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Version 1

Simple Step toy Increase Gibson Assembly Efficiency V.1

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Protocol status: Working

We use this protocol and it's working

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Abstract


The Gibson assembly solution 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-Nagel Catalog #740609.10**

Troubleshooting



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