SYBR Staining - Jenn's Method

Jennifer Brum

Abstract

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Guidelines

** It is IMPORTANT to use a buffer that matches what the virus is resuspended in as it helps keep the virus heads intact. If you are looking at a CsCl gradient fraction that has not been dialyzed, we find that as the diluent using CsCl with a similar density helps keep the viruses intact. If the virus has been dialyzed, use the same formulation as the final buffer. Use of MTN (0.6M NaCl, 0.1M Tris-Cl, pH 7.5, 0.1M MgCl2) that has been 0.02 µm filtered works very well for many viruses (better than TE).

Note: Avoid vortexing or pipetting virus samples up and down as this may cause capsids to burst and release DNA which will decrease virus count and cause messy background.

Protocol

Step 1.
Remove 10,000x SYBR-Gold and Anti-fade solutions from -20°C freezer. Keep SYBR in the dark.

NOTES
VERVE Team 06 Aug 2015
Wear gloves and use filter forceps to handle filters.

Step 2.
Keep SYBR in the dark.

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Step 3.
Have 0.02 µm filtered TE, dilution buffer and water on hand enough to make dilutions and wet filters.

Step 4.
Prepare dilutions of sample.** Mix by inverting tube up and down.

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Best to make slightly more than 1 ml so pipetting will be accurate. Avoid pipetting up and down.

Step 5.
Label frosted slides and get out coverslips.

Step 6.
Cut scotch tape in half for taping down coverslips.

Step 7.
Turn off the lights.

Step 8.
Dilute SYBR 1:10 using 5 µl in 45µl TE.

Step 9.
Put 10 µl drop of Anti-fade onto coverslips.

Step 10.
Label petri dishes: mark in half and use arrow or number to designate top.

Step 11.
Pipet 2.5 µl diluted SYBR on to top and bottom halves of petri dish (2 drops per dish) and add 97.5 µl TE.

NOTES

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2 drops per dish.

Step 12.
Turn vacuum pump on, but do not turn on vacuum to tower supports.

Step 13.
Pipet water onto top of frosted support.

Step 14.
Place a backing filter on top of tower support and open vacuum to evenly lay down filter.

Step 15.
Turn off vacuum.

Step 16.
Place another drop of water on top of filter and place 25 mm 0.02 µm Anodisc filter on top.

Step 17.
Turn on vacuum. Clamp tower to support.

Step 18.
Pipet in sample bringing tip very close to filter.

Step 19.
Let filter dry (30 sec).

DURATION

00:00:30

Step 20.
With vacuum still running, carefully remove filter and place on drop of SYBR in petri dish.

Step 21.

Stain for 15 min.

DURATION

00:15:00

Step 22.

Turn off vacuum.

Step 23.

Repeat steps 18 - 23 until all samples are stained.

Step 24.

After staining, place 0.5 ml water on backing filter.

Step 25.

Pick up stained filter with forceps and place on top of water droplet.

NOTES

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Jenn does not use water – she puts anodisc filter on top of dry backing filter.

Step 26.

Turn on vacuum for 30 sec to dry filter.

DURATION

00:00:30

Step 27.

Remove filter with vacuum still on and place coverslip with anti-fade on top.

Step 28.

Set down forceps and place filter and coverslip onto slide. Tape down one end.

Step 29.

Put 2 coverslips per slide and tape in the middle to hold down coverslips.

Step 30.

Examine immediately or place in freezer for 1 hr to improve staining.

DURATION

01:00:00