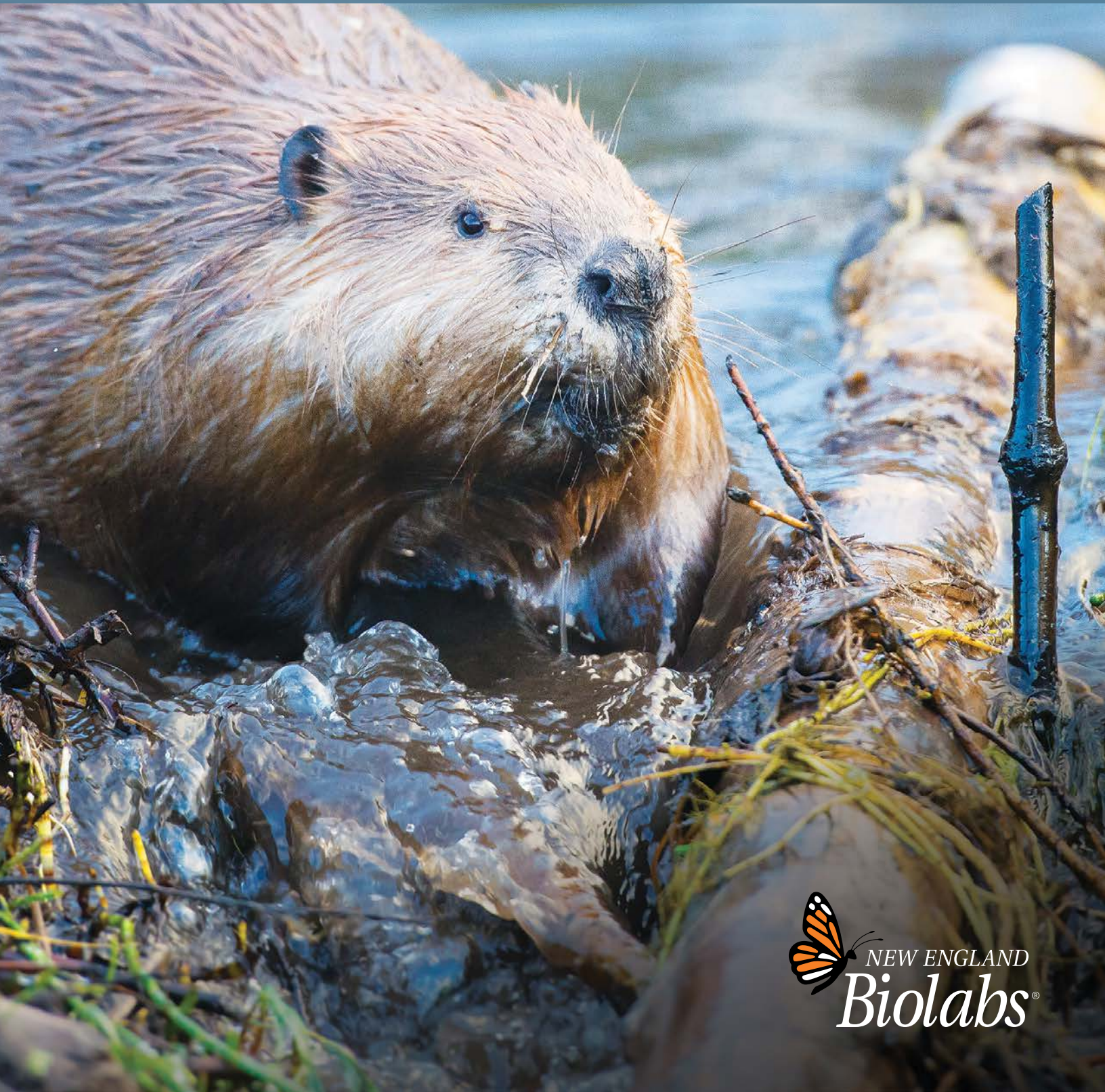


 Now includes Authenticase®

# DNA Assembly & Synthetic Biology

TOOLS FOR DESIGN, ASSEMBLY, MANIPULATION, AND EXPRESSION



 NEW ENGLAND  
**Biolabs**®





# DNA Assembly & Synthetic Biology – Tools to support DNA design, assembly, manipulation, and expression

The goal of synthetic biology, in which genes and proteins are viewed as parts or devices, is redesigning and/or assembling them in novel ways to create a new and useful functionality. These projects often rely on the ordered assembly of multiple DNA sequences to create large, artificial DNA structures, and methods have evolved to simplify this process.

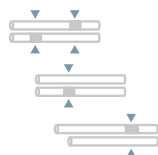
New England Biolabs offers many products and tools to support synthetic biology and protein engineering workflows, including those utilizing automation for higher throughput.

Find more information on the following:



## DNA Assembly

---



## Error-Correction

---



## Site-Directed Mutagenesis

---



## Genome Editing

---



## Amplification

---

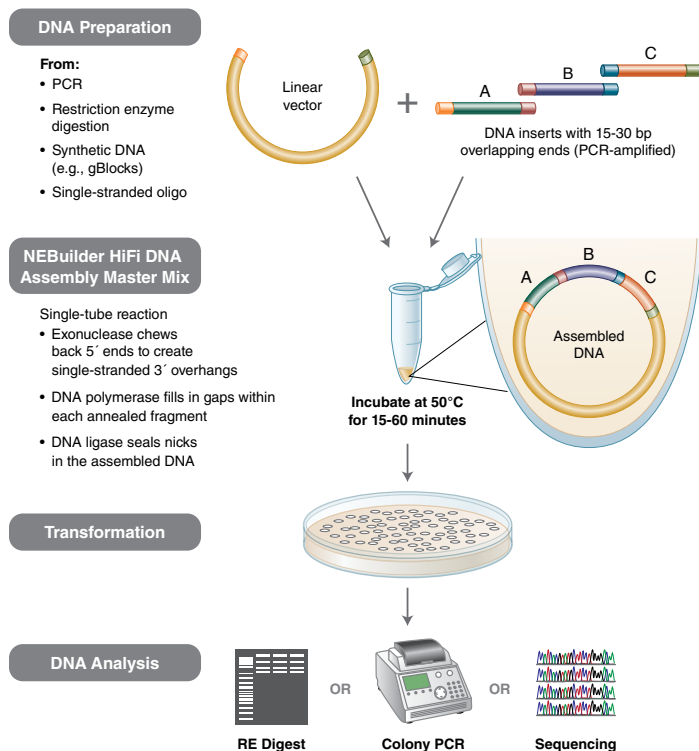


## Cell-free Protein Expression

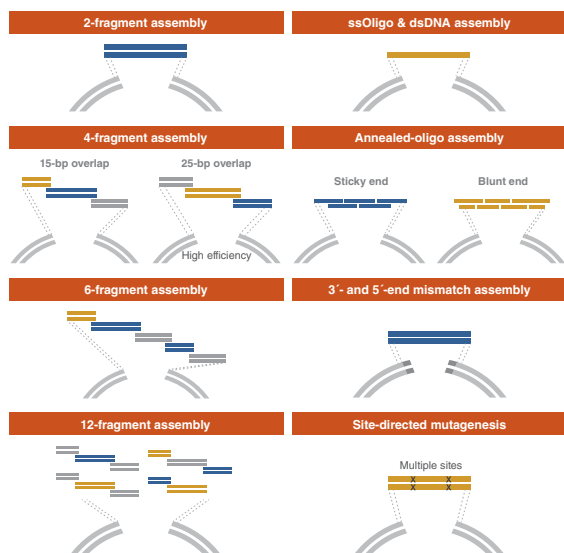
# NEBuilder<sup>®</sup> HiFi DNA Assembly

NEBuilder HiFi DNA Assembly enables virtually error-free joining of DNA fragments, even those with 5'- and 3'-end mismatches. Available with and without competent *E. coli*, this flexible kit enables simple and fast seamless cloning utilizing a proprietary high-fidelity polymerase. Make NEBuilder HiFi your first choice for DNA assembly and cloning.

## Overview of the NEBuilder HiFi DNA Assembly cloning method



NEBuilder HiFi DNA Assembly can be used for a variety of DNA assembly methods.



## RECOMMENDED PRODUCTS

**NEBuilder HiFi DNA Assembly Cloning Kit**  
(NEB #E5520)

**NEBuilder HiFi DNA Assembly Master Mix**  
(NEB #E2621)

**NEBuilder HiFi DNA Assembly Bundle for Large Fragments**  
(NEB #E2623)

- Simple and fast seamless cloning in as little as 15 minutes
- Use one system for both "standard-size" cloning and larger gene assembly products (up to 12 fragments and 22 kb)
- DNA can be used immediately for transformation or as template for PCR or RCA
- Adapts easily for multiple DNA manipulations, including site-directed mutagenesis
- Enjoy less screening/re-sequencing of constructs, with virtually error-free, high-fidelity assembly
- Use NEBuilder HiFi in successive rounds of assembly, as it removes 5'- and 3'-end mismatches
- Bridge two ds-fragments with a synthetic ssDNA oligo for simple and fast construction (e.g., linker insertion or gRNA library)
- No PCR clean-up step required
- No licensing fee requirements from NEB for NEBuilder products

## TOOLS & RESOURCES

Visit [NEBuilderHiFi.com](https://NEBuilderHiFi.com) to find:

- Online tutorials to help with assembly and primer design
- Application notes utilizing NEBuilder HiFi
- Comparisons against In-Fusion<sup>®</sup> Snap Assembly and GeneArt<sup>®</sup> Gibson Assembly<sup>®</sup>



## NEBuilder Assembly Tool

Get started designing primers at [NEBuilder.neb.com](https://NEBuilder.neb.com)



## NEBuilder Protocol Calculator

Generate a custom protocol at [NEBuilderCalculator.neb.com](https://NEBuilderCalculator.neb.com)

DOWNLOAD THE NEB AR APP\*

How does  
NEBuilder HiFi DNA  
Assembly work?



\*see back cover for details

# Golden Gate Assembly

The efficient and seamless assembly of DNA fragments, commonly referred to as Golden Gate assembly (1,2) refers to multiple inserts being assembled into a vector backbone using only the sequential (3) or simultaneous (4) activities of a single Type IIS restriction enzyme and T4 DNA Ligase. Golden Gate has enabled single inserts, the cloning of inserts from diverse populations enabling library creation, and multi-module assemblies. NEB has made extraordinary improvements that touch every application of the Golden Gate technology.

## Type IIS Restriction Enzymes for Golden Gate Assembly

NEB offers more Type IIS (i.e., recognize asymmetric DNA sequences and cleave outside of their recognition sequence) restriction enzymes than any other supplier, many of which are used in Golden Gate Assembly. These enzymes, along with our extensive data on ligase fidelity, allows complex **50+ fragment assemblies** with high efficiency, >95% accuracy, and low backgrounds.

PRODUCT	NEB #	PRODUCT	NEB #
BbsI	R0539	BtgZI	R0703
BbsI-HF®	R3539	Esp3I	R0734
BsaI-HF®v2	R3733	PaqCI®	R0745
BsmBI-v2	R0739	SapI	R0569
BspQI	R0712		

## RECOMMENDED PRODUCTS

### NEBridge® Golden Gate Assembly Kits (BsaI-HFv2 or BsmBI-v2) (NEB #E1601, NEB #E1602)

- Seamless cloning – no scar remains following assembly
- Includes destination plasmid with T7/SP6 promoters
- Ordered assembly of multiple fragments (2-50+) in a single reaction
- Can also be used for cloning single inserts and library preparations
- Efficient with regions with high GC content and areas of repeats
- Compatible with a broad range of fragment sizes (< 100 bp to > 15 kb)

### NEBridge Ligase Master Mix (NEB #M1100)

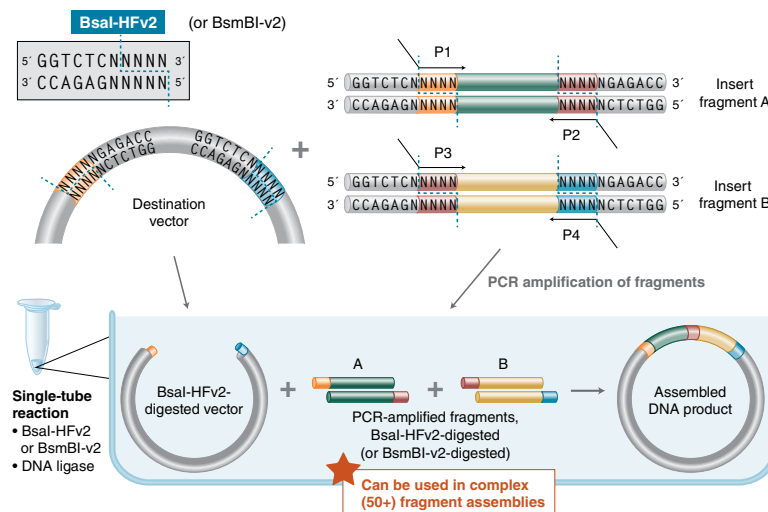
- Optimized for efficient and accurate Golden Gate Assembly
- Convenient 3X Master Mix format
- Flexibility to use with NEB Type IIS restriction enzymes

## TOOLS & RESOURCES

Visit [www.neb.com/GoldenGate](http://www.neb.com/GoldenGate) to find:

- Publications and protocols related to ligase fidelity and Golden Gate Assembly
- Access to the **NEBridge Ligase Fidelity Tools** to facilitate the design of high-fidelity Golden Gate Assemblies
- View our webinar: Listen to DAD when constructing high-complexity Golden Gate Assembly Targets
- View our *MoClo Overhang Standards Usage Guidelines* and our tutorial, *Domestication and Golden Gate Assembly*

## Golden Gate Assembly Workflow for complex assemblies



In its simplest form, Golden Gate Assembly requires a Type IIS recognition site, in this case, BsaI-HFv2 (GGTCTC), or BsmBI-v2 (CGTCTC) added to both ends of a dsDNA fragment. After digestion, these sites are left behind, with each fragment bearing the designed 4-base overhangs that direct the assembly.

## References:

1. Engler, C. et al. (2008) *PLoS ONE*, 3: e3647.
2. Engler, C. et al. (2009) *PLoS ONE*, 4: e5553.
3. Lee, J.H. et al. (1996) *Genetic Analysis: Biomolecular Engineering*, 13: 139-145.
4. Padgett, K.A. and Sorge, J.A. (1996) *Gene*, 168, 31-35.



## NEBridge Golden Gate Assembly Tool

Get started designing your experiments and primers at [GoldenGate.neb.com](http://GoldenGate.neb.com)

How does  
Golden Gate  
Assembly work?



# NEBridge® Ligase Fidelity Tools

## Enabling the design of high-fidelity Golden Gate Assemblies

Research at NEB has led to increased understanding of ligase fidelity, including the development of a comprehensive method for profiling end-joining ligation fidelity to predict which overhangs have improved fidelity (5). This research led to Data-optimized Assembly Design (DAD) which allows careful choice of overhang sets and is especially important for achieving complex and high-fidelity Golden Gate Assemblies.

The NEBridge Ligase Fidelity tools utilize DAD to enable the design of new assemblies, including complex high fragment number assemblies, and the evaluation, modification, or expansion of existing assemblies, and more.



View our growing list of publications and learn more here:  
[www.neb.com/ligasefidelity](http://www.neb.com/ligasefidelity)

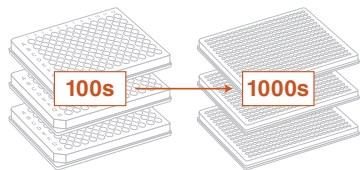


**Featured publication:** Sikkema A.P. et al. (2023)  
High-Complexity One-pot Golden Gate Assembly. *Current Protocols*.  
<https://doi.org/10.1002/cpz1.882>

Find detailed protocols for applying NEB Data-optimized Assembly Design (DAD) and ligase fidelity data to Golden Gate Assembly design using the NEBridge Ligase Fidelity Tools.

## High-throughput Cloning and DNA Assembly

High-throughput cloning is a molecular biology method of assembling large numbers of DNA sequences, such as genes, open reading frames (ORFs) or highly repetitive gRNAs, to create libraries and enable screening of constructs, protein expression or protein function. With the integration and adoption of automation, researchers can scale up and increase throughput to hundreds or thousands of reactions, save time and money with rapid workflows and miniaturized volumes, and improve reproducibility with automated complex mixing that reduces manual errors.



NEBuilder® HiFi DNA Assembly and NEBridge® Golden Gate Assembly are leading the way in the next generation of cloning. Both methods are amenable to high-throughput workflows and scale up using automation platforms such as the Echo® 525 Liquid Handler from Labcyte®, Inc., the Opentrons OT-2 liquid handler, and the mosquito® LV from SPT Labtech. These tools enable the rapid interrogation of a more expansive set of custom designs. High efficiency and accuracy (>95%) with both cloning methods ensures you can confidently and quickly progress with accurate constructs.

Please refer to the DNA Assembly Selection Chart (page 11) for additional help determining which product is best suited for your needs.

Learn more here: [neb.com/automatewithconfidence](http://neb.com/automatewithconfidence)

Try our suite of free, online tools at [ligasefidelity.neb.com](http://ligasefidelity.neb.com)



### NEBridge Ligase Fidelity Viewer®

Evaluate existing assemblies and visualize overhang ligation preferences



### NEBridge GetSet®

Predict high-fidelity overhang sets for new assemblies and expand existing assemblies



### NEBridge SplitSet®

Split a DNA sequence into multiple fragments for scarless high-fidelity assembly

## RECOMMENDED PRODUCTS

### NEBuilder HiFi DNA Assembly Master Mix (NEB #E2621)

- Perform less sequencing and screening of constructs with high-fidelity, virtually error-free assembly
- Enjoy compatibility with synthetic dsDNA fragments, such as gBlocks™, and ssDNA oligos
- Save time by avoiding PCR clean-up, simplifying your workflow
- Supports miniaturization with nanoliter scale volumes

### NEBridge Golden Gate Assembly Kits (NEB #E1601, NEB #E1602)

- Experience high efficiency within regions of high GC content and areas of repeats
- Supports miniaturization
- Enjoy compatibility with synthetic dsDNA fragments, such as gBlocks
- Find flexibility with **NEBridge Ligase Master Mix (NEB #M1100)** and your choice of Type IIS restriction enzymes
- Design complex, high-fidelity Golden Gate Assemblies easily with our online tools

#### References:

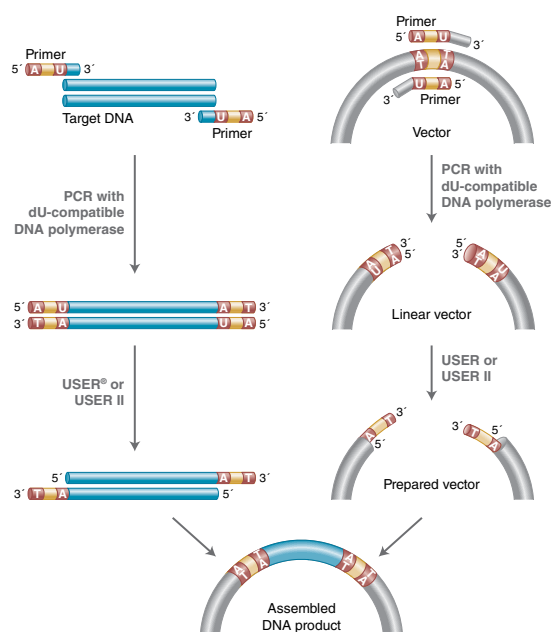
(5) Potapov, V. et al. (2018) *ACS Synth. Biol.* DOI: 10.1021/acssynbio.8b00333.



# USER® Enzyme

The USER-friendly DNA engineering method enables multiple PCR fragment assembly, nucleotide sequence alteration and directional cloning. Target DNA molecules and cloning vector are generated by PCR with 6-10 bases of homology between the neighboring fragments. PCR primers contain a single deoxyuracil residue (dU) flanking the 3' end of the homology region, and can accommodate nucleotide substitutions, insertions and/or deletions. The primers are then used to amplify the vector and target DNA with discrete overlapping fragments that incorporate a dU at each end. Subsequent treatment of PCR fragments with USER Enzyme creates a single nucleotide gap at each dU, resulting in PCR fragments flanked with ss-extensions that allow seamless and directional assembly of customized DNA molecules into a linearized vector. Multi-fragment assemblies and/or various mutagenic changes can be performed in a single experiment.

## DNA assembly with USER Enzyme or Thermolabile USER II Enzyme



# Gibson Assembly®

Gibson Assembly enables multiple, overlapping DNA fragments to be joined in a single-tube isothermal reaction, with no additional sequence added (scarless). The Gibson Assembly Master Mix includes three different enzymatic activities that perform in a single buffer. The assembled, fully-sealed construct is then transformed into NEB 5-alpha competent *E. coli*. The entire protocol, from assembly to transformation, takes just under two hours.

## RECOMMENDED PRODUCTS

### USER Enzyme (NEB #M5505)

### Thermolabile USER II Enzyme (NEB #M5508)

- Seamless and directional assembly
- Multiple fragment assemblies and/or mutations can be performed in a single experiment
- USER assembly is performed at 37°C or room temperature (no need for thermocycler)
- USER method can be used for assembly of small fragments (< 100 bp) or oligo duplexes and for sequences with end repeats.

### Q5U® Hot Start High-Fidelity DNA Polymerase (NEB #M0515)

- Modified version of Q5 High-Fidelity Polymerase that possesses 3' → 5' exonuclease activity
- Enables the ability to read and amplify templates containing uracil and inosine bases

## RECOMMENDED PRODUCTS

### Gibson Assembly Cloning Kit (NEB #E5510)

### Gibson Assembly Master Mix (NEB #E2611)

- Visit [NEBGibson.com](http://NEBGibson.com) to learn more

# Synthetic Biology/DNA Assembly Selection Chart

	NEBuilder HiFi DNA Assembly NEB #E2621 NEB #E5520 NEB #E2623	NEB Gibson Assembly NEB #E2611 NEB #E5510	NEBridge Golden Gate Assembly Kits (BsaI-HFv2/BsmBI-v2) NEB #E1601 NEB #E1602  NEBridge Ligase Master Mix NEB #M1100	USER Enzyme NEB #M5505  Thermolabile USER II Enzyme NEB #M5508
<b>Properties</b>				
Removes 5' or 3' End Mismatches	★★★	★	N/A	N/A
Assembles with High Fidelity at Junctions	★★★	★★	★★★	★★★
Tolerates Repetitive Sequences at Ends	★	★	★★★	★★★
Generates Fully Ligated Product	★★★	★★★	★★★	NR
Joins dsDNA with Single-stranded Oligo	★★★	★★	NR	NR
Assembles Low Amounts of DNA with High Efficiency	★★★	★★	★★	★★
Accommodates Flexible Overlap Lengths	★★★	★★★	★	★★
<b>Applications</b>				
2 Fragment Assembly (Simple cloning)	★★★	★★★	★★★	★★★
3-6 Fragment Assembly (one pot)	★★★	★★★	★★★	★★★
7-11 Fragment Assembly (one pot)	★★★	★★	★★★	★★★
12-50+ Fragment Assembly (one pot) <sup>(1)</sup>	★	★	★★★	NR
Template Construction for <i>In vitro</i> Transcription	★★★	★★★	★★★	★★★
Synthetic Whole Genome Assembly	★★★	★	★★★	★
Multiple Site-directed Mutagenesis	★★★	★★	★★	★★
Library Generation	★★★	★★★	★★★	★★
Metabolic Pathway Engineering	★★★	★★	★★★	★★★
TALENs	★★	★★	★★★	★★
Short Hairpin RNA (shRNA) Cloning	★★★	★★	★	★
gRNA Library Generation	★★★	★★	★	★
Large Fragment (> 10 kb) Assembly	★★★	★★★	★★★	★★
Small Fragment (< 100 bp) Assembly	★★★	★	★★★	★★★
Use in Successive Rounds of Restriction Enzyme Assembly	★★★	★	NR	★

<sup>(1)</sup> Please visit [neb.com/GoldenGate](http://neb.com/GoldenGate) for more information

KEY	
★★★	Optimal, recommended product for selected application
★★	Works well for selected application
★	Will perform selected application, but is not recommended
N/A	Not applicable to this application
NR	Not recommended

## Enzymes for Innovation



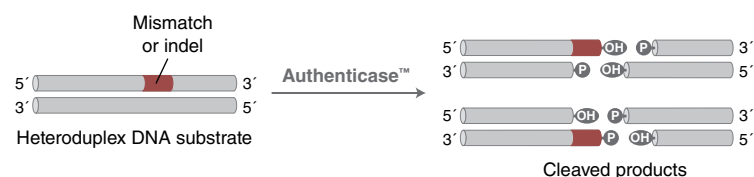
Enzymes for Innovation (EFI) is a unique program initiated by NEB to provide enzymes with interesting activities and unique properties to the scientific community, in hopes of enabling the discovery of new and innovative applications.

Learn more at [www.neb.com/enzymesforinnovation](http://www.neb.com/enzymesforinnovation)

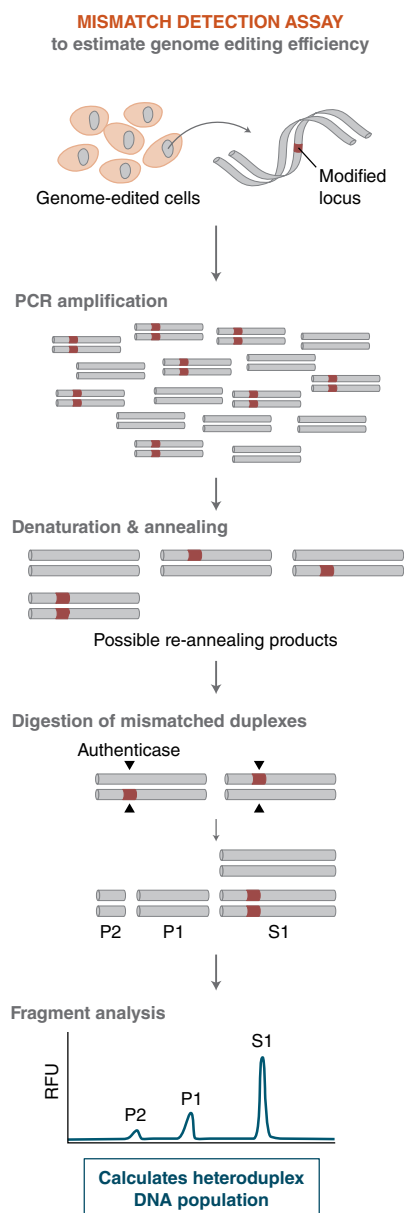
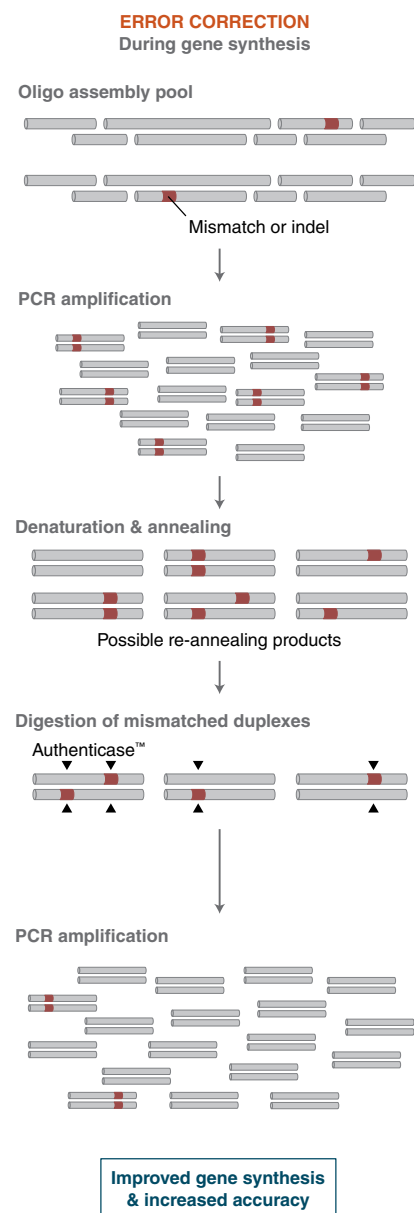
# Authenticase<sup>®</sup>

Authenticase is a proprietary mixture of structure-specific nucleases capable of recognizing and cleaving outside mismatch and indel (insertion and/or deletion) regions, ranging from 1-10 base pairs (bp) on double-stranded DNA. The formulation has limited non-specific activity on homoduplex regions of DNA. Authenticase can be used as an error-correction reagent in oligo-based PCR gene assembly by enzymatically removing “mistakes” prior to the final renaturation and amplification step (i.e., removes mismatch/indel errors caused by oligonucleotide synthesis). Alternatively, Authenticase can replace T7 Endonuclease I in the mismatch detection assay used to assess the efficiency of genome editing.

## Mechanism of Authenticase



## Applications of Authenticase



## RECOMMENDED PRODUCT

### Authenticase (NEB #M0689)

- Recognizes indels (insertions and/or deletions) as well as single base mismatches: C/C, T/C, A/C, T/G, G/G, T/T and A/A
- Applications:
  - Error-correction in oligonucleotide synthesis
  - Mismatch Detection Assay
- Reduces number of colonies that need to be screened and saves time with gene synthesis
- Performs better than T7 Endonuclease I to assess efficiency of gene editing



# Site-Directed Mutagenesis

Reliably make single or multiple point mutations, perform combinatorial mutagenesis, or create mutant libraries in a high-throughput workflow using our site-directed mutagenesis products.

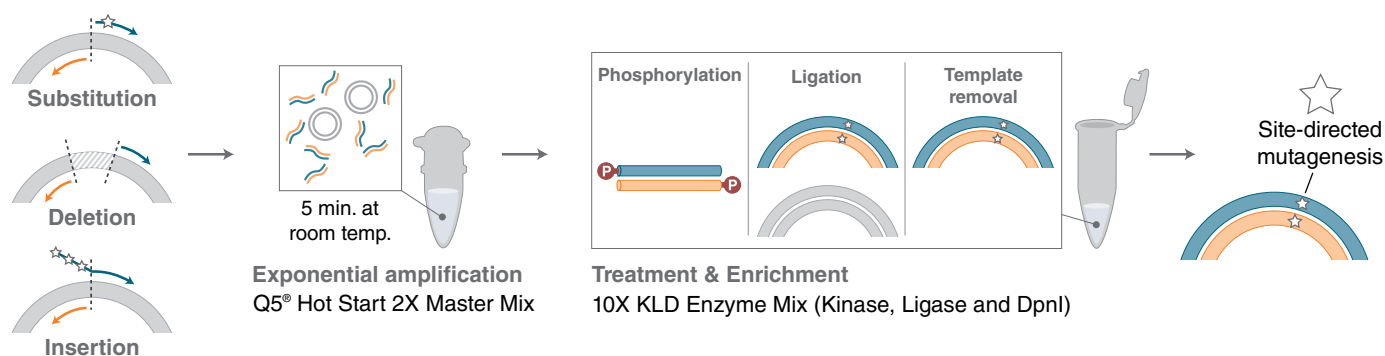
For single point mutations, whether substitutions, insertions, or deletions, we recommend using the **Q5® Site-Directed Mutagenesis Kit** or individual components, **Q5 Hot Start High-Fidelity 2X Master Mix** and **KLD Enzyme Mix**. These master mix formats are compatible with automation and ready to miniaturize for scaling up to high-throughput workflows. **Q5 Hot Start DNA Polymerase** offers the added benefit of room temperature setup and reduces screening of correct mutants due to its high accuracy.

For mutations at multiple sites, or combinatorial mutagenesis, we recommend **NEBuilder HiFi DNA Assembly**.



**NEBaseChanger®**

To begin, use our free online tool to design primers for any of these methods at [nebasechanger.neb.com](https://nebasechanger.neb.com)



## RECOMMENDED PRODUCTS

**Q5 Site-Directed Mutagenesis Kit (NEB #E0554)**

**Q5 Site-Directed Mutagenesis Kit (Without Competent Cells) (NEB #E0552)**

**Q5 Hot Start High-Fidelity 2X Master Mix (NEB #M0494)**

**KLD Enzyme Mix (NEB #M0554)**

- Generation of substitutions, insertions, or deletions in plasmid DNA
- Non-overlapping primer design ensures robust, exponential amplification and generates a high % of desired mutations from a wide range of templates

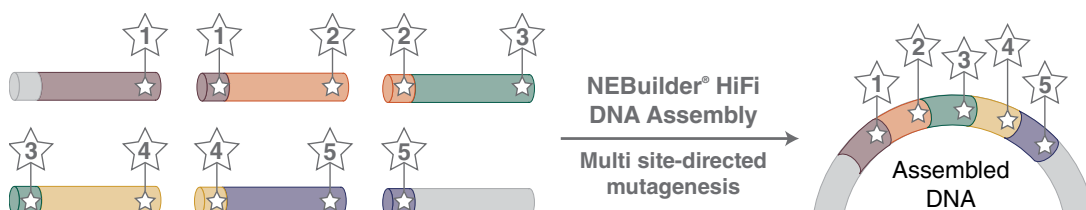
## Multi-site mutagenesis

Using **NEBuilder HiFi DNA Assembly (NEB #E2621)**, perform multi-site mutagenesis or combinatorial mutagenesis for diverse multi-site mutant library creation and screening.

## RECOMMENDED PRODUCT

**NEBuilder HiFi DNA Assembly (NEB #E2621)**

- Create multiple mutations at the same time in a single reaction
- Design primers with NEBaseChanger



# CRISPR/Cas Genome Editing

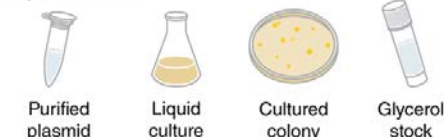
The simplicity of the CRISPR nuclease system (nuclease and guide RNA), makes this system attractive for laboratory use. Breaks activate repair through error prone Non-Homologous End Joining (NHEJ) or Homology Directed Repair (HDR). In the presence of a donor template with homology to the targeted locus, the HDR pathway may operate, allowing for precise mutations to be made. In the absence of a template, NHEJ is activated, resulting in insertions and/or deletions (indels), which disrupt the target locus (1,2).

New England Biolabs provides reagents to support a broad variety of CRISPR/Cas genome editing approaches. From introduction of Cas nucleases and single guide RNA (sgRNA) on plasmids, to direct introduction of Cas nuclease ribonucleoprotein (RNP) and detection of edits using next generation sequencing or enzymatic mutation detection, NEB provides reagents that simplify and shorten genome editing workflows.

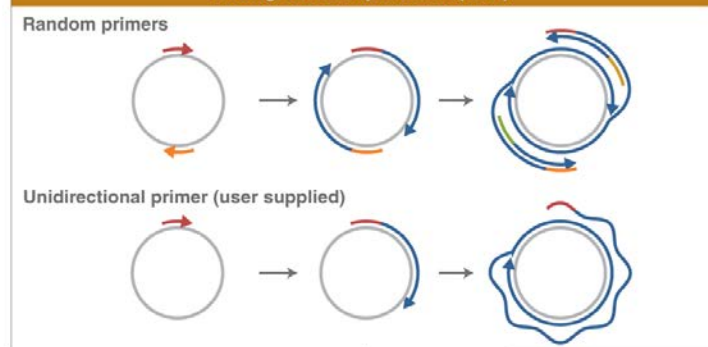
## phi29-XT RCA Kit

This new kit includes everything you need for rolling circle amplification (RCA) and features phi29-XT DNA Polymerase, an engineered polymerase with improved thermostability and sensitivity. The kit delivers high yields of DNA products from a variety of starting materials including purified circular DNA or bacterial cells and enables multiple downstream applications without further processing.

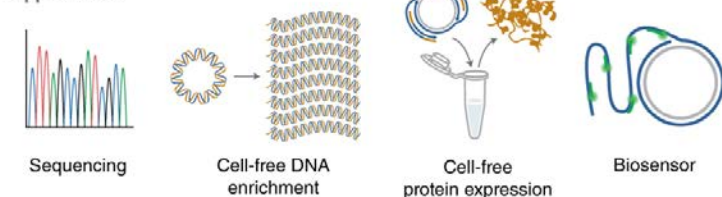
### Input materials



### Rolling Circle Amplification (RCA)



### Applications



### References:

1. Overbye-Petersen, S., et al. (2013) *Proc. Natl. Acad. Sci. U.S.A.*, 110, 19860–19865.
2. Gong, C., et al. (2005) *Nat. Struct. Mol. Biol.*, 12, 304–312.

### RECOMMENDED PRODUCTS

**EnGen® Spy Cas9 HF1**  
(NEB #M0667)

**EnGen® Spy Cas9 NLS**  
(NEB #M0646)

**EnGen Spy Cas9 Nickase**  
(NEB #M0650)

**EnGen Spy dCas9 (SNAP-tag®)**  
(NEB #M0652)

**EnGen Lba Cas12a (Cpf1)**  
(NEB #M0653)

**EnGen Sau Cas9**  
(NEB #M0654)

**Cas9 Nuclease, *S. pyogenes***  
(NEB #M0386)

**EnGen sgRNA Synthesis Kit, *S. pyogenes***  
(NEB #E3322)

**EnGen Mutation Detection Kit**  
(NEB #E3321)

### TOOLS & RESOURCES

Visit [www.neb.com/GenomeEditing](http://www.neb.com/GenomeEditing) to find:

- Up-to-date listing of products and protocols to support this application
- Tips for planning your Cas9 experiment
- Strategies for sgRNA template construction for Cas9 gene editing
- Protocols for measuring targeting efficiency with the T7 Endonuclease I Assay

### RECOMMENDED PRODUCT

**phi29-XT RCA Kit (NEB #E1603)**

- Generates high product yield in a short reaction time with improved thermostability and sensitivity
- Amplify from as little as 1 fg of circular DNA input
- Flexible protocol offers compatibility with different types of sample input material
- Kit includes dNTPs and exonuclease-resistant random primers

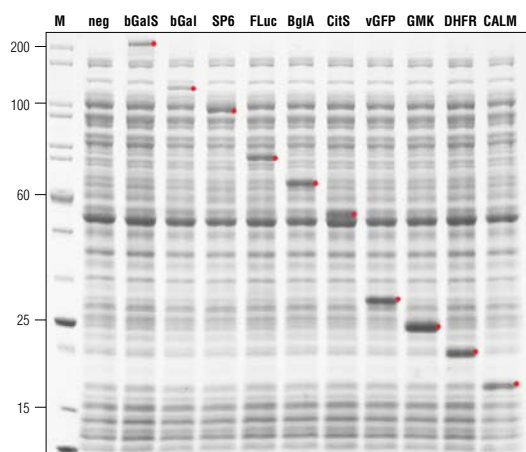
# Cell-free Protein Expression

## NEBExpress® Cell-free *E. coli* Protein Synthesis System

The NEBExpress Cell-free *E. coli* Protein Synthesis System is a coupled transcription/translation system designed to synthesize proteins encoded by a DNA or mRNA template under the control of a T7 RNA Polymerase promoter. The system offers high expression levels, the ability to produce high molecular weight proteins, scalability, and is cost effective for high-throughput expression applications. The speed and robustness of the system facilitates protein synthesis in applications such as protein engineering, mutagenesis studies and enzyme screening.

The NEBExpress Cell-free *E. coli* Protein Synthesis System is a bacterial extract-based cell-free expression system containing all the components required for protein synthesis, only requiring the user add target template DNA. It contains a combination of a highly active cell extract from a genetically engineered strain, a reaction buffer, and an optimized T7 RNA Polymerase, which together yield robust expression of a wide variety of protein targets ranging from 17 to 230 kDa.

The NEBExpress Cell-free *E. coli* Protein Synthesis System can be used to express a wide range of proteins



## PURExpress® In Vitro Protein Synthesis Kit

A rapid method for gene expression analysis, PURExpress is a cell-free transcription/translation system reconstituted from purified components necessary for *E. coli* translation. Synthesize a wide range of proteins free of modification or degradation by simply mixing two tubes followed by adding template DNA. With results available in only a few hours, PURExpress saves valuable laboratory time and is ideal for high throughput technologies. Product selection includes the original kit, with all components in two tubes, as well as options for protein translation experiments, protein synthesis/ribosomal display experiments and synthesis with modified amino acids.

### RECOMMENDED PRODUCTS

#### NEBExpress Cell-free *E. coli* Protein Synthesis System (NEB #E5360)

- Express proteins in a cell-free system, without the need to grow bacteria in traditional expression systems
- Synthesize high yields of protein (typically 0.5 mg/ml)
- Protein can be synthesized and visualized in approximately 2–4 hours
- Synthesize target proteins ranging from 17 to 230 kDa
- Templates can be plasmid DNA, linear DNA, or mRNA
- RNase contamination can be inhibited by the supplied RNase inhibitor, eliminating clean-up steps
- Flexible reaction conditions achieve maximum yield; protein synthesis can be sustained for 10 hours at 37°C or up to 24 hours at lower temperatures
- Reactions can be miniaturized or scaled up to yield milligram quantities of protein

#### PURExpress In Vitro Protein Synthesis Kit (NEB #E6800)

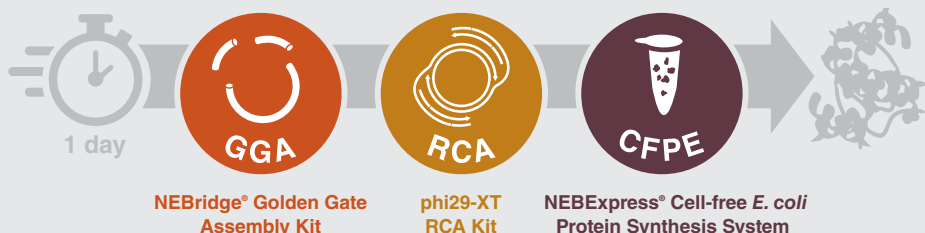
- Minimal nuclease and protease activity for stability of synthesized protein and encoding target
- Visualize synthesized protein directly on a Coomassie-stained gel
- Protein expression in approximately 2 hours
- Templates can be plasmid DNA, linear DNA or mRNA
- Transcription/translation components can be removed by affinity chromatography

Visit [neb.com/proteinexpression](http://neb.com/proteinexpression) to view the entire portfolio of protein expression and purification tools.

## Featured Application Note



Download our application note  
[www.neb.com/appnote-DNAtoProtein](http://www.neb.com/appnote-DNAtoProtein)



Accelerating DNA Construction to Protein Expression:  
A Rapid 1-Day Workflow Using NEBridge® Golden Gate Assembly



# Ordering Information

## DNA Assembly and Amplification

PRODUCT	NEB #	SIZE
NEBuilder HiFi DNA Assembly Cloning Kit	E5520S	10 reactions
NEBuilder HiFi DNA Assembly Master Mix	E2621S/L	10/50 reactions
NEBuilder HiFi DNA Assembly Bundle for Large Fragments	E2623S	20 reactions
Gibson Assembly Cloning Kit	E5510S	10 reactions
Gibson Assembly Master Mix	E2611S/L	10/50 reactions
NEBridge Golden Gate Assembly Kit (Bsal-HFv2)	E1601S/L	20/100 reactions
NEBridge Golden Gate Assembly Kit (BsmBI-v2)	E1602S/L	20/100 reactions
NEBridge Ligase Master Mix	M1100S/L	50/250 reactions
USER Enzyme	M5505S/L	50/250 units
Thermolabile USER II Enzyme	M5508S/L	50/250 units
Q5U Hot Start High-Fidelity DNA Polymerase	M0515S/L	100/500 units
phi29-XT RCA Kit	E1603S/L	100/500 reactions

## Type IIS Restriction Enzymes

PRODUCT	NEB #	SIZE
BbsI	R0539S/L	300/1,500 units
BbsI-HF	R3539S/L	300/1,500 units
BsaI-HFv2	R3733S/L	1,000/5,000 units
BsmBI-v2	R0739S/L	200/1,000 units
BspQI	R0712S/L	500/2,500 units
BtgZI	R0703S/L	100/500 units
Esp3I	R0734S/L	300/1,500 units
PaqCI®	R0745S/L	200/1,000 units
SapI	R0569S/L	250/1,250 units

## Genome Editing Workflows

PRODUCT	NEB #	SIZE
EnGen Spy Cas9 NLS	M0646T/M	400/2,000 pmol
EnGen Mutation Detection Kit	E3321S	25 reactions
EnGen sgRNA Synthesis Kit, <i>S. pyogenes</i>	E3322V/S	10/20 reactions
EnGen Spy Cas9 Nickase	M0650S/T	400/700 pmol
EnGen Spy dCas9 (SNAP-tag®)	M0652S/T	400/700 pmol
EnGen Spy Cas9 HF1	M0667T/M	500/2,500 pmol



Download the **NEB AR App** for iPhone®, iPad® or Android™. Scan the augmented reality butterfly icon located on the corner of the page to find videos, tutorials and immersive experiences.

One or more of these products are covered by patents, trademarks and/or copyrights owned or controlled by New England Biolabs, Inc. The use of trademark symbols does not necessarily indicate that the name is trademarked in the country where it is being read; rather, it indicates where the document was originally developed. For more information, please email us at [busdev@neb.com](mailto:busdev@neb.com). The use of these products may require you to obtain additional third party intellectual property rights for certain applications.

Your purchase, acceptance, and/or payment of and for NEB's products is pursuant to NEB's Terms of Sale at [www.neb.com/support/terms-of-sale](http://www.neb.com/support/terms-of-sale). NEB does not agree to and is not bound by any other terms or conditions, unless those terms and conditions have been expressly agreed to in writing by a duly authorized officer of NEB.

GENEART® is a registered trademark of Life Technologies, Inc. GIBSON ASSEMBLY® is a registered trademark of Synthetic Genomics, Inc. IN-FUSION® is a registered trademark of Clontech Laboratories, Inc. BIOBUILDER® is a registered trademark of BioBuilder, Inc. IPHONE® and IPAD® are registered trademarks of Apple, Inc. ANDROID™ is a trademark of Google, Inc.

© Copyright 2024, New England Biolabs, Inc.; all rights reserved.



## Genome Editing Workflows (continued)

PRODUCT	NEB #	SIZE
EnGen Lba Cas12a (Cpf1)	M0653S/T	400/2,000 pmol
EnGen Sau Cas9	M0654S/T	70/400 pmol
Cas9 Nuclease, <i>S. pyogenes</i>	M0386S/L/M	70/300/600 pmol
HiScribe™ T7 mRNA Kit with CleanCap® Reagent AG	E2080S	20 reactions
HiScribe T7 ARCA mRNA Kit (with or without tailing)	E2060S/ E2065S	20 reactions
HiScribe T7 High Yield RNA Synthesis Kit	E2040S	50 reactions
HiScribe T7 Quick High Yield RNA Synthesis Kit	E2050S	50 reactions
T7 Endonuclease I	M0302S/L	250/1,250 units

## Protein Expression

PRODUCT	NEB #	SIZE
NEBExpress Cell-free <i>E. coli</i> Protein Synthesis System	E5360S/L	10/100 reactions
NEBExpress GamS Nuclease Inhibitor	P0774S	75 µg
PURExpress <i>In Vitro</i> Protein Synthesis Kit	E6800S/L	10/100 reactions
PURExpress Δ Ribosome Kit	E3313S	10 reactions
PURExpress Δ (aa, tRNA) Kit	E6840S	10 reactions
PURExpress Δ RF123 Kit	E6850S	10 reactions
PURExpress Disulfide Bond Enhancer	E6820S	50 reactions
<i>E. coli</i> Ribosome	P0763S	1 mg

## DNA Ligases & Modifying Enzymes

PRODUCT	NEB #	SIZE
<i>Thermos aquaticus</i> (Taq) DNA Ligase	M0208S/L	2,000/10,000 units
T4 DNA Ligase	M0202S/L/T/M	20,000/100,000 units
T7 DNA Ligase	M0318S/L	100,000/750,000 units
Salt-T4® DNA Ligase	M0467S/L	20,000/100,000 units
Hi-T4™ DNA Ligase	M2622S/L	20,000/100,000 units
T5 Exonuclease	M0363S/L	1,000/5,000 units
NEBridge Ligase Master Mix	M1100S/L	50/250 reactions
Authenticase	M0689S/L	25/125 reactions

## Competent Cells

PRODUCT	NEB #	SIZE
NEB 5-alpha Competent <i>E. coli</i> (High Efficiency)	C2987I/P/ H/R/U	6 x 0.2 ml/1 x 96 well/ 20 x 0.05 ml/1 x 384 well/ 96 x 0.05 ml
NEB 10-beta Competent <i>E. coli</i> (High Efficiency)	C3019I/P/H	6 x 0.2 ml/1 x 96 well/ 20 x 0.05 ml
NEB Stable Competent <i>E. coli</i> (High Efficiency)	C3040I/H	6 x 0.2 ml /20 x 0.05 ml

## DNA Ladders

PRODUCT	NEB #	SIZE
Quick-Load® Purple 1 kb Plus DNA Ladder	N0550S/L	250-750 gel lanes
Quick-Load Purple 100 bp DNA Ladder	N0551S/L	125/375 gel lanes
Quick-Load Purple 1 kb DNA Ladder	N0552S/L	125/375 gel lanes
Quick-Load Purple 50 bp DNA Ladder	N0556S	125-250 gel lanes

**NEB supplies reagents, free of charge, to participants in both iGEM and BioBuilder®. Visit [www.neb.com/promoting-science-education](http://www.neb.com/promoting-science-education)**



New England Biolabs, Inc., 240 County Road, Ipswich, MA 01938-2723 Telephone: (978) 927-5054  
Toll Free: (USA Orders) 1-800-632-5227 (USA Tech) 1-800-632-7799 Fax: (978) 921-1350 e-mail: [info@neb.com](mailto:info@neb.com)

**For a complete list of NEB subsidiaries and distributors, please go to:**  
**[www.neb.com/international-support](http://www.neb.com/international-support)**