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🌐 PhageFISH for DIG-labelled bacterial probes

📖 [PLOS One](#)

✓ Peer-reviewed method

📁 In 1 collection

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Protocol status: Working

We use this protocol and it's working

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Abstract

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

Attachments



[627-1301.docx](#)

32KB












Materials

Reagents

- 1% paraformaldehyde
- PBS
- 0.01M HCl
- 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









Fix liquid samples to glass slides


- 1 Place liquid sample in a  30-50 μL droplet on poly-L-lysine coated slide.
- 2 Dry in warm incubator for approx.  00:30:00 or until the droplet has dried out. 
- 3 OPTIONAL: if sample is very dilute add several droplets and repeat drying procedure. 
- 4 Add 1% paraformaldehyde to cover the sample area. 
- 5 Incubate at  Room temperature for  01:00:00 . 

- 6 Aspirate the paraformaldehyde off.
- 7 Rinse samples in PBS for  00:01:00 . 







Fix faecal samples to glass slides

- 8 Mix a small faecal sample with  10-20 μL PBS (1X) and vortex thoroughly. 
- 9 Allow suspension to settle for  00:05:00 . 
- 10 Take  10 μL of the supernatant and place on coated glass slide.
- 11 Smear the droplet over the slide using a cover slip.

12 Allow the sample to dry – this should not take more than  00:10:00 . 10m

13 Overlay the slides with 1% paraformaldehyde. Ensure the whole sample area is covered (approx.  1 mL).

14 Incubate for  01:00:00 at  Room temperature or  Overnight at  4 °C . 1h



15 Aspirate off excess paraformaldehyde.

16 Wash in PBS for  00:01:00 . 1m

Note

FREEZING POINT



Permeabilise cells

17 Add lysozyme to permeabilisation buffer. 


18 Overlay samples with permeabilisation buffer.

19 Incubate  On ice for  01:00:00 . 1h







20 Wash samples in PBS for  00:05:00 . 5m





21 Wash samples in sterile water for  00:01:00 . 1m



Inactivate peroxidases


- 22 Incubate samples in for . 10m 
- 23 Wash samples in PBS for . 5m 
- 24 Wash samples in sterile water for . 1m 
- 25 Wash samples in 96% ethanol for . 1m 
- 26 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 hybridisation buffer to cover the filters.
- 28 Transfer to a humidity chamber with hybridisation buffer soaked paper towels.
- 29 Incubate for at hybridisation temperature____. 1h 
- 30 Mix gene hybridisation buffer with of each probe. Vortex to mix. 
- 31 Place one droplet of probe mix on a petri dish for each filter.
- 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 🌡️ 85 °C .


1h


- 34 Immediately place the humidity chamber at hybridisation temperature 🕒 Overnight .

1h


- 35 Wash filters.

1h


- 35.1 Wash filters in gene washing buffer I 🕒 00:01:00 . (1/3)


1m
- 35.2 Wash filters in gene washing buffer I 🕒 00:01:00 . (2/3)

1m
- 35.3 Wash filters in gene washing buffer I 🕒 00:01:00 . (3/3)

1m
- 35.4 Wash filters in gene washing buffer I 🕒 00:30:00 at 🌡️ 42 °C .

30m
- 36 Wash filters.

1h


- 36.1 Wash filters in gene washing buffer II for 🕒 00:01:00 . (1/3)


1m
- 36.2 Wash filters in gene washing buffer II for 🕒 00:01:00 . (2/3)

1m
- 36.3 Wash filters in gene washing buffer II for 🕒 00:01:00 . (3/3)











1m
- 36.4 Wash filters in gene washing buffer II for 🕒 01:30:00 at 🌡️ 42 °C .

1h 30m
- 37 Wash filters in PBS for 🕒 00:01:00 .








1m



Antibody binding

- 38 Place filters in a petri dish and add antibody blocking solution to cover the filters. Incubate for  00:30:00 .   30m
- 39 Move filters to antibody binding solution and incubate for  01:30:00 . 1h 30m 
- 40 Wash filters. 
- 40.1 Wash filters in antibody washing solution for  00:01:00 . 1m
- 40.2 Wash filters in antibody washing solution for  00:10:00 . (1/3) 10m
- 40.3 Wash filters in antibody washing solution for  00:10:00 . (2/3) 10m
- 40.4 Wash filters in antibody washing solution for  00:10:00 . (3/3) 10m

CARD amplification

- 41 Mix  1 mL amplification buffer with  10 μ L H_2O_2 and  2 μ L Alexa tyramides (488). Vortex to mix. 
- 42 Place filters in a petri dish and cover with probe mix by spotting droplets of  30-100 μ L .
- 43 Wash filters. 
- 43.1 Wash filters in PBS for  00:01:00 1m

43.2 Wash filters in PBS for  00:05:00 . 5m

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






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



44 Wash filters in sterile water for  00:01:00 . 1m



45 Wash filters in 96% ethanol for  00:01:00 . 1m

Remove RNases


46 Add  10.8 mL sterile water,  1.2 mL Tris-HCl (1M, pH 8),  15 µL RNase I, and  30 µL RNase A to a 15ml falcon tube. 

47 Place filters in the RNase solution and incubate for  01:00:00 at  37 °C . 1h



48 Wash filters in PBS for  00:05:00 . 5m



49 Repeat wash. 

50 Wash filters in sterile water for  00:01:00 . 1m



Gene hybridisation

51 Cover samples with hybridisation buffer.

52 Transfer to a humidity chamber with formamide soaked paper towels at the corresponding concentration.

53 Incubate for  01:00:00 at hybridisation temperature (approx.  46 °C).

1h




54 Mix  1 mL gene hybridisation buffer with  1 µL of each probe. Vortex to mix.



55 Cover the samples with the hybridisation buffer-probe mix.

56 Place the dish back in the humidity chamber and incubate for  01:00:00 at  85 °C .

1h

57 Immediately place the humidity chamber at hybridisation temperature  Overnight .

1h

Note

OVERNIGHT



58 Wash filters.



58.1 Wash filters in gene washing buffer I for  00:01:00 . (1/3)



1m

58.2 Wash filters in gene washing buffer I for  00:01:00 . (2/3)

1m

58.3 Wash filters in gene washing buffer I for  00:01:00 . (3/3)

1m

58.4 Wash filters in gene washing buffer I for  00:30:00 at  42 °C .

30m










59 Wash filters.










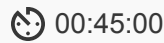


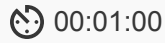

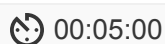

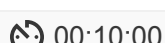





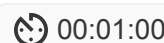


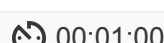


- 59.1 Wash filters in gene washing buffer II for  00:01:00 . (1/3) 1m
- 59.2 Wash filters in gene washing buffer II for  00:01:00 . (2/3) 1m
- 59.3 Wash filters in gene washing buffer II for  00:01:00 .(3/3) 1m
- 59.4 Wash filters in gene washing buffer II for  01:30:00 at  42 °C . 1h 30m
- 60 Wash filters in PBS for  00:01:00 . 1m



Antibody binding

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

- 62 Move filters to antibody binding solution and incubate for  01:30:00 . 1h 30m

- 63 Wash filters. 
- 63.1 Wash filters in antibody washing solution for  00:01:00 . 1m
- 63.2 Wash filters in antibody washing solution for  00:10:00 . (1/3) 10m
- 63.3 Wash filters in antibody washing solution for  00:10:00 . (2/3) 10m
- 63.4 Wash filters in antibody washing solution for  00:10:00 . (3/3) 10m

CARD amplification



- 64 Mix  1 mL amplification buffer with  10 μL H_2O_2 and  2 μL Alexa tyramides (594). Vortex to mix. 
- 65 Place filters in a petri dish and cover with probe mix by spotting droplets of  30-100 μL .  45m
Incubate at  37 $^\circ\text{C}$ for  00:45:00 . 
- 66 Wash filters. 
- 66.1 Wash filters in PBS for  00:01:00 .  1m
- 66.2 Wash filters in PBS for  00:05:00 .  5m
- 66.3 Wash filters in PBS for  00:10:00 at  46 $^\circ\text{C}$.  10m
- 66.4 Wash filters in PBS for  00:10:00 at  46 $^\circ\text{C}$.  10m
- 67 Wash filters in sterile water for  00:01:00 .  1m

- 68 Wash filters in 96% ethanol for  00:01:00 .  1m


Note

OPTIONAL FREEZING POINT

Staining

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

- 70 Apply  5-10 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  $-20\text{ }^{\circ}\text{C}$.