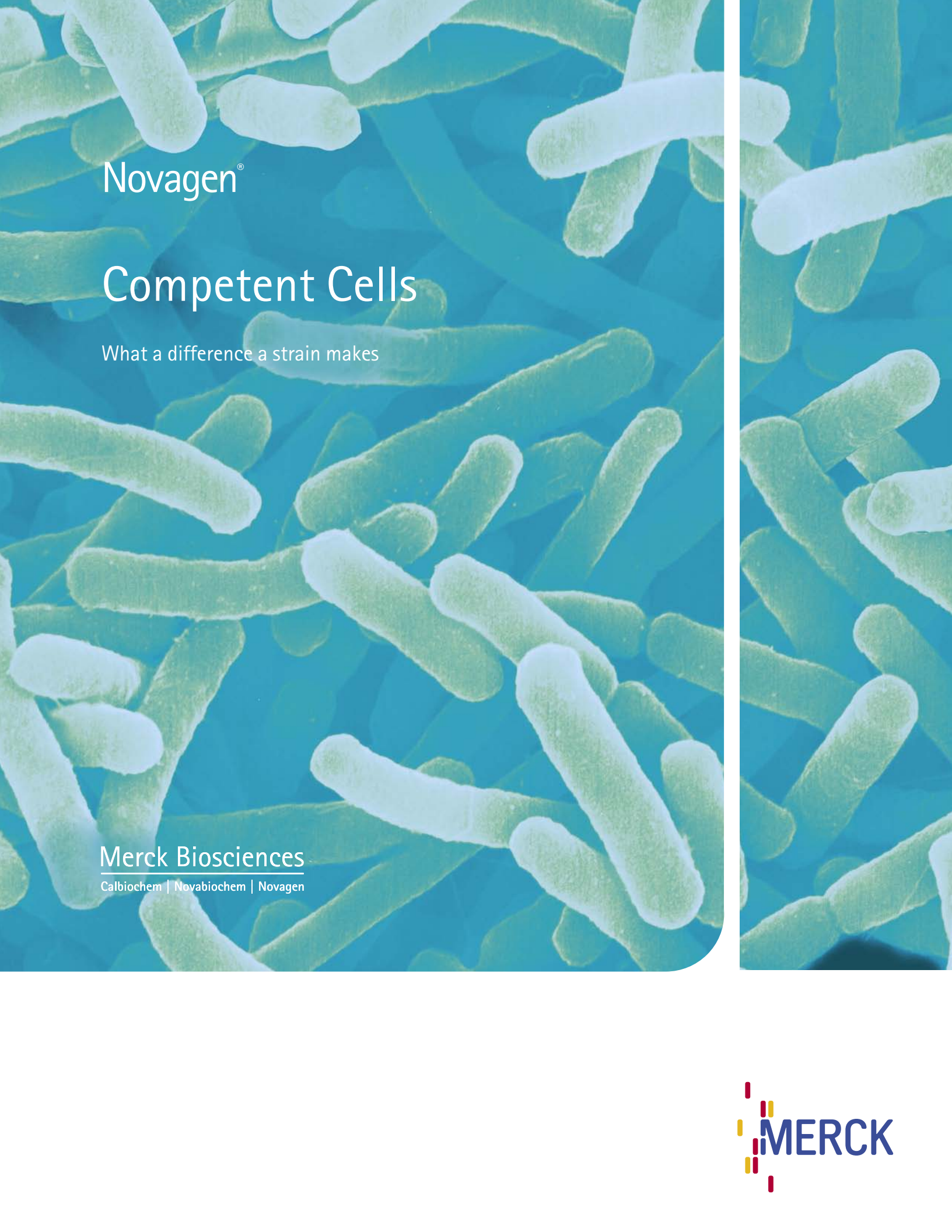


A scanning electron micrograph (SEM) of numerous E. coli cells. The cells are rod-shaped and appear in various orientations, some showing flagella. They are set against a dark blue background.

Novagen®

# Competent Cells

What a difference a strain makes

Merck Biosciences

Calbiochem | Novabiochem | Novagen



## Inactive proteins?

Express active folded proteins with disulfide bonds in *E. coli*.

## Codon bias?

Express mammalian proteins more efficiently in *E. coli* without tedious codon optimization. Use a bacterial host system that supplies 7 rare codon tRNAs.

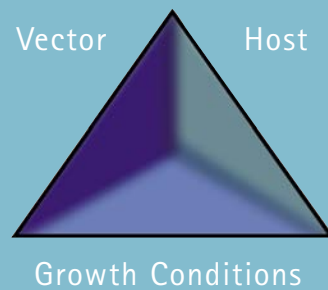
## Insoluble protein?

Fine tune your expression levels to avoid aggregation.

## What a difference a strain makes!

Novagen competent cells embody the widest selection available for protein expression and offer fundamental strains for cloning applications. We verify the phenotype and purity of each strain and guarantee its transformation efficiency. To meet your needs for maximized yield and activity of target proteins, we offer expression strains that allow stringent control over basal expression levels, enable disulfide bond formation in the cytoplasm, and alleviate codon usage incompatibilities. Chemically competent NovaBlue strains are an excellent choice for routine cloning. For cloning applications that require highest transformation efficiencies, our electrocompetent cell strains have a genotype optimized to construct large, complex libraries. See for yourself what a difference a strain makes!

## Features Determining Vector-Host Compatibility



Three factors influence protein expression: the expression vector, host cell, and growth/induction conditions. Changing one or more of these factors can dramatically influence expression levels and target protein solubility.

## Vector-Host Relationship

Any number of systems may be suitable for expression of analytical amounts of some proteins for screening, yet only one combination of vector, host strain, and culture conditions may work best for other proteins, for activity assays, and for larger-scale production. If you need a high yield of active protein, it is worth testing a matrix of vector, host, and culture conditions to find the optimal result. To do this, it helps to know more about the target protein and also to empirically determine expression optima by using Novagen competent cell sets, Quarters™ Competent Cells and QuarterPack™ Competent Cell Arrays.

## Vector-Host Compatibility

You can use Novagen host strains with many different expression vectors, as long as the plasmid replicon and antibiotic-resistance markers are distinct from corresponding elements carried by the host.

## Protein Expression Troubleshooting Guide

| Symptom           | Possible Problem                        | Solution                                     | Suggested Host                                                |
|-------------------|-----------------------------------------|----------------------------------------------|---------------------------------------------------------------|
| No protein        | <i>E. coli</i> codon usage (codon bias) | Supply rare tRNAs                            | Rosetta™                                                      |
| Truncated protein |                                         |                                              | Rosetta 2<br>Rosetta-gami™ 2<br>Rosetta-gami B<br>RosettaBlue |
| Insoluble protein | Reduction of disulfide bonds            | Minimize reduction in cytoplasm              | Origami™ 2<br>Rosetta-gami 2<br>Rosetta-gami B                |
|                   | Too much expression                     | Attenuate expression (titrate IPTG)          | Tuner™<br>Rosetta-gami B                                      |
| No activity       | Misfolded protein                       | Minimize reduction in cytoplasm              | Origami™ 2<br>Rosetta-gami 2<br>Rosetta-gami B                |
|                   |                                         | Attenuate expression (titrate IPTG)          | Tuner<br>Rosetta-gami B                                       |
| Cell death        | Toxic protein                           | More stringent control over basal expression | pLysS hosts                                                   |
| No colonies       | High basal expression                   | More stringent control over basal expression | pLysS hosts                                                   |



# Expression

## A variety of expression hosts

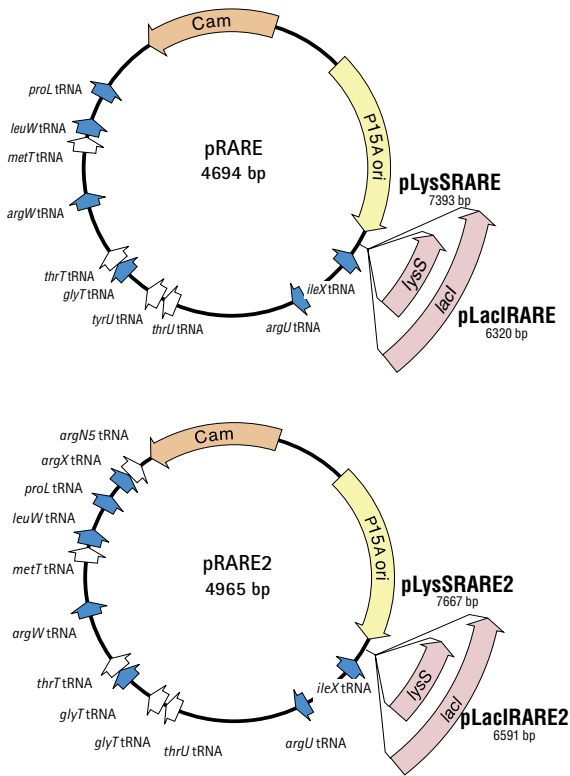
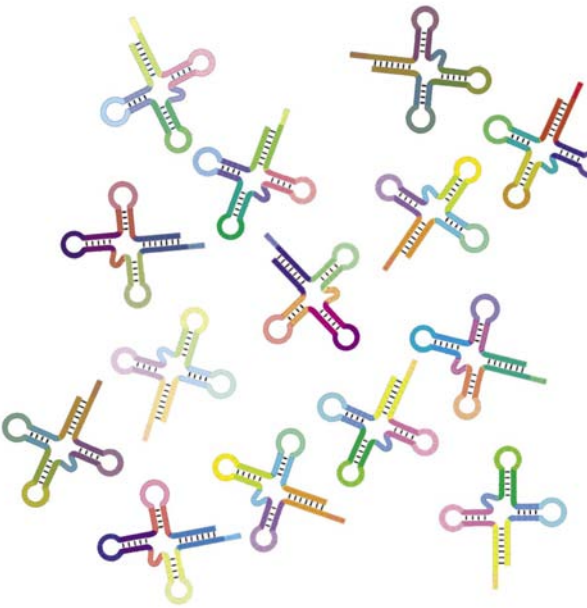
Expression host strains in many different versions can be used with a variety of protein expression systems. For production of protein from target genes cloned in T7 expression vectors, lysogens of  $\lambda$ DE3 carry a chromosomal copy of the T7 RNA polymerase gene under the control of the *lacUV5* promoter. Look for a strain having a pLysS designation; these hosts carry a plasmid that encodes T7 lysozyme, a natural inhibitor of T7 RNA polymerase. Use these strains to suppress basal expression of T7 RNA polymerase before induction, and thereby, stabilize recombinants in pET, pRSF, and pCDF and pCOLA vectors, which encode target proteins that affect cell growth and viability. For expression from *E. coli* promoters such as *tac*, *lac*, *trc*, and *p<sub>L</sub>*, or for T7-based expression by infection with  $\lambda$ CE6, versions of these host strains that lack T7 RNA Polymerase also are available.

## BL21—still the gold standard

For routine protein expression, **BL21** is an ideal starting point. First commercialized in 1990, the Novagen BL21 strain has remained the gold standard among expression hosts ever since. BL21 and its derivatives are deficient in both *lon* and *ompT* proteases (1). The parental strain, **B834** is a methionine auxotroph that allows high specific activity labeling of target proteins with <sup>35</sup>S-methionine or selenomethionine for crystallography studies (2). **BLR**, the *recA*<sup>-</sup> derivative of BL21, may help stabilize target plasmids containing repetitive sequences or whose products may cause the loss of the DE3 prophage (3, 4). **Tuner™**, the *lacZY* deletion mutant of BL21, enables adjustable levels of protein expression throughout all cells in a culture. Its *lac* permease (*lacY*) mutation allows uniform entry of IPTG into all cells in the population, which produces a concentration-dependent, homogeneous level of induction. By adjusting the IPTG concentration, expression can be regulated from very low levels up to robust, fully induced levels commonly associated with pET hosts. Lower level expression may enhance the solubility and activity of difficult target proteins.

# Seven rare tRNAs

**Rosetta™** and **Rosetta 2** host strains are BL21 derivatives designed to enhance the expression of eukaryotic proteins that contain codons rarely used in *E. coli*. By supplying these rare tRNAs, the Rosetta strains provide for “universal” translation, which would otherwise be limited by the codon usage of *E. coli*. The original Rosetta strains carry the pRARE plasmid (5) and supply tRNAs for the codons AUA, AGG, AGA, CUA, CCC, and GGA on a chloramphenicol-resistant plasmid. Rosetta 2 strains carry the pRARE2 plasmid and supply a seventh rare tRNA for CGG. In the pLysS and pLacI derivatives of these strains, the rare tRNA genes are present on the same plasmids that carry the T7 lysozyme and *lac* repressor genes, respectively.



Rare codons in *E. coli*

| Amino acid | Codon | Fraction in all genes <sup>6</sup> | Fraction in Class II <sup>7</sup> |
|------------|-------|------------------------------------|-----------------------------------|
| Arg        | AGG   | 0.022                              | 0.003                             |
| Arg        | AGA   | 0.039                              | 0.006                             |
| Arg        | CGG   | 0.098                              | 0.008                             |
| Arg        | CGA   | 0.065                              | 0.011                             |
| Arg        | CGU   | 0.378                              | 0.643                             |
| Arg        | CGC   | 0.398                              | 0.330                             |
| Gly        | GGG   | 0.151                              | 0.044                             |
| Gly        | GGA   | 0.109                              | 0.020                             |
| Gly        | GGU   | 0.337                              | 0.508                             |
| Gly        | GGC   | 0.403                              | 0.428                             |
| Ile        | AUA   | 0.073                              | 0.006                             |
| Ile        | AUU   | 0.507                              | 0.335                             |
| Ile        | AUC   | 0.420                              | 0.659                             |
| Leu        | UUG   | 0.129                              | 0.034                             |
| Leu        | UUA   | 0.131                              | 0.055                             |
| Leu        | CUG   | 0.496                              | 0.767                             |
| Leu        | CUA   | 0.037                              | 0.008                             |
| Leu        | CUU   | 0.104                              | 0.056                             |
| Leu        | CUC   | 0.104                              | 0.080                             |
| Pro        | CCG   | 0.525                              | 0.719                             |
| Pro        | CCA   | 0.191                              | 0.153                             |
| Pro        | CCU   | 0.159                              | 0.112                             |
| Pro        | CCC   | 0.124                              | 0.016                             |

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## Enhanced disulfide bond formation

**Origami™ 2** host strains are K-12 derivatives that have mutations in both the thioredoxin reductase (*trxB*) and glutathione reductase (*gor*) genes, which greatly enhance disulfide bond formation in the cytoplasm. Unlike the original Origami strains, the Origami 2 strains are kanamycin sensitive; like the original strains, the *gor* mutation is still selected for by tetracycline. To reduce the possibility of disulfide bond formation between molecules, hosts containing the *trxB/gor* mutation are recommended only for the expression of proteins that require disulfide bond formation for proper folding.

**Origami B** host strains are derived from a *lacZY* mutant of BL21 to enable precise control of expression levels by adjusting the concentration of IPTG. In addition to *trxB/gor* mutations these strains include the *lon* and *ompT* deficiencies of BL21 which increase protein stability.

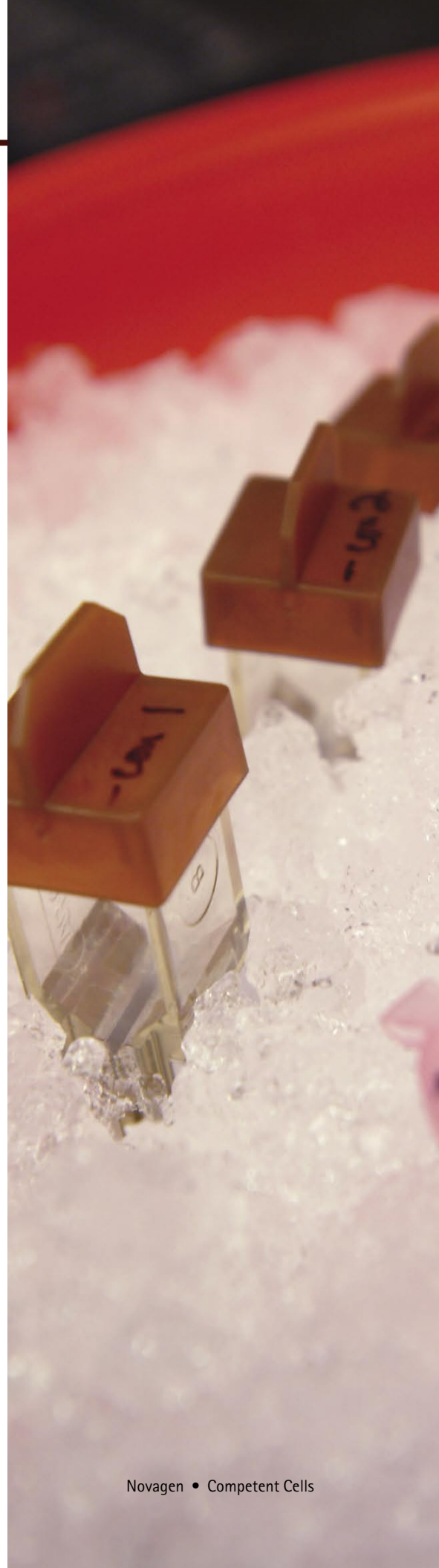
**Rosetta-gami™ 2** host strains combine the advantages of Rosetta™ 2 and Origami 2 strains to alleviate codon bias and enhance disulfide bond formation in the cytoplasm when heterologous proteins are expressed in *E. coli*. These *trxB/gor* mutants are compatible with kanamycin-resistant vectors, and carry the chloramphenicol-resistant pRARE2 plasmid, which supplies seven rare tRNAs.



# Cloning

## High-efficiency electrocompetent cells

**NovaXG and NovaXGF' Zappers™ Electrocompetent Cells** combine favorable genotype with high transformation efficiency for the most demanding cloning applications. NovaXG features deletion of genes involved in restriction of methylated DNA, [ $\Delta(mcrC-mrr)$ ], and *recA endA* mutations, which facilitate high yields of excellent quality plasmid DNA. The *lacZ*  $\Omega$  fragment is expressed from the chromosome and allows blue/white screening for recombinants by *lacZ*  $\alpha$ -complementation with appropriate vectors. NovaXGF' cells have the same genotype as NovaXG, but harbor an F' which confers tetracycline resistance and allows for infection by M13 for ssDNA production. Because the F' carries the *lacI<sup>f</sup>* repressor gene, addition of IPTG is required for blue/white screening of recombinants in these cells. Both strains are manufactured for high transformation efficiency ( $> 1 \times 10^{10}$  cfu/ $\mu$ g) by electroporation to deliver a maximum number of transformants, which is especially important when working with limited amounts of DNA or when constructing large or complex libraries. The cells are packaged in a convenient two transformations per tube format to minimize thawing of excess cells.





## Chemically competent cells

**NovaBlue Competent Cells** are designed for ultimate convenience and reliability in plasmid transformation. NovaBlue is a K-12 strain ideally suited as an initial cloning host due to its high transformation efficiency, blue/white screening capability (with appropriate plasmids), and *recA endA* mutations, which result in high yields of excellent-quality plasmid DNA. The cells are grown and made chemically competent by an optimized procedure. Select **NovaBlue GigaSingles™** for applications requiring higher transformation efficiencies or **NovaBlue Singles™** for more routine cloning applications. **Veggie™** **NovaBlue Singles** are maintained and manufactured with media and reagents derived from nonanimal sources, making these cells ideally suited for applications in which animal-free materials are desired. **NovaBlue T1<sup>R</sup>** have the same features as NovaBlue Singles, with the added benefit of being resistant to T1 and T5 phage.

| NovaBlue Competent Cells Format | Transformation Efficiency (cfu/μg) | Reaction Size | Application                                                           |
|---------------------------------|------------------------------------|---------------|-----------------------------------------------------------------------|
| GigaSingles™                    | > 1 × 10 <sup>9</sup>              | 50 μl         | High-efficiency cloning                                               |
| Singles™                        | > 1.5 × 10 <sup>8</sup>            | 50 μl         | Routine cloning                                                       |
| Veggie™                         | > 1.5 × 10 <sup>8</sup>            | 50 μl         | Applications requiring nonanimal-derived materials<br>Routine cloning |
| HT96™                           | > 1.0 × 10 <sup>8</sup>            | 96 x 20 μl    | High-throughput cloning                                               |
| T1 <sup>R</sup>                 | > 1.5 × 10 <sup>8</sup>            | 50 μl         | T1/T5 Phage resistant<br>Routine cloning                              |

# Overnight Express

High-level protein expression without the need to monitor cell growth

**Two Overnight Express™ Autoinduction Systems** are available, both featuring high-level protein production in the pET and other IPTG-inducible bacterial expression systems without the need to monitor cell growth or add an inducer. Cell mass and target protein yield are often increased several-fold as compared with conventional protocols using induction with IPTG.

## Overnight Express Protocol

- Prepare medium
- Inoculate with a single colony
- Incubate 8 to 24 hours
- Harvest target protein

## Features

- High cell densities and protein expression levels
- No need to monitor cell growth rate or add inducer
- Ideal for pET Expression System or other IPTG-inducible bacterial systems
- Induction of numerous expression clones simultaneously
- Compatible with cultures grown in flasks, culture tubes, and deep-well plates
- Minimal sample handling
- Minimal lot-to-lot variability

## Additional features of Overnight Express Autoinduction System 2

- Complete chemically defined medium
- Ideal for selenomethionine labeling of proteins to be crystallized for x-ray diffraction studies

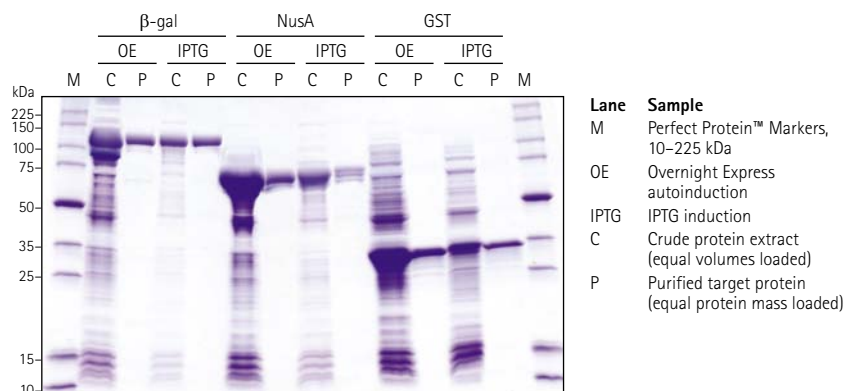
| Product                                   | Size   | Cat. No. |
|-------------------------------------------|--------|----------|
| Overnight Express™ Autoinduction System 1 | 1 kit* | 71300-3  |
|                                           | 1 kit† | 71300-4  |
| Overnight Express Autoinduction System 2  | 1 kit* | 71366-3  |
|                                           | 1 kit† | 71366-4  |

\* includes enough reagents to induce 1 liter

† includes enough reagents to induce 5 liters

## Available separately:

| Product            | Size   | Cat. No. |
|--------------------|--------|----------|
| L-Selenomethionine | 250 mg | 561505   |
|                    | 1 g    |          |



## Expression and purification of target proteins from cultures induced with Overnight Express versus IPTG

pET recombinants encoding  $\beta$ -gal, NusA, and GST His•Tag® fusion proteins were transformed into BL21(DE3). Protein expression was induced in parallel cultures either by Overnight Express System 1 or 1 mM IPTG. Cells were harvested by centrifugation and extracted with BugBuster® HT Protein Extraction Reagent plus rLysozyme™ Solution. Equal volumes (7  $\mu$ l) of the extract were analyzed by SDS-PAGE (10–20% gradient gel) and Coomassie blue staining (C lanes). The remainder of the extract was used for robotic affinity purification using Ni-NTA His•Bind® Resin. Samples (2  $\mu$ g) of the purified fraction were loaded on the gels (P lanes).



## Antibiotics

| Product                    | Size  | Cat. No. |
|----------------------------|-------|----------|
| Carbenicillin              | 5 g   | 69101-3  |
| Chloramphenicol            | 25 g  | 220551   |
|                            | 100 g |          |
|                            | 500 g |          |
| Kanamycin Sulfate          | 5 g   | 420311   |
|                            | 25 g  |          |
| Tetracycline Hydrochloride | 10 g  | 58346    |
|                            | 25 g  |          |
|                            | 50 g  |          |

## Accessory Products

| Product                    | Size     | Cat. No. |
|----------------------------|----------|----------|
| ColiRollers™ Plating Beads | 1 pkg    | 71013-3  |
|                            | 5 pkg    | 71013-4  |
| Veggie™ Peptone            | 500 g    | 71280-3  |
| Veggie Yeast Extract       | 500 g    | 71279-3  |
| HT96 Isothermal Block      |          | 71195-3  |
| 100 mM IPTG Solution       | 15 ml    | 70527-3  |
| X-Gal Solution             | 3 x 1 ml | 71077-3  |

For more information about these products visit our website at [www.merckbiosciences.com](http://www.merckbiosciences.com)



### Need a larger package size?

All of our competent cells, antibiotics, and Overnight Express™ Autoinduction Systems can be custom packaged to suit your needs. Just ask us!

# Optimized packaging



Novagen competent cells are featured in many different packaging formats. In addition to the Standard 0.2-ml volumes in 20- and 50-reaction kit sizes, several strains are available as Singles™ Competent Cells, single-use, 50-µl volumes for extra convenience and efficiency. Quarters™ Competent Cells consist of 24 wells in a 3 × 8-well configuration that makes up one “quarter” section of a 96-well plate. Each well contains 20 µl competent cells. Quarters sections are ideal for high-throughput screening using multiple strain genotypes for optimization of target protein expression. The BL21(DE3) expression strain and the NovaBlue cloning host are available as HT96™ configurations, which contain 20-µl volumes of competent cells per well in an automation-compatible, 96-well format. For other HT96 configurations or other special packaging needs, contact our Bulk Department.

## Host Features Determining Vector Compatibility

| Host Strain            | Extrachromosomal Replicon(s) in Host | Host Drug Resistance(s) |
|------------------------|--------------------------------------|-------------------------|
| pLysS-containing cells | P15A                                 | Cam                     |
| pLacI-containing cells | P15A                                 | Cam                     |
| Rosetta™               | P15A                                 | Cam                     |
| Rosetta 2              | P15A                                 | Cam                     |
| Origami™ 2             | F                                    | Tet + Str*              |
| Rosetta-gami™ 2        | P15A + F                             | Cam + Tet + Str*        |
| Rosetta-gami           | P15A + F                             | Cam + Kan + Tet+ Str*   |
| BL21                   | none                                 | none                    |
| NovaBlue               | F                                    | Tet                     |
| Origami B              | none                                 | Kan + Tet               |
| RosettaBlue™           | P15A + F                             | Cam + Tet               |
| Rosetta-gami B         | P15A                                 | Cam + Kan + Tet         |
| Tuner™                 | none                                 | none                    |
| BLR                    | none                                 | Tet                     |
| HMS174                 | none                                 | Rif                     |

\* These strains carry a mutation in ribosomal protein (rspl) conferring resistance to streptomycin; however, streptomycin is not necessary to maintain strain genotype.

## Competent Cell Kit Configurations

| Kit Component     | Standard Kits |            | Singles™   |            | Quarters   | QuarterPack™ Array | HT96™      |                  | Electrocompetent Cells |            |
|-------------------|---------------|------------|------------|------------|------------|--------------------|------------|------------------|------------------------|------------|
|                   | 0.4 ml        | 1 ml       | 11 rxn     | 22 rxn     |            |                    | 1 plate    | 4 plates         | 10 rxn                 | 20 rxn     |
| Competent Cells   | 2 × 0.2 ml    | 5 × 0.2 ml | 11 × 50 µl | 22 × 50 µl | 24 × 20 µl | 4 × (24 × 20 µl)   | 96 × 20 µl | 4 × (96 × 20 µl) | 5 × 50 µl              | 10 × 50 µl |
| Test Plasmid      | 10 µl         | 10 µl      | 10 µl      | 10 µl      | 10 µl      | 10 µl              | 10 µl      | 2 × 10 µl        | 10 µl                  | 10 µl      |
| SOC Medium        | 2 × 2 ml      | 4 × 2 ml   | 2 × 2 ml   | 4 × 2 ml   | 2 × 2 ml   | 14 ml              | 14 ml      | 4 × 14 ml        |                        |            |
| 8-cap Strip       |               |            |            |            | pkg/12     | pkg/12             | pkg/12     | 4 × (pkg/12)     |                        |            |
| Reagent Reservoir |               |            |            |            | 1          | 1                  | 1          | 4                |                        |            |
| HT96 Lids         |               |            |            |            |            |                    | 1          | 4                |                        |            |

## Competent Cell Selection

| Protein Expression Strains | Subtype    | Singles<br>11 reactions | Singles<br>22 reactions | Standard<br>0.4 ml | Standard<br>1.0 ml | Quarters™<br>24 reactions |
|----------------------------|------------|-------------------------|-------------------------|--------------------|--------------------|---------------------------|
| B834                       | (DE3)      |                         |                         | 69041-3            | 69041-4            |                           |
|                            | (DE3)pLysS |                         |                         | 69042-3            | 69042-4            |                           |
| BL21*                      | (DE3)      | 70235-3                 | 70235-4                 | 69449-3            | 69449-4            | 71158-3                   |
|                            | (DE3)pLysS | 70236-3                 | 70236-4                 | 69450-3            | 69450-4            | 71159-3                   |
|                            |            |                         |                         | 69451-3            | 69451-4            | 71160-3                   |
| BLR                        | (DE3)      |                         |                         | 69052-3            | 69052-4            |                           |
|                            | (DE3)pLysS |                         |                         | 69053-3            | 69053-4            |                           |
|                            |            |                         |                         | 69956-3            | 69956-4            |                           |
| HMS174                     | (DE3)      |                         |                         | 69452-3            | 69452-4            |                           |
|                            | (DE3)pLysS |                         |                         | 69453-3            | 69453-4            |                           |
|                            |            |                         |                         | 69454-3            | 69454-4            |                           |
| Origami™ 2                 | (DE3)      | 71408-3                 | 71408-4                 | 71344-3            | 71344-4            |                           |
|                            | (DE3)pLysS | 71409-3                 | 71409-4                 | 71345-3            | 71345-4            |                           |
|                            |            |                         |                         | 71346-3            | 71346-4            |                           |
| Origami B                  | (DE3)      |                         |                         | 70836-3            | 70836-4            | 71162-3                   |
|                            | (DE3)pLysS |                         |                         | 70837-3            | 70837-4            | 71163-3                   |
|                            |            |                         |                         | 70839-3            | 70839-4            | 71164-3                   |
| Rosetta™                   | (DE3)      |                         |                         | 70953-3            | 70953-4            | 71166-3                   |
|                            | (DE3)pLysS |                         |                         | 70954-3            | 70954-4            | 71167-3                   |
|                            |            |                         |                         | 70956-3            | 70956-4            | 71168-3                   |
| Rosetta 2                  | (DE3)      | 71400-3                 | 71400-4                 | 71402-3            | 71402-4            |                           |
|                            | (DE3)pLysS | 71401-3                 | 71401-4                 | 71397-3            | 71397-4            |                           |
|                            |            |                         |                         | 71403-3            | 71403-4            |                           |
| RosettaBlue™               | (DE3)      |                         |                         | 71058-3            | 71058-4            |                           |
|                            | (DE3)pLysS |                         |                         | 71059-3            | 71059-4            |                           |
|                            |            |                         |                         | 71034-3            | 71034-4            |                           |
| Rosetta-gami™ 2            | (DE3)      |                         |                         | 71350-3            | 71350-4            |                           |
|                            | (DE3)pLysS |                         |                         | 71351-3            | 71351-4            |                           |
|                            |            |                         |                         | 71352-3            | 71352-4            |                           |
| Rosetta-gami B             | (DE3)      |                         |                         | 71135-3            | 71135-4            | 71170-3                   |
|                            | (DE3)pLysS |                         |                         | 71136-3            | 71136-4            | 71171-3                   |
|                            |            |                         |                         | 71137-3            | 71137-4            | 71172-3                   |
| Tuner™                     | (DE3)      |                         |                         | 70622-3            | 70622-4            |                           |
|                            | (DE3)pLysS |                         |                         | 70623-3            | 70623-4            |                           |
|                            |            |                         |                         | 70624-3            | 70624-4            |                           |

\*Also available – HT96, 1 Plate: 71012-3; HT96, 4 Plates: 71012-4



Need a larger package size?

Competent cells, antibiotics, and Overnight Express™ autoinduction systems can be custom packaged to suit your needs.

E-mail us today:  
customer.service@merckbio.com

| Cloning Strain           | Singles<br>11 rxn | Singles<br>22 rxn | GigaSingles™<br>11 rxn                                         | GigaSingles<br>22 rxn | Standard<br>0.4 ml | Standard<br>1.0 ml | HT96<br>1 plate | HT96<br>4 plates | Electrocompetent<br>10 rxn | Electrocompetent<br>20 rxn |
|--------------------------|-------------------|-------------------|----------------------------------------------------------------|-----------------------|--------------------|--------------------|-----------------|------------------|----------------------------|----------------------------|
| NovaBlue                 | 70181-3           | 70181-4           | 71227-3                                                        | 71227-4               | 69825-3            | 69825-4            | 71011-3         | 71011-4          |                            |                            |
| NovaXG                   |                   |                   |                                                                |                       |                    |                    |                 |                  | 71315-3                    | 71315-4                    |
| NovaXGF'                 |                   |                   |                                                                |                       |                    |                    |                 |                  | 71317-3                    | 71317-4                    |
| NovaBlue T1 <sup>R</sup> | 71318-3           | 71318-4           |                                                                |                       |                    |                    |                 |                  |                            |                            |
| Veggie™ NovaBlue         | 71251-3           | 71251-4           | Veggie NovaBlue Singles 11 rxn; Veggie NovaBlue Singles 22 rxn |                       |                    |                    |                 |                  |                            |                            |

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