Material and Equipment

1 Equipment:

Fixogum: Marabu Fixo gum, 290117000

Hybridization chamber: Corningâ , 2551

Staining box: Neolab, E-1701 E-1702

2 Chemicals:

Dig Easy: Roche, 1 796 895

Ficollâ 400: Sigma, 26873-85-8

Polyvinylpyrrolidon: Roth, 4606.1

BSA Fraktion V: PAA, K41-401-100

Cot1-DNA: Gibco BRL, 15279-011

3 Solutions:

50X Denhardt´s:

- ·Ficoll 400: · 1% (w/v)
- ·Polyvinylpyrrolidon: ·1% (w/v)
- ·BSA Fraktion V: ·1% (w/v)

steril filtration before storage at –20°C

2x Hybridization Buffer:

- ·2,5xDIG-EASY: ·2xDIG-Easy
- ·100xDenhardt`s: ·10xDenhardts
- ·cot1-DNA 20ng/µl: ·2ng/µl

Storage at –20°C

2,5x DIG EASY: add 25,6mL H_2O to original DigEasy bottle, solve and storage at –20°C

20% SDS (w/v): Heat 200g SDS in 900mL MQ-H_2O to 68°C and solve; check pH7.2 with HCl and fill to 1000ml; do not autoclave; store at RT;

20xSSC: 175,3g NaCl and 88,2g Na-Citrat in 1000ml MQ-H_2O; pH7.0; autoclave, store at RT

Washing Buffer 1: 1X SSC, 0,1%SDS, store at RT

Washing Buffer 2: 0.1X SSC, 0,1%SDS, store at RT

Procedure

1. Prepare a (shaking) water bath to 37°C; prepare a plastic box with 37°C water for the chambers
2. Clean Arrays and Coverslip with air pressure and check rubber gasket in corning chamber
3. Denaturate labeled sample in 1x Hybridzation Buffer for 2min at 65°C
4. Add 50µl (depends on array size) labeled sample to the slide and cover the array with a coverslip
5. Remove carefully air bubbles and fix the coverslip on both ends with small dot of Fixogum; dry Fixogum for two minutes (light protection!)
6. Fill 10µl 1x DIG-EASY in both hollows of the corning chamber and transfer the slide; close the chamber and use the box for all chambers
7. Incubate Slides for 14h at 37°C in the water bath with slowly shaking (70rpm)
8. Prepare a (shaking) water bath to 25°C; prepare 1xSSC/0,1%SDS and 0,1xSSC/0,1%SDS-Buffer in staining glass boxes
9. Remove Slides from Corning chambers and transfer them to staining glass boxes; Wash 15min at 25°C to remove the coverslip (use a pipett to remove them carefully)
10. Wash 2 times for 10min in 1xSSC/0,1%SDS, than one time for 10min in 0,1xSSC/0,1%SDS
11. Transfer the slides continuously through a serial of 70% Ethanol, 95% Ethanol and 100% Isopropanol
12. Remove slides immediately from Isopropanol and dry with air pressure
13. Store Slides dusty-free at RT until Scanning
14. Scan Slides with Axon 4000B Scanner (PMT voltage settings: 532: 540; 635: 700; 10µm Resolution)

Version	Tracking of changes	Name	Date