



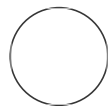
MAR 30, 2023

## Sequencing of construct

In 1 collection

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### ABSTRACT

This protocol describes sequencing of construct.

### ATTACHMENTS

[404-874.docx](#)

### MATERIALS

#### Materials

Fw primer (CMV FW)  
 Rv primer (BGH RV)  
 sodium acetate (Carl Roth)  
 EtOH (VWR Chemicals BDH Prolabo)  
 Terminator Sequencing buffer  
 MilliQ-H<sub>2</sub>O  
 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**

### OPEN ACCESS

**DOI:**  
[dx.doi.org/10.17504/protocols.io.3byl4jy7jlo5/v1](https://dx.doi.org/10.17504/protocols.io.3byl4jy7jlo5/v1)

**Protocol Citation:** Pascale Baden, michela.deleidi 2023. Sequencing of construct. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.3byl4jy7jlo5/v1>

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

**Created:** Mar 28, 2023

**Last Modified:** Mar 30, 2023

**PROTOCOL integer ID:**  
 79541

**Keywords:** Sequencing-PCR, Sodium acetate precipitation, Load Sequencing-plate

## PCR to amplify region of interest

1



A	B
Master mix	1x
H <sub>2</sub> 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 1M 3 Molarity (M) 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  $\mu$ L 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  $\mu$ L 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-H<sub>2</sub>O to pellet and vortex 15-20 min (speed 0-1).




## Sequencing-PCR

14



A	B
	1x sample
H <sub>2</sub> O	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  $\mu$ L 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  $\mu$ L MilliQ-H<sub>2</sub>O to pellet and vortex 15-20min (speed 0-1).




#### Note


**Note:** COVER! Light sensitive!

## Load Sequencing-plate

29 Add  10  $\mu$ L Hi-Di Formamide (Applied Biosystems).



30  7  $\mu$ L DNA after purification (Step 28).

**31** Store in  4 °C until sequencing.