Agilent Custom Mutation Detection Tiling Microarrays

Alex Ward and David Gresham June 2008

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 100bp is based on a protocol developed by Justin Borevitz.

1. Preparing genomic DNA by sonication
   - Dilute 5ug of genomic DNA in a total volume of 200uL dH2O
   - Sonicate DNA using program 4 on the Botstein sonicator (power=1, duration= 0.5s, total=15s)
   - Run 15uL on a 1% gel to confirm sonication of median size of ~600bp
   - Concentrate DNA in a Zymo-5 column
   - Add 1mL of DNA binding buffer to 200uL sample
   - Add 600uL aliquots to column and spin making sure to empty collection tube between spins,
   - Wash as per Zymo directions and elute in 25uL 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 200uL PCR tube, add on ice:
     - 72ul DNA in ddH2O (1000ng DNA)
     - 60ul 2.5X random primer solution
   - Denature by heating for 8min at 99° in PCR block, fast ramp cool down to 4° for 8min, 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 3ul Klenow fragment
     - Mix gently but thoroughly by pippetting
     - Incubate at 25° for 16hr (in PCR machine using 16at25)
   - Add 15ul stop buffer the next morning downstairs.
   - Transfer the solution to 1.5ml eppi-tube, add:
     - 16ul 3M sodium acetate (pH5.2), mix
     - 400ul cold ethanol
     - Mix by inverting the tube
     - Place at -20° for 1-2h
     - Centrifuge at maximum for 10min to pellet DNA
     - Carefully remove the supernatant
     - Wash with 500ul 80% cold ethanol at room temperature
     - Centrifuge at maximum speed for 10min
     - Carefully remove the supernatant
     - Dry the pellet at room temp
     - Resuspend the pellet in 50ul ddH2O
     - Run 5uL on 2% agarose gel (upstairs in lab is ok). There should be a discrete band centered at 100bp 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 1350uL of distilled water to 10x blocking agent, leave for 60 minutes at room temperature to reconstitute (store at -20C 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