Stripping protocol for Affymetrix tiling gene chips

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Method Article

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Abstract

Introduction

Tiling microarrays integrate the genome in an unbiased fashion and provide a much higher genomic resolution compared to tradition microarrays. Affymetrix tiling microarray platform is one of the most popular tiling array platforms available, and has been widely applied in the studies of transcriptome, transcriptional and epigenetic regulations. Similar to all Affymetrix gene chip families, the tiling microarrays are designed for one time use only. Various stripping strategies have been described in other microarray platforms. Here we describe a method for reusing the Affymetrix tiling gene chips by simple stripping procedures.

Reagents

Stripping buffer: 1% SDS. Wash buffer A: Non-Stringent Wash Buffer \(6X SSPE, 0.01% Tween-20) \(1 L). Storage buffer: 0.06X SSPE. Pre-hybridization buffer 2X: \(16.6% 12X MES, 35.4% 5M NaCl, 8% 0.5M EDTA, 0.2% 10% Tween-20)

Equipment

Scanner: GeneChip® Scanner 3000 7G. Fluidics: GeneChip® Fluidics Station 450. Hybridization Oven: GeneChip® Hybridization Oven 645.

Procedure

1. Pre-warm the stripping buffer at 65°C. 2. Pre-warm the hybridization oven at 65°C. 2. Fill the tiling chip with 200 µl pre-warmed stripping buffer. 3. Place the chip in hybridization oven at 65°C. Rotate 20 min at 60 rpm. 4. Remove stripping buffer. Strip again with another 200 µl of stripping buffer for 10 min. 5. Repeat step 4 for one more time. 6. Remove the stripping buffer and fill with 200 µl of wash buffer A to wash. Place the chip in the hybridization oven at 65°C. Rotate 5 min at 60 rpm. 7. Remove the wash buffer and repeat step 6 twice. 8. Fill with 250 µl storage buffer. Scan chip to check stripping efficiency. 9. Scan the chip to check the scanned image. 10. If no signal is detected on the image, proceed to hybridize with the target \(re-probe). 11. Change the oven temperature to 45°C. 12. Replace the storage buffer with pre-hybridization buffer. Place the chip in the hybridization oven at 45°C. Rotate 5 min at 60 rpm. 13. If not re-probed immediately, the chip can be stored at 4°C overnight.

Timing

1 hour

Critical Steps

The use of fresh probes for re-probing is suggested.

Troubleshooting

If the scanned image is not clean, repeat the stripping step 3 for another 10 min.

Anticipated Results

The correlation coefficient between the first hybridization \(without stripping) and re-hybridization \(stripped) should be above 0.9. The range we have is around 0.96.