Institute for Systems Biology Dual-Color Hybridization Protocol

- Take 5 ug of total RNA or 0.5-1 ug of polyA alexa labeled probe per dye color and combine in an eppendorf tube.

- Speed-vac the samples down to less than 5 ul (try not to dry them completely)

- Add 40 ul of hybridization buffer to probe and mix well by pipeting.

  Hybridization Buffer

  36.2 ul DIG Easy Hyb
  1.8 ul SS DNA
  1.8 ul tRNA
  0.13 ul dT(50)

- Heat probe to 95 degrees C for 3 minutes and put immediately on ice for 2 minutes.

- Pipet probe onto slide and cover with a clean coverslip.

- Hybridize in a humid chamber at 42 degrees C for 15 hours.

- Remove coverslip by placing in a shaking petri-dish with 30 mls of 42 degree 1X SSC,0.2%SDS for 5 minutes.

- Remove slide, wipe the back, and place in Molecular Dynamics Automated Slide Processor for further washing.

Washes:

2 x 5 minute wash in 1X SSC, 0.2% SDS (3ml) at 42 degrees C
2 x 5 minute wash in 0.1X SSC, 0.2% SDS (3ml) at 25 degrees C
2 x 5 minute wash in 0.1X SSC (3.5ml) at 25 degrees C

Remove slide from processor, dip in water for 2 seconds and blow dry with compressed air.

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