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Version 2

MoClo reaction V.2

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MoClo

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Protocol status: In development

We are still developing and optimizing this protocol

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Keywords: moclo, dna, moclo reaction molecular cloning system

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 μ L
- T4 DNA ligase - 1 μ L
- BSA (10X) - 1.5 μ L
- T4 DNA ligase buffer (Thermo) 1.5 μ L
- Sterilized H₂O - up to 15 μ L

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

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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!
	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 = Bsal