ARIA

Gibson Assembly

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Introduction

Gibson Assembly facilitates plasmid cloning in a one-pot reaction. In this context, a single fragment has been joined to the backbone plasmid.

Materials

- Backbone plasmid
- DNA Insert
- Gibson Assembly Master Mix (2X)
- Deionized H_2O

Procedure

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1. Prior to reaction assembly, DNA samples must be diluted to a determined concentration that contemplates 0.02–0.5 pmols of total DNA quantity.

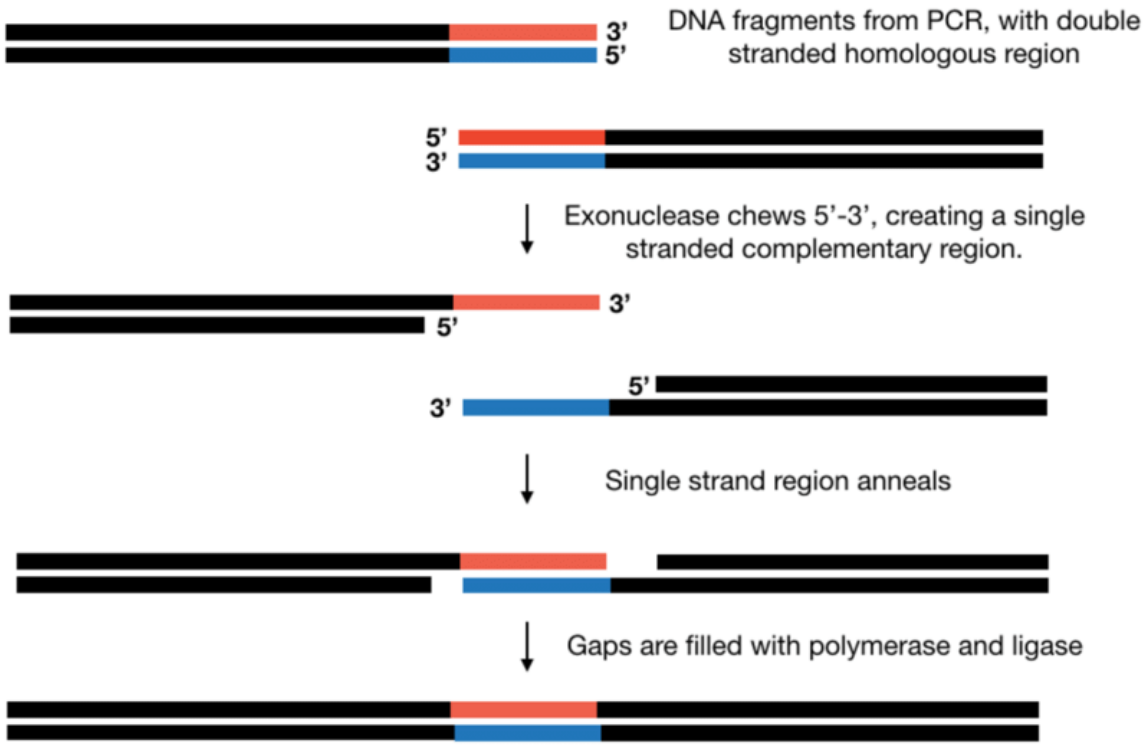
2. Mix the previously noted components as follows:

    - x µL DNA insert
    - x µL Backbone Plasmid
    - 10 µL Gibson Assembly Master Mix
    - 10-2x µL Deionized H_2O (or up to 20 µL)

3. Incubate samples in a thermocycler at **50°C for 15 minutes**

4. Store samples on ice or at -20°C for subsequent transformation

    Further detail of the steps of Gibson Assembly cloning [2]



Bibliography

[1] New England Biolabs. (2012, 25 septiembre). *Gibson Assembly® Master Mix – Assembly (E2611) | NEB*. https://international.neb.com/protocols/2012/09/25/gibson-assembly-master-mix-assembly

[2] Cutts, E., & Vannini, A. (2018). Troubleshooting biGBac: a practical guide v1. *protocols.io*. Published. https://doi.org/10.17504/protocols.io.q3ydypw