Gibson assembly

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Abstract

Gibson is a PCR-based multi-fragment assembly technique. So whether PCR works has large impacts on final product.

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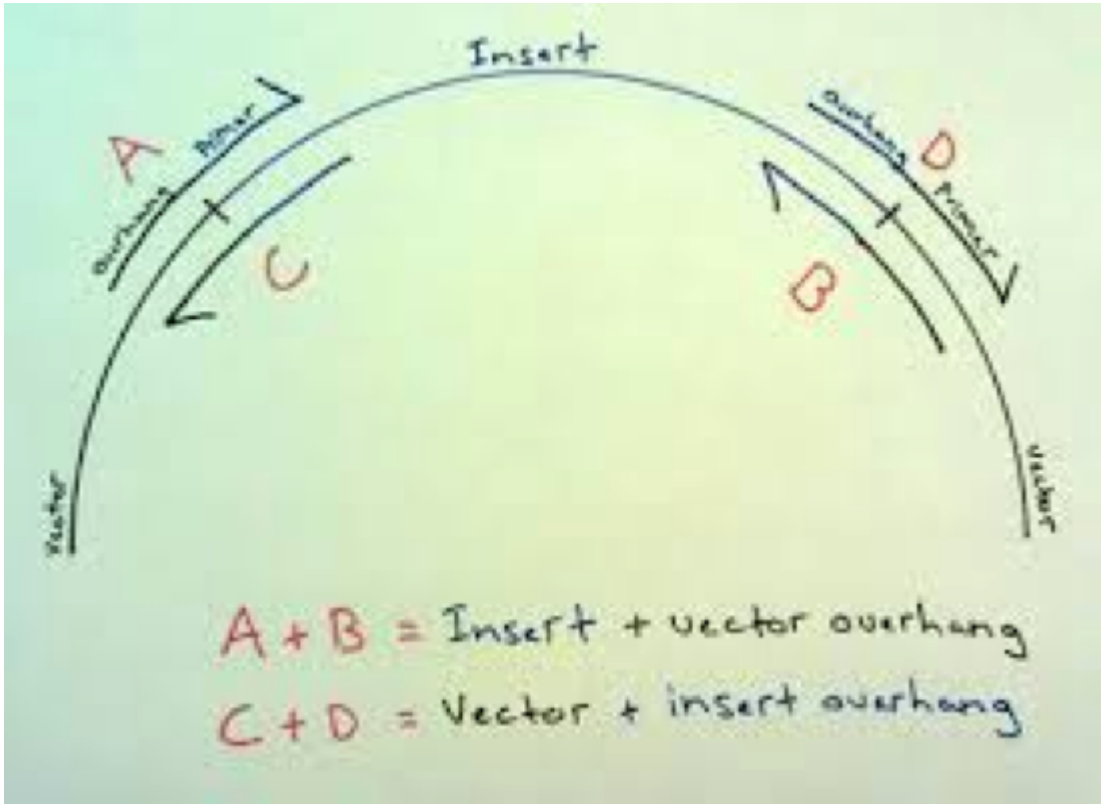
Materials

Gibson Assembly Master Mix - 10 rxns E2611S by New England Biolabs

Protocol

Primer design

Step 1.



Step 2.

Construct a complete map of insert in the desired destination vectors in Snapgene or Ape.

Step 3.

Identify the connection region between inserts and vector.

Step 4.

Design primer for amplifying insert and vectors. Target melting temperature Tm set to 60 or higher.

Step 5.

Order primers A, B, C and D.

Notes: A has two parts. Starting from 5', the overhang is the reverse complement of Rev primer of backbone, and the primer region is a standard primer to amplify insert (Fwd primer of insert). B is same and has reverse complement of backbone Fwd primer followed by standard Rev primer of insert.

So primer A= Backbone Rev primer (rev complement)+ Insert Fwd primer

Primer B= Backbone Fwd primer (rev complement)+ Insert Rev primer

Primer C is reverse complement of A.

Primer D is reverse complement of B.

Step 6.

For more fragment to assemble, increase number of primers correspondingly.

PCR

Step 7.

Amplify insert with A and B primers. Amplify backbone with C and D primers. Use 25 or 50 uL reaction volume. Use DMSO or nested/ touchdown PCR if necessary.

Step 8.
Add 0.25 uL DpnI and incubate at 37 C for 1hr (or longer depending on your paranoid) to remove template.

Step 9.
Run a gel to verify the size of PCR product.

Step 10.
Use PCR cleanup or Gel extraction to get clean product.

Step 11.
Quantify the concentration by Nanodrop

Gibson Assembly

Step 12.

NOTES

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For 2 or 3 fragments assembly, use 0.02-0.5 pmols of DNA. For more fragments, use 0.2-1.0 pmols.

pmols=(weight in ng) x 1000 / (base pairs x 650 daltons)

Step 13.
Put insert and vectors into reaction and add DI water to 10 uL.

Step 14.
Add 10 uL 2X Gibson Master mix.

Step 15.
Incubate at 50 C for 15-60 minutes depending on the fragments number. 2-3 fragments use 15 mins. 4-6 use 60 mins.

Step 16.
Store in -20 C.

Transformation

Step 17.
Mix 1 uL of reaction and 10 uL of competent cells, do not vortex. Keep on ice for 30 mins.

Step 18.
Heat shock in 42 C water bath for 45 sec.

Step 19.
Keep on ice for 5 mins.

Step 20.
Add 300 uL SOC (pre-warm to Room temperature). Put in 250 rpm shaker in 37 C for 1hr.

Step 21.
Use 50-100 uL of recovered solution to plate out depending on copy number and plate size.

Step 22.
Harvest colony next day and pick single colony for liquid culture in corresponding antibiotics.

Step 23.
Sequencing and digestion to verify the assembly.