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New England Biolabs
Product Specification

Product Name:	*NEBridge™ Ligase Master Mix*
Catalog #:	*M1100S/L*
Concentration:	*3X Concentrate*
Shelf Life:	*24 months*
Storage Temp:	*-20°C*
Composition (1X):	*Proprietary*
Specification Version:	*PS-M1100S/L v1.0*
Effective Date:	*30 Sep 2021*

Assay Name/Specification (minimum release criteria)
Functional Testing (Assembly) - A 15 µl reaction containing 75 ng pGGAselect (Golden Gate destination plasmid, CamR), 75 ng each of 5 plasmids carrying fragments of a gene encoding *laclZ*, 1 µl of BsaI-HF v2 and 5 µl NEBridge Ligase Master Mix is incubated for 30 cycles of 37°C for 1 minute, 16°C for 1 minute, and then at 60°C for 5 minutes to linearize any remaining plasmid. Successfully assembled fragments result in *laclZ* gene in the pGGAselect vector and yield blue colonies on Cam/XGAL/IPTG agar plates. Transformation of T7 Express Competent *E. coli* (High Efficiency, NEB #C2566) with 2 µl of the assembly reaction yields >250 colonies and > 80% blue colonies when 5% of transformation is plated.
Functional Testing (Ligation and Transformation, Blunt Ends) - After a 15 minute ligation of linearized, dephosphorylated LITMUS 28 containing blunt EcoRV ends and a mixture of compatible insert fragments, transformation into chemically competent NEB 5-alpha competent *E. coli* (high efficiency) cells yields a minimum of 10^6 recombinant transformants per µg plasmid DNA.
Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in NEBuffer 1 containing 1 µg of CIP-treated Lambda-HindIII DNA and a minimum of 10 µl of NEBridge™ Ligase Master Mix incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Date 30 Sep 2021

Derek Robinson
Director, Quality Control