

## New England Biolabs Product Specification

*Product Name:* NEBuilder® HiFi DNA Assembly Master Mix  
*Catalog #:* E2621G  
*Kit Components:* NEBuilder® HiFi DNA Assembly Master Mix (M5520)  
NEBuilder® Positive Control (N2611)

*Shelf Life:* 18 months  
*Storage Temp:* -20°C  
*Specification Version:* PS-E2621G v2.0  
*Effective Date:* 15 May 2024

### Assay Name/Specification (minimum release criteria)

**Functional Testing (NEBuilder® HiFi DNA Assembly)** - 10 µl of 2X NEBuilder® HiFi DNA Assembly Master Mix was incubated with 0.05 pmol each of 6 DNA fragments (4 fragments of 1,000 bp, one fragment of 1,152 bp with 80 bp overlap, and a vector of 3,373 bp with a 20 bp overlap) in a final volume of 20 µl at 50°C for 60 minutes. NEB® 5-alpha Competent *E. coli* (High Efficiency) were transformed with 2 µl of the assembled products. Successfully assembled fragments produce an intact lacZ gene in the pACYC184 vector, and yield blue colonies on an IPTG/Xgal/Chloramphenicol plate when incubated overnight at 37°C after transformation. Greater than 100 blue colonies were observed when 1/10 of the outgrowth (500 µl) was spread on a plate.

\* **Individual Product Component Note** - Standard Quality Control Tests are performed for each component included in NEBuilder® HiFi DNA Assembly Master Mix and meet the designated specifications.

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Date 15 May 2024

Nancy Considine  
Quality Approver

