DNA Assembly & Synthetic Biology

TOOLS TO SUPPORT DESIGN AND DNA ASSEMBLY

UPDATE 2018/19

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. Recent advances in the production of biochemicals and biofuels, and a new understanding of the minimal genome, benefit from synthetic biological approaches. 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)	**NEB Gibson® Assembly** (NEB #E5510) (NEB #E2611)	**NEB Golden Gate Assembly Mix** (NEB #E1600)	**USER® Enzyme** (NEB #M5505)
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 with High Efficiency with Low Amounts of DNA	★★★	★★	★★	★★
Accommodates Flexible Overlap Lengths	★★★	★★★	★	★★
APPLICATIONS				
Simple Cloning (1-2 Fragments)	★★★	★★★	★★★	★★★
4-6 Fragment Assembly	★★★	★★★	★★★	★★★
6-11 Fragment Assembly	★★★	★★	★★★	★★★
12-24 Fragment Assembly(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

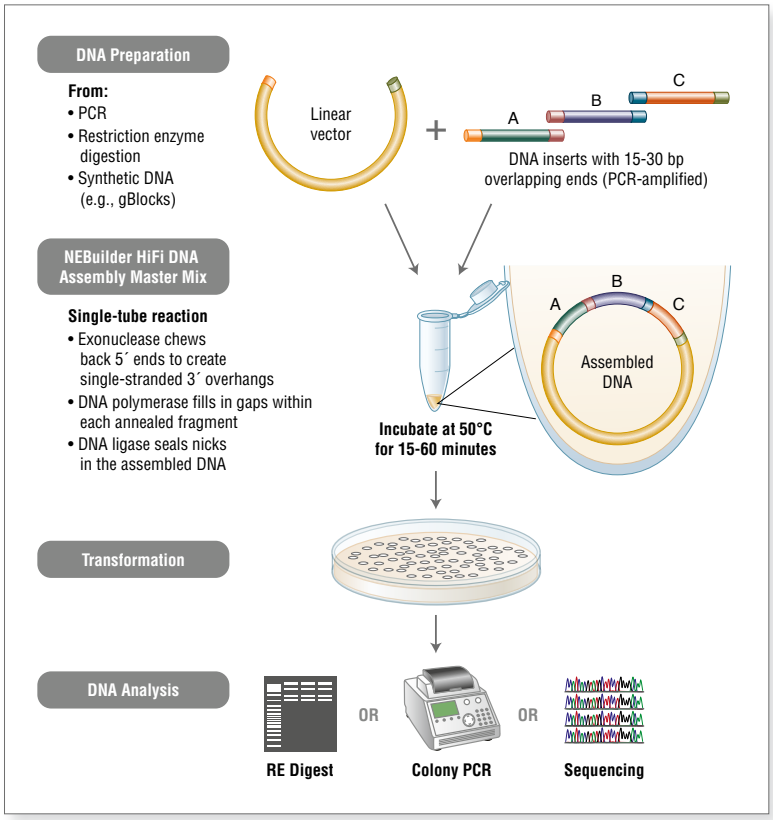
★★★ Optimal product for selected application
★★ Works well for selected application
★ Will perform selected application, but is not recommended
N/A Not applicable to this application
NR Not recommended
(1) Please visit **neb.com/GoldenGate** for more information

NEB offers several online tools to aid in DNA assembly. Look for the icons on the following pages.

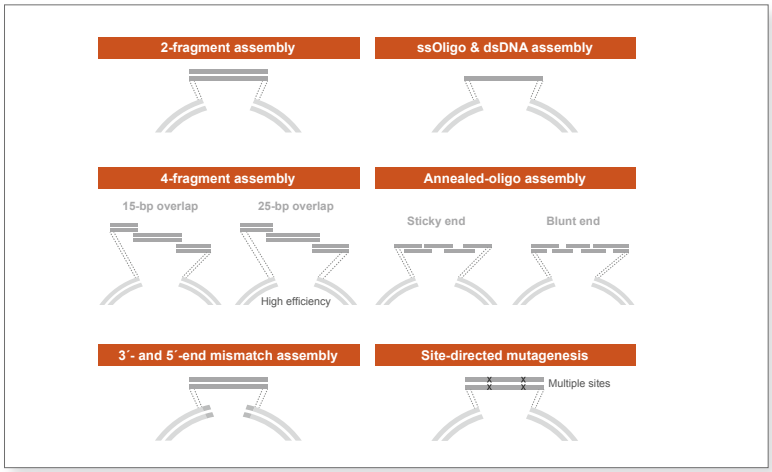
NEBuilder® HiFi DNA Assembly

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



Not your average DNA assembly reagent



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 20 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 NEB 5-alpha Competent *E. coli*
- NEBuilder HiFi DNA Assembly Bundle for Large Fragments includes NEB 10-beta Competent *E. coli*

TOOLS & RESOURCES

Visit NEBuilderHiFi.com to find:

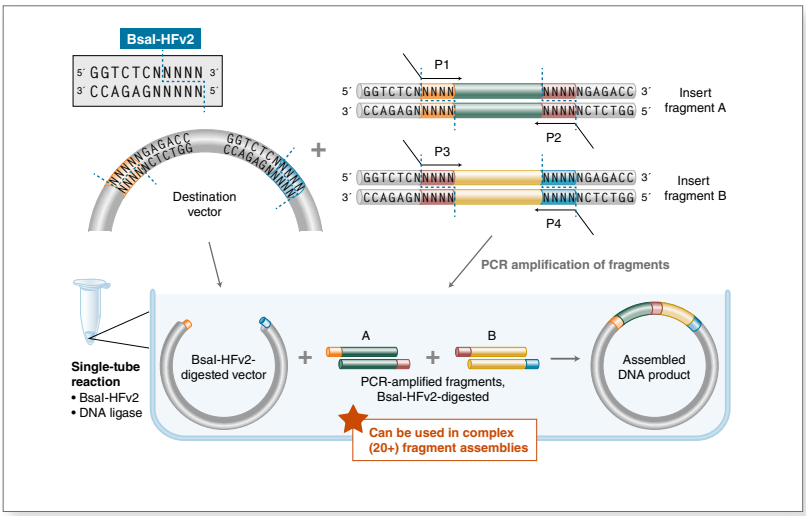
- Online tutorials to help with assembly and primer design
- Application notes utilizing NEBuilder HiFi
- Access to **NEBuilder Assembly Tool**, our online primer design tool

For help with designing primers, try NEBuilder Assembly Tool at **NEBuilder.neb.com**

Golden Gate Assembly

The efficient and seamless assembly of DNA fragments, commonly referred to as Golden Gate assembly (1,2), has its origins in 1996 when, for the first time, it was shown that multiple inserts could 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. Since this pioneering work, Golden Gate has enabled single inserts, the cloning of inserts from diverse populations enabling library creation, and multi-module assemblies. We now have made extraordinary improvements that touch every application of the Golden Gate technology.

NEB Golden Gate workflow



In its simplest form, Golden Gate Assembly requires a Type IIS recognition site, in this case, BsaI (GGTCTC), 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.

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.

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. NEB is pleased to introduce two new restriction enzymes for use in Golden Gate: Esp3I, an isoschizomer of BsmBI that is recommended for use at 37°C and is supplied with CutSmart® Buffer, and the improved BsaI-HFv2, optimized for Golden Gate Assembly. This enzyme, along with the ligase fidelity data, allows complex 20+ fragment assemblies with high efficiency, > 90% accuracy and low backgrounds.

The NEB Golden Gate Assembly Mix incorporates digestion with BsaI and ligation with T4 DNA Ligase into a single reaction, and can be used to assemble up to 12 fragments in a single step.

RECOMMENDED PRODUCTS

NEB Golden Gate Assembly Mix (NEB #E1600)

- Seamless cloning – no scar remains following assembly
- Fast (5 min) protocols for routine, single-insert cloning
- High efficiencies for cloning during library creation
- Ordered assembly of up to 12 fragments in a single reaction
- Efficient with regions with high GC content and areas of repeats
- Compatible with a broad range of fragment sizes (< 100 bp to > 15 kb)

Type IIS Enzymes used in Golden Gate

- BsaI (NEB #R0535)
- BsaI-HF®v2 (NEB #R3733)
- BbsI (NEB #R0539)
- BbsI-HF (NEB #R3539)
- BsmBI (NEB #R0580)
- Esp3I (NEB #R0734)

TOOLS & RESOURCES

Visit www.neb.com/GoldenGate to find:

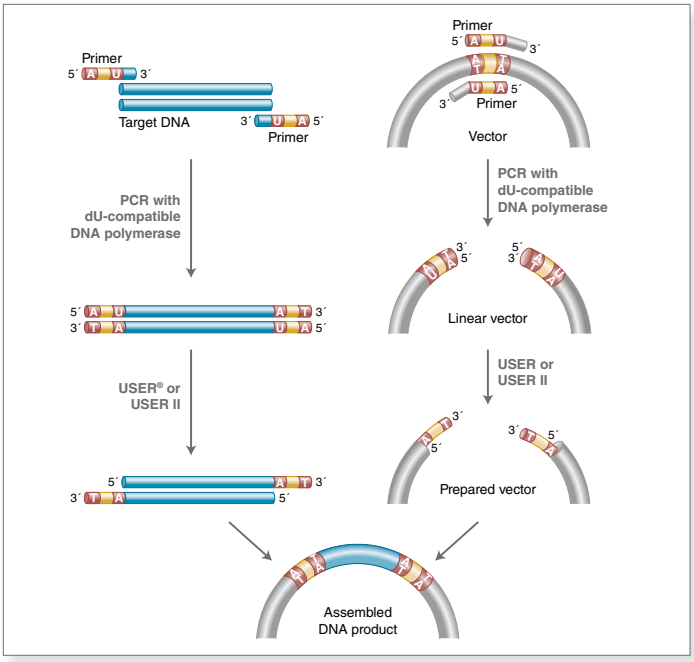
- Publications and protocols related to ligase fidelity and Golden Gate Assembly
- A video tutorial on the Golden Gate Assembly workflow
- Access to the **NEB Golden Gate Assembly Tool**
- View our webinar: **Fidelity and bias in end-joining ligation: Enabling complex, multi-fragment Golden Gate DNA Assembly at www.neb.com/NEBTVwebinars**

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) *bioRxiv*, 32297; doi: https//doiorg/10.1101/322297.

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.

BioBrick® Assembly

With the BioBrick synthetic biology approach, DNA fragments encoding proteins, promoters, ribosome binding sites, etc., have been standardized and are contained in a "parts" registry of plasmids with identical restriction sites flanking the "payload" of the part. By employing standardized flanking sites that are not contained within the coding sequence of the part, they can be ligated in any order to create a novel "device". By choosing restriction sites with compatible ends that destroy the recognition site when ligated to one another, parts can be combined together and the original flanking sites re-used for the next round of assembly. Despite the limitations of introducing a sequence scar for every ligation event and the multiple rounds of assembly required to fabricate a device, a wide assortment of exciting systems have been designed and built.

RECOMMENDED PRODUCTS

BioBrick Assembly Kit (NEB #E0546)

- The BioBrick Assembly Kit was developed in partnership with Ginkgo BioWorks™
- The BioBrick approach has been used to build a wide variety of novel biological systems

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.

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.

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)

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

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

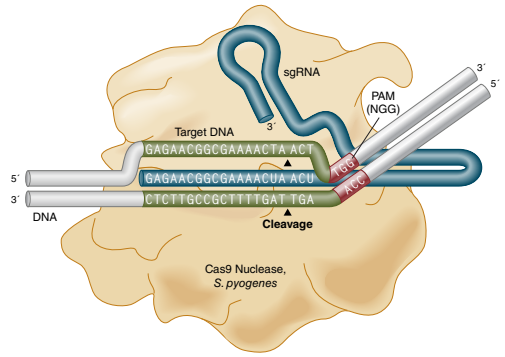
EnGen Mutation Detection Kit
(NEB #E3321)

TOOLS & RESOURCES

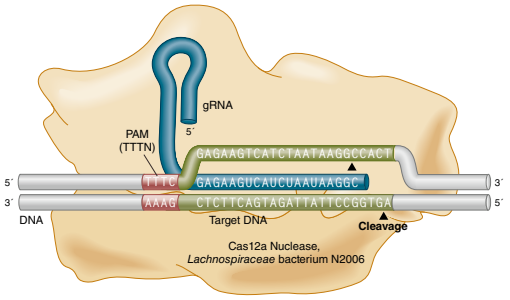
Visit www.neb.com/GenomeEditing to find:

- 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

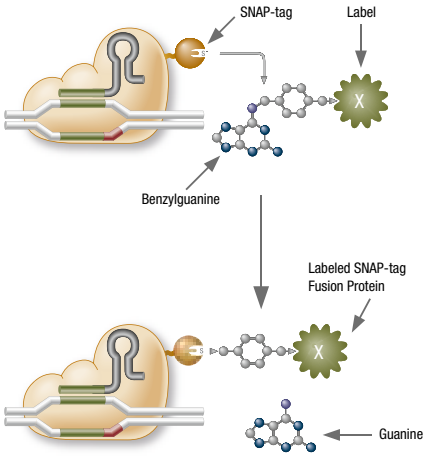
EnGen Cas9 Nuclease, *S. pyogenes*
Standard genome editing



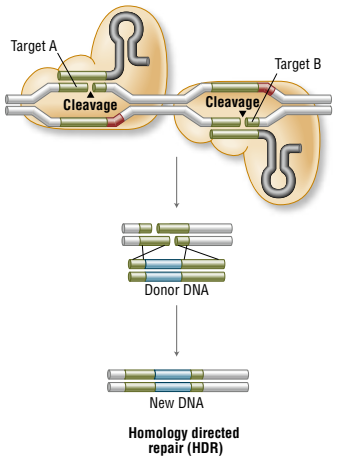
EnGen Lba Cas12a, *Lachnospiraceae* bacterium N2006
AT-rich PAM, expanded temperature range



EnGen Spy dCas9 (SNAP-tag)
in vivo labeling and target enrichment



EnGen Spy Cas9 Nickase
increased specificity homology directed repair, dual guide sequence



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 (described below). 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)

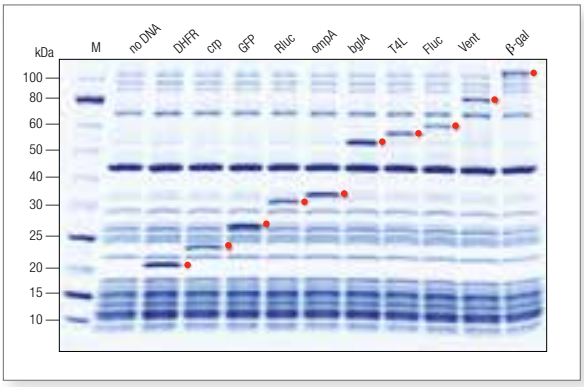
Gibson Assembly Master Mix (NEB #E2611)

- Assemble multiple fragments and transform in just under two hours
- Clone into any vector with no additional sequence added
- Gibson Assembly Cloning Kit includes NEB 5-alpha Competent *E. coli*
- No PCR cleanup step required
- Visit **NEBGibson.com** for online tutorials and application notes

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.

Protein expression using the PURExpress *In Vitro* Protein Synthesis Kit from NEB



Reactions were carried out according to manual recommendations. Red dot indicates protein of interest. Marker M is the Protein Ladder (NEB #P7703).

RECOMMENDED PRODUCTS

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

PURExpress Δ Ribosome Kit (NEB #E3313)

PURExpress Δ (aa, tRNA) Kit (NEB #E6840)

PURExpress Δ RF123 Kit (NEB #E6850)

PURExpress Disulfide Bond Enhancer (NEB #E6820)

- Suitable for circular or linear DNA template
- Visualize synthesized protein directly on a Coomassie stained gel
- Protein expression in approximately 2 hours
- Transcription/translation components can be removed by affinity chromatography

Ordering Information

Ordering Information

PRODUCT	NEB #	SIZE
NEBuilder HiFi DNA Assembly Cloning Kit	E5520S	10 reactions
NEBuilder HiFi DNA Assembly Master Mix	E2621S/L/X	10/50/250 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 Mix	E1600S	15 reactions
USER Enzyme	M5505S/L	50/250 units
Thermolabile USER II Enzyme	M5508S/L	50/250 units
BioBrick Assembly Kit	E0546S	50 reactions
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		
EnGen Spy Cas9 NLS	M0646T/M	400/2,000 pmol
EnGen Mutation Detection Kit	E3321S	25 reactions
EnGen sgRNA Synthesis Kit	E3322S	20 reactions
EnGen Spy Cas9 Nickase	M0650S/T	400/700 pmol
EnGen Spy dCas9 (SNAP-tag®)	M0652S/T	400/700 pmol
EnGen Lba Cas12a (Cpf1)	M0653S/T	400/2,000 pmol
Cas9 Nuclease, *S. pyogenes*	M0386S/L/M	70/300/600 pmol
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

PRODUCT	NEB #	SIZE
RESTRICTION ENZYMES		
BsaI	R0535S/L	1,000/5,000 units
BsaI-HFv2	R3733S/L	1,000/5,000 units
BbsI	R0539S/L	300/1,500 units
BbsI-HF	R3539S/L	300/1,500 units
BsmBI	R0580S/L	200/1,000 units
Esp3I	R0734S/L	300/1,500 units
DNA LIGASES & MODIFYING ENZYMES		
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
T5 Exonuclease	M0363S/L	1,000/5,000 units
COMPETENT CELLS		
NEB 5-alpha Competent *E. coli* (High Efficiency)	C2987H/I/P	20 x 0.05 ml/6 x 0.2 ml/ 1 x 96 well plate
NEB 10-beta Competent *E. coli* (High Efficiency)	C3019H/I	20 x 0.05 ml/6 x 0.2 ml
NEB Stable Competent *E. coli* (High Efficiency)	C3040H/I	20 x 0.05 ml/6 x 0.2 ml
DNA LADDERS		
Quick-Load® Purple 1 kb Plus DNA Ladder	N0550S	125-250 gel lanes
Quick-Load Purple 100 bp DNA Ladder	N0551S	125 gel lanes
Quick-Load Purple 1 kb DNA Ladder	N0552S	125 gel lanes

Germany & Austria
New England Biolabs GmbH
Brüningstr. 50, Geb B852
65926 Frankfurt/Main, Germany
Tel: +49/(0)69/305-23140
Fax: +49/(0)69/305-23149

Free Call: 0800/246 5227 (Germany)
Free Call: 00800/246 52277 (Austria)
info.de@neb.com

www.neb-online.de

France
New England Biolabs France SAS
Genopole Campus 1, Bâtiment 6
5 rue Henri Desbruères
91030 Evry cedex, France
Tel.: 0800 100 632 (Customer Service)
Tel.: 0800 100 633 (Technical Service)
FAX.: 0800 100 610
info.fr@neb.com

www.neb-online.fr

RECOMMENDED RESOURCES

Programming Life: Inquiry & Engineering Through Synthetic Biology

- Follow the evolution of synthetic biology by visiting our feature article at **www.neb.com/SynBioFeature**

NEB supplies reagents, free of charge, to participants in both iGEM and BioBuilder®.

- Since its inception in 2004, **iGEM** has evolved into a highly successful vehicle for training and showcasing a new generation of biological engineers using the synthetic biology framework
- At **BioBuilder**, synthetic biology challenges are presented an accessible way, giving everyone a chance to experience authentic and meaningful scientific problem solving. The **BioBuilderClub** supports small teams of high school students and teachers who want to use out-of-classroom time to design, build and/or test synthetic living systems.
- For more information visit **www.neb.com/promoting-science-education**

*Visit **Q5PCR.com** for ordering information.

nqa. ISO 9001 Registered Quality Management
nqa. ISO 14001 Registered Environmental Management
nqa. ISO 13485 Registered Medical Devices