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Simple Step to Increase Gibson Assembly Efficiency V.2

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

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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

MATERIALS

Gibson Assembly Master Mix - 50 rxns **New England Biolabs** **Catalog #**E2611L

NucleoSpin® Gel and PCR Clean-up **Macherey-Nagal** **Catalog #**740609.10

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