

2X Gibson Assembly Master Mix
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Prepare Isothermal Start Mix

1.5 g PEG₈₀₀₀
3 ml 1 M Tris-HCl, pH 8.0
150 μ l 2 M MgCl₂

Put on tube rotator until PEG is in solution

2X Gibson Assembly Master Mix

405 μ l Isothermal Start Mix
25 μ l 1 M DTT
20 μ l 25 mM dNTPs
50 μ l NAD⁺ (NEB Cat. # B9007S)
1 μ l T5 exonuclease (NEB Cat. # M0363S – 10 units/ μ l)
31.25 μ l Phusion High Fidelity DNA Polymerase (NEB Cat. # M0530S – 2 units/ μ l)
250 μ l Taq Ligase (NEB Cat. # M0208S – 40 units/ μ l)
467.75 μ l H₂O

Mix by pipetting gently. Make 100 μ l aliquots.