

# Protocol for assembling annealed DNA oligonucleotides and a double-stranded DNA vector using NEBuilder HiFi DNA Assembly (NEB #E2621)

## Materials Required but not Supplied

### NEBuilder® HiFi DNA Assembly Bundle for Large Fragments

- Nuclease-free Water (NEB #B1500)

### NEBuilder® HiFi DNA Assembly Master Mix

- Nuclease-free Water (NEB #B1500)

### NEBuilder® HiFi DNA Assembly Cloning Kit

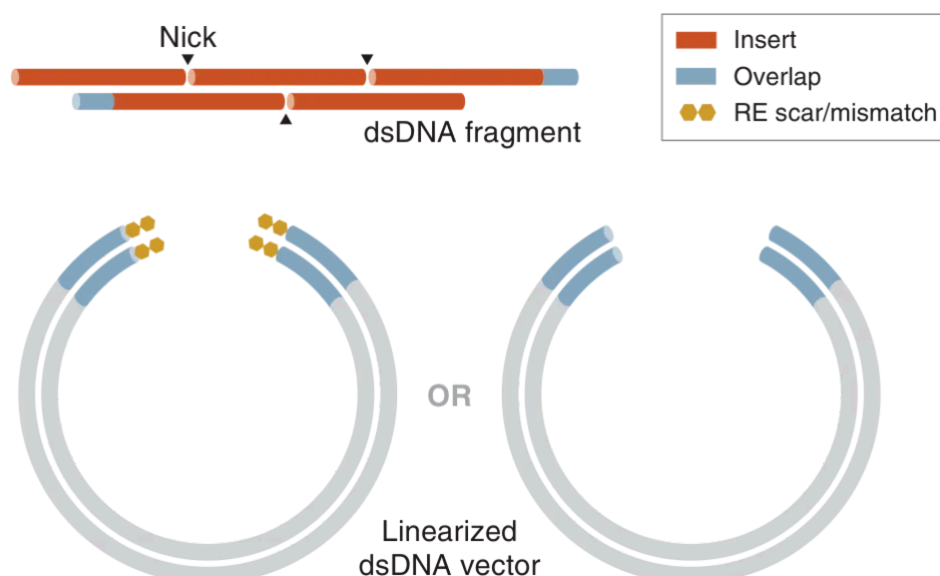
- Nuclease-free Water (NEB #B1500)

## Overview

The following method allows you to anneal short overlapping single-stranded DNA oligos (generally 60 nucleotides (nt) each) to assemble a longer double-stranded DNA (dsDNA) fragment. The annealed nicked dsDNA fragment can then be combined and assembled with a linearized vector fragment. This annealed oligo protocol provides an alternative to short, synthesized dsDNA, such as gBlocks™.

This protocol is recommended for the assembly of the following types of DNA fragments:

- Annealed short DNA oligos forming a nicked dsDNA fragment
- dsDNA vector linearized by PCR or restriction digest



## Annealed DNA Oligo Design

Short, annealed ssDNA oligos (60 nt each) should be designed with 30 nt overlaps with adjacent complementary oligos. When annealed, the overlapping oligos will form a nicked dsDNA fragment with no gaps, and ssDNA vector overlaps at each end. Please note that DNA oligos with 5' phosphates are not required.

## DNA Quantities

This protocol uses a 1:50 (vector:insert) molar ratio with 0.02 picomoles of vector and 1 picomole of annealed oligos.

## Protocol

1. Prepare oligos for annealing by adding 1  $\mu$ l of each oligo (100  $\mu$ M stock) to a final concentration of 0.2  $\mu$ M (0.2 pmol/ $\mu$ l) using 1X NEBuffer r2.1\*. This can be done by combining 1  $\mu$ l of each 100  $\mu$ M oligo stock in a single tube with an appropriate volume of buffer for a total volume of 500  $\mu$ l.

| Component                                            | 500 $\mu$ l Volume                                | Final Conc. Or Amount                         |
|------------------------------------------------------|---------------------------------------------------|-----------------------------------------------|
| Overlapping Oligos (100 $\mu$ M stock concentration) | 1 $\mu$ l of each oligo, for a total of X $\mu$ l | 0.2 $\mu$ M (0.2 pmol/ $\mu$ l) of each oligo |
| 1X NEBuffer r2.1*                                    | Buffer volume = (500 $\mu$ l - X $\mu$ l)         | 1X                                            |

*\*Note: you can also use TE buffer (10 mM Tris, 0.1 mM EDTA; pH 8.0) supplemented with 50 mM NaCl as an annealing buffer.*

2. Heat the oligo mixture solution at 100°C for 3 min and allow to cool at room temperature for 20 min. The annealed oligos are ready to assemble.
3. Set-up the following reaction on ice:

| Component                                   | 20 $\mu$ l Reaction | Final Conc. Or Amount*             |
|---------------------------------------------|---------------------|------------------------------------|
| Annealed Oligo Mixture (0.2 pmol/ $\mu$ l)  | 5 $\mu$ l           | 1 pmol (1:50 vector: insert ratio) |
| Linearized Vector (0.02 pmol/ $\mu$ l)*     | 1 $\mu$ l           | 0.02 pmol                          |
| Nuclease-free Water                         | 4 $\mu$ l           |                                    |
| NEBuilder HiFi DNA Assembly Master Mix (2X) | 10 $\mu$ l          | 1X                                 |

\* [NEBioCalculator](#) can help with DNA mass-to-molar quantity conversions for both ssDNA and dsDNA.

4. Incubate the reaction at 50°C in a thermocycler for 60 min. Transform 2  $\mu$ l of assembled mix into 50  $\mu$ l of NEB 5-alpha Competent *E. coli* (High Efficiency) ([NEB #C2987](#)) following the recommended protocol.

## Notes:

- \* If you are working with large plasmids >10 kb in size we recommend NEB® 10-beta Competent *E. coli* (High Efficiency) ([NEB #C3019H](#)). If your plasmid or insert contain repetitive sequences, we recommend NEB® Stable Competent *E.*

*coli* (High Efficiency) ([NEB #C3040H](#)).

- For selection of transformed competent cells, we recommend LB plates with an appropriate antibiotic.
- For generating PCR Products, we recommend Q5<sup>®</sup> High-Fidelity DNA Polymerase ([NEB #M0491](#)) or related products, such as Q5 Hot Start High-Fidelity DNA Polymerase ([NEB #M0493](#)) or Q5 Hot Start High-Fidelity 2X Master Mix ([NEB #M0494](#))

## Resources:

- [NEBBUILDER<sup>®</sup> Protocol Calculator](#)
- [Comparison of DNA Assembly Reaction Types](#)
- [NEBioCalculator](#)
- [High throughput cloning and automation solutions](#)