Gibson assembly

Introduction

Check this before usage: https://www.neb.com/protocols/2012/09/25/gibson-assembly-master-mix-assembly

Materials

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Procedure

1. * Optimized cloning efficiency is 50–100 ng of vectors with 2–3 fold of excess inserts. Use 5 times more of inserts if size is less than 200 bps. Total volume of unpurified PCR fragments in Gibson Assembly reaction should not exceed 20%.

Table1

	2-3 Fragment Assembly	4-6 Fragment Assembly	Positive Control**
Total Amount of Fragments	0.02–0.5 pmols* X µl	0.2–1 pmols* X µl	10 µl
Gibson Assembly Master Mix (2X)	10 µl	10 µl	10 µl
Deionized H2O	10-X µl	10-X µl	0 µl
Total Volume	20 µl***	20 µl***	20 µl
* Optimized cloning efficiency is 50–100 ng of vectors with 2–3 fold of excess inserts. Use 5 times more of inserts if size is less than 200 bps. Total volume of unpurified PCR fragments in Gibson Assembly reaction should not exceed 20%.	** Control reagents are provided for 5 experiments.	*** If greater numbers of fragments are assembled, additional Gibson Assembly Master Mix may be required.	

2. Incubate samples in a thermocycler at 50°C for 15 minutes when 2 or 3 fragments are being assembled or 60 minutes when 4-6 fragments are being assembled. Following incubation, store samples on ice or at -20°C for subsequent transformation.

3. Note: Extended incubation up to 60 minutes may help to improve assembly efficiency in some cases (for further details see FAQ section).