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Manual hybridization on slides , using lifterslips

Equipment:

Waterbath 42°C
Heatingblock 95°C

Prehybmix:

Volume 65-70µl for POP 2 chip
5X SSC (sterilefiltered)
50% Formamide (deionized)
5X Denhardts
100 µg/ml Calf Thymus DNA

Hybmix:

Volume 75-80 µl for POP 2 chip

Target		39,4µl
Blocker		2µl
20X SSC	(20%)	16µl
100% Formamide	(25%)	20µl
10% SDS	(0,33%)	2,6µl
		≈ 80µl

Washbuffers:

1. 1X SSC, 0.03%SDS
2. 0.2X SSC
3. 0.05X SSC

Blocker:

Polyadenylic acid (5´) 5mg/ml SIGMA P-9403
Ribonucleic acid, transfer 5mg/ml SIGMA R-5636

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Prehybridization:

Add 5- 8µl 25% Formamide in the small wells of the hybridization chamber.

Wash the Lifter slip before use:

i) dH_20 x 2
ii) Et-OH 95-100%
iii) Blow-dry with $N_{2(g)}$

Place the cover slip on the slide and gently apply the hybridization mix (65-70μl), from one corner until the entire slide is covered.
Directly place in chamber, close and put in 42°C waterbath.
Incubate 30-50 min.
Open chamber and wash slide with tapwater until the lifterslip comes off.
Directly place the slide in 2-propanol and dry under nitrogen gas.

Hybridization:

Remember to set the heatingblock at 95°C
Wash the Lifter Slip before use, as previous.
Keep "darkness" when you start working with the hybmix.
Denature hybmix 2min at 95°C
Place on ice 15-20 sec, vortex and spin down, place on 42°C heatingblock.
Apply hybmix (75-80µl) on slide and place a coverslip on top, like for the prehybridisation
Directly place in chamber, close and put in 42°C waterbath
Incubate over night or ca 18 hours

Washing:

Wash at roomtemperature.
5 min with slow shaking in each washingbuffer.
Dry under nitrogen gas.