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MoClo reaction V.2

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

Molecular cloning system

Materials

Reagents for level 1:

- DNA part - 75 ng / each GB basic part
- Destination vector - 50 ng circularized plasmid, 25 ng linearized plasmid
- TSII endonuclease (Bsai) - 1 µL
- T4 DNA ligase - 1 µL
- BSA (10X) - 1.5 µL
- T4 DNA ligase buffer (Thermo) 1.5 µL
- Sterilized H20 - up to 15 µL

N° Cycles	Time (minutes)	T (°C)
1	10	37
25	3	37
	4	16
1	10	50
1	10	80

Troubleshooting

1 **Fill in Setup Sheet (tab below)**

2 **Dilutions for Parts and Destination Vectors**

Follow instructions on "Dilutions_PRINT" sheet
If dilutions are already done, skip to "Reaction Set-up" below.

3 **Reaction Set-up**

3.1
- Turn on PCR machine
- Thaw DNA samples
- Thaw 10x NEB ligase buffer on ice

3.2
- Obtain 1 PCR tube per reaction and label
- Add the following to each tube in order:
- 2µL of NEB ligation buffer (10x)

3.3
- ___ uL of each part
- ___ uL destination vector
- ___ µL sterile H2O
- 0.5 µL of Promega T4 ligase enzyme

3.4
- Close PCR tubes and spin them to collect liquid at the bottom of tube

3.5
- Incubate as follows in PCR machine:
  Level 1 or 2: 25 cycles of [37°C 1.5min, 16°C 3min], 50°C 5min, 80°C 10min, hold at 4°C
- Use in transformation or store at -20°C until use

4

Tube Label	MoClo Level	Promoter	RBS	Ntag	Ntag2	CDS	Ctag	Ctag2	Terminator	Vector	10x Ligase Buffer	Vol. Pro	Vol. RBS
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2	#DIV/0!	#DIV/0!
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2	#DIV/0!	#DIV/0!
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2	#DIV/0!	#DIV/0!
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2	#DIV/0!	#DIV/0!
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2	#DIV/0!	#DIV/0!
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2	#DIV/0!	#DIV/0!
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2	#DIV/0!	#DIV/0!
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2	#DIV/0!	#DIV/0!
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2	#DIV/0!	#DIV/0!

*Level 1 = BsaI