240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350

www.neb.com info@neb.com

New England Biolabs Product Specification

Product Name: NEBuilder® HiFi DNA Assembly Cloning Kit

Catalog #: E5520S

Kit Components: NEBuilder® HiFi DNA Assembly Master Mix (M5520) — Store at -20°C

NEBuilder® Positive Control (N2611) — Store at -20°C

NEB® 5-alpha Competent E. coli (High Efficiency) (C2987) — Store at -80°C

SOC Outgrowth Medium (B9020) — Store at 4°C

pUC19 Vector (N3041) — Store at -20°C

Shelf Life: 12 months

Storage Temp: Multi-temperature Specification Version: PS-E5520S v2.0 Effective Date: 11 Jul 2018

Assay Name/Specification (minimum release criteria)

Functional Testing (NEBuilder® HiFi DNA Assembly) - 10 µl of 2X NEBuilder® HiFi DNA Assembly Cloning Kit 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 μ 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 Cloning Kit and meet the designated specifications.

Date 11 Jul 2018

Derek Robinson Director of Quality Control





