Jun 20, 2019

Fabrication of DNA constructs by Gibson Assembly and Golden Gate reactions V.2

Tamara Matute[1], Isaac Nuñez[1], Peter Von Dassow[1], Fernan Federici[1]

[1]PUC

Working dx.doi.org/10.17504/protocols.io.4fcgtiw

Protist Research to Optimize Tools in Genetics (PROT-G) SynBioUC Chile

Fernan Federici

ABSTRACT

We used this protocol to build a series of codon optimized BFP expressing vectors in the context of the EMS initiative from Gordon and Betty Moore foundation. A complete list of files corresponding to the DNA parts and vectors used in this study can be found at our Drive and zenodo database.

Introduction and rationale

1 Golden Gate assembly creates vectors by combining vectors containing level 0 parts (e.g. promoters, CDSes, terminators) and an acceptor vector. The first step involves the creation of libraries of level 0 parts by 'domesticating' DNA sequences of interest (e.g a promoter or a new fluorescent protein).

Domestication of DNA sequences into level 0 part vectors

2 Level 0 parts for Golden Gate reactions were generated by Gibson Assembly. Parts can be generated by PCRing natural sequences (e.g genomic DNA) or existing vectors; or by fully synthesising (e.g. gblocks from IDT) DNA fragments of interest.

For PCRed parts, reactions were performed with Phusion® High-Fidelity DNA Polymerase following this conditions:
Thermocycling
98°C x 30 sec
(98°C x 10 sec + 60°C x 30 sec + 72°C x 2 min) x 35 cycles
72°C x 10 min
4°C

PCR mix:
1ul primer forward Gibson
1ul primer reverse Gibson
1,33ul template (concentration: 1ng/ul)
8 ul Buffer HF 5X
0,8ul dNTPs (10uM)
0,4ul Phusion® High-Fidelity DNA Polymerase (2U/ul)
27,46 ul H20

Commericlaly synthetisised DNA fragments were ordered containing the 20-30bp overhangs used for Gibson assembly into the level 0 part vector.

Gibson assembly step for the creation of level 0 part vector

3 Desired PCR products were identified by gel electrophoresis and extracted with the purification kit Wizard SV Gel & PCR Clean-Up System (Promega). DNA concentration were corroborated with a Take3™ Micro-Volume Synergy™ HTX before performing Gibson reactions.
The volumes used for each part were calculated by eq 1, 2 and 3.

Eq.1

$$X\,[pMol/\mu l] = \frac{Concentration\,[ng/\mu l] * 1000}{650\,[\frac{gr/mol}{bp}] * Lenght\,[bp]}$$

Eq.2

$$Vol_{ratio\ i} = \frac{X}{pmol_{req}}$$

Volume ratio [ul] = pmol/required pmol
where required pmol is:
0,01 for parts >2500 bp
0,03 for parts >200 bp and <2500 bp
0,05 for parts <200 bp

Volume to be added was calculated as:
Eq.3

$$Vol_{piece\ i} = \frac{Vol_{total} * Vol_{ratio\ i}}{\sum_{i}^{n} Vol_{ratio\ i}}$$

1,5µl of parts were combined with 4,5µl of Gibson Master Mix in ice. This reaction was incubated at 50°C for 1 hour.

Reagents:
Gibson Mix (1.33X):
100 ul 5X Isothermal Buffer
2 ul T5 Exonuclease (1 U/ul)
6,25 ul Phusion® High-Fidelity DNA Polymerase (2U/ul)
50 ul Taq DNA ligase (40U/ul)
216,75 ul H20

Store at -20°C.

5X Isothermal Buffer:
25% PEG-8000
500 mM Tris-HCl pH 7,5
50 mM MgCl2
50 mM DTT
1 mM dATP
1 mM dTTP
1 mM dCTP
1 mM dGTP
5 mM NAD
H20
Store at -80°C

Transformation of Level 0 donor vectors

4 These reactions were transformed into chemically competent TOP10 cells prepared by the OOW protocol (http://www.openwetware.org/wiki/TOP10_chemically_competent_cells), minipreped and sequenced. These level 0 parts were stored as vectors for the following Golden Gate reactions.

Golden Gate assembly of promoters and fluorescent proteins combinations

5 Final combinatorial assemblies of promoters, CDSes and terminators here described were produced by Golden Gate. For this, a general aceptor vector was created that accepts level 0 parts for the assembly of transcriptional units (e.g. promoter-CDS-terminator of interest).

Eq.4

$$Vol_{required}\,[ul] = \frac{X * Length * 650}{[concentration] * 10^6}$$

The assembly was performed as follows:
(37°C x 3 min + 16°C x 5 min) * 40 cycles.
50°C x 5 min.
80°C x 10 min
4°C

Transformation of Golden Gate reactions

6 3µl of these reactions were used to transform TOP10 Escherichia coli cells following the step described above.

Positive colonies were identified by colony PCR and sequenced.