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Cloning with Golden Gate

In 1 collection

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Abstract

This protocol details the cloning and transformation.

Materials

PCR mix:

	A	B	C
	Component	**Final conc.**	**1Rxn (uL)**
	H2O		Up to 25ul
	T4 DNA ligase buffer	1X	2.5
	T4 DNA ligase (2,000U/ul)		0.5
	Plasmid (100ng/ul)	2.5ng	2.5
	SapI (10U/ul)	20U	2
	Insert (ul)	1:2 mol ratio	1.09
	Total		25

Digestion mix:

	A	B	C	D	E	F
			Total samples #	2	Err	1.05
	Component	Final conc.	1Rxn (uL)	Total		
	H2O		3	6.3		
	rCutSmart Buffer		1	2.1		
	SapI (10U/ul)	10U	1	2.1		
	Total		5	#REF!		

Wild-type target assembly

1d

1 **Golden Gate assembly:**

- If the wild-type insert is obtained from PCR amplification, ensure the PCR product is cleaned.

	A	B	C
	Component	**Final conc.**	**1Rxn (uL)**
	H2O		Up to 25ul
	T4 DNA ligase buffer	1X	2.5
	T4 DNA ligase (2,000U/ul)		0.5
	Plasmid (100ng/ul)	2.5ng	2.5
	SapI (10U/ul)	20U	2
	Insert (ul)	1:2 mol ratio	1.09
	Total		25

1.1 A negative control reaction with H2O instead of insert is recommended.

1.2 Set the thermal cycler for the program below:

	A	B	C
	Step	**Temperature**	**Time (hh:mm:ss)**
	1	37°C	00:05:00
	2	16°C	00:05:00
	4	Go to step 1 for 10-15 total cycles	
	5	60°C	00:15:00
	6	85°C	00:15:00
	7	4°C	hold

Note

SapI enzyme is less thermostable. Consider adding 1 µL of SapI enzyme after 2 hours.

2 Made additional digestion mix.

		Total samples #	2	Err	1.05
Component	Final conc.	1Rxn (uL)	Total		
H2O		3	6.3		
rCutSmart Buffer		1	2.1		
SapI (10U/ul)	10U	1	2.1		
Total		5	#REF!		

2.1 Aliquot 5 µL of digestion mix to each of the golden gate reactions.

2.2 Set the thermal cycler for the program below:

A	B	C
Step	**Temperature**	**Time (hh:mm:ss)**
1	55°C	At least 2 hours
2	80°C	00:15:00
4	Hold at 4C	

3 Purify each reaction with Zymo Clean & Concentrate -5 kit.

4 **Transformation:**

Add 1 µL - 2 µL of DNA from sample reaction and negative control to chemical competent E. coli cells by following NEB stable competent cell transformation protocol.

> Note
>
> Quick transformation without an outgrowth period is adequate. It will reduce transformation efficiency.

4.1 Consider to add Puc 19 and H_2O as controls to the transformation process.

5 Incubate overnight at 30 °C for 24:00:00 . 1d

6 **Colony selection and mini prep:**

Check negative control plate to ensure the background colony is less than 10% of the colony counts from the sample plate.

7 Pick 2-3 colonies for an overnight growth in 3 mL of LB + amp (1:1000) per colony for mini prep.

8 Sequence confirmation by nanopore sequencing.