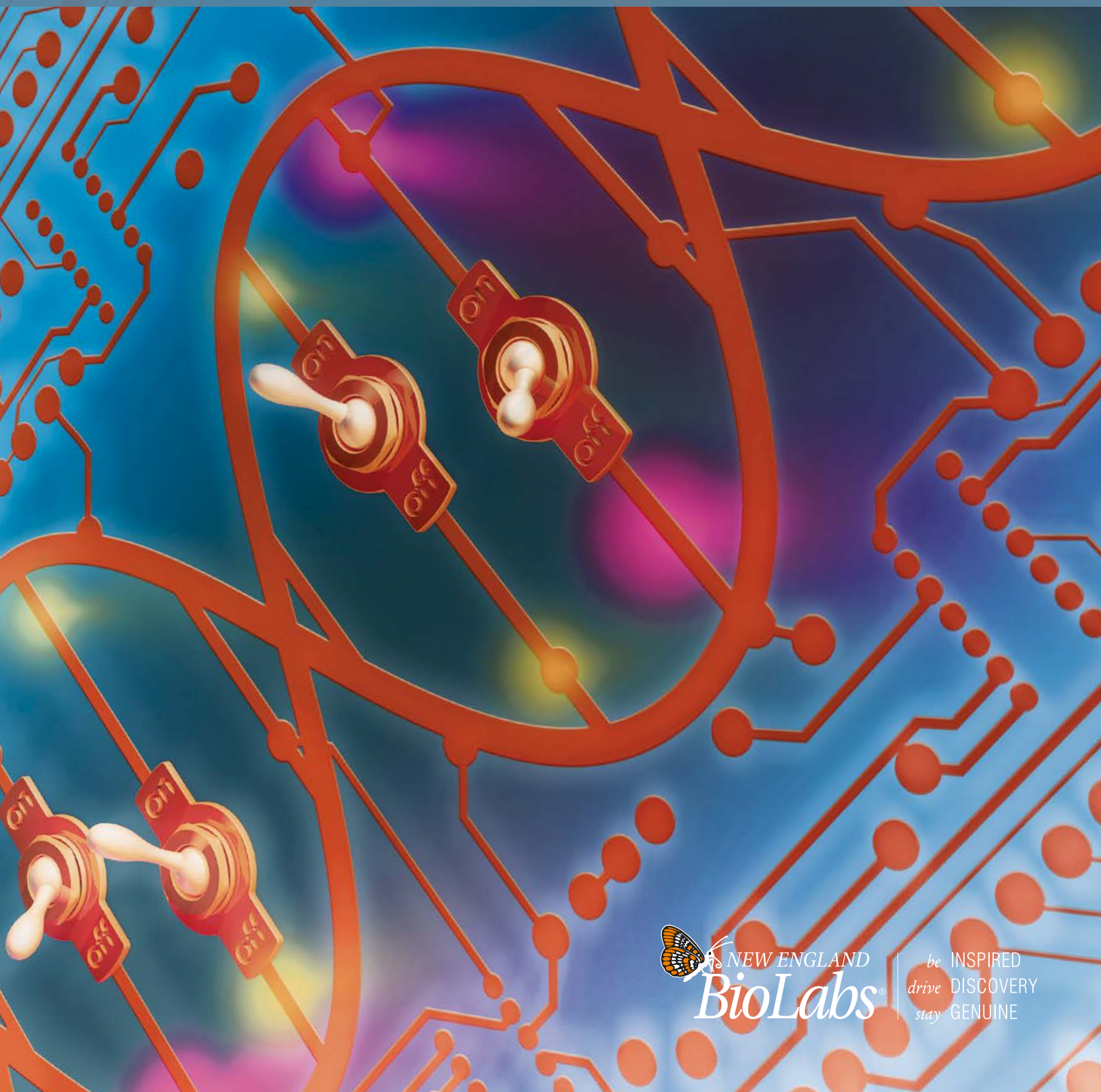
Now includes NEBridge® Ligase Master Mix

DNA Assembly & Synthetic Biology

TOOLS TO SUPPORT DESIGN AND DNA ASSEMBLY



NEW ENGLAND BioLabs®

be INSPIRED
drive DISCOVERY
stay GENUINE

DNA Assembly & Synthetic Biology – Tools to support your design and assembly

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 now offers several products that can be used for DNA assembly and cloning. Use this chart to determine which product would work best to assemble your DNA.

	NEBuilder® HiFi DNA Assembly (NEB #E2621) (NEB #E5520) (NEB #E2623)	**Gibson Assembly®** (NEB #E5510) (NEB #E2611)	**NEB Golden Gate Assembly Kit (BsaI-HF®v2, BsmBI-v2) and NEBridge® Ligase Master Mix*** (NEB #E1601) (NEB #E1602) (NEB #M1100)	**USER® Enzyme** (NEB #M5505) **Thermolabile USER II Enzyme** (NEB #M5508)
PROPERTIES				
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	★★★	★★★	★	★★
APPLICATIONS				
2 Fragment Assembly (simple cloning)	★★★	★★★	★★★	★★★
3-6 Fragment Assembly (one pot)	★★★	★★★	★★★	★★★
7-11 Fragment Assembly (one pot)	★★★	★★	★★★	★★★
12-50+ Fragment Assembly (one pot)(1)	★	★	★★★	NR
Template Construction for *In vitro* Transcription	★★★	★★★	★★★	★★★
Synthetic Whole Genome Assembly	★★★	★	★★★	★
Multiple Site-directed Mutagenesis	★★★	★★	★★	★★
Library Generation	★★★	★★★	★★★	★★
Metabolic Pathway Engineering	★★★	★★	★★★	★★★
TALENs	★★	★★	★★★	★★
Short Hairpin RNA Cloning (shRNA)	★★★	★★	★	★
gRNA Library Generation	★★★	★★	★	★
Large Fragment (> 10 kb) Assembly	★★★	★★★	★★★	★★
Small Fragment (< 100 bp) Assembly	★★★	★	★★★	★★★
Use in Successive Rounds of Restriction Enzyme Assembly	★★★	★	NR	★

KEY

★★★ Optimal, recommended product for selected application

★★ Works well for selected application

★ Will perform selected application, but is not recommended

(1) Please visit **neb.com/GoldenGate** for more information

N/A Not applicable to this application

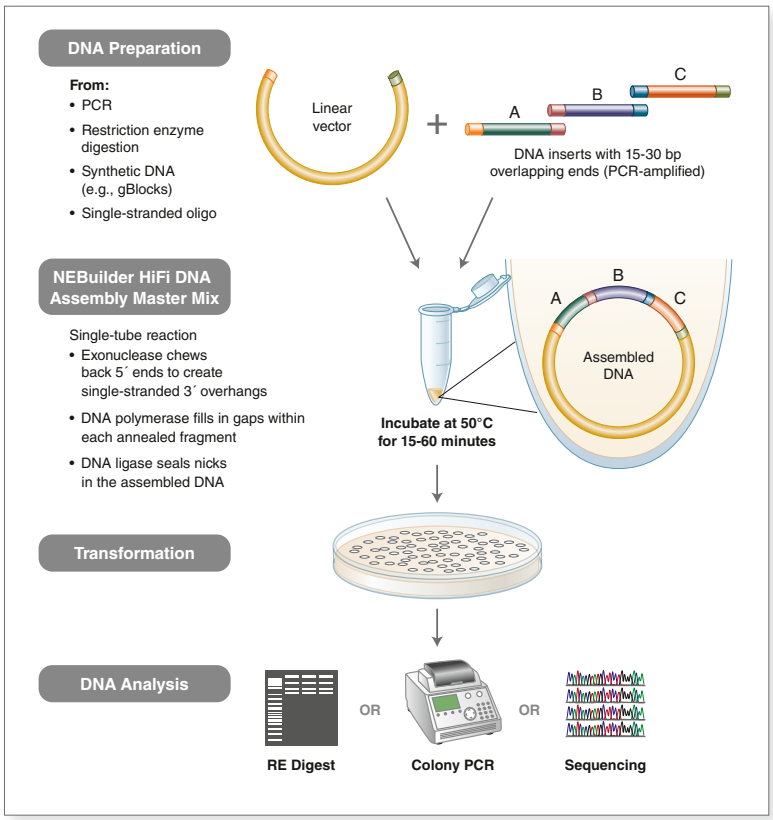
NR Not recommended

NEW
MORE STABLE FORMULATION

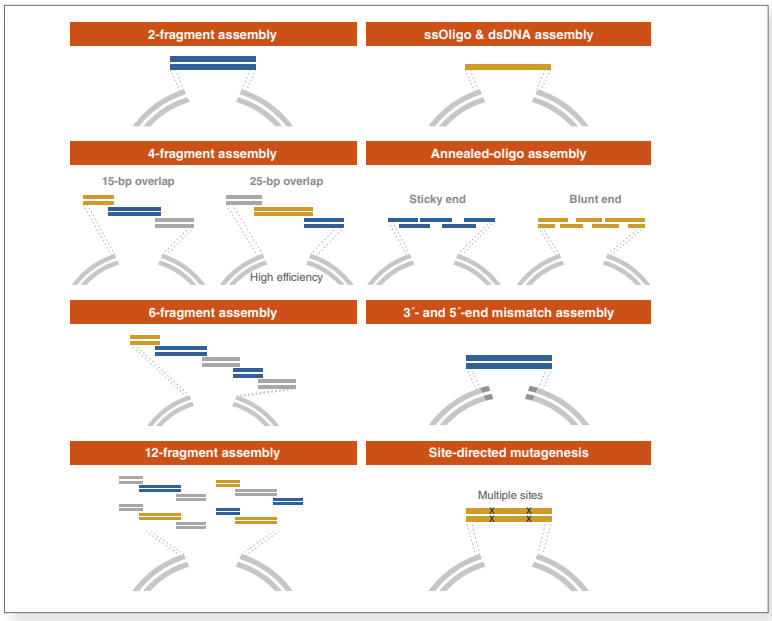
NEBuilder® HiFi DNA Assembly

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 11 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 licensing fee requirements from NEB for NEBuilder products
- NEBuilder HiFi DNA Assembly Cloning Kit includes the NEBuilder HiFi DNA Assembly Master Mix and NEB 5-alpha Competent *E. coli*
- NEBuilder HiFi DNA Assembly Bundle for Large Fragments includes the NEBuilder HiFi DNA Assembly Master Mix and NEB 10-beta Competent *E. coli* for assemblies larger than 15 kb.

TOOLS & RESOURCES

Visit NEBuilderHiFi.com to find:

- Online tutorials to help with assembly and primer design
- Application notes utilizing NEBuilder HiFi
- Access to **NEBuilder Assembly Tool**, our online primer design tool
- Comparisons against In-Fusion® Snap Assembly and GeneArt® Gibson Assembly®

DOWNLOAD THE NEB AR APP*

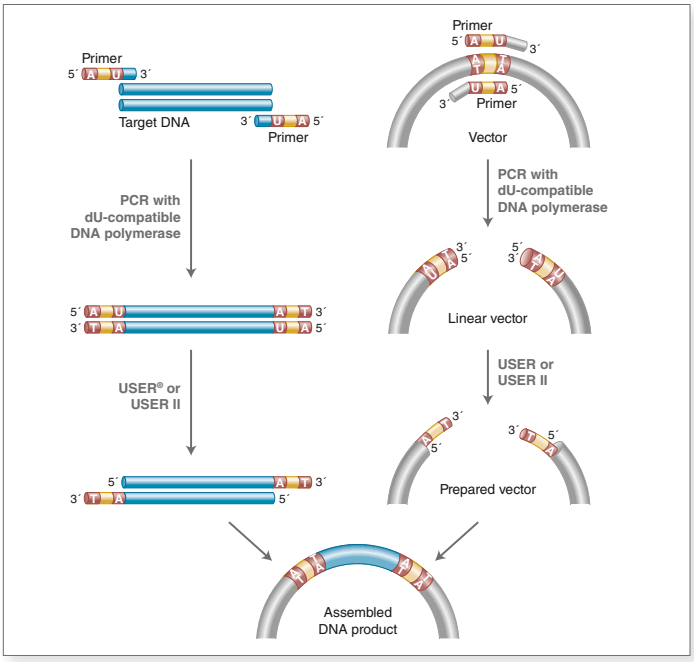
How does NEBuilder HiFi DNA Assembly work?

*see back cover for details

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



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

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**

Golden Gate Assembly

The efficient and seamless assembly of DNA fragments, commonly referred to as Golden Gate assembly (1,2) multiple inserts to be 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.

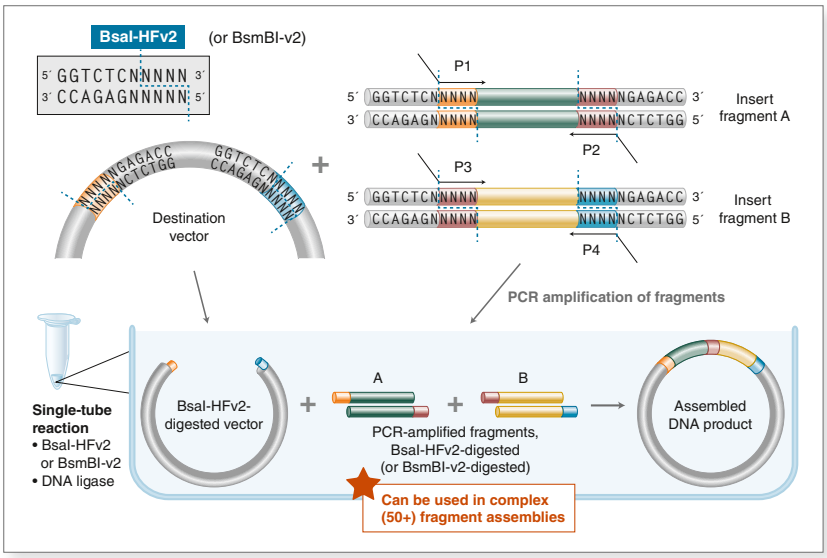
Advances in Ligase Fidelity

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

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 the ligase fidelity data, allows complex **50+ fragment assemblies** with high efficiency and low backgrounds.

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.

RECOMMENDED PRODUCTS

NEB 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 of 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
- Use with NEB Type IIS restriction enzymes

Type IIS Enzymes used in Golden Gate

- BbsI (NEB #R0539)
- BbsI-HF (NEB #R3539)
- BsaI-HFv2 (NEB #R3733)
- BsmBI-v2 (NEB #R0739)
- BspQI (NEB #R0712)
- BtgZI (NEB #R0703)
- Esp3I (NEB #R0734)
- PaqCI (NEB #R0745)
- SapI (NEB #R0569)

TOOLS & RESOURCES

Visit www.neb.com/GoldenGate to find:

- Publications and protocols related to ligase fidelity and Golden Gate Assembly
- Access to **NEBridge Golden Gate Assembly Tool**, for help with designing your experiment at **GoldenGate.neb.com**
- Access to the **NEBridge Ligase Fidelity Tools** to facilitate the design of high-fidelity Golden Gate Assemblies
- View our webinar: Fidelity and bias in end-joining ligation: Enabling complex multi-fragment Golden Gate DNA Assembly
- View our *MoClo Overhang Standards Usage Guidelines* and our tutorial, *Domestication and Golden Gate 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.
5. Potapov, V. et. al. (2018) *ACS Synth. Biol.* DOI: 10.1021/acssynbio.8b00333.

How does Golden Gate Assembly work?

Gibson Assembly®

Gibson Assembly enables multiple, overlapping DNA fragments to be joined in a single-tube isothermal reaction, with no additional sequence added (scar-less). 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

Gibson Assembly Cloning Kit (NEB #E5510)

Gibson Assembly Master Mix (NEB #E2611)

- Visit **NEBGibson.com** to learn more

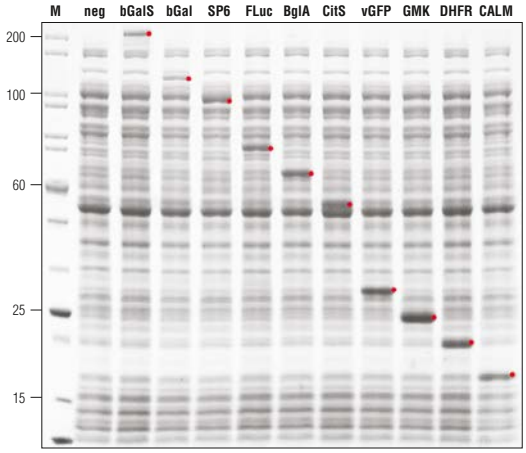
Cell-free Expression

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 contains all the components required for protein synthesis, except for the target template DNA. It is 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



50 µl reactions containing 250 ng template DNA were incubated at 37°C for 3 hours. The red dot indicates the protein of interest. M = Unstained Protein Standard, Broad Range (NEB #P7717), "neg" = negative control, no DNA

RECOMMENDED PRODUCTS

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

- 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)

- Suitable for circular or linear DNA template
- 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

PURExpress® *In Vitro* Protein Synthesis Kit

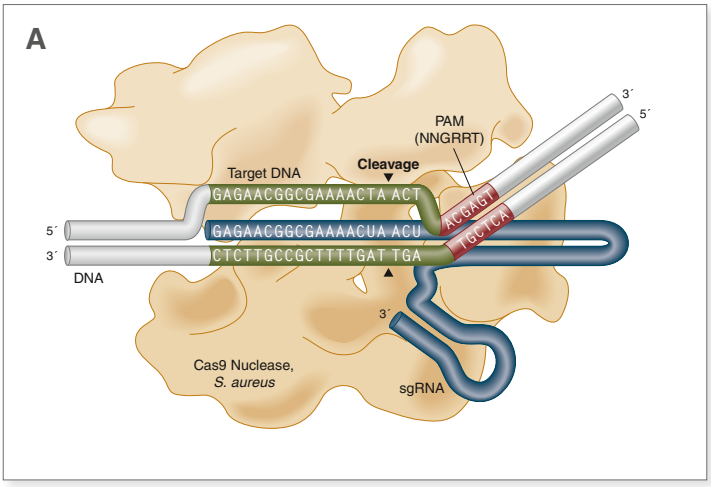
A rapid method for gene expression analysis, PURExpress is a novel cell-free transcription/translation system reconstituted from purified components necessary for *E. coli* translation. Synthesize a wide range of proteins free of modification or degradation by simply mixing two tubes followed by the addition of 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.

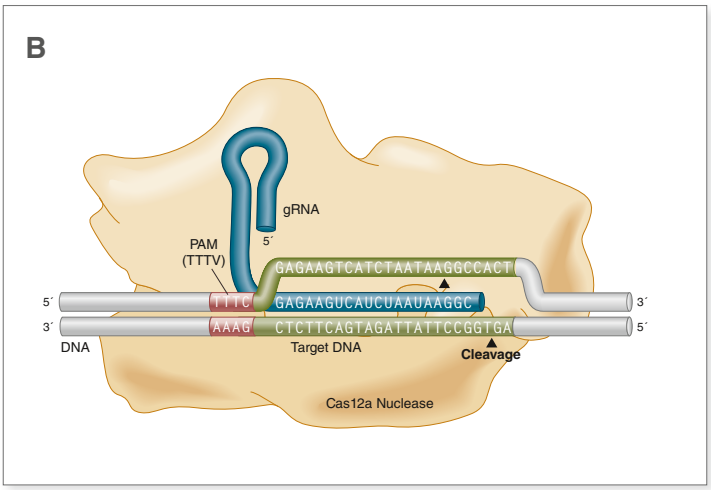
CRISPR/Cas Genome Editing

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.

Schematic representation of Cas9 Nuclease, *S. aureus* (A) and Lba Cas12a, *Lachnospiraceae* bacterium N2006 (B) sequence recognition and DNA cleavage





RECOMMENDED PRODUCTS

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 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

References:
1. Overballe-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.

Ordering Information

DNA Assembly

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
NEB Golden Gate Assembly Kit (BsaI-HFv2)	E1601S/L	20/100 reactions
NEB Golden Gate Assembly Kit (BsmBI-v2)	E1602S/L	20/100 reactions
NEBridge Ligase Master Mix	M1100S	50 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

Protein Expression

PRODUCT	NEB #	SIZE
NEBExpress Cell-free *E. coli* Protein Synthesis System	E5360S/L	10/100 reactions
NEBExpress GamS Nuclease Inhibitor	P0774S	75 µg
PURExpress *In Vitro* 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
E. coli Ribosome	P0763S	1 mg

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, *S. pyogenes*	E3322V/S	10/20 reactions
EnGen Spy Cas9 Nickase	M0650S/T	400/700 pmol
EnGen Spy dCas9 (SNAP-tag®)	M0652S/T	400/700 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.

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, *S. pyogenes*	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

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

DNA Ligases & Modifying Enzymes

PRODUCT	NEB #	SIZE
Thermos aquaticus (*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	50 reactions

Competent Cells

PRODUCT	NEB #	SIZE
NEB 5-alpha Competent *E. coli* (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 *E. coli* (High Efficiency)	C3019I/P/H	6 x 0.2 ml/1 x 96 well/ 20 x 0.05 ml
NEB Stable Competent *E. coli* (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

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