WCSB, Wageningen University	IBISBA-SOP-WU22
	Version 1.0

EPP - Standard Operating Procedure

(only for selected experiments intended to transfer results from one lab to the other)

Title: *Golden gate cloning*

distribution list			
changes to prior version:			
	name	signature	date
Experimenter 1	Rita Volkers		19/3/2019

Instruction

Golden gate cloning

1. Introduction / Purpose

This is a general description of golden gate cloning. It gives an example of one way to do it, but many other ways are possible as well. These can be found on the internet.

Keywords: Cloning - golden gate

2. Equipment and chemicals

2.1. Equipment

- PCR machine

2.2. Chemicals

- T4 ligase
- A type IIS restriction enzyme
- An appropriate buffer
- MQ water
- Any additive that is needed for the chosen procedure

2.4. Other materials

- The parts that need to be ligated

4. Procedures

Many different protocols can be found online. In the Yeast Toolkit, the following is done for example:

T4 ligase buffer:1.5 µl
T4 ligase:0.8 µl
BsmBI or *BsaI*:1.0 µl
10X BSA:1.5 µl
MQ:to a total volume of 15 µl
Parts:depends on concentration

Program in PCR machine:

37 °C – 5 minutes
37 °C – 3 minutes + 16 °C – 4 minutes --> 25 cycles
50 °C – 5 minutes
80 °C – 5 minutes
10 °C – hold

Transform up to 5 µl of this mixture to *E. coli* and select for correct colonies.

5. Remarks / troubleshooting

With Golden Gate cloning it is possible to construct plasmids out of multiple fragments in one reaction.

It is most efficient if the fragments ('parts') are on plasmids ('part plasmids') themselves, but the parts can be PCR products as well.

It basically is a restriction and ligation reaction at the same time. An example of the use of Golden Gate is the Yeast Toolkit described here:

Lee ME, DeLoache WC, Cervantes B, Dueber JE; A Highly Characterized Yeast Toolkit for Modular, Multipart Assembly, 2015, *ACS Synth. Biol.* 4:975-986

Golden Gate makes use of type IIs restriction enzymes that cut DNA outside of their recognition site. The overhang thus can be designed by the user. Below in red underlined: recognition site. In green: cut site.

GCATGCTCTCATCGACGCTGAAGCTGA
CGTACGAGAGTAGCTGCGACTTCGACT

Example:

PCR gene of interest or other part with primers that contain recognition site for type IIs enzyme and a 4-base unique overhang.

GCATGCTCTCANNNN----------
Gene of interest
----------NNNNACTCTCGTACG

Red underlined: BsmBI recognition site
Green bold: unique overhang
Green dotted: primer
Black: any base

Each part has overhangs that can ligate to only one overhang of another part.

Therefor the parts can only ligate in one way, resulting in the correct plasmid.

Sometimes, a backbone plasmid is used to ligate the parts into. In this case, a dropout gene (GFP for example) is cut out of the backbone during the process to make selection of colonies easier. In the case of GFP, colonies that have the correct inserts are white and the incorrect ones are yellow.

6. Biosafety

No biosafety issues were associated with this protocol.

7. Rererences

Lee ME, DeLoache WC, Cervantes B, Dueber JE; A Highly Characterized Yeast Toolkit for Modular, Multipart Assembly, 2015, ACS Synth. Biol. 4:975-986

8. Acknowledgements

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