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Simple Step toy Increase Gibson Assembly Efficiency V.1

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1 Works for me dx.doi.org/10.17504/protocols.io.7kzhkx6

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ABSTRACT

The Gibson assembly solutoin is extremely toxic to some bacteria cells.

Doing a solution change after assembly and before transforming the bacteria will significantly improve bacteria survival. This increases 10-100x the amount of colonies.

MATERIALS

NAME	CATALOG #	VENDOR
Gibson Assembly Master Mix - 50 rxns	E2611L	New England Biolabs
NucleoSpin® Gel and PCR Clean-up	740609.10	Macherey and Nagel

Do Normal Assembly

1 Do a normal Gibson assembly. Incubate in the PCR machine 60 min @ 50C

2 Remove the assemblies. Do a PCR quickchange/ gel extract column. This will remove the Gibson Assembly solution but keep the 100ng (or however much) of DNA.

Elute in 15u -20ull TE or water

3 Take the 15-20ul assembled plasmid and heat shock your bacteria. 30 second heat shock. 2 min ice. Add 200ul media and shake for 45 min. Carb can be plated immediatly.

Plate

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