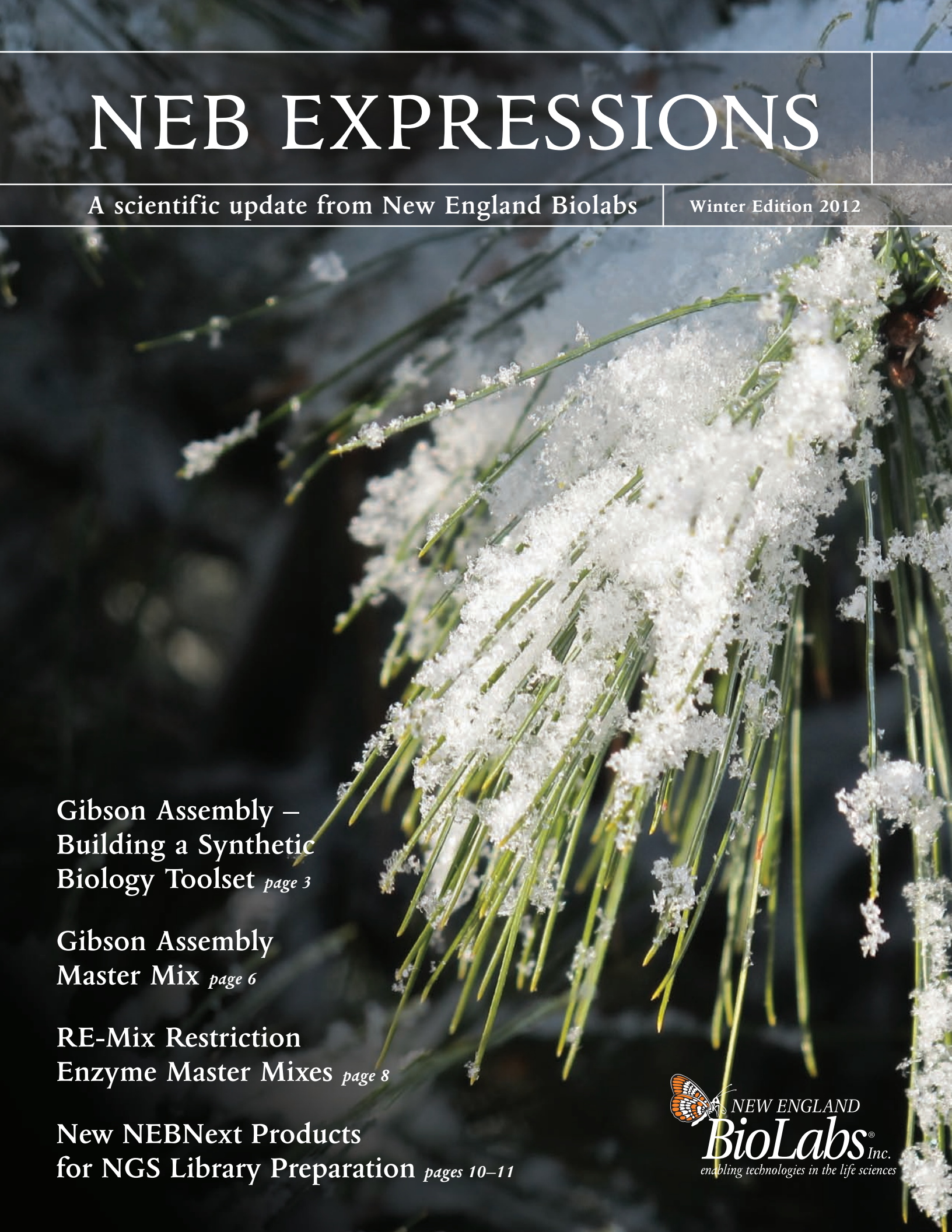
# NEB EXPRESSIONS

A scientific update from New England Biolabs | Winter Edition 2012





NEW ENGLAND BioLabs® Inc.
*enabling technologies in the life sciences*

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# Upcoming Tradeshows

## Visit the NEB booth at the following meetings:

- **ABRF (Association for Biomolecular Resource Facilities)** Orlando, FL
  March 17–20
  http://conf.abrf.org/
  Booth 205
- **AACR (American Association for Cancer Research)** Chicago, IL
  March 31–April 4
  http://www.aacr.org/default.aspx
  Booth 727
- **Experimental Biology** San Diego, CA
  April 21–24
  http://www.experimentalbiology.org
  Booth 1210

# A Letter from NEB

Dear Researcher,

Innovation is more than just change for change's sake. Innovation is the intelligent modification of existing processes for improved outcomes. NEB has been developing innovative tools for molecular biology research for nearly 40 years, and continues to be a leader in the research, discovery and development of life sciences reagents. We take our commitment to the advancement of science seriously, and we strive to offer cutting edge resources to take your research to the next level. To that end, we have joined with Synthetic Genomics to offer the Gibson Assembly Master Mix to facilitate assembly of DNA fragments in your lab. This one-step, isothermic approach to *in vitro* recombination allows for the joining of multiple fragments with overlapping ends into a single recombinant molecule. Check out the feature article in this edition of NEB expressions to learn more about synthetic biology and the innovative Gibson Assembly method of fragment assembly.

This edition of NEB expressions also highlights our new product line of RE-Mix Master Mixes, offering true one-step simplicity in your enzyme digestions. The RE-Mix line of Master Mixes offers speed and simplicity, but also offers the flexibility to digest your DNA for 5 minutes or overnight — with no loss of product.

Wishing you continued success in your research,

New England Biolabs

*Pine branches covered with snow on the New England Biolabs' campus. Photographed by Wendy Geary.*



# Gibson Assembly™ – Building a Synthetic Biology Toolset

**In the quest to create the first bacterial cell controlled by a synthetic genome, the J. Craig Venter Institute (JCVI), with support from Synthetic Genomics, Inc. (SGI), developed a variety of powerful new DNA synthesis and assembly methodologies (1–5) to manipulate large, complex DNAs. These methods include a simple, one-step isothermal *in vitro* recombination technology capable of joining DNAs ranging from relatively short oligonucleotides to fragments hundreds of kilobases in length. This approach, commonly referred to as "Gibson Assembly," is now being used in laboratories around the world to construct DNA fragments. It has the potential to improve upon traditional cloning methods and opens up a range of innovative and ultimately very useful real-world applications.**

Daniel G. Gibson, Ph.D., Synthetic Genomics, Inc. and Salvatore Russello, Ph.D., New England Biolabs, Inc.

## Introduction:

The use of recombinant DNA technology began soon after the discovery of DNA ligase and restriction endonucleases. Soon after, advent of the polymerase chain reaction (PCR) opened up new possibilities for amplification of specific DNA sequences from a complex mixture of genomic DNA. These technologies have been a mainstay in the modern scientific laboratory for several decades and remain useful methods for cloning potentially valuable or interesting DNA today. However, as scientists seek to work with larger DNA fragments, conduct extensive re-engineering of genetic elements, synthesize whole genomes and move towards automated approaches, the technologies required to manipulate DNA also need to evolve.

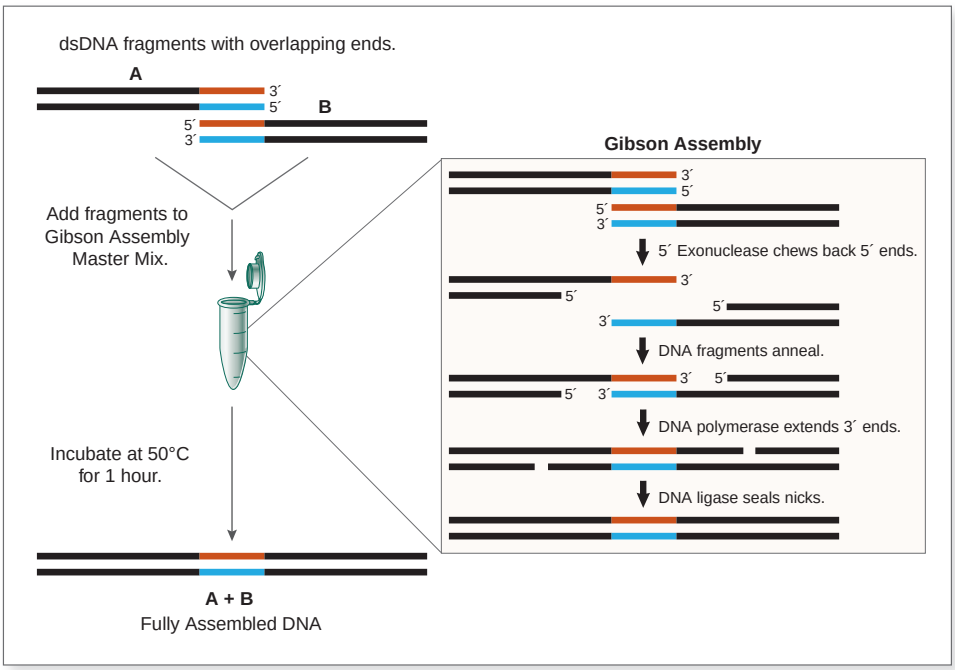
Investigators at the J. Craig Venter Institute (JCVI) have developed a number of *in vitro* enzymatic strategies to assemble short oligonucleotides into larger double-stranded DNA constructs (1-4). In 2003, JCVI made a significant advancement in the production of a synthetic genome by assembling the 5,386 bp genome of phiX174, a virus that infects bacteria, in just 14 days (5). This approach involved joining synthetic oligonucleotides by polymerase cycling assembly, and subsequently amplifying them by PCR (5-6). The unprecedented speed with which this was completed laid the foundation for constructing larger and more complex genomes.

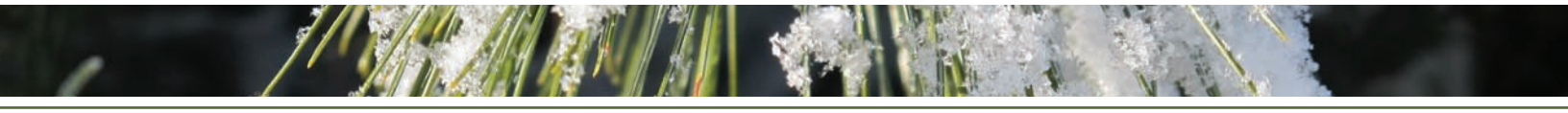
In 2004, JCVI began synthesizing the *Mycoplasma genitalium* genome. It was found that overlapping DNA molecules could be efficiently joined using three enzyme specificities: (i) exonuclease activity, that chews back the ends of DNA fragments and exposes ssDNA overhangs that can anneal to their ssDNA complement; (ii) DNA polymerase activity, that fills gaps in the annealed products, and (iii) DNA ligase activity, that covalently seals the resulting nicks in the assembly. A two-step thermocycle-based *in vitro* recombination method utilizing these enzymes was used to join 101 overlapping DNA cassettes into four parts of the *M. genitalium* genome, each between 136 kb and 166 kb in size. This milestone marked the first assembly of a genome derived from a free-living organism. At 582,970 bp, this synthetic genome was the largest chemically defined DNA structure synthesized in a laboratory, and was 18 times larger than any DNA that had previously been synthesized (4).

Since then, two additional *in vitro* recombination methods have been developed by JCVI to join and clone DNA molecules larger than 300 kb in a single step (2-4). The simplest of these methods is Gibson Assembly, a one-step isothermal approach that utilizes the same three enzymatic activities described previously. This method can be used to join both ssDNA and dsDNAs.

Gibson Assembly has become the most commonly used of the *in vitro* assembly methods discussed above, as it is easy-to-use, flexible and needs little or no optimization, even for large, complex assemblies. All that is required is input DNA with appropriate overlaps, and an appropriate mix of the three enzymes – the Gibson Assembly Master Mix. DNA fragments are added to the master mix and incubated at 50°C for 1 hour; the resulting assembly product is a fully sealed dsDNA suitable for a range of downstream applications (Figure 1). JCVI has used Gibson Assembly to rapidly synthesize the entire 16,520 bp mouse mitochondrial genome from 600 overlapping 60-base oligonucleotides (3). It was also used in combination with yeast assembly to synthesize the 1.1 Mbp *Mycoplasma mycoides* genome, which was then activated in a recipient cell to produce the first synthetic cell (1).

Figure 1. Overview of Gibson Assembly.





## Applications of Gibson Assembly:

**Cloning.** Gibson Assembly eliminates the need to engineer restriction enzyme cut sites within DNA when assembling fragments together. DNA molecules are designed such that neighboring fragments contain a 20-40 bp overlapping sequence. If the DNA fragments originate from PCR products, the overlapping sequence is introduced at the 5′ ends of the primers used in the amplification reaction (Figure 2). DNA fragments can also be assembled with restriction enzyme digested or PCR amplified vector to form circular products suitable for cloning, or for use in downstream applications, such as rolling circle amplification (RCA). To produce these vectors by PCR, each primer needs to include an overlap with one end of the vector, a restriction site (e.g., Not I) not present within the insert or inserts to enable it to be released from the vector, and an overlap with the ends of the DNA fragment assembly or insert. JCVI has been using this approach to combine DNA fragments with vectors, which are then transformed into *E. coli*. One or more fragments have been routinely assembled with general cloning vectors, such as pUC19, and assembled into NEB's pTYB1 expression vector (NEB #N6701). The latter approach was used to express several methylase genes, which aided the genome transplantation efforts at JCVI (8).

**Assembly of large DNA constructs.** Laboratories worldwide are beginning to explore the use of synthetic biology approaches in the production of pharmaceuticals, industrial compounds, antibiotics, cosmetics and alternative energy sources (7). This often requires the assembly of a genetic pathway consisting of multiple enzymes and their associated regulatory elements. Although template DNA is still required, Gibson Assembly simplifies construction of these types of molecules from component fragments. A long stretch of desirable DNA sequence (e.g., a 40 kb genetic pathway) can be broken down into several overlapping PCR products (e.g., eight, 5 kb pieces), which can then be amplified by conventional PCR and combined using Gibson Assembly. This approach has been used to move genetic pathways from one organism to another and to rapidly swap genes, promoters, terminators and ribosome binding sites. DNAs up to ~1 Mbp have been assembled *in vitro* using Gibson Assembly (2).

**Assembly of chemically-synthesized oligonucleotides into dsDNA fragments.** Gibson Assembly can also be used to directly assemble oligonucleotides into a cloning vector, such as pUC19 (3). A common problem observed when chemically synthesizing long stretches of oligonucleotides is the introduction of errors (9). To ensure that error-free molecules are obtained at a reasonable efficiency, a strategy employed by SGI and JCVI involves the assembly of only eight to twelve 60-base oligonucleotides (with 30 bp overlaps) at one time. The resulting dsDNA molecules are sequence-verified and assembled into larger DNA fragments using the same approach. Because assembly itself does not generally introduce new errors, the final assembled product can be retrieved at high efficiencies. Using this approach, many of the costly and time consuming steps currently used to synthesize DNA, including PCR and an error correction, are eliminated.

**Site-directed mutagenesis.** Gibson Assembly can also be used to make rapid changes to DNA fragments, including substitutions, deletions and insertions. To use Gibson Assembly for mutagenesis, the desired changes are introduced into the PCR primers, within the overlapping sequences at assembly points (Figure 3). To modify a DNA sequence in this way, two PCR primers are required: the first contains the desired nucleotide changes, and the second contains the reverse complement of the first primer at the overlapping region. Following amplification and assembly of the fragments, the designed changes are incorporated into the final product. The number of changes that can be made at once depends on the number of fragments simultaneously assembled. For example, an eight-piece assembly, which contains eight assembly points, provides eight opportunities to introduce changes in the DNA sequence. Because the method can be used to assemble large DNA fragments, mutations can rapidly be made to very large pieces of DNA. For example, eight modifications can be introduced into an 80 kb DNA molecule following the assembly of eight 10 kb PCR fragments. This site-directed mutagenesis strategy was used during synthesis of the *M. mycoides* genome. The cassettes comprising the synthetic genome were ordered based on an imperfect draft sequence, which resulted in small differences between the synthetic cassettes and the desired *M. mycoides* genome sequence. The sequences of 16 cassettes were successfully edited using this approach (1).

**Combinatorial synthesis of DNA Fragments.** In the near future, chromosomes will be designed and synthesized for processes ranging from biofuel production to pharmaceutical manufacture. Bacteria and plants often carry out syntheses that far exceed what can be readily achieved by the best organic chemists. The genes that control desirable pathways can be chemically synthesized, placed in artificial chromosomes, and "installed" in suitable host cells, including bacteria, yeast or plant cells. These multigene pathways can be constructed in a combinatorial fashion, such that each member of the library has a different combination of gene variants. Using screening and selection methods, cells bearing the pathway with the desirable trait (highest yield of a compound, for instance) can be obtained. The engineered host organism then becomes a biologic factory used to manufacture the product specified by the synthetic pathway. Gibson Assembly has the potential to be used to produce combinatorial libraries of synthetic or semisynthetic chromosomes carrying thousands of genes. Figure 4 demonstrates the combinatorial assembly of cassettes produced from 60-mer oligonucleotides. Here, 1,024 ($2^{10}$) variants of a 1 kb gene, containing 10 single nucleotide changes, are produced from 30 sequence-verified cassettes.

## Moving Forward

**Synthetic & Minimal Cells.** For the past 17 years, the genomes of many organisms have been sequenced and deposited in databases. It has recently been shown that it is possible to reverse this process and synthesize bacterial cells from digitized information (1). In order to realize this vision, researchers at JCVI needed tools and technologies to sequence, synthesize and transplant genomes. Although many hurdles needed to be overcome, synthetic cells can now be produced in the laboratory. As proof of concept, the 1.08 Mbp *M. mycoides* JCVI-syn1.0 genome was designed, synthesized

Figure 2. PCR-Generated Vector and Insert Assembly.

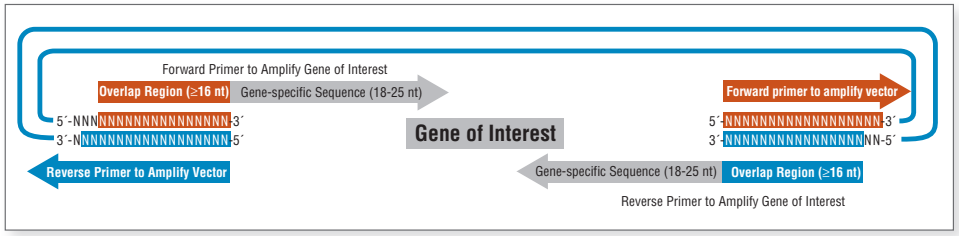
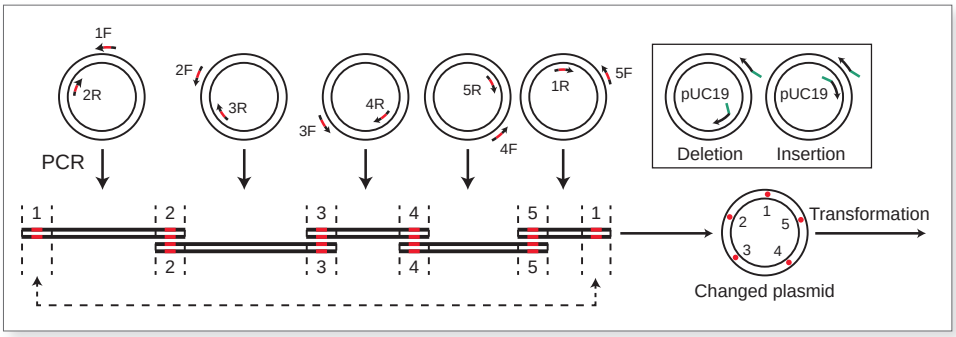


Figure 3. Introducing changes to a desired DNA using Gibson Assembly.




*In the example above, five changes were introduced into a DNA plasmid using Gibson Assembly. Each primer contains a change introduced by PCR. The overlapping PCR fragments with each change at the assembly point are then combined using Gibson Assembly. (credit: Mikkel Algire, JCVI).*

and assembled, starting from the digitized genome sequence, and transplanted into a *Mycoplasma capricolum* recipient cell to create new *M. mycoides* cells controlled only by the synthetic chromosome. The only DNA present in the cells is the designed synthetic DNA, including "watermark" sequences, and other designed gene deletions and polymorphisms and mutations acquired during the building process. The new cells have the expected phenotypic properties and are capable of continuous self-replication (1). The *M. mycoides* genome is currently the largest chemically defined DNA structure that has been synthesized in a laboratory. It is almost twice as large as the synthetic *M. genitalium* genome reported in 2008, and more than an order of magnitude larger than any reported DNA sequence synthesized outside JCVI. What has been learned in this "proof of concept" experiment can now be applied to designing and producing new organisms with useful properties.

Further, researchers at JCVI have already begun working on their ultimate objective: to synthesize a minimal cell with only the machinery necessary for independent life. Now that a living cell can be produced from a synthetic genome, components of a synthetic genome can be removed and transplanted in an iterative fashion until only the essential genes are present and the genome is as small as possible. This will help to better understand the function of every gene in a cell and what DNA is required to sustain life in its simplest form. Gibson Assembly is one of the core technologies that will be used to achieve these goals.

## Conclusion

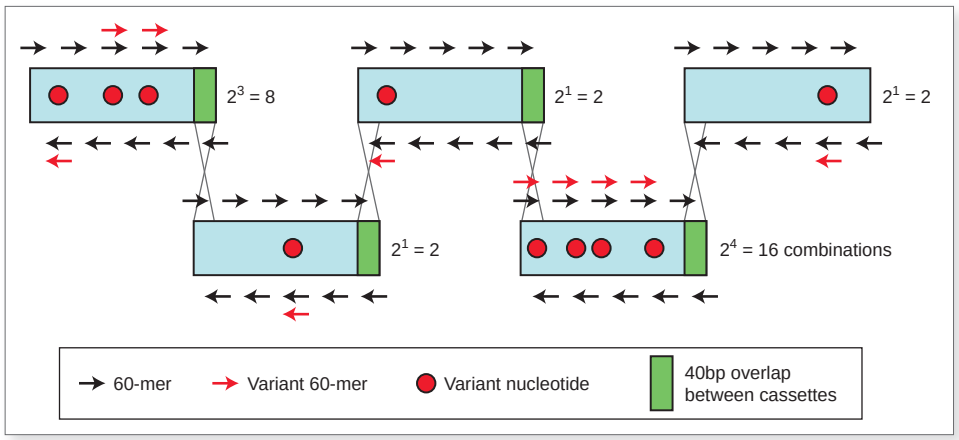
Gibson Assembly is a simple and robust method that enables the simultaneous production of many different combinations of genes and pathways, accelerating the progress of synthetic biology. Furthermore, this powerful technology has the potential to help turn DNA sequence into genes and pathways useful in the production of biofuels, industrial compounds, pharmaceuticals and vaccines. The synthesis of genes and pathways, and even small genomes, has been made easier with Gibson Assembly, helping to move the field of synthetic biology forward. As the power of DNA sequencing increases and sequencing costs decrease, DNA databases will continue to fill with novel genes and pathways waiting to be identified, optimized and expressed in a heterologous host organism. It is time to better understand how to turn these DNA sequences into useful applications.

The ability to quickly construct whole genes and genomes has the potential to accelerate research in a variety of other fields. This capability may also make it possible to quickly respond to emerging threats, and may allow researchers to understand how "life" works. The power of large scale DNA synthesis will dramatically impact the way research is done and vastly accelerate the pace of science. The Gibson Assembly Master Mix provides a new and powerful tool for biotechnology, whose most far-reaching benefits may not yet even be envisioned.

References
1. Gibson, D.G. et al. (2010) *Science*, 239, 52–56.
2. Gibson, D.G. et al. (2009) *Nature Methods*, 6, 343–345.
3. Gibson, D.G. et al. (2010) *Nature Methods*, 7, 901–903.
4. Gibson, D.G. et al. (2008) *Science*, 319, 1215–1220.
5. Smith et al. (2003) *PNAS*, 100, 15440–15445.
6. Stemmer, W.P. et al. (1995) *Gene*, 164, 49–53.
7. Endy, D. (2005) *Nature*, 438, 449–453.
8. Lartique, C. et al. (2009) *Science*, 325, 1693–1696.
9. Carr et al. (2004) *Nucleic Acids Res.* 32, e162.



Figure 4. Combinatorial gene synthesis.




*Ten overlapping 60-mer oligonucleotides are recombined into five cassettes (light blue rectangles). Following sequence verification of these cassettes, they are recombined (indicated by the X) into the full-length gene in a second stage. Recombination of the cassettes is possible because 40 bp overlaps have been added to the cassettes. Within each cassette, there are between 1 and 4 positions in which 2 nucleotides are acceptable. At these locations, a variant 60-mer oligonucleotide can also be assembled. This equates to 2-16 different combinations for each cassette. In order to produce every different combination ($2^3 \times 2^1 \times 2^1 \times 2^4 \times 2^1 = 1{,}024$) of the full-length gene, every different cassette can be pooled and assembled into a cloning vector to form a circular DNA, cloned into E. coli, and then sequenced.*

# Gibson Assembly™ Master Mix

*Gibson Assembly was developed by Dr. Daniel Gibson and his colleagues at the J. Craig Venter Institute and licensed to NEB by Synthetic Genomics, Inc. It allows for the successful assembly of multiple DNA fragments, regardless of fragment length or end compatibility. It has been rapidly adopted by the synthetic biology community due to its ease-of-use, flexibility and suitability for large DNA constructs.*

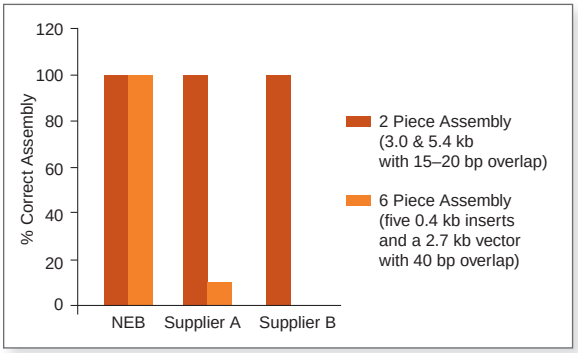
Gibson Assembly efficiently joins multiple overlapping DNA fragments in a single-tube isothermal reaction (1,2). The Gibson Assembly Master Mix includes three different enzymatic activities that perform in a single buffer (see Figure 1, page 3):

- The exonuclease creates a single-stranded 3´ overhang that facilitates the annealing of fragments that share complementarity at one end.
- The polymerase fills in gaps within each annealed fragment.
- The DNA ligase seals nicks in the assembled DNA.

The end result is a double-stranded, fully-sealed DNA molecule that can serve as template for PCR, RCA or a variety of other molecular biology applications, including direct transformation. The method has been successfully used by Gibson's group and others to assemble oligonucleotides, DNA with varied overlaps (15–80 bp) and fragments hundreds of kilobases long (1–3).

In contrast to other methods, Gibson Assembly is suitable for a wide range of different types of assemblies, including large numbers of DNA fragments. The figure below shows that Gibson Assembly outperforms other methods for assembly of six fragments.

Assembly of DNA fragments using methods from three different suppliers.




*The assemblies were conducted in accordance with the manufacturer's recommended protocols. Following assembly and transformation, a portion of the resulting colonies were tested for the presence of a PCR fragment corresponding to a correct, fully assembled construct, by colony PCR, using primers that amplify the entire fragment. While all three vendors' methods perform a 2 fragment assembly well, the Gibson Assembly Master Mix outperforms other suppliers for larger numbers of fragments.*

## Ordering Information

| PRODUCT | NEB # | SIZE |
|---|---|---|
| Gibson Assembly Master Mix | E2611S/L | 10/50 reactions |

*Gibson™ is a trademark of Synthetic Genomics, Inc.*
*gBlocks™ and Ultramer™ are trademarks of Integrated DNA Technologies, Inc.*

## Advantages

- Increased number of successful assembly products, particularly for longer or greater number of fragments
- Flexible sequence design with no need to engineer cloning sites (scarless cloning)
- Does not require clean up of PCR products prior to assembly
- Complex assembly achieved in 1 hour
- DNA can be used immediately for transformation, or as template for PCR or RCA
- Easily adapted for multiple DNA manipulations, including site-directed mutagenesis, insertions and deletions

1. Gibson, D.G. et al. (2009) *Nature Methods*, 343–345.
2. Gibson, D.G. et al. (2010) *Nature Methods*, 901–903.
3. Gibson, D.G. Personal communication.

## Looking for Synthetic Gene Fragments?

Integrated DNA Technologies (IDT) offers a range of synthetic gene products manufactured using Ultramer™ oligonucleotides, the highest fidelity next generation synthesis technology available. gBlocks™ Gene Fragments are linear, high-fidelity, double-stranded genomic blocks up to 500 bp in length, and are ideal for use with the Gibson Assembly method. Integrated DNA Technologies also offers complete custom synthetic genes, delivered in a vector ready for use in a variety of applications. For more information, visit www.idtdna.com.

*Manufactured by New England Biolabs, Inc. under license from Synthetic Genomics, Inc.*

SYNTHETIC GENOMICS®

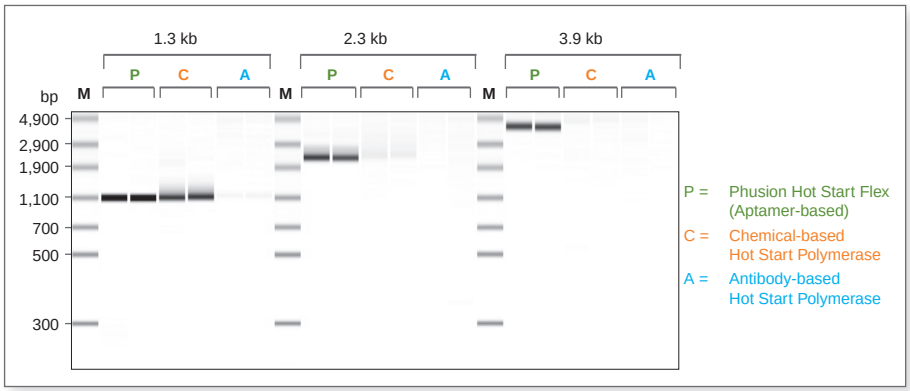
# Phusion® Hot Start Flex DNA Polymerase

Phusion Hot Start Flex DNA Polymerase delivers additional flexibility to reaction setup and cycling conditions. In contrast to chemically modified or antibody-based hot start polymerases, Phusion Hot Start Flex utilizes an innovative synthetic aptamer. This structure binds to the polymerase through non-covalent interactions, blocking activity during reaction setup. The polymerase is activated during normal cycling conditions, allowing reactions to be set up at room temperature. Phusion Hot Start Flex does not require a separate high temperature activation step, shortening reaction times and increasing ease-of-use. Phusion Hot Start Flex is an ideal choice for high specificity amplification over a flexible range of annealing temperatures and offers robust amplification on a wide variety of templates.

## Advantages

- **Robust Reactions** – maximum success with minimal optimization
- **Extreme Fidelity** – highest of any available thermostable polymerase (50X greater than *Taq*)
- **High Speed** – dramatically reduced extension times
- **High Yield** – increased product yield using minimal amount of enzyme
- **Versatile** – can be used for routine PCR, as well as long or difficult templates
- **Hot Start** – flexible formulation allows room temperature reaction setup and no activation step

Phusion Hot Start Flex DNA Polymerase delivers robust amplification for a wide range of templates.




*All amplicons are from human Jurkat template except for the 1.3 kb C. elegans amplicon. Amplicon sizes are indicated above the gel. All reactions were conducted in duplicate according to manufacturers' instructions using 30 cycles and visualized via microfluidic LabChip® analysis.*

## Ordering Information

| PRODUCT | NEB # | SIZE |
|---|---|---|
| Phusion® Hot Start Flex DNA Polymerase | M0535S/L | 100/500 units |
| Phusion® Hot Start Flex 2X Master Mix | M0536S/L | 100/500 rxns (50 µl vol) |
| Phusion® High-Fidelity DNA Polymerase | M0530S/L | 100/500 units |
| Phusion® High-Fidelity PCR Master Mix with HF Buffer | M0531S/L | 100/500 rxns (50 µl vol) |
| Phusion® High-Fidelity PCR Master Mix with GC Buffer | M0532S/L | 100/500 rxns (50 µl vol) |
| Phusion® PCR Kit | E0553S/L | 50/200 rxns (50 µl vol) |
| **COMPANION PRODUCTS** | | |
| Phusion® HF Buffer Pack | B0518S | 6.0 ml |
| Phusion® GC Buffer Pack | B0519S | 6.0 ml |
| Detergent-free Phusion® HF Buffer Pack | B0520S | 6.0 ml |
| Detergent-free Phusion® GC Buffer Pack | B0521S | 6.0 ml |

*Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. Phusion DNA Polymerase is now manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific.*
*Phusion® is a registered trademark and property of Thermo Fisher Scientific.*
*For licensing information, visit www.neb.com.*

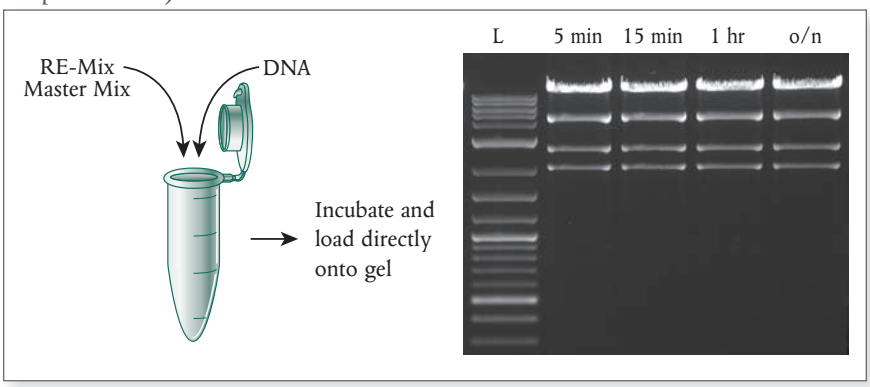
# RE-Mix™ Restriction Enzyme Master Mixes

For over 35 years, New England Biolabs has been developing innovative solutions for molecular biology applications. A respected leader in the field of restriction enzyme biology, NEB is pleased to offer the first selection of restriction enzyme master mixes that bring total simplicity and convenience to restriction enzyme digests. RE-Mix Restriction Enzyme Master Mixes contain enzyme, buffer, BSA and loading dye; all that is required is the addition of DNA and water. All RE-Mixes are Time-Saver qualified and will digest DNA in 15 minutes. Unlike other "fast" restriction enzymes, they can also be used in overnight reactions without any unwanted cleavage. With easy-to-follow protocols and flexible reaction conditions, RE-Mix enzymes from NEB offer maximum convenience.

## Advantages

- **Convenience** – easy-to-use master mix format contains enzyme, buffer, BSA and loading dye
- **Simple** – just add DNA and water
- **Time-Saver qualified** – digests substrate DNA in 15 minutes
- **Flexibility** – obtain the same results whether you choose to digest for 15 minutes or overnight
- **Low vial price**

Simple & fast – Just add DNA!




*pXba DNA was digested with EcoRV-HF™ RE-Mix™ according to the recommended protocol. Lane L is the TriDye™ 2-Log DNA Ladder (NEB #N3270). The same results are obtained whether incubated for 5–15 minutes, 1 hour or overnight.*

# FAQ Spotlight – RE-Mix Master Mixes

**Q: Can I use RE-Mix Mster Mixes in a double digest?**

**A:** *Yes, RE-Mix Master Mixes can be used together in a double digest reaction. For this, we recommend setting up a 40 µl reaction containing 2 µl of each RE-Mix Master Mix.*

**Q: If the restriction enzyme that I am using requires BSA, do I need to add it to the reaction when using RE-Mix?**

**A:** *RE-Mix Master Mixes already contain enzyme, buffer, BSA and loading dye. No additional BSA is needed.*

**Q: Do I need to stop the reaction or add loading dye prior to agarose gel electrophoresis?**

**A:** *RE-Mix Master Mixes already contain a loading dye and can be loaded directly onto an agarose gel.*

Visit **www.NEBREMIX.com** for more information or to download a datacard

Ordering Information

| PRODUCT | NEB # | # OF REACTIONS |
|---|---|---|
| AgeI-HF™ RE-Mix™ | R5552S | 25 |
| AscI RE-Mix™ | R5558S | 50 |
| BstEII-HF™ RE-Mix™ | R5162S | 100 |
| EcoRI-HF™ RE-Mix™ | R5101S | 500 |
| EcoRV-HF™ RE-Mix™ | R5195S | 200 |
| KpnI-HF™ RE-Mix™ | R5142S | 200 |
| MfeI-HF™ RE-Mix™ | R5589S | 25 |
| NcoI-HF™ RE-Mix™ | R5193S | 50 |
| NheI-HF™ RE-Mix™ | R5131S | 50 |
| NotI-HF™ RE-Mix™ | R5189S | 25 |
| PacI RE-Mix™ | R5547S | 25 |
| SalI-HF™ RE-Mix™ | R5138S | 100 |
| ScaI-HF™ RE-Mix™ | R5122S | 50 |
| SpeI RE-Mix™ | R5133S | 50 |
| XbaI RE-Mix™ | R5145S | 150 |
| XhoI RE-Mix™ | R5146S | 200 |

*RE-Mix™ is a trademark of New England Biolabs, Inc.*



# High-Fidelity (HF) Restriction Enzymes

Gone are the days of buffer compatibility analysis and sub-optimal enzyme conditions! HF restriction enzymes are all 100% active in a single buffer: NEBuffer 4. This simplifies double digest reactions and eliminates the need for setting up sequential digests. HF enzymes share the same site specificity and cleavage efficiency as their wild-type counterparts, but offer the increased flexibility and convenience that you need in your laboratory. Whether you have 5 minutes or want to leave your reaction overnight, restriction enzyme digests with HF enzymes produce the same, reliable results. Set up your digests with reaction-specific enzyme concentrations, without fear of risking product integrity to star activity. HF enzymes offer added flexibility and functionality without added cost – they're available for the same price-per-unit as the standard version.

Three new HF's have been added to our growing list:

| PRODUCT | NEB # | SIZE |
|---|---|---|
| BmtI-HF™ | R3658S/L | 300/1,500 units |
| BstEII-HF™ | R3162S/L | 2,000/10,000 units |
| SpeI-HF™ | R3133S/L | 500/2,500 units |

Visit **www.neb.com/HF** for a complete list of HF Enzymes available.

## Benefits of HF Restriction Enzymes:

- Engineered for performance under a wide range of conditions
- Reduced star activity eliminates unwanted cleavage
- One buffer convenience with no loss of performance
- Time-Saver qualified for 5-15 minute digests
- Added flexibility without added cost


New Products

# New NEBNext® Products for the Illumina Sequencing Platforms

## NEBNext Singleplex Oligos for Illumina®

## NEBNext Multiplex Oligos for Illumina® (Index Primers 1-12)

New England Biolabs now supplies two new NEBNext modules that enable singleplex or multiplex library construction of up to 12 samples:

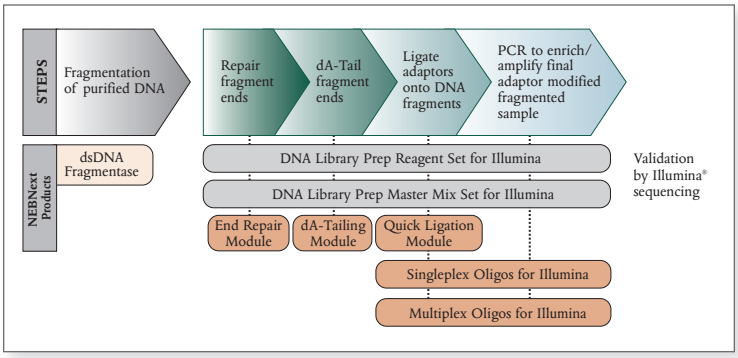
- The NEBNext Singleplex Oligos Module contains the required adapter for ligation to end-repaired, dA-tailed DNA, as well as primers for singleplex library construction.
- The NEBNext Multiplex Oligos Module contains the required adapter for ligation and the primers required for library amplification by PCR. The multiplex set enables incorporation of the barcodes at the PCR amplification step.

Oligo modules are compatible with existing NEBNext reagent sets and modules for library preparation of DNA, ChIP-Seq and mRNA libraries for sequencing with all current Illumina instruments.

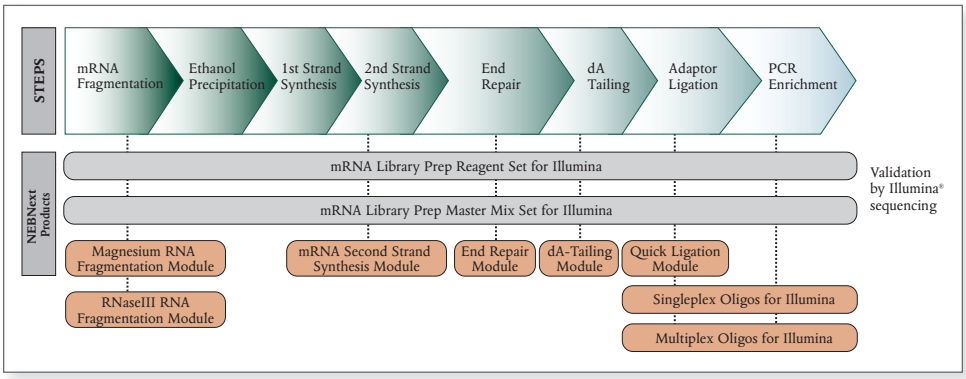
### Advantages

- High library yields
- Efficient adaptor ligation
- Module format enables flexibility in library prep workflow
- Low price per reaction
- Singleplex kit is also multiplex compatible

DNA Library Prep Workflow for Illumina.




Conversion of purified mRNA to DNA followed by library preparation.

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Ordering Information

| PRODUCT | NEB # | SIZE |
|---|---|---|
| NEBNext Singleplex Oligos for Illumina | E7350S/L | 12/60 rxns |
| NEBNext Multiplex Oligos for Illumina (Index Primers 1–12) | E7335S/L | 24/96 rxns |

## NEBNext Multiplex Small RNA Library Prep Set for Illumina (1-12)

## NEBNext Small RNA Library Prep Set for Illumina

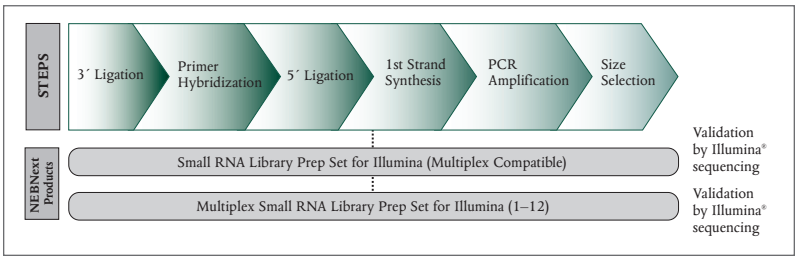
### (Multiplex Compatible)

These new NEBNext sets include oligos, enzymes, buffers and protocols for construction of small RNA libraries for single read and paired-end read sequencing, using all current Illumina sequencing instruments.

- The NEBNext Multiplex Small RNA Library Prep Set enables multiplexing of up to 12 samples.
- The NEBNext Small RNA Library Prep Set is suitable for singleplex sequencing, but is also multiplex compatible.

The NEBNext Fast DNA Library Prep Sets for Ion Torrent streamline library preparation workflow

Novel NEBNext Small RNA library preparation workflow for Illumina.




Ordering Information

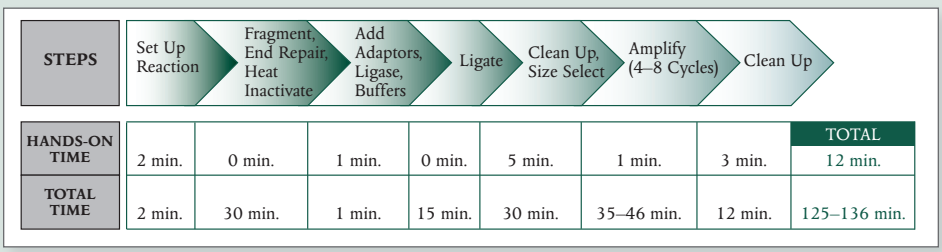
| PRODUCT | NEB # | SIZE |
|---|---|---|
| NEBNext Multiplex Small RNA Library Prep Set for Illumina (1–12) | E7300S/L | 24/96 rxns |
| NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible) | E7330S/L | 24/96 rxns |

## Advantages

- Novel protocol offers improved yields and substantially reduced adaptor-dimer formation
- Enables multiplexing
- Low price per reaction

# Now Available for the Ion Torrent™ Platform

The NEBNext Fast DNA Library Prep Sets for Ion Torrent streamline library preparation workflow and decrease hands-on-time. NEBNext sets are available with or without fragmentation reagents.




| | | | | | | | | TOTAL |
|---|---|---|---|---|---|---|---|---|
| HANDS-ON TIME | 2 min. | 0 min. | 1 min. | 0 min. | 5 min. | 1 min. | 3 min. | 12 min. |
| TOTAL TIME | 2 min. | 30 min. | 1 min. | 15 min. | 30 min. | 35–46 min. | 12 min. | 125–136 min. |

| PRODUCT | NEB # | SIZE |
|---|---|---|
| NEBNext Fast DNA Fragmentation & Library Prep Set for Ion Torrent | E6285S/L | 10/50 rxns |
| NEBNext Fast DNA Library Prep Set for Ion Torrent | E6270S/L | 10/50 rxns |

## Advantages

- Fast (just over 2 hours) with minimal hands-on-time
- High yield
- Simple protocol
- Streamlined workflow
- Compatible with a broad range of sample input (100 ng – 1 µg)
- Optional enzyme-based fragmentation
- Multiplex compatible

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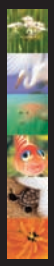

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- genomics and bioinformatics
- DNA sequencing and fingerprinting
- RNAi, siRNA and microarrays

No previous experience in molecular biology is required or expected. For additional information, course dates and to fill out an application, visit the Summer Workshop website: http://www.science.smith.edu/neb.