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.

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

<table>
<thead>
<tr>
<th>PROPERTIES</th>
<th>NEBuilder HiFi DNA Assembly (NEB #E2621)</th>
<th>Gibson Assembly (NEB #E5510)</th>
<th>NEB Golden Gate Assembly Kit (BsaI-HFv2) (NEB #E1601)</th>
<th>USER® Enzyme (NEB #M5505)</th>
<th>NEBuilder HiFi DNA Assembly (NEB #E5520)</th>
<th>Gibson Assembly (NEB #E2611)</th>
<th>NEB Golden Gate Assembly Kit (BsaI-HFv2) (NEB #E1601)</th>
<th>USER® Enzyme (NEB #M5508)</th>
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</thead>
<tbody>
<tr>
<td>Removes 5’ or 3’ End Mismatches</td>
<td>***</td>
<td>*</td>
<td>N/A</td>
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<td>Assembles with High Fidelity at Junctions</td>
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<td>Tolerates Repetitive Sequences at Ends</td>
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<td>Generates Fully Ligated Product</td>
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<td>Joins dsDNA with Single-stranded Oligo</td>
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<tr>
<td>Assembles with High Efficiency with Low Amounts of DNA</td>
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<tr>
<td>Accommodates Flexible Overlap Lengths</td>
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### APPLICATIONS

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<tr>
<th>APPLICATIONS</th>
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<th>Gibson Assembly (NEB #E5510)</th>
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<th>USER® Enzyme (NEB #M5508)</th>
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<tr>
<td>Simple Cloning (1-2 Fragments)</td>
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<td>4-6 Fragment Assembly (one pot)</td>
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<td>7-11 Fragment Assembly (one pot)</td>
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<td>12-24 Fragment Assembly (one pot)(1)</td>
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<td>Template Construction for In vitro Transcription</td>
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<td>Synthetic Whole Genome Assembly</td>
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<td>Multiple Site-directed Mutagenesis</td>
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<td>Library Generation</td>
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<td>Metabolic Pathway Engineering</td>
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<td>TALENs</td>
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<td>Short Hairpin RNA Cloning (shRNA)</td>
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<td>gRNA Library Generation</td>
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<td>Large Fragment (&gt; 10 kb) Assembly</td>
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<td>Small Fragment (&lt; 100 bp) Assembly</td>
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<tr>
<td>Use in Successive Rounds of Restriction Enzyme Assembly</td>
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</tbody>
</table>

### 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

---

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

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

Explore and Discover

Download the NEB Augmented Reality (AR) app and enjoy videos, tutorials and experiences by scanning the icons.

*see back cover for details
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](http://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

**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

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

In its simplest form, Golden Gate Assembly requires a Type IIS recognition site, in this case, BsaI-HFv2 (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.

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. 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.

How does Golden Gate Assembly work?

REFERENCES

**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**

![Diagram of DNA assembly with USER Enzyme or Thermolabile USER II Enzyme](image)

**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.
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 (2,3).

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.

References:

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

A. EnGen Spy Cas9 NLS
   Standard genome editing

B. EnGen Spy Cas9 Nickase
   Increased specificity homology directed repair, dual guide sequence

C. EnGen Lba Cas12a (Cpf1)
   Alternative Cas Nuclease, Lachnospiraceae bacterium ND2006

D. EnGen Sau Cas9
   Alternative Cas Nuclease, S. aureus
## Ordering Information

**PRODUCT** | **NEB #** | **SIZE**
--- | --- | ---
NEBuilder HI-FI DNA Assembly Cloning Kit | E5526S | 10 reactions
NEBuilder HI-FI DNA Assembly Master Mix | E2621S/L | 10/50 reactions
NEBuilder HI-FI 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
USER Enzyme | MS5065S | 50/250 units
Thermolabile USER II Enzyme | MS5068L | 50/250 units
Q5 Hot Start High-Fidelity DNA Polymerase | M0515S/L | 100/500 units
BioBrick Assembly Kit | E0546S | 50 reactions
PURExpress In Vitro Protein Synthesis Kit | E68005L | 10/100 reactions
PURExpress Δ Ribosome Kit | E3313S | 10 reactions
PURExpress Δ (aa, RNA) Kit | E6840S | 10 reactions
PURExpress Δ RF123 Kit | E6850S | 10 reactions
PURExpress Dilisulide Bond Enhancer | E6820S | 50 reactions
E. coli Ribosome | P0763S | 1 mg

**GENOME EDITING WORKFLOWS**

- EnGen Spy Car9 NLS | M06467/M | 400/2,000 pmol
- EnGen Mutation Detection Kit | E3321S | 25 reactions
- EnGen sgRNA Synthesis Kit, S. pyogenes | E3322S | 20 reactions
- EnGen Spy Car9 Nickase | M06565S/T | 400/700 pmol
- EnGen Spy dCas9 (SNAP-tag) | M06525S/T | 400/700 pmol
- EnGen Lba Cas12a (Cpf1) | M06535S/T | 400/2,000 pmol
- EnGen Sau Car9 | M06545S | 70/400 pmol
- Cas9 Nuclease, S. pyogenes | M03865S/L/M | 70/300/600 pmol
- HiScribe T7 ARCA mRNA Kit (with or without tailing) | E2065S/ | 20 reactions
- HiScribe T7 High Yield RNA Synthesis Kit | E2040S | 50 reactions
- HiScribe T7 Quick High Yield RNA Synthesis Kit | E2040S | 50 reactions
- T7 Endonuclease I | M03025S/L | 250/1,250 units

**RESTRICTION ENZYMES**

- Bsal | R0535S/L | 1,000/5,000 units
- Bsal-HF2 | R37335S/L | 1,000/5,000 units
- BbsI | R0535S | 300/1,500 units
- BbsI-HF | R3359S/L | 300/1,500 units
- BsmBI | R0580S | 200/1,000 units
- Esp3I | R0734S/L | 300/1,500 units

**DNA LIGASES & MODIFYING ENZYMES**

- Thermos aquaticus (Tag) 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 | M0333S/L | 1,000/5,000 units

**COMPETENT CELLS**

- NEB S-alpha Competent E. coli (High Efficiency) | C2987/ | 6 x 0.2 ml/1 x 96 well/ 20 x 0.05 ml/1 x 384 well/ 96 x 50 µl
- NEB 10-beta Competent E. coli (High Efficiency) | C3019H | 20 x 0.05 ml/6 x 0.2 ml
- NEB Stable Competent E. coli (High Efficiency) | C3040H | 20 x 0.05 ml/6 x 0.2 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 | N0553S/L | 125-375 gel lanes
- Quick-Load Purple Low Molecular Weight DNA Ladder | N0552S | 125-375 gel lanes

**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](http://www.neb.com/SynBioFeature)
  - N.E.B. 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](http://www.neb.com/promoting-science-education)