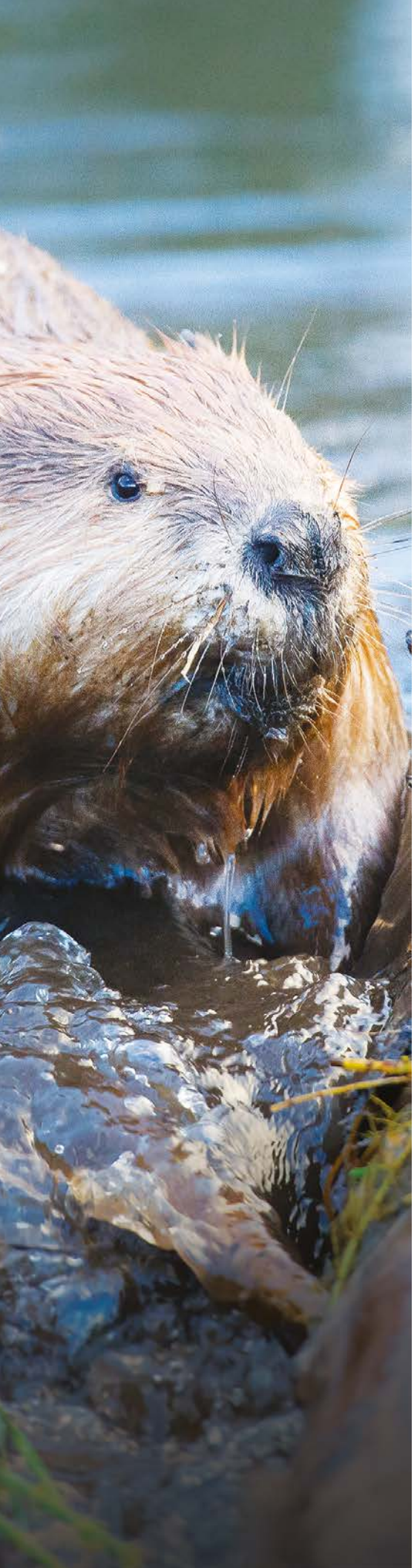
Now includes Authenticase® and EnGen® SpRY Cas9 Nuclease

DNA Assembly & Synthetic Biology

TOOLS FOR DESIGN, ASSEMBLY, MANIPULATION, AND EXPRESSION

NEW ENGLAND Biolabs®



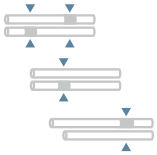
DNA Assembly & Synthetic Biology – Tools to support DNA design, assembly, manipulation, and expression

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

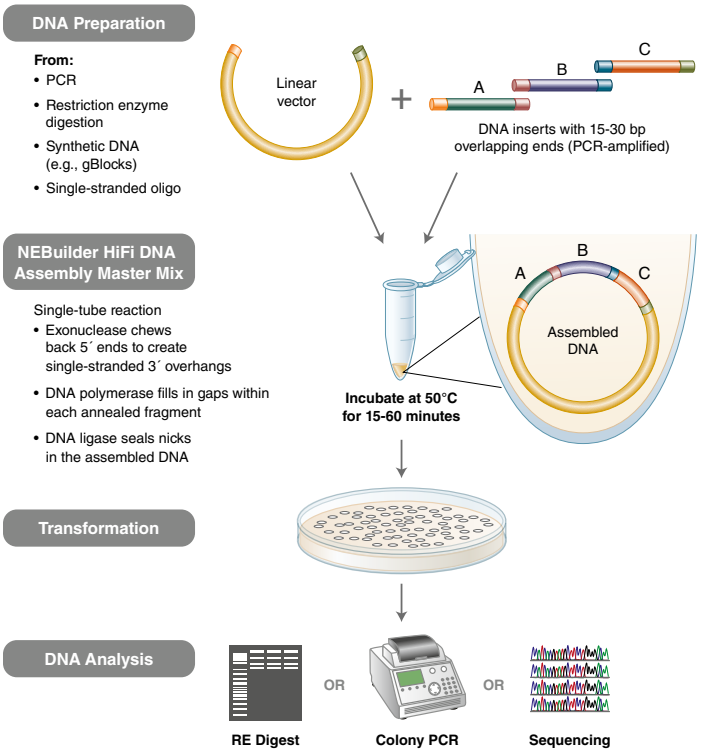
Amplification

Cell-free Protein Expression

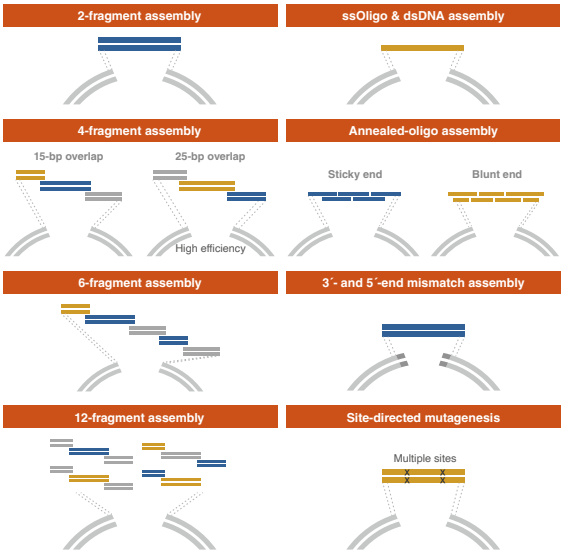
NEBuilder® HiFi DNA Assembly

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 to find:

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

NEBuilder Assembly Tool

Get started designing primers at NEBuilder.neb.com

NEBuilder Protocol Calculator

Generate a custom protocol at NEBuilderCalculator.neb.com

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.

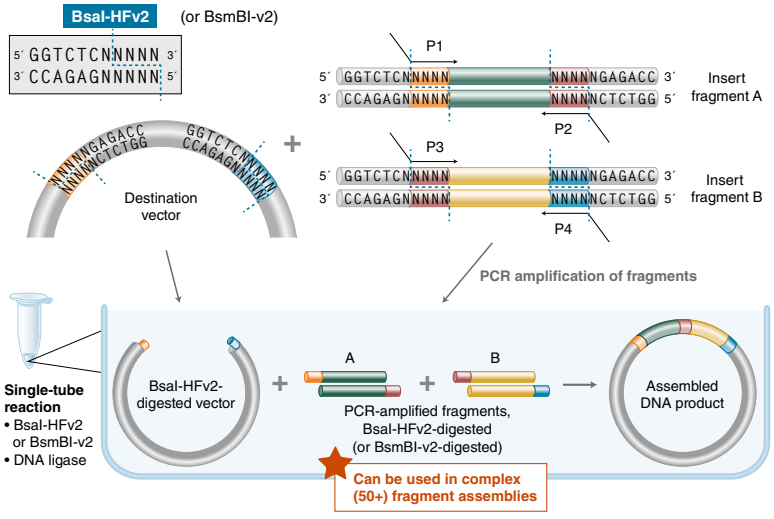
Type IIS Restriction Enzymes for Golden Gate Assembly

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 #
BbsI	R0539
BbsI-HF®	R3539
BsaI-HF®v2	R3733
BsmBI-v2	R0739
BspQI	R0712

PRODUCT	NEB #
BtgZI	R0703
Esp3I	R0734
PaqCI®	R0745
SapI	R0569

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

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 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*

NEBridge Golden Gate Assembly Tool

Get started designing your experiments and primers at GoldenGate.neb.com

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® 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

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.

Try our suite of free, online tools at **ligasefidelity.neb.com**

NEBridge Ligase Fidelity Viewer®

Evaluate existing assemblies and visualize overhang ligation preferences

NEBridge GetSet®

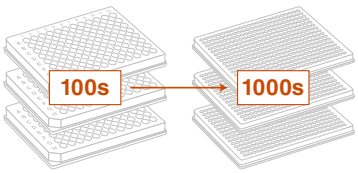
Predict high-fidelity overhang sets for new assemblies and expand existing assemblies

NEBridge SplitSet®

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

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**

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)

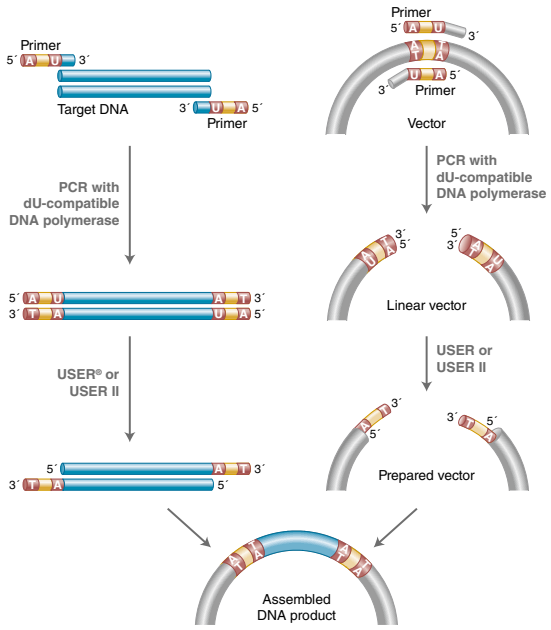
- Experience high efficiency within regions of high GC content and areas of repeats
- Supports miniaturization
- Enjoy compatibility with synthetic dsDNA fragments, such as gBlocks
- Find flexibility with **NEBridge Ligase Master Mix (NEB #M1100)** and your choice of Type IIS restriction enzymes
- Design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



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

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

Gibson Assembly Cloning Kit (NEB #E5510)

Gibson Assembly Master Mix (NEB #E2611)

- Visit 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
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 (shRNA) Cloning	★★★	★★	★	★
gRNA Library Generation	★★★	★★	★	★
Large Fragment (> 10 kb) Assembly	★★★	★★★	★★★	★★
Small Fragment (< 100 bp) Assembly	★★★	★	★★★	★★★
Use in Successive Rounds of Restriction Enzyme Assembly	★★★	★	NR	★

[1] Please visit **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

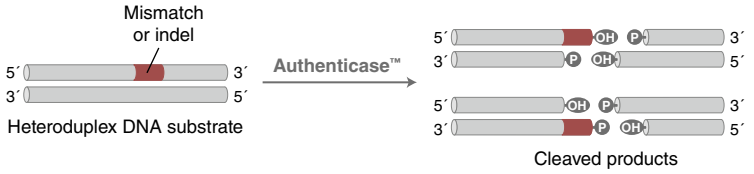
Enzymes for Innovation (EFI) is a unique program initiated by NEB to provide enzymes with interesting activities and unique properties to the scientific community, in hopes of enabling the discovery of new and innovative applications.

Learn more at **www.neb.com/enzymesforinnovation**

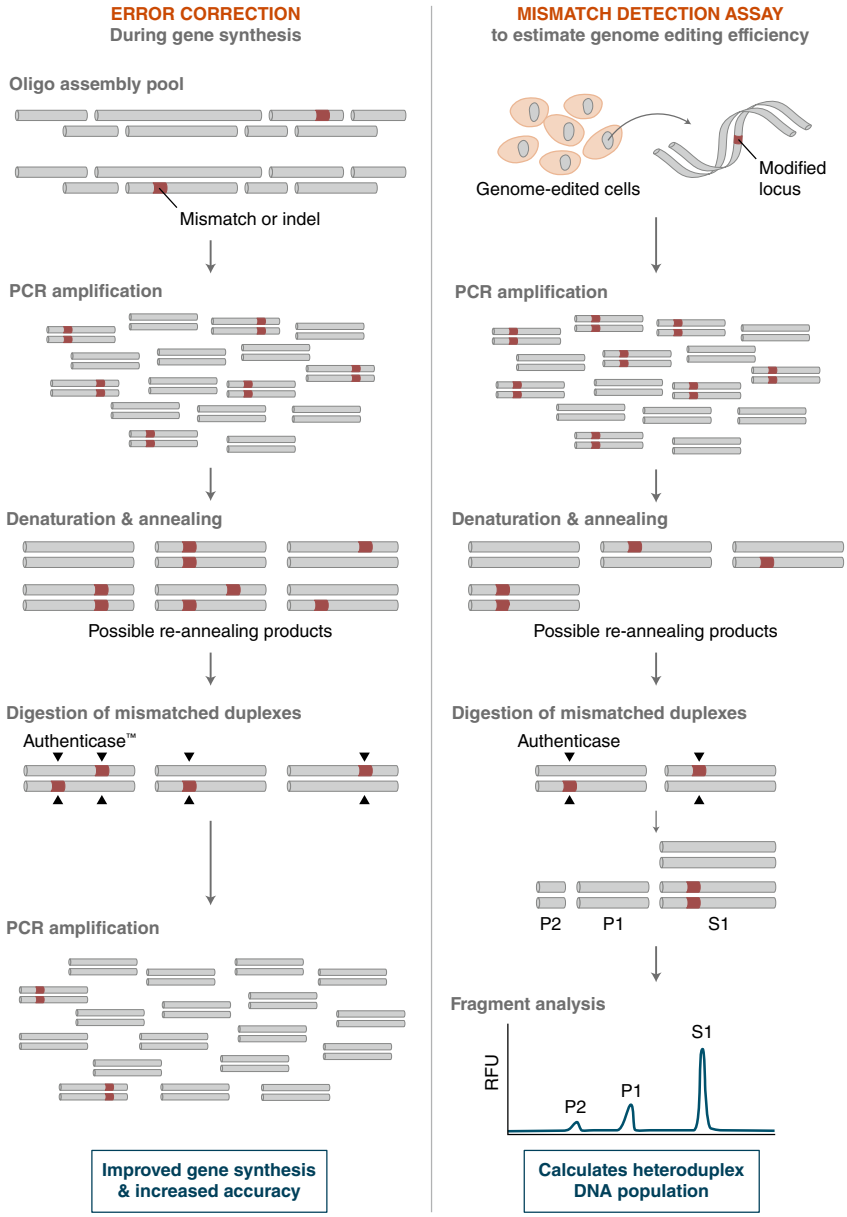
Authenticase®

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**

RECOMMENDED PRODUCTS

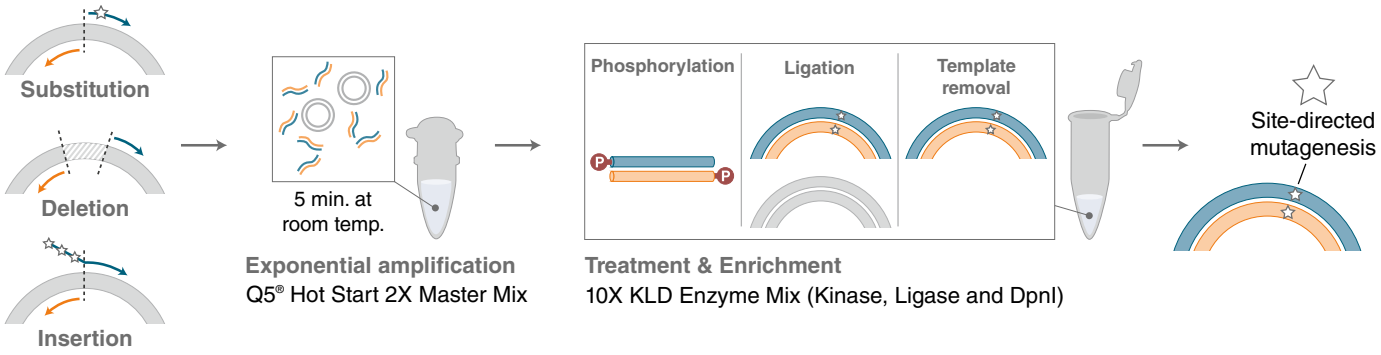
Q5 Site-Directed Mutagenesis Kit (NEB #E0554)

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



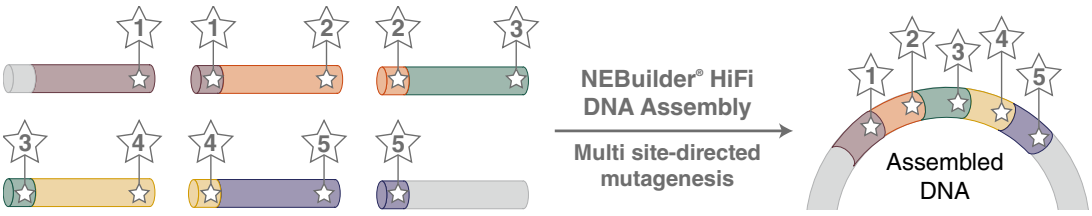
Multi-site mutagenesis

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)

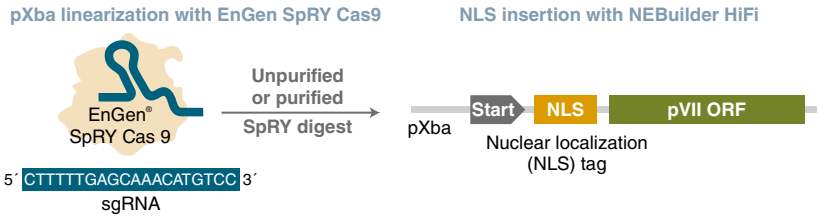
- Create multiple mutations at the same time in a single reaction
- Design primers with NEBaseChanger



EnGen® SpRY Cas9 Nuclease

EnGen SpRY Cas9 from *Streptococcus pyogenes* is an engineered RNA-guided DNA endonuclease that catalyzes site-specific cleavage of double-stranded DNA (dsDNA) and can be used to streamline large construct cloning workflows. Targeting requires an ~100 nucleotide single guide RNA (sgRNA) with complementarity to the 20 nucleotide region immediately upstream of a protospacer adjacent motif (PAM) on the dsDNA substrate. Unlike the canonical 5´-NGG-3´ PAM of wild-type Spy Cas9, SpRY Cas9 is essentially PAMless *in vitro*, requiring a PAM of 5´-NNN-3´. DNA cleavage by EnGen SpRY Cas9 produces a double-stranded break occurring 3 nucleotides upstream of the PAM. EnGen SpRY Cas9 contains Simian virus 40 (SV40) T antigen nuclear localization sequence (NLS) on the C-terminus of the protein.

Use in conjunction with the EnGen sgRNA Synthesis Kit, *S. pyogenes* to quickly and easily transcribe high yields of sgRNA in a single 30-minute reaction; the EnGen Mutation Detection Kit or Authenticase for detection of on-target genome editing events; and NEBuilder HiFi DNA Assembly Master Mix for cloning and DNA Assembly.



Schematic depicting the linearization of pXba (22,563 bp) and cloning strategy. 1 µg of pXba plasmid was linearized downstream of the start codon of the pVII orf with 50 nM EnGen SpRY Cas9 and 50 nM sgRNA in 1X NEBuffer r3.1 for 1 hour at 37°C. The reactions were either spin column purified or left unpurified before proceeding to DNA assembly. The NEBuilder HiFi DNA Assembly kit was used to insert an oligonucleotide encoding a nuclear localization signal (NLS) tag to pVII according to the recommended protocol.

RECOMMENDED PRODUCTS

EnGen SpRY Cas9 Nuclease (NEB #M0669)

- Eliminate sequence constraints for dsDNA targeting with non-specific PAM (5´-NNN-3´ PAM)
- Digest large plasmids in cloning workflows successfully

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

EnGen Mutation Detection Kit (NEB #E3321)

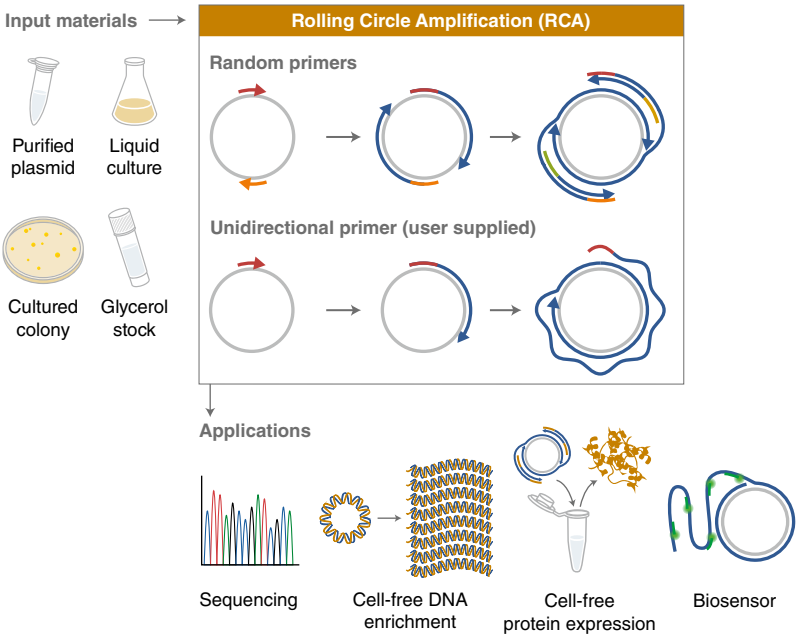
Authenticase (NEB #M0689)

NEBuilder HiFi DNA Assembly Master Mix (NEB #E2621)

Visit www.neb.com/GenomeEditing to view the entire portfolio of CRISPR/Cas genome editing tools.

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.



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

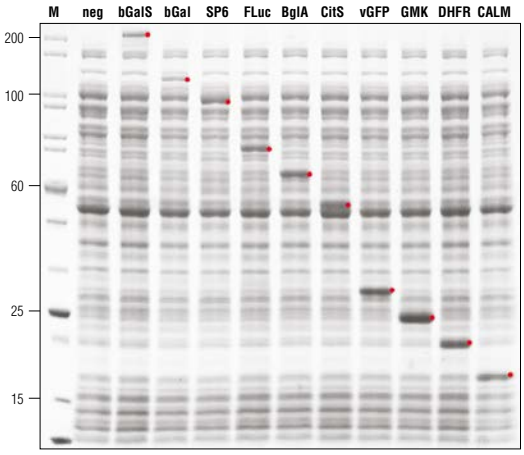
Cell-free Protein Expression

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



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

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 www.neb.com/proteinexpression to view the entire portfolio of protein expression and purification tools.

Featured Application Note

NEBridge® Golden Gate Assembly Kit | phi29-XT RCA Kit | NEBExpress® Cell-free *E. coli* Protein Synthesis System

Download our application note
www.neb.com/appnote-DNAtoProtein

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

Ordering Information

PRODUCT	NEB #	SIZE
DNA Assembly and Amplification		
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 (BsaI-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		
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		
EnGen SpRY Cas9	M0669T/M	500/2,500 pmol
EnGen Mutation Detection Kit	E3321S	25 reactions
EnGen sgRNA Synthesis Kit, *S. pyogenes*	E3322V/S	10/20 reactions
T7 Endonuclease I	M0302S/L	250/1,250 units
Protein Expression		
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
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
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		
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		
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

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