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## New England Biolabs Product Specification

Product Name: NEBuilder® HiFi DNA Assembly Master Mix

Catalog #: E2621S/X/L

Kit Components: NEBuilder® HiFi DNA Assembly Master Mix (M5520)

NEBuilder® Positive Control (N2611)

Shelf Life: 18 months
Storage Temp: -20°C

Specification Version: PS-E2621S/X/L v3.0

Effective Date: 15 May 2024

## Assay Name/Specification (minimum release criteria)

Functional Testing (NEBuilder® HiFi DNA Assembly) -  $10 \,\mu$ l 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  $\,\mu$ l at 50°C for 60 minutes. NEB® 5-alpha Competent *E. coli* (High Efficiency) were transformed with 2  $\,\mu$ l 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  $\,\mu$ l) 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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Namy Gundan

Date 15 May 2024

Nancy Considine Quality Approver





