Feb 01, 2023

PhageFISH for DIG-labelled bacterial probes

PLOS One

Peer-reviewed method

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.kqdg3931pg25/v1

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DOI: dx.doi.org/10.17504/protocols.io.kqdg3931pg25/v1

Protocol Citation: Line Jensen Ostenfeld, Saria Otani 2023. PhageFISH for DIG-labelled bacterial probes. **protocols.io https://dx.doi.org/10.17504/protocols.io.kqdg3931pg25/v1**

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

Created: January 25, 2023

Last Modified: February 01, 2023

Protocol Integer ID: 75844

Keywords: PhageFISH, DIG-labelled bacterial probes

Funders Acknowledgements:
NovoNordisk
Grant ID: NNF16OC0021856

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. 30m

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 . 1h

6 Aspirate the paraformaldehyde off.

7 Rinse samples in PBS for 00:01:00 . 1m

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 . 5m

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 [M] 0.01 Molarity (M) HCl for 00:10:00 . 10m

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

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

25 Wash samples in 96% ethanol for 00:01:00 . 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 100 µL hybridisation buffer to cover the filters.

28 Transfer to a humidity chamber with hybridisation buffer soaked paper towels.

29 Incubate for 01:00:00 at hybridisation temperature_____. 1h

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

31 Place one droplet of 30-100 µL 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.

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.

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 µL H_2O_2 and 2 µL Alexa tyramides (488). Vortex to mix.

42 Place filters in a petri dish and cover with probe mix by spotting droplets of 30-100 µL .

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 . Incubate at 37 °C for 00:45:00 . 45m

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 °C . 10m

66.4 Wash filters in PBS for 00:10:00 at 46 °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 °C .