Gambierdiscus Whole Cell Hybridization

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Abstract

Protocol for labeling Gambierdiscus cells with FISH probes in PIG.

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Before start

Turn on the incubator and set to correct temperature according to probe.
Get probes and Poly A out of freezer to thaw in dark location.

Protocol

Step 1.
Prepare Prehybridization/ Hybridization Buffer
The Promega filtration manifold has a 14-sample capacity. The following recipe prepares buffer for 15 samples. In a 50 mL centrifuge tube labeled “prehybridization” buffer add:
20.4 mL Milli-Q water
6.0 mL 25X SET
300 µL 10% IGEPAL CA-630
300 µL Poly A 10 mg/mL (3-10 freezer in Styrofoam box)
3.0 mL Formamide* (in flammable refrigerator/freezer in 3-30)

AMOUNT
20 ml Additional info:

ANNOTATIONS
Chris Upton 11 Sep 2015
Is the amount supposed to be 30ml??

This makes a 5X SET solution, is this right?

Step 2.
Prepare hybridization buffer:
Probe working stock concentration = 200 ng/µL.
For each sample, use 1 mL buffer + 10 µL working stock probe. Thus, for 14 samples, transfer 14 mL buffer into a 15 mL tube labeled “hybridization buffer and add 140 µL probe (14 x 10 µL).

Step 3.
Prepare 0.2X SET Wash
For 15 samples (1 mL per sample):
120 µL 25X SET

14.880 mL Milli-Q water
20ul of Calcifluor (3-30 fridge)

Step 4.
Place Whatman Cyclopore filter (5 µm, 25 mm), shiny-side up, on the filter unit base with minimal vacuum applied (2.5" Hg = 65 mm Hg). With continued vacuum, wet filter with Milli-Q, add the o-ring and the chimney. Tighten by only turning base of the filter chimney! Discard blue backing filter.

Step 5.
Label the towers with the appropriate sample information. Include: Site ID and number, Sampling Month and Hybridization Date. (Use a sticky label that can later be placed onto a microscope slide).

Step 6.
Mix sample well (inverting tube 6 times) and remove an aliquot of sample and place onto the membrane (record volume to sample used).

Step 7.
Filter each tower to near dryness.

Step 8.
EMPTY CONTENTS OF FILTRATION MANIFOLD (THE "PIG") INTO METHANOL WASTE CARBOY!

Step 9.
Add 1 mL prehybridization buffer to each tower.

Step 10.
Prehybridize the cells for 5 minutes at room temperature.

Step 11.
Filter each sample to near dryness.

Step 12.
Add 1 mL hybridization buffer containing the oligonucleotide probe.

Step 13.
Cap the tubes and place the filter manifold into a large black plastic bag containing a wet paper towel to help minimize evaporation. Fold over the bag and seal it with a binder clip.

Step 14.
Place the filter manifold and the tube of 0.2X SET into the incubator and allow the samples to hybridize for an hour at the probe's hybridization temperature.

Step 15.
After incubation, filter each sample to near dryness.

Step 16.
Add 1.0 mL 0.2X SET (50˚C) to each sample (wash step) and incubate for 5 minutes at room temperature.

Step 17.
Filter each sample to near dryness.

Step 18.
While the vacuum is on, remove the chimney (loosen by only turning base of filter chimney!). Remove the filter from the fritted base and place it on a microscope slide using forceps (minimize the amount of filter surface area that forceps come into contact with).

Step 19.
Add 25-30 µL glycerol/SET solution in equal drops to the filter and mount with a cover slip. Add the label to the slide.

Step 20.

Store prepared slides cold and dark. View filters on a fluorescence microscope with the appropriate filter set. Counts should be completed within 1-2 days of staining.

Step 21.
EMPTY CONTENTS OF FILTRATION MANIFOLD (THE “PIG”) INTO FORMALIN WASTE CARBOY!