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Hybridization of labeled community DNA to the FGA

Solutions:

1. Make up a 3x SSC buffer solution (enough for 20 µL per slide)

Prehydridize the slides:

1. Prepare prehybridization solution (50 mL per Coplin jar, a Coplin jar will hold 5 slides):
   - 5x SSC (12.5 mL 20x SSC)
   - 0.1% SDS (250 µL 20% SDS)
   - 0.1 mg mL^{-1} BSA (100 µL 50 mg/mL BSA)
   - DI water [37.15 mL (qs 50 mL)]
2. Warm prehybridization solution to 42°C
3. Immerse array slides in prehybridization solution for 45-60 min at 42°C
4. Transfer slides to 0.1x SSC (250 µL 20x SSC in 50 mL DI water) and incubate at room temperature 5 min
5. Repeat step 4
6. Transfer slides to room temperature DI water for 30 sec
7. Dry slides with Woosh Duster
8. If the slides do not look clean, repeat steps 4 through 7

Prepare items for microarray assembly

1. Preheat coverslips (22 x 601), slides, tips, hybridization chambers, and 3x SSC at **60°C** for at least **20** min. *Use the heating oven in the dark room.*
2. Heat the heatblock to 60°C *(flip the block to the flat surface before starting to set up the arrays)*
3. Set the waterbath to the appropriate hybridization temperature
4. Rehydrate labeled DNA with nuclease-free water (*the volume depends on the amount of DNA. Ideally, 3-5 µg of DNA should be used for each microarray. No more than 7.56 µL of sample should be added (see hybridization solution volumes below.)* Or rehydrate sample with hybridization buffer.
5. Combine the following into a PCR tube (hybridization solution) *(need a volume of 40 µL for manual hybridization*):

	Final Concentration	Volume per array
Formamide	(50%)	20 µL
20 x SSC	(3x)	6 µL
10 % SDS	(0.3%)	1.2 µL
Herring sperm DNA (10 µg/µL)	(0.7 µg/µg)	2.8 µL
DTT (0.1M)	(0.86 mM)	0.34 µL
Sample/water		9.66 µL

6. Denature hydridization solution at 95°C for at least 3-5 min and then maintain the temperature at 60°C. *Use a termocycler.*
7. **At this point all solutions, slides, tips, etc. should be maintained at 60°C, until the arrays are placed into the waterbath.**

8. Remove a hybridization chamber, slide, and cover slip from the heating oven and place on the 60°C heat block.
9. Using the tips from the heating oven, add 17.5 µL of hot 3x SSC to the two wells in the hybridization chamber.
10. There are two ways to apply sample to the arrays:
    a. Pipette the hybridization buffer onto the slide approximately 1/3 the way down from the non-barcoded side. Add the coverslip, avoiding air bubbles. *Gently lean the coverslip onto the slide and slowly lower the coverslip until it touches the droplet on the slide, then release the coverslip. The array starts about 4 mm from the bottom (the end without the barcode) of the slide.*
    b. Preassemble the array with lifter slip in place (*the arrays and hybridization chambers can be assembled beforehand and placed into the heating oven to warm up*). Slowly pipette the entire sample solution onto the surface of the slide beginning at the edge of the Lifter slip (side w/o barcode). The solution should fill all the space between the slide and the Lifter slip by capillary action.
11. Seal the chamber and immediately immerse it in the pre-warmed waterbath
12. Incubate over night (~12-15 h).

Posthybridization procedure:

Wash buffer I

20 x SSC	25 mL
20 % SDS	2.5 mL
water	472.5 mL

Wash buffer II

20 x SSC	2.5 mL
20 % SDS	2.5 mL
water	495 mL

Wash buffer III

20 x SSC	2.5 mL
water	497.5 mL

<u>To reduce handling time of the slides at room temperature after hybridization, take out only one hybridization chamber at a time from the water bath.</u>

1. Immediately disassemble the chamber and remove the coverslip by immersion of the slide in 50°C Wash Buffer I – *do not agitate*.
2. Once the coverslip falls off, transfer the slide to fresh 50°C Wash Buffer I.
3. Incubate at 50°C for 5 min
4. Transfer slides to room temperature Wash Buffer II and incubate with gentle shaking for 5 min
6. Transfer to Wash Buffer III and incubate with gentle shaking for 5 min
7. Dry slides using whoosh duster
8. Scan