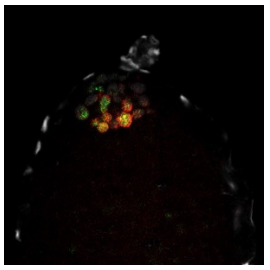
May 21, 2018

Whole mount DNA fluorescence in situ hybridisation (DNA-FISH) on Planococcus citri tissue



DOI
dx.doi.org/10.17504/protocols.io.p9vdr66

Maria Kupper[1]

[1]University of Montana

Mealybug Team

Maria Kupper
Institute of Molecular Biology

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.p9vdr66

Protocol Citation: Maria Kupper 2018. Whole mount DNA fluorescence in situ hybridisation (DNA-FISH) on Planococcus citri tissue. **protocols.io https://dx.doi.org/10.17504/protocols.io.p9vdr66**

Manuscript citation:

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

Created: May 21, 2018

Last Modified: May 21, 2018

Protocol Integer ID: 12309

Keywords: DNA-FISH, whole-mount, tissue, Planococcus citri, probes, tissue of planococcus citri, planococcus citri tissue this protocol, whole mount dna fluorescence in situ hybridisation, planococcus citri, whole mount dna fluorescence, planococcus, citri tissue, bacteriome tissue, citri endosymbiont, dna fish, stained tissue, dissected tissue, dna, use of different probe, situ hybridisation, other tissue, different probe

Abstract

This protocol describes how to perform DNA FISH on dissected tissue of Planococcus citri. While the aim of this experiment was to distinguish localisation of the P. citri endosymbionts in ovarian and bacteriome tissue, the protocol can be easily adjusted for other tissues or the use of different probes.
The probes used here were designed according to the works of von Dohlen et al. (Nature, 2001) and Lopez-Madrigal et al. (PLOS ONE, 2013).
In brief, tissues were isolated in 1xPBS and transferred to various fixatives. After fixation samples were dehydrated and pre-incubated in hybridisation buffer before adding probes. After hybridisation, samples were washed and counter stained with Hoechst. FInally, stained tissues were transferred to microscopy slides, briefly air-dried and mounted in FLuorSave Reagent (Millipore).

Materials

MATERIALS

- 4% paraformaldehyde/1XPBS solution
- 1XPBS
- Ethanol
- FluorSave™ Reagent **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**345789
- Apexbio Technology LLCSupplier Diversity Partner HOECHST 34580 100MG **Fisher Scientific Catalog #**50-115-0629
- Carnoys fixative
- Hybridisation buffer
- Washing buffer

Troubleshooting

Dissection and fixation

1 Depending on required tissue samples Planococcus citri individuals of the respective developmental stage were isolated from a culture and tissues were isolated in ice-cold 1xPBS (here ovaries from virgin or mated females; as well as bacteriomes from 2nd or 3rd instars).
After mild washing of the tissue in ice-cold 1xPBS, samples were transferred to 1.5ml tubes with three different fixatives:
- 4% PFA in 1xPBS (PFA)
- 4% PFA in 1xPBS supplemented with 1% Triton X-100 (PFA-Triton)
-Carnoy's fixative (Ethanol-Chloroform-Acetic acid; 6:3:1)
Fixation was performed at 4ºC, over night.

Washing and Dehydration

2 PFA and PFA-Triton fixed samples were washed three times each 5 minutes in 1xPBS. Floating tissues were pelleted by mild centrifugation to max. 8000xg for a few seconds. Additionally, reagents were never removed completely; instead a small residue amount was left in the tube to prevent tissues from desiccation.
Each samples was then supplied to an increasing ethanol series at RT. Incubation in ethanol lasted 30 minutes and was repeated twice for each EtOH concentration (30%; 50%; 70%; 90 - Carnoy's fixed samples only 70-90%).
Samples can be stored in 90% EtOH at 4ºC till further use.

Pre-hybridisation and hybridisation

3 All samples were pre-incubated 30 minutes at 46ºC in hybridisation buffer (35% formamide, 900 mM NaCl, 20 mM Tris/HCl pH 7.5, 0.2% SDS). Hybridisation buffer was carefully removed (samples tend to float in SDS containing solution) and fresh buffer containing 500 nM of each required oligonucleotide probe was added.
Probes used to detect mealybug symbionts:
- b91 (5'-GCCTTAGCCCGTGCTGCCGTAC-3'); Cy5 modified 5'-end (Tremblaya princeps)
- g630 (5'- CGAGACTCTAGCCTATCAGTTTC-3'); Cy3 modified 5'end (Moranella endobia)
Samples were incubated in hybridisation buffer with probes for 2-3 hours at at 46ºC in the dark.

Washing and counterstaining

4 Subsequent to hybridisation samples were washed in washing buffer (70 mM NaCl, 20 mM Tris/HCl pH7.5, 5 mM EDTA, 0.01% SDS) for 30 minutes at 48ºC in the dark.

Next, washing buffer was carefully removed and 1xPBS with Hoechst (1:1000) was added. Samples were counterstained for 30 minutes at RT in the dark.

Mounting and sample analysis

5 Predecending to mounting, samples were washed twice for 10 minutes in 1xPBS and once for 10 minutes in dH_2O. Tissue samples were then transferred to microscopy slides and slightly air-dried to fix them to the slides.
Samples were then mounted in FluorSave ^{TM} Reagent and further analysed with a confocal microscope Zeiss LSM 880 (Carl Zeiss Microscopy GmbH, Germany). Single and stack pictures were taken with the Zeiss ZEN Imaging Software and further processed using ImageJ 1.51n.