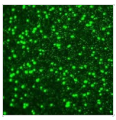

Typing E. coli ATCC 11303 with fluorescently stained virus.

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ABSTRACT

Please contact Dr. Steven Wilhelm (wilhelm@utk.edu) with any additional information regarding this protocol.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

1 Grow a 4 hour culture of ATCC 11303 in Fe-free LB by inoculating ATCC 11303 from an overnight culture grown in Fe-Free media into fresh media.

04:00:00 ATCC 11303 culture

2 Add 4 hour ATCC 11303 culture into a sterile 1.5 mL tube.

100 µl of 4 hour ATCC 11303 culture

3 Add YO-PRO stained T5.

0.5 µl YO-PRO

NOTE

add 10 ul DAPI, if needed.

4 Allow culture to sit in the dark at room temperature.

00:15:00

5 Add 500 µl of 10 mM $MgSO_4$.

500 µl of 10 mM

6 In centrifuge, spin cells down at ~ 10,000 rpm, for 3 minutes.

00:03:00 at 10,000 rpm

7 Gently remove the supernatant with a pipette, then re-suspend the bacterial pellet in 1.5 ml of 10 mM $MgSO_4$.

1.5 ml of 10 mM

8 Filter the sample onto 0.2 µm pore-size, black polycarbonate filter.

9 Rinse the filter gently, three times, with 1 ml of 10 mM $MgSO_4$.

1 ml of 10 mM

10 Finally, with a clean slide and cover glass, pipet a drop of glycerol or immersion oil in the middle of the glass slide and place the cover glass on top of it. Let the glycerol spread out evenly under the cover glass, removing any bubbles. Remove the cover slip from the slide, place the filter on the slide and replace the cover slip.