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Gibson Assembly Cloning

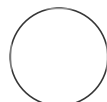
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Protocol status: Working
We use this protocol and it's working

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ABSTRACT

Gibson assembly requires a vector backbone and one or more inserts that have been PCR amplified. The inserts should have 15-20 base pairs of overlap at ligation sites, so primers used to amplify the inserts should contain a tail overhang with this homology. The vector backbone should be linear.

A Gibson reaction uses a master mix that can be homemade or commercially purchased. Add the appropriate amount of master mix, then your vector and insert at 2-3 fold molar excess for a 20 µl reaction. Exact ratios will need to be optimized based on the size of your vector and inserts and how many inserts you have. Incubate this reaction at 50°C for 60 minutes. Then transform this reaction into DH5 alpha (or competent cell of choice) and plate on LB agar with appropriate antibiotic.

PROTOCOL integer ID:
87471

Keywords: ASAPCRN

MATERIALS

PEG-8000
Tris-HCl pH 7.5
MgCl₂
DTT
dNTPs [NEB, N0447S]
NAD
10U/μl T5 exonuclease [NEB, M0663S]
2U/μl Phusion polymerase [Thermo, F530S]
40U/μl Taq Ligase [NEB, M0208S]
Sterile water

Competent cells (DH5α) [Thermo, 18258012]
PCR machine
LB, LB agar
antibiotic of choice

Prepare Gibson master mix

1 Make 5 ml of 5x reaction buffer




1.1	25% PEG-8000	1.25 g
	500 mM Tris-HCl pH 7.5	2.5 ml
	50 mM MgCl ₂	0.25 ml of 1M
	50 mM DTT	0.25 ml of 1M
	1 mM each dNTPs	0.05 ml each of 100mM
	5 mM NAD	0.5 ml of 50 mM

2 Prepare master mix

2.1	Master Mix
	320 μl 5X Reaction Buffer (above)
	0.64 μl 10U/μl T5 exonuclease
	20 μl 2U/μl Phusion
	160 μl 40U/μl Taq Ligase
	700 μl Water





- 3 Use master mix as 1.5x. Snapfreeze in  15 µL aliquots and store at  -80 °C .

Gibson assembly

- 4 Prepare linearized vector backbone by restriction enzyme digestion or PCR amplification.
- 5 Prepare inserts by PCR amplification. Ensure that each insert has 15-20 base pairs of overlap with other inserts/vector at ligation sites, added by primer design.
- 6 Take a 15 ul aliquot of 1.5x Gibson master mix on ice. Add 50-100 ng of vector with 2-3 fold molar excess of PCR inserts, and sterile water if necessary, to make total reaction volume up to  20 µL .
- 7 Mix well and let incubate in PCR machine for  01:00:00 at  50 °C 1h

Plasmid preparation

1h

- 8 After incubation is complete, transform into competent cells (DH5α) and plate onto LB agar with appropriate antibiotic. Let this grow at  37 °C  Overnight .
- 9 Pick a single colony to grow in 5 ml LB culture at  37 °C  Overnight and purify plasmid to check sequencing.