Agilent Custom Mutation Detection Tiling Microarrays

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This protocol describes labeling and hybridizing DNA to custom mutation detection arrays made by Agilent. The protocol was used for our study of mutation detection using the Agilent platform, but is generally applicable to any two-color array hybridization experiment in which one aims to co-hybridize two DNA samples. The DNA labeling process, which results in labeled fragments of approximately 100 bp is based on a protocol developed by Justin Borevitz.

1. Preparing genomic DNA by sonication
   - Dilute 5 ug of genomic DNA in a total volume of 200 µL dH2O
   - Sonicate DNA using program 4 on the Botstein sonicator (power=1, duration= 0.5 s, total=15 s)
   - Run 15 µL on a 1% gel to confirm sonication of median size of ~600 bp
   - Concentrate DNA in a Zymo-5 column
   - Add 1 mL of DNA binding buffer to 200 µL sample
   - Add 600 µL aliquots to column and spin making sure to empty collection tube between spins,
   - Wash as per Zymo directions and elute in 25 µL water.
   - Determine DNA concentration using fluorimeter. You need at least 1 g to label

1. Labeling DNA with Cy3 and Cy5 using non-persmissive Klenow labeling with the Invitrogen BioPrime Labeling
   - In 200 µL PCR tube, add on ice:
     - 72 ul DNA in ddH2O (1000 ng DNA)
     - 60 ul 2.5X random primer solution
   - Denature by heating for 8 min at 99° in PCR block, fast ramp cool down to 4° for 8 min, centrifuge briefly. (PCR program = BioPrime)
   - Do the following steps downstairs in array facility
     - Add: 13 ul 10X dUTP mixture
     - Add: 2 ul Cy-labeled dUTP
     - Mix briefly
     - Add 3 ul Klenow fragment
     - Mix gently but thoroughly by pipetting
     - Incubate at 25° for 16 hr (in PCR machine using 16 at 25)
   - Add 15 ul stop buffer the next morning downstairs.
   - Transfer the solution to 1.5 ml eppi-tube, add:
     - 16 ul 3M sodium acetate (pH 5.2), mix
     - 400 ul cold ethanol
     - Mix by inverting the tube
     - Place at -20° for 1-2 h
     - Centrifuge at maximum for 10 min to pellet DNA
     - Carefully remove the supernatant
     - Wash with 500 ul 80% cold ethanol at room temperature
   - Centrifuge at maximum speed for 10 min
   - Carefully remove the supernatant
   - Dry the pellet at room temp
   - Resuspend the pellet in 50 ul ddH2O
   - Run 5 ul on 2% agarose gel (upstairs in lab is ok). There should be a discrete band centered at 100 bp fragments (as shown in this example <Link> . If not repeat labeling procedure.)
   - Quantitate DNA using the nanodrop. Ensure good dye incorporation.
   - Store sample at -20°.

Preparation of Hybridization cocktail
   - Add 1350 ul of distilled water to 10X blocking agent q, leave for 60 minutes at room temperature to reconstitute (store at -20°C thereafter)

For each hybridization reaction add in an eppendorf tube:
- 200ng of each labeled DNA sample and water to a total volume of 208uL in water (ensure that there are at least 2.5pmol of dye in each channel. Usually our labeling results in 4-10pmol dye per channel)
- 52uL of 10X Blocking Agent
- 260 uL of 2X Hi-RPM Hybridization Buffer

Final volume = 520uL

- Mix sample well.
- Heat at 95C for 5 minutes
- Incubate at 37C for 30 minutes
- Quick spin to collect sample at bottom of tube.

Hybridization to array

- Slowly dispense 490uL onto array
- Add gasket slide and hybe chamber
- Place in hybe oven at 60C
- Rotate at 20rpm
- Allow hybridization to occur for 20 hours.

Wash

- Wash array using standard Agilent wash A and wash B solutions at room temperature
- Rinse slides in acetonitrile
- Scan at 5um resolution using extended dynamic range scan mode.

Feature Extraction

- Scan slide using the protocol Bot_MutationDetection_CGH-v4.95_Feb07_2
- Dye normalization lists have been created for each slide design and are automatically used for normalization