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# PhageFISH for DIG-labelled bacterial probes

In 1 collection

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**ABSTRACT** 

This protocol details about PhageFISH for DIG-labelled bacterial probes.

**ATTACHMENTS** 

627-1301.docx

**MATERIALS** 

### Reagents

- 1% paraformaldehyde
- PBS
- 0.01M HCI
- sterile water
- 96% ethanol
- permeabilisation buffer
- hybridisation buffer
- gene washing buffer I
- gene washing buffer II
- amplification buffer
- Alexa tyramides (488)
- Tris-HCl
- RNase I
- RNase A
- antibody-blocking solution
- antibody binding solution
- antibody washing solution
- Alexa tyramides (594)
- SlowFade Gold
- DAPI dye

# OPEN BACCESS

#### DOI:

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protocols.io

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

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**PROTOCOL** integer ID:

75844

**Keywords:** PhageFISH, DIGlabelled bacterial probes

### Fix liquid samples to glass slides



- Take  $\underline{\underline{L}}$  10  $\mu \underline{L}$  of the supernatant and place on coated glass slide.
- 11 Smear the droplet over the slide using a cover slip.
- Allow the sample to dry this should not take more than 00:10:00

10m

- Overlay the slides with 1% paraformaldehyde. Ensure the whole sample area is covered (approx. 1 mL ).
- Incubate for 01:00:00 at Room temperature or Overnight at 4 °C

1h



- 15 Aspirate off excess paraformaldehyde.
- 16 Wash in PBS for **(5)** 00:01:00

Note

### **FREEZING POINT**

### **Permeabilise cells**

17 Add lysozyme to permeabilisation buffer.



18 Overlay samples with permeabilisation buffer.

19 Incubate 8 On ice for 01:00:00



Wash samples in PBS for 00:05:00



Wash samples in sterile water for (5) 00:01:00 .



21

# **Inactivate peroxidases**

22 Incubate samples in [M] 0.01 Molarity (M) HCl for 00:10:00

10m

5m



1m

Wash samples in sterile water for 00:01:00

Po

Wash samples in 96% ethanol for 00:01:00

1m



Allow slides to dry on blotting paper or filter paper.

## rRNA hybridisation of DIG-labelled probes

- 27 Place filters in a petri dish and spot up to  $\underline{\text{A}}_{100 \, \mu \text{L}}$  hybridisation buffer to cover the filters.
- Transfer to a humidity chamber with hybridisation buffer soaked paper towels.
- 29 Incubate for 01:00:00 at hybridisation temperature\_\_\_\_\_.

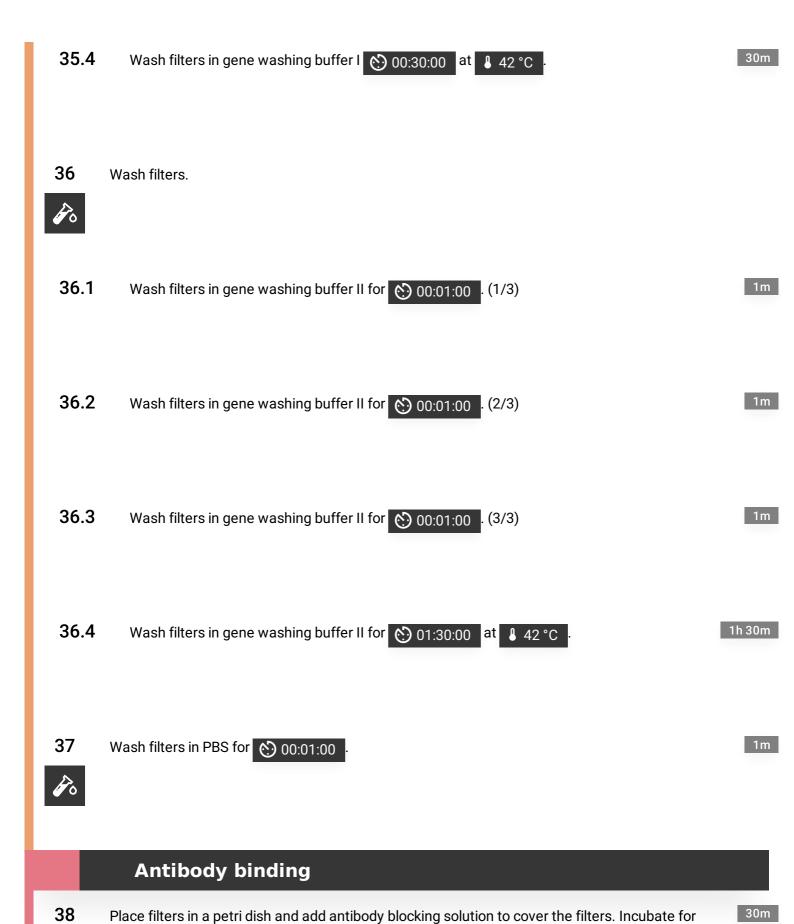
1.



30 Mix  $\underline{A}$  1 mL gene hybridisation buffer with  $\underline{A}$  1  $\mu$ L of each probe. Vortex to mix.

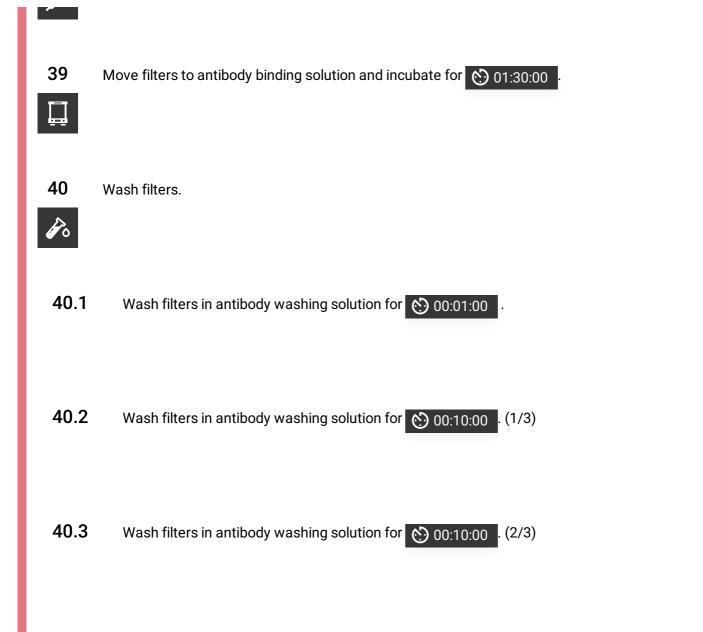


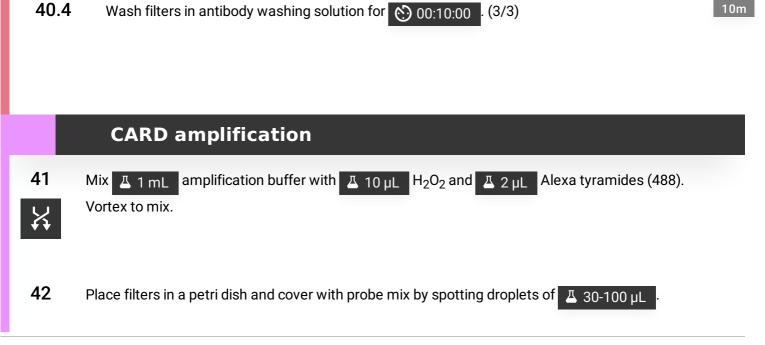
31 32 Place the filters face down in the probe mix droplets. 33 Place the dish back in the humidity chamber and incubate for 01:00:00 at 8 85 °C 34 Immediately place the humidity chamber at hybridisation temperature 💍 Overnight 35 Wash filters. 35.1 Wash filters in gene washing buffer I 00:01:00 . (1/3) 1m 35.2 1m Wash filters in gene washing buffer I 00:01:00 . (2/3) 35.3 Wash filters in gene washing buffer I (5) 00:01:00 . (3/3)





**©** 00:30:00





1h 30m

1m

10m

43 Wash filters.



43.1 Wash filters in PBS for © 00:01:00

1m

Wash filters in PBS for 00:05:00

5m

43.3 Wash filters in PBS for  $\bigcirc$  00:10:00 at  $\bigcirc$  46 °C . (1/2)

10m

43.4 Wash filters in PBS for  $\bigcirc$  00:10:00 at  $\bigcirc$  46 °C . (2/2)

10m

Wash filters in sterile water for 00:01:00

1m

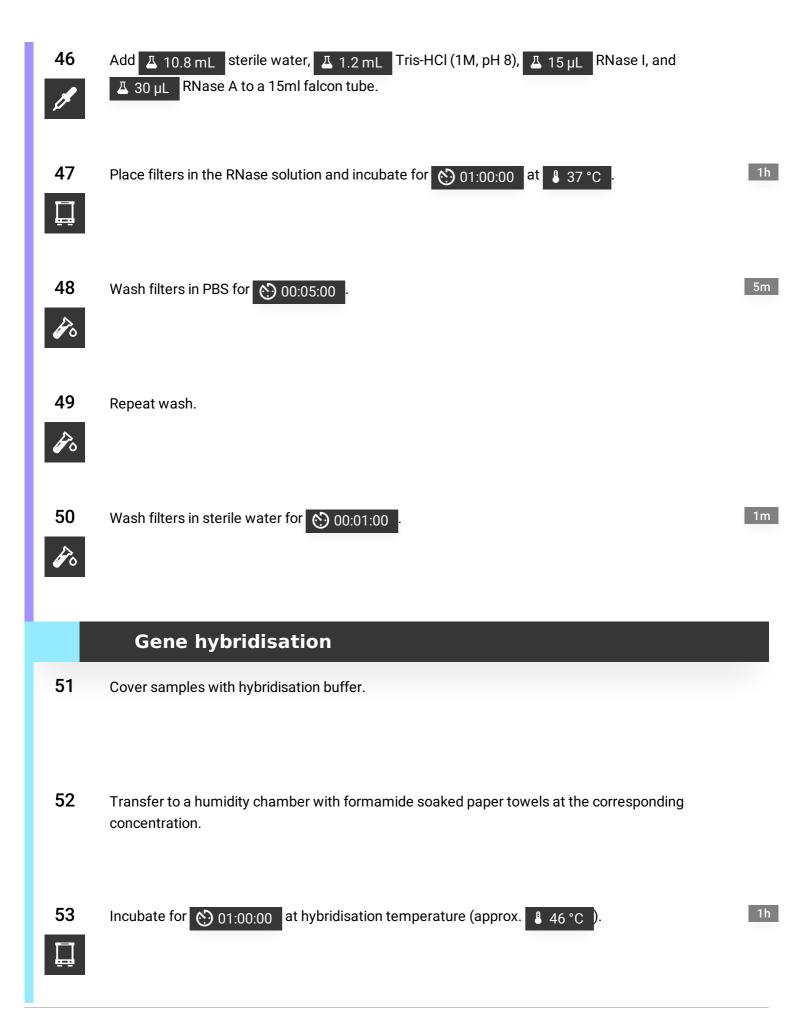
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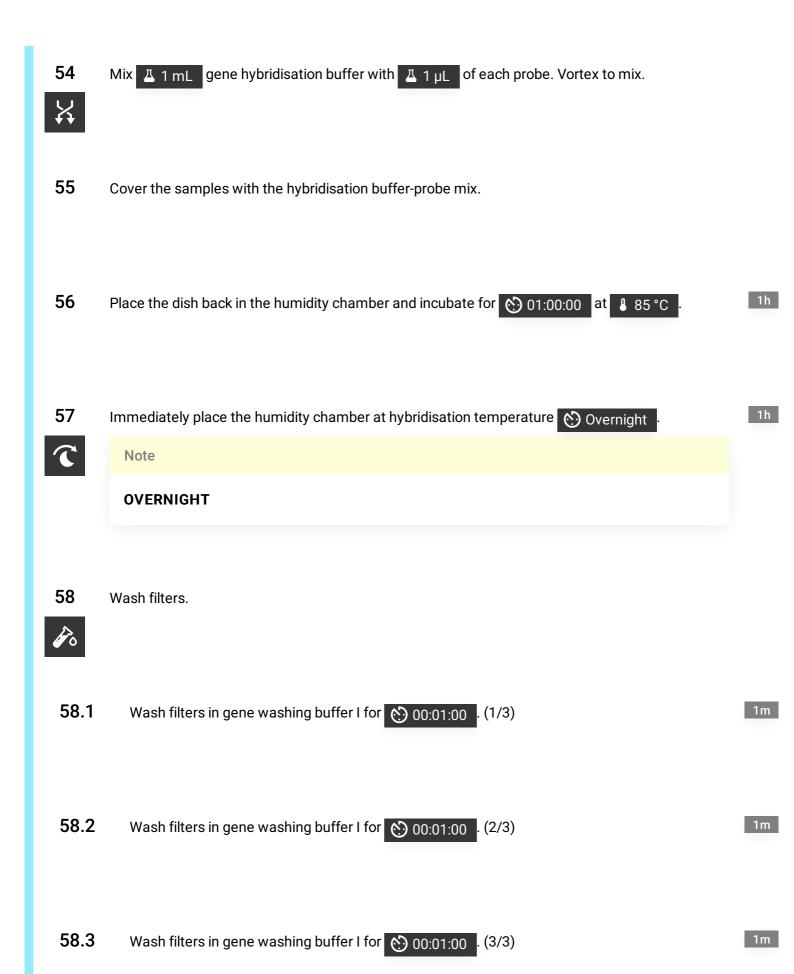
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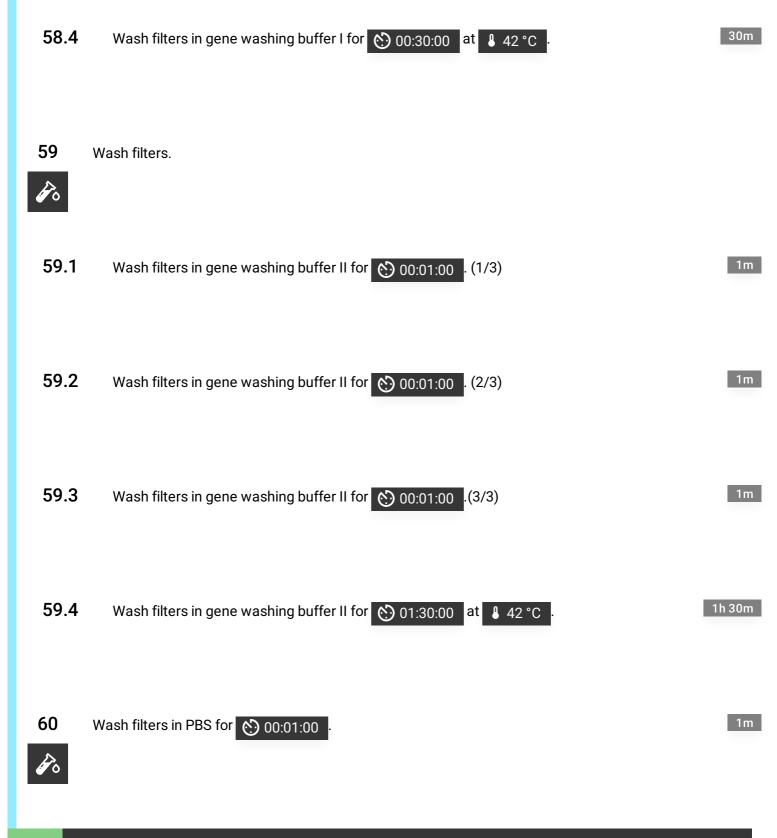
Wash filters in 96% ethanol for 👏 00:01:00

1m

### Remove RNases









61 Place filters in a petri dish and add antibody-blocking solution to cover the filters. Incubate for **©** 00:30:00



Move filters to antibody binding solution and incubate for 01:30:00

1h 30m



Wash filters.



Wash filters in antibody washing solution for 00:01:00

1m

Wash filters in antibody washing solution for 00:10:00 . (1/3)

10m

Wash filters in antibody washing solution for 00:10:00 . (2/3)

10m

Wash filters in antibody washing solution for 00:10:00 . (3/3

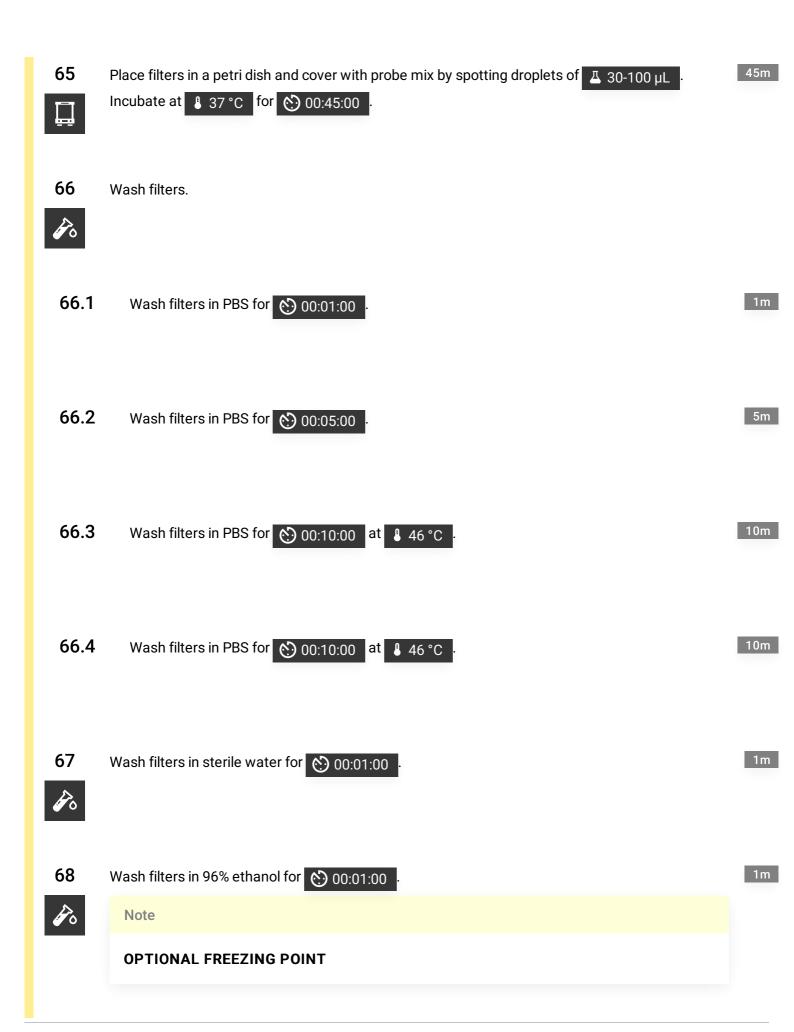
10m

## **CARD** amplification

Mix  $\blacksquare$  1 mL amplification buffer with  $\blacksquare$  10  $\mu$ L H<sub>2</sub>O<sub>2</sub> and  $\blacksquare$  2  $\mu$ L Alexa tyramides (594). Vortex to mix.



64



## **Staining**

Mix Δ 1 mL SlowFade Gold with Δ 1 μL Δ 5 mg/mL DAPI dye.



- 70 Apply  ${\tiny \bot}$  5-10  ${\tiny µL}$  mix in droplets to each slide.
- 71 Apply coverglass and carefully press down to seal sample with minimal air bubbles.
- 72 Seal with clear nail polish on all edges of the sample.
- **73** Allow to cure completely.
- **74** Store at 4 -20 °C