# Fabrication of DNA constructs by Gibson Assembly and Golden Gate reactions

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## Abstract

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## Protocol

### Introduction and rationale

**Step 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

**Step 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

**Step 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 50ºC 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

**Step 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

**Step 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

**Step 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.