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Sequencing of construct

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Protocol status: Working

We use this protocol and it's working

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Keywords: Sequencing-PCR, Sodium acetate precipitation, Load Sequencing-plate, ASAPCRN, construct, construct this protocol, protocol

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-H₂O

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
H ₂ O	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 . 
 
- 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 . 

- 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 . 

- 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. 

- 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-H₂O 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.