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Sequencing of construct

In 4 collections

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes sequencing of construct.

Attachments

404-874.docx
52KB

Materials

Materials

Fw primer (CMV FW)
Rv primer (BGH RV)
sodium acetate (Carl Roth)
EtOH (VWR Chemicals BDH Prolabo)
Terminator Sequencing buffer
MilliQ-H_2O
Hi-Di Formamide (Applied Biosystems)

GoTaq(R) DNA Polymerase, 500u **Promega Catalog #**M3005

dNTP Set 100 mM Solutions **Thermo Fisher Scientific Catalog #**R0182

BigDye™ Terminator v3.1 Cycle Sequencing Kit **Thermo Fisher Catalog #**4337455

PCR to amplify region of interest

1

	A	B
	Master mix	**1x**
	H2O	13.3
	5x Colorless Reaction Buffer (Promega, #M3005)	4
	10mM dNTPs (ThermoFisher, #R0182)	0.4
	Fw primer (CMV FW)	0.6
	Rv primer (BGH RV)	0.6
	GoTaq Polymerase (Promega, #M3005)	0.1
	Σ	**19 µl**

Mix 19 µL MM with 1 µL DNA (50 ng).

PCR Program

2

94°C -> 5 min
94°C -> 30 sec
60°C* -> 30 sec } 10 x
72°C -> 1 min
94°C -> 30 sec
50°C -> 30 sec } 25 x
72°C -> 1 min
72°C -> 7 min
4°C -> endless

* -1°C /cycle

Sodium acetate precipitation

3 Add 50 µL sodium acetate (1 mL [M] 3 Mass Percent Na Acetate (Carl Roth) + 24 mL 100% EtOH (VWR Chemicals BDH Prolabo)) to 20 µL PCR product.

4 Mix well and centrifuge at 3200 rcf, 4°C, 00:45:00 . 45m

5 Remove supernatant (pat plate on paper).

6 Add 100 µL 70% EtOH onto pellet.

7 Centrifuge at 3200 rcf, 4°C, 00:15:00 . 15m

8 Remove supernatant (pat plate on paper).

9 Add 100 µL 70% EtOH onto pellet.

10 Centrifuge at 3200 rcf, 4°C, 00:15:00 . 15m

11 Remove supernatant (pat plate on paper).

12 Centrifuge upside down max. 600 rcf, 4°C, 00:01:00 (top of sample down on tissue paper) to remove EtOH. 1m

13 Add 15 µL MilliQ-H2O to pellet and vortex 15-20 min (speed 0-1).

Sequencing-PCR

14

	A	B
		1x sample
	H2O	5.3 µl
	5x Terminator Sequencing buffer	3.3 µl
	BigDye v3.1 (ThermoFisher, #4337455)	1.4 µl
	Σ	10 µl

Add 5 µL MM.

15 Add 4 µL DNA from step 13.

16 Add 1 µL primer FW or RV.

Sequencing program

17

94°C -> 5 min
94°C -> 10 sec
60°C -> 4 min
4°C -> endless
29 x

Sodium acetate precipitation

18 Add 25 µL sodium acetate to 10 µL PCR product from.

19 Mix well and centrifuge at 3200 rcf, 4°C, 00:45:00 .

45m

20 Remove supernatant (pat plate on paper).

21 Add 100 µL 70% EtOH onto pellet.

22 Centrifuge at 3200 rcf, 4°C, 00:15:00 . 15m

23 Remove supernatant (pat plate on paper).

24 Add 100 µL 70% EtOH onto pellet.

25 Centrifuge at 3200 rcf, 4°C, 00:15:00 . 15m

26 Remove supernatant (pat plate on paper).

27 Centrifuge upside down max. 600 rcf, 4°C, 00:01:00 (top of sample down on tissue paper) to remove EtOH. 1m

28 Add 15 µL MilliQ-H_2O to pellet and vortex 15-20min (speed 0-1).

Note

Note: COVER! Light sensitive!

Load Sequencing-plate

29 Add 10 µL Hi-Di Formamide (Applied Biosystems).

30 7 µL DNA after purification (Step 28).

31 Store in 4 °C until sequencing.