

## SPECTRAL KARYOTYPING – Mouse

1. Warm an aliquot of SKY Paint (**Vial 1** ~10 $\mu$ l) briefly, then flash spin to pellet any precipitate.
2. Pipet 8-10 $\mu$ l onto metaphase spreads. Avoid causing bubbles (if a bubble occurs, it may be popped with the corner of a coverslip)
3. Gently overlay a 22x22 coverslip
4. Denature on a heat block (80°C). Incubation times can be from 30sec to 5min, and must be determined empirically.
5. Seal edges with rubber cement, and incubate 24hrs in a humidified chamber at 37°C, protected from light.
6. Carefully remove rubber cement and coverslip and wash as follows:
  - I. Three washes, five minutes each, in 2XSSC (pH 7)/50% deionized formamide at 45°C
  - II. Two washes, five minutes each in 1XSSC (pH 7) at 45°C
  - III. One wash, two minutes, in 4XSSC (pH7)/0.1% Tween 20 at 45°C.
7. Remove slide from wash and tap off excess liquid. Add 80 $\mu$ l Blocking Solution (**Vial 2**)
8. Incubate 30 min in a humidified chamber at 37°C, protected from light
9. Remove coverslip and tap off excess liquid. Add 100-120 $\mu$ l diluted **Vial 3** and cover with a new coverslip (22x40)  
(To dilute, add 500 $\mu$ l 4XSSC (pH7) to a 5 $\mu$ l aliquot of **Vial 3** (Cy5 staining reagent))
10. Incubate 30-45 min in a humidified chamber at 37°C protected from light
11. Wash three times, two minutes each, in 4XSSC/0.1% Tween 20 at 45°C.
12. Remove from wash and tap off excess liquid. Add 100-120 $\mu$ l diluted **Vial 4** and cover with a new coverslip (22x40)  
(To dilute, add 500 $\mu$ l 4XSSC (pH7) to a 2.5 $\mu$ l aliquot of **Vial 4** (Cy5.5 staining reagent).)
13. Incubate 30-45 min in a humidified chamber at 37°C protected from light
14. Wash three times, 2 minutes each, in 4XSSC (pH 7)/0.1% Tween 20, at 45°C.
15. Tap off excess liquid and allow to drain for 30 seconds. Tap gently again.
16. Apply 20-30 $\mu$ l **Vial 5** (anti-fade with DAPI). (Can also use Vectashield with DAPI)
17. Apply a coverslip (number 1.5) (22x40 or 22x60)
18. Squeeze out excess antifade by gently pressing down on the coverslip. Remove extruded anti-fade by aspiration with a Pasteur pipet fitted to a vacuum line.
19. Seal edges of coverslip with nail polish and allow to dry a minimum of 30 min at room temp, protected from light, before proceeding with microscopy. Slides may be stored at -20°C.

## Reagents and solutions:

1. Vial 1 – SKY paint. When a new kit arrives, Vial 1 should be briefly warmed and gently agitated (flicking it works) a few times. Then flash spin and aliquot reagent into 8-10 $\mu$ l aliquots, avoiding any precipitate at the bottom of the vial. Aliquots should be kept at -20°C, protected from light.
2. Vial 3 – Cy5 staining reagent. When a new CAD kit arrives, Vial 3 should be dispensed into 5 $\mu$ l aliquots and stored at -20°C, protected from light. Just before use, add 500 $\mu$ l 4XSSC (pH 7) directly to a Vial 3 aliquot. This will be sufficient reagent for 5 slides, or more).
3. Vial 4 – Cy5.5 staining reagent. When a new CAD kit arrives, Vial 4 should be dispensed into 2.5 $\mu$ l aliquots and stored at -20°C, protected from light. Just before use, add 500 $\mu$ l 4XSSC (pH 7) directly to a Vial 4 aliquot. This will be sufficient reagent for 5 slides, or more).
4. Vial 2 and Vial 5 may be kept at 4°C protected from light.
5. Wash Solutions:

### Washing solution I

#### **2XSSC (pH7)/50% DI-Formamide**

Add 75ml DI-Formamide (American Bioanalytical AB00600) to a 250ml graduated cylinder. Add 75ml 4XSSC (pH7). Make fresh and preheat prior to use.

### Washing Solution II

#### **1XSSC (pH 7)**

Add 25ml 4XSSC (pH 7) to a graduated cylinder. Add ultrafiltered (Nanopure) water to bring volume to 100 ml. Make fresh and preheat prior to use.

### Washing Solution III

#### **4XSSC (pH 7)/0.1% Tween 20**

Add 100ml 20XSSC (pH 7) to a graduated cylinder. Bring volume to 500ml with ultrafiltered (Nanopure) water. Add 500 $\mu$ l Tween 20 (Fisher BP337-100). Be slow and careful because Tween is very viscous, so it takes a while to draw it into the pipet and a while to dispense it. Rinse the pipet tip a few times in the solution after the Tween is initially dispensed. Make fresh and preheat prior to use.