can be used to visualize the pellet. Resuspend in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)

Note: User Protocols for the products mentioned above are available at www.emdchemicals.com

Ligation

1. For each reaction, assemble the following components in a microcentrifuge tube:

1 µl (~0.02 pmol)	**A**ccep**T**or™ Vector (50 ng/µl)
0.5 – 4.0 µl (0.1 – 0.2 pmol)	PCR product (or 2 µl **A**ccep**T**or™ Control Insert)
X µl	Nuclease-free Water to a total of 10 µl
5.0 µl	Clonables™ 2X Ligation Premix
10 µl	total volume

   Mix gently by stirring with a pipet tip.

   Optional but highly recommended: Prepare a positive control ligation to test the efficiency of cloning. Use 2 µl (30 ng = 0.22 pmol) of the **A**ccep**T**or™ Vector Control Insert provided with the kit in place of the amplified product. The **A**ccep**T**or™ Vector Control Insert is a 212 bp PCR product amplified with *Taq* DNA polymerase.

   Also prepare a negative control ligation, omitting the PCR product.
2. Incubate the ligation reactions at 16°C for 30 min. (Incubation can be performed from 15 min to 2 h; the number of recombinants may be increased 2–3 fold by incubating for 2 h vs. 30 min)

   Note: Insert:vector molar ratios between 5:1 and 10:1 are appropriate. To calculate ng/pmol for a given target, number bp in DNA X 650 = pg/pmol. (e.g. a 500 bp fragment is 350 ng/pmol, therefore use 50 ng for 0.15 pmol).

Transformation of NovaBlue Singles™ Competent Cells

NovaBlue enables blue/white screening, yields high quality plasmid preps for mapping and sequencing, and does not express T7 RNA polymerase. It is a preferred host strain for the isolation of recombinant plasmids under non-expressing conditions. In the following steps, 1 µl of the ligation reaction is used to transform NovaBlue Singles™ Competent Cells. Up to 5 µl of the ligation reaction containing high quality reagents can be added to NovaBlue Singles™ Competent Cells without reducing transformation efficiency. Transformants are selected for ampicillin resistance (pETBlue™-1 or pSTBlue-1) or kanamycin resistance (pSTBlue-1).

Note: When selecting for transformants by ampicillin resistance (expression of β-lactamase), we recommend using carbenicillin. Carbenicillin is less sensitive to drop in pH of the medium that normally occurs during bacterial growth. Use ampicillin or carbenicillin at 50 µg/ml. When selecting transformants by kanamycin resistance (expression of aminoglycoside 3'-phosphotransferase), use 30 µg/ml kanamycin.

Note:For blue/white screening of recombinants, plates must include IPTG (80 µM), X-gal (70 µg/ml), and tetracycline (12.5 µg/ml). IPTG induces expression of the β-galactosidase activity and X-gal is a chromogenic stubstrate. Tetracycline maintains the F'-containing lacZΔM15, thus eliminates the background of colonies that are white because of having lost the F' rather than because of having an insert. If plates containing X-gal and IPTG are not available, these reagents can be spread on plates and allowed to soak in for at least 30 minutes prior to plating. To overlay, use 35 µl of 50 mg/ml X-gal in dimethyl formamide and 20 µl 100 mM IPTG (in water) per 100 mm plate (25 ml agar).

1. For each transformation, remove a tube of NovaBlue Singles™ Competent Cells from the freezer. (Include one extra sample for Test Plasmid positive control, if desired). Immediately place the tubes on ice, so that all but the cap is surrounded by ice. Allow the cells to thaw on ice for ~2–5 min.
2. Verify that cells have thawed. Gently flick cells 1–2 times to evenly resuspend. Do not vortex.
3. Add 1 µl of a ligation reaction or 1µl test plasmid DNA (0.2 ng) directly to the cells. Mix and return tube to ice, making sure that tube is surrounded by ice except for the cap. Repeat for additional samples.

   Note: Adding more of the ligation reaction generally will not produce more transformants; more than 2 µl of ligation reaction can inhibit transformation. However, transformation efficiencies can be increased several fold by diluting the ligation reaction 5-fold with TE or water, or by extracting the ligation reaction twice with 1:1 TE-buffered phenol:CIAA (24:1 chloroform:isoamyl alcohol), once with CIAA, ethanol precipitating in the presence of NaOAc, and resuspending in TE or water.
4. Leave tubes on ice for 5 min.
5. Place tubes for exactly 30 sec in a 42°C water bath; do not shake.
6. Place on ice for 2 min.
7. Add 250 µl of room temperature SOC Medium to each tube.
8. Determine the requirement of an outgrowth step based on the selected antibiotic marker.

   a. If selecting for amp^R, no outgrowth step is required, although more colonies may be obtained with 30–60 minute outgrowth. Proceed to Step 9.

   b. If using pSTBlue-1, you may select either for kan^R or for amp^R. For selection with kanamycin, an outgrowth step is required. Shake at 200–250 rpm at 37°C for 30–60 min prior to plating. Proceed to Step 9.
9. Plate 20–100 µl transformation mixture (1–5 µl for Test Plasmid) directly on LB agar media containing 50 µg/ml carbenicillin (pETBlue™ or pSTBlue) or 30 µg/ml kanamycin (pSTBlue), plus 15 µg/ml tetracycline, 70 µg/ml X-gal and 80 µM IPTG. If plating less than 50 µl, apply 50 µl of SOC to the plate then add the transformation mixture to the SOC cushion. Spread.
10. Let the plates sit on the bench for several minutes to allow excess liquid to be absorbed, then invert and incubate overnight at 37°C (preferably 15–18 h).

    Note: The appropriate amount of transformation mixture to plate will vary with the efficiency of both the ligation and the transformation. Plate 20 µl and 100 µl of the transformation mixture to ensure that one of the plates will contain a sufficient number of isolated colonies for screening. For the Test Plasmid, plate 1 µl of the transformation mixture.

    Spread until the sample is evenly distributed on the plate using ColiRollers™ Plating Beads, a sterile bent glass rod, or specialized spreader. Do not continue spreading after sample is absorbed, as overspreading can kill cells.

    ColiRollers™ Plating Beads (Cat. No. 71013) are specially treated glass beads that gently and evenly distribute cells without use of spreader, alcohol, or flame.

Screening

Blue/White Phenotype

The **A**ccep**T**or™ Vectors provide for blue/white screening of transformants to differentiate those having recombinant plasmids. When plated on X-gal/IPTG indicator plates, colonies with recombinant plasmids (vector plus insert) are white while those with vector alone are blue. The vectors encode a functional *lacZ* α-peptide that complements the *lacZ* ω-fragment expressed by the host strain (*lacZ*ΔM15 on F' in NovaBlue). The resulting β-galactosidase activity can cleave the chromogenic substrate X-gal to yield a blue colony phenotype. Since the cloning site is within the α-peptide open reading frame (ORF), inserts disrupt this ORF, thereby preventing the production of functional α-peptide and resulting in the white colony phenotype.

Note: To increase the visual distinction between blue and white colonies, enhance blue color development by placing plates in a 4°C refrigerator for a few hours after colonies have grown to desired size.

Transformations will yield both blue and white colonies. Blue colonies from the negative control ligation (vector but no insert) contain self-ligated vector with no insert. The very small number of white colonies that arise from the negative control result from ligating vector DNA having damaged ends. Transformation using ligations of vector + insert (or Positive Control Insert) should produce 10–50-fold more white colonies than transformations using negative control ligation but a similar number of blue colonies. Number and color of colonies in transformations of control ligations are an indication of the cloning efficiency. Choose several white colonies to screen.

In addition to blue and white phenotypes, a light blue phenotype sometimes occurs in transformations of vector + insert ligations. Also, white colonies may eventually develop a light blue center or "bullseye" appearance during prolonged incubation or storage at 4°C. These can be tentatively counted as having insert, as we have found that more than 90% of light blue colonies do contain inserts of the expected size. Presumably, a small amount of functional α-peptide is produced in these recombinants by an alternative means such as ribosomal frameshifting, second site translational initiation, or as an α-peptide fusion protein.

Rapid Screening by Colony PCR

White colonies can be further screened by colony PCR. Presence and size of the insert can be determined by using vector-specific primers flanking the cloning site. The expected sizes of PCR products for vector alone and vector plus the 212 bp Positive Control Insert are listed in the following table.

AccepTor™ Vector	Vector Primers (5' & 3')	Expected PCR Product	
		No Insert	+ 212 bp Insert
pSTBlue™-1	T7 promoter & U-19-mer	231 bp	443 bp
	R-20mer & U-19mer	258 bp	470 bp
pETBlue™-1	pETBlueUP + pETBlueDOWN	157 bp	369 bp
	pETBlueT7UP + pETBlueDOWN	179 bp	391 bp

To determine insert orientation, use one of the vector-specific primers (above) with 5 pmol of an insert-specific primer. Plasmid with no insert or the incorrect orientation will give no PCR product; those with the correctly oriented insert will give the expected PCR product.

Colony PCR

1. Pick a colony from an agar plate using a 200 µl pipet tip or sterile toothpick. Choose colonies that are at least 1 mm in diameter and collect as many cells as possible. If a copy of the colony is desired, touch the pipet tip to a plate before transferring the bulk of the colony to the tube in the next step.
1. Transfer the bacteria to a tube containing 50 µl of sterile water. Vortex to disperse the pellet.
2. Place the tubes in boiling water or a heat block at 99°C for 5 min to lyse the cells and denature DNases. (Use lid lock on caps to prevent caps from opening).
3. Centrifuge at 12,000 × *g* for 1 min to remove cell debris.
4. Transfer 10 µl of the supernatant to a fresh 0.5 ml tube for PCR. Leave on ice until use.

5. Each PCR reaction requires the following:

31.8 µl	Nuclease-free water
1 µl	dNTP mix (10 mM each dATP, dCTP, dGTP, dTTP)
1 µl	5' primer, approx. 5 pmol/µl
1 µl	3' primer, approx. 5 pmol/µl
5 µl	10X buffer (10X = 100 mM Tris-HCl pH 8.8 at 25°C, 500 mM KCl, 15 mM $MgCl_2$, 1% Triton X-100)
0.25 µl	1.25 U Nova*Taq*™ DNA polymerase
40 µl	total volume

   Mix reaction components in a single tube (master mix) scaled according to the number of reactions desired. Multiply the volumes by X.5, where X is the number of reactions to allow slight excess.
6. Add 40 µl of the master mix to each tube of 10 µl sample. Mix gently. Cap tubes and put samples in a thermal cycler. Process for 35 cycles, as follows:

   94°C 1 min
   55°C 1 min
   72°C 2 min

   Include a final extension of 72°C for 5 min.
7. To analyze reaction products, add 5 µl of 10X loading dye and load 10–25 µl per lane on a 1% agarose gel containing 0.5 µg/ml ethidium bromide. Include a lane with an appropriate DNA marker as a size standard.

Analysis of Recombinants by *In Vitro* Transcription/Translation

If the target insert encodes an open reading frame, *in vitro* transcription/translation can be used to verify that recombinants will produce the desired protein product. As template for the *in vitro* transcription/translation reaction, use PCR-generated DNA (as above) or isolated recombinant plasmid.

To PCR amplify for *in vitro* transcription/translation, the 5' primer should be upsteam of the T7 promoter to provide sufficient space for T7 RNA polymerace to initiate transcription at the T7 promoter. (T7 promoter primer will not allow efficient transcription initiation at the T7 promoter, as it is too close to the DNA end.) For subsequent *in vitro* transcription/translation, use R-20mer & U-19mer primers for pSTBlue-1 clones or pETBlueT7UP + pETBlueDOWN primers for pETBlue™-1 clones. Alternatively use R-20mer or pETBlueT7UP as the 5' upstream primer and a 3' insert-specific primer.

In addition to plasmids and colonies, ligation reactions can also be used as templates to generate PCR products suitable for transcription/translation. For amplification, the same protocol as colony PCR can be used; templates can be single colonies, 1–10 ng purified plasmid DNA, or 1 µl of a ligation reaction. Use 1–2 µl or 2–4 µl of the completed reaction as the template for STP3, T7 or EcoPro™ T7 respectively. The PCR product should be purified using standard PCR clean-up protocols (e.g. SpinPrep™ PCR Clean-Up Kit, See User Protocol TB290) prior to transcription/translation analysis.

The PCR product can serve as a suitable template in a Single Tube Protein™ System 3 (STP3™, T7) or EcoPro™ T7 system for *in vitro* transcription/translation analysis of the target protein. STP3 is a linked reaction in which transcription by a bacteriophage RNA polymerase is directly followed by translation in an optimized rabbit reticulocyte lysate (See User Protocol TB206). The EcoPro™ System employs a proprietary fractionated *E. coli* extract to perform coupled transcription and translation in one step (See User Protocol TB278).

DNA Isolation and Sequencing

Isolate plasmid DNA from candidate recombinants for analysis and verification before transformation of an expression host. Standard mini prep methods may be used to isolate DNA suitable for sequencing or restriction enzyme analysis. The **A**ccep**T**or™ vectors contain the high copy pUC origin of replication and produce DNA yields similar to other pUC-based plasmids. Note that host strain NovaBlue is *recA endA* deficient and therefore is recommended for high-quality plasmid preparations.

*Note: Cloning into the **A**ccep**T**or™ vector typically destroys the EcoRV site: therefore, EcoRV is not a useful enzyme for restriction mapping of recombinant plasmids.*

Because the **A**ccep**T**or™ vectors contain an f1 origin of replication, it is also possible to prepare single-stranded plasmid DNA from strain NovaBlue by infection with a single-stranded DNA helper phage. The required helper phage (strain R408 or M13KO7) and protocols for infection and DNA isolation are available from a number of commercial suppliers. The NovaBlue host strain carries an F' and is therefore suitable for helper phage infection.

Verify DNA by sequence, prior to expression experiments. Detailed protocols for sequencing with double stranded or single stranded templates are available from manufacturers of sequencing kits. Primers for sequencing are indicated on the vector maps available at www.merck4biosciences.com.

Protein expression with pETBlue™-1 Recombinants

For IPTG-inducible expression of target genes in *E. coli* from pETBlue™-1 vectors, transform plasmid DNA prepared from NovaBlue into an expression host which supplies inducible expression of T7 RNA polymerase. Suitable hosts include the Tuner™(DE3)pLacI host (supplied in the pETBlue™ Kits), Origami™2(DE3)pLacI, Origami B(DE3)pLacI, or Rosetta™2(DE3)pLacI host strains (available separately). Since the pETBlue™ vectors contain T7*lac* promoter but do not contain a copy of the *lacI* gene, they require an additional source of Lac repressor to suppress basal expression of target genes. The pLacI plasmid carried by these recommended hosts serves as the source for additional Lac repressor.

After the target plasmid DNA is prepared from NovaBlue and structure verified, transform ~1 ng plasmid into an expression host (e.g., 1 µl of 1:50 dilution of a mini prep). Tuner(DE3)pLacI Competent Cells are provided in 0.2 ml aliquots. The standard transformation reaction calls for 20 µl cells (each tube contains enough cells for 10 transformations.

Procedure: Transformation of Tuner(DE3)pLacI Competent Cells

1. Remove competent cell tube from freezer. Immediately place tube on ice. Ensure that all but the cap is surrounded by ice. Allow cells to thaw on ice for ~5 minutes.
2. For each transformation, place a 1.5-ml snap-cap polypropylene tube on ice to pre-chill.
   (Optional: include a tube for a Test Plasmid transformation to determine transformation efficiency).
3. Verify that cells have thawed. Gently flick tube to evenly resuspend cells. Do not vortex.
4. Pipet 20 µl aliquots of thawed cells into each pre-chilled tube.
5. Add 1 µl of the pETBlue™-1 recombinant plasmid (~1 ng/µl) or 1 µl (0.2 ng) Test Plasmid directly to the cells. Stir gently to mix and return tube to ice, making sure that tube is surrounded by ice except for the cap. Repeat for additional samples.
6. Incubate tubes on ice for 5 min.
7. Heat tubes for exactly 30 sec in a 42°C water bath; do not shake.
8. Place the tubes on ice for 2 min.
9. Add 80 µl of room temperature SOC Medium to each tube.
10. Incubate at 37°C while shaking at 250 rpm for 60 min.
11. Plate 5–50 µl cells on LB agar media containing 50 µg/ml carbenicillin (or ampicillin) and 34 µg/ml chloramphenicol.
12. Let plates sit on bench for several min to allow excess liquid to be absorbed, then invert and incubate overnight at 37°C.

Protein Expression

Target genes in pETBlue™-1 **A**ccep**T**or™ vector can be expressed in *E. coli*, provided that the inserted gene is in the sense orientation relative to the T7*lac* promoter of the vector and that insert contains an ATG translational start codon at its 5' end. Since *E. coli* RNA polymerase does not recognize the T7 promoter, transcription requires a source of T7 RNA polymerase. Expression hosts for pETBlue™-1 are λDE3 lysogens and carry the pLacI plasmid. λDE3 is a clone of T7 RNA polymerase under the control of the lacUV5 promoter (2,3) thus providing for IPTG inducible expression of T7 RNA polymerase and of the cloned gene.

To express the protein from the cloned gene, inoculate growth medium (plus carbenicillin and chloramphenicol) with Tuner™ (DE3)pLacI carrying the pETBlue™-1 recombinant clone. Shake at the desired temperature (typically 37°C or 30°C) until OD_{600} is ~0.6–1.0. Add IPTG to 1 mM and continue incubation 3–5 hr. Harvest cells by centrifugation.

Detailed procedures for protein expression from pETBlue™-1 recombinants as well as for cell lysis and fractionation are described in the pETBlue™ System Manual, TB249, available at www.merck4biosciences.com.

References

1. Clark, J.M. (1988) *Nucleic Acids Res.* **16**, 9677–9686.
2. Studier, F. W. and Moffatt, B. A. (1986) *J. Mol. Biol.* **219**, 61–68.
3. Studier, F. W., et. al. (1990) *Meth. Enzymol.* **185**, 60-89.

Bacterial Strain Non-distribution Agreement

By purchase of the Origami™ 2, Origami™ B, Rosetta™, Rosetta 2, RosettaBlue™, Rosetta-Gami™ 2, or Rosetta-Gami™ B host strains and acceptance of the following terms, Novagen® grants a limited license to use the Origami™ 2, Origami™ B, Rosetta, Rosetta 2, RosettaBlue™, Rosetta-Gami™ 2, or Rosetta-Gami™ B host strains for the cloning and expression of genes. The intent of this license is not to limit the research use of these materials, but to protect against unauthorized commercial distribution of the strains by third parties.

1. The Origami™ 2, Rosetta, Rosetta 2, RosettaBlue™, Rosetta-Gami™ 2, or Rosetta-Gami™ B host strains or any derivative therefrom is not to be offered for resale or distributed outside your laboratory.
2. Gene clones and libraries in the Origami™ 2, Rosetta, Rosetta 2, RosettaBlue™, Rosetta-Gami™ 2, or Rosetta-Gami™ B host strains may be distributed for research purposes only, provided that the recipient acknowledge the foregoing condition.
3. Commercial customers must obtain a research license agreement from Brookhaven Science Associates before purchasing DE3 lysogens of host strains Origami™ 2, Rosetta, Rosetta 2, RosettaBlue™, Rosetta-Gami™ 2, or Rosetta-Gami™ B.

The initial purchaser may refuse to accept the above conditions by returning the kit unopened and the enclosed materials unused. By accepting or using the kit or the enclosed materials, you agree to be bound by the foregoing conditions.

Appendix A: Vector Map References

Please visit http://www.merck4biosciences.com and download TB214 and TB258 for the pSTBlue™-1 Vector Map and the pETBlue™-1 Vector Map, respectively.