untitled protocol

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Abstract

Citation: Yuan Yao untitled protocol. **protocols.io**
dx.doi.org/10.17504/protocols.io.qaddsa6
Published: 22 May 2018

Protocol

Step 1.
Incubate E.coli cells 1:100 in 10 ml LB medium with antibiotics.

Step 2.
OD600=0.4 , collect all cells at 4 ℃, 8000 rpm for 10 min.

Step 3.
Wash with 1 ml 1*PBS one time.

Step 4.
Resuspend in 500 ul 1 * PBS and sonicate.

Step 5.
Spin at 10000 rpm for 15 min.

Step 6.
Transfer supernatant into a new 1.5 ml tube and sit on ice.

Step 7.
Load into 120% SDS-PAGE gel , run at 200v for 45 min.

Step 8.
Get the PVDF membrane and filter paperPVDF: Put in methanol (5s) then in transfer bufferFilter: transfer bufferGel: transfer buffer

Step 9.
Sit on Room Temperature for 20 min.

Step 10.
Transfer membrane at 25 V for 30 min.

Step 11.

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Blook woth 5% skim milk RT for 1 hour.

Step 12.
1:1000 Ab1 DnaA Ab.

Step 13.
4 ℃ over night rotation.

Step 14.
Wash the membrane with 1* PBST for 3 times (15 min , 10 min, 5 min).

Step 15.
Dillute AB2 1:10000 in 50 ml PBST.

Step 16.
Incubate with Ab2 for 1 h.

Step 17.
Wash the membrane with 1* PBST for 3 times (15 min , 10 min, 5 min).

Step 18.
Mix solution 1 and 2 (1:1).

Step 19.
Incubate the membrane with Mix solution for 5 s.

Step 20.
Develop the membrane with ChemiDoc MP Imaging system (BIO-RAD).

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