

CHAPTER 12

Enhanced microarray hybridization using surface acoustic wave mixing

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1. INTRODUCTION

Microarray technology relies on efficient and specific hybridization to thousands of different target elements that comprise the microarray. Microarray hybridization presents several challenges. First, it is important to find conditions that promote specific hybridization across potentially highly variant targets. Differences in target length and GC content (particularly in the case of cDNA microarrays) can lead to the use of 'compromise' conditions that maximize the number of specific hybridization events and minimize the number of cross-hybridization events without ever achieving completely optimal conditions. Oligonucleotide microarrays allow rational target design, which can partially overcome this challenge by allowing a choice of targets with a very narrow range of lengths and GC content.

Another challenge common to virtually all microarray platforms is that very little mixing occurs during the hybridization reaction, particularly when a sample is placed under a cover slip and hybridized to a glass substrate. The use of a cover slip has the advantage of maintaining a low hybridization volume and therefore a high probe concentration, but any mixing of the solution is limited to the rate of diffusion (1). It has been suggested that, using a passive hybridization process, any given element or spot on an 18 mm × 54 mm microarray is exposed to less than 0.3% of the available probe molecules in solution (1). Furthermore, a typical DNA probe molecule diffuses only 1–3 mm during a static 24 h hybridization (2) and, as such, equilibrium is not reached for weeks after the hybridization has been initiated (3, 4). This problem is generally best dealt with via modifications to the hybridization apparatus. Cover slips that have raised edges (e.g. LifterSlip; Erie Scientific) provide a thin 20–100 µm lumen and

therefore allow increased volumes and potentially greater mixing; however, this approach alone does not provide full mixing.

The issue of limited mixing of the sample is often not apparent when measuring highly expressed messages, as the local concentration of probe molecules may be sufficient to obtain strong signals. The situation is much more critical with respect to low-abundance messages, where the lack of diffusion and mixing may cause a local depletion of probe molecules near the cognate microarray target elements (1). This may, in part, explain why signals from low-intensity spots tend to be more variable than high-intensity spots. Oligonucleotide microarrays present an additional level of concern with respect to different binding efficiencies and kinetics for sequences that are perfectly complementary to probe molecules versus sequences that have one or more mismatches. Dai and colleagues (5) demonstrated that, under standard hybridization conditions with 60mer oligonucleotide microarrays and a complex sample, it takes longer for perfectly matched targets to reach equilibrium than targets bearing a sequence mismatch.

Another common hardware design used to mitigate the effects of limited sample mixing involves injecting the hybridization solution into a closed cassette containing the microarray and mixing the solution inside the cassette lumen using a rotary mixing device. To facilitate mixing of the hybridization solution on the rotary device, an air bubble is introduced into the lumen of the cassette. Whilst this simple method is relatively effective and proven, it does have some limitations: (i) generally large sample volumes (~200 μ l) are required (6); (ii) if the air bubble becomes trapped during the incubation, portions of the microarray can dry out leading to very high background; and (iii) the air bubble causes oxidation of dye molecules at the sample-bubble interface leading to slightly elevated background and weaker signals, particularly when using extended hybridization times.

Mechanical mixing systems have also been developed and adopted in many laboratories. Mechanical mixing systems generally involve the pumping of sample and wash buffers in and out of the hybridization chamber. Again, one of the limitations of this type of system is that hybridization volumes need to be increased to ~200 μ l, which reduces hybridization signals relative to the use of smaller volumes. Although mixing is expedited with mechanical mixing systems, the mixing can be uneven across the slide surface, with a greater effect at the periphery of the slide (proximal to the inlet/outlet) and a reduced effect towards the middle of the slide.

One novel method of enhancing microarray sample mixing involves the use of acoustic waves to pulse the hybridization solution across the microarray surface. This technology has been incorporated into the SlideBooster and ArrayBooster instruments provided by Advantix. Here,

we present methods that take advantage of the mixing provided by the SlideBooster instrument, with slightly different methods recommended for cDNA and oligonucleotide microarrays.

2. METHODS AND APPROACHES

Protocol 6

Hybridization to oligonucleotide microarrays^a

Equipment and Reagents

- Nuclease-free dH₂O (Sigma)
- Yeast tRNA (10 mg/ml; Invitrogen)
- Calf thymus DNA (10 mg/ml; Sigma)
- DIG Easy Hyb solution (Roche)
- BSA Fraction V solution (12.5 mg/ml; Sigma)
- 20× Sodium chloride/sodium citrate buffer (SSC)
- 10% Sodium dodecyl sulfate (SDS)
- 24 × 60 mm M-Series LifterSlips (Erie Scientific)
- Printed oligonucleotide microarrays (60–70mers)
- Powder-free nitrile gloves
- AdvaSon (Advalytix)
- AdvaHum 101 for nonformamide-based hybridization buffers (Advalytix)
- SlideBooster (Advalytix)

Method

1. Obtain the dry cDNA samples from the previous protocol (*Protocol 5*, step 13).
2. Resuspend each cDNA sample in 5 µl of nuclease-free dH₂O.
3. Prepare the hybridization solution by mixing 100 µl of DIG Easy Hyb solution, 5 µl of 10 mg/ml yeast tRNA, 5 µl of 10 mg/ml calf thymus DNA, and 4 µl of 12.5 mg/ml BSA. Incubate the hybridization solution at 65°C for 3 min and cool to room temperature.
4. Add 90 µl of hybridization solution (step 3) to each 5 µl cDNA sample (step 2).
5. Incubate the probe mixture at 65°C for 3 min and cool to room temperature.
6. Pipette 15 µl of AdvaSon coupling fluid onto each agitation position of the SlideBooster instrument.
7. Position the microarray slide such that the agitation chips are directly beneath the hybridization area.
8. While wearing powder-free nitrile gloves, push down on the edges of the slide and move gently back and forth to ensure complete contact between the slide and the agitation chips.
9. Load 500 µl of AdvaHum 101 into the humidifying reservoirs located at each end of the chamber (1000 µl total).

10. Close each lid on the SlideBooster instrument, making certain that the lid latch has snapped closed firmly.
11. Load the program for each chamber and press 'Start' to pre-heat the hybridization chambers on the instrument. Follow the on-screen instructions and use the following parameters: Mix Power = 27, Pulse/Pause Ratio = 3:7, Temperature = 37°C, Processing Time = 16 h.
12. Place the LifterSlip on to the array.
13. Slowly pipette the sample at the edge of the LifterSlip allowing the solution to be drawn underneath the LifterSlip by capillary action.

Note

^aOnce the labeled cDNA has been prepared, the hybridization protocol required depends on the type of microarray being used. For oligonucleotide microarrays, we have had excellent success with the DIG Easy Hyb solution from Roche. Hybridization is carried out overnight with continual mixing at 37°C. Microarrays of cDNAs require a slightly different protocol. We have found that AdvaHyb is a more appropriate hybridization buffer when using the SlideBooster instrument. To enhance specificity, the cDNA microarray hybridization protocol uses a 10 min incubation at 75°C with mixing and then a gradual temperature reduction to 42°C for the overnight hybridization.

Protocol 7

Hybridization to cDNA microarrays

Equipment and Reagents

- Nuclease-free dH₂O (Sigma)
- Yeast tRNA (10 mg/ml; Invitrogen)
- Calf thymus DNA (10 mg/ml; Sigma)
- DIG Easy Hyb solution (Roche)
- BSA Fraction V solution (12.5 mg/ml; Sigma)
- 20× SSC
- 10% SDS
- 24 × 60 mm M-Series LifterSlips (Erie Scientific)
- Printed cDNA microarrays
- Powder-free nitrile gloves
- Heating block set at 42°C
- AdvaHyb (Advalytix)
- AdvaSon (Advalytix)
- AdvaHum 102 for formamide-based hybridization buffers (Advalytix)
- SlideBooster (Advalytix)

Method

1. Pre-heat the AdvaHyb solution at 42°C until the precipitate has dissolved.
2. Obtain the purified dry cDNA samples from a previous protocol (*Protocol 5*, step 13). Resuspend the samples in 4.5 µl of 10 mg/ml yeast tRNA and 4.5 µl of 10 mg/ml calf thymus DNA.
3. To each resuspended cDNA sample, add 81 µl of pre-heated AdvaHyb solution from step 1 above. Mix by vortexing.
4. Denature the sample at 95°C for 3 min.
5. Cool the sample to 42°C (5 min).
6. Load 15 µl of AdvaSon coupling fluid onto each SlideBooster agitation chip, one location per microarray hybridized.
7. Position the microarray slide such that the agitation chip is located directly beneath the hybridization area.
8. While wearing powder-free nitrile gloves, push down on the edges of the slide while moving the slide back and forth to ensure complete contact between the slide and the agitation chips.
9. Load 500 µl of AdvaHum 102 into the humidifying reservoirs located at either end of each chamber, for a total volume of 1000 µl per chamber.
10. Close the lid and make sure the catch has snapped closed firmly. This step is very important. Failure to snap the catch closed firmly will lead to a failed experiment.
11. Load the program written for each chamber and push 'Start' to pre-heat the chambers. The on-screen instructions should read as follows: Mix Power = 27; Pulse/Pause Ratio = 3 : 7; Temperature = Initial Step – 1 min at 42°C with mixing; Step 1: 10 min at 75°C with mixing; passively cools to 42°C; and Final Step: 900 min at 42°C with mixing.
12. Place the LifterSlip on to the array.
13. Slowly pipette the sample at the edge of the LifterSlip allowing the solution to be drawn underneath the LifterSlip by capillary action.

Protocol 10

Hybridization to Agilent microarrays

Equipment and Reagents

- Whole human genome oligonucleotide microarrays (Agilent)
- SlideBooster (Advalytix)
- AdvaSon (Advalytix)
- AdvaHum 101 (Advalytix)
- 24 × 60 mm LifterSlips (Erie Scientific)

Method

1. Load 20 µl of AdvaSon coupling buffer onto each agitation chip.
2. Fill each humidifying chamber with 500 µl of AdvaHum 101.
3. Place a microarray on top of the agitation chip and heat the chambers to 65°C using the following program: Mix Power = 27; Pulse/Pause Ratio = 3:7; Temperature = 65°C; and Processing time = 17 h.
4. Place a 24 × 60 mm LifterSlip onto the microarray and load 100 µl of hybridization solution from the previous protocol (*Protocol 9*, step 4) under the LifterSlip.
5. Initiate the SlideBooster program and allow the microarrays to hybridize for 17 h^a.

Note

^aCpG island microarrays have been used for chromatin immunoprecipitation (ChIP) analysis. ChIP assays help to identify binding sites for transcription factors and other DNA-binding proteins. Whilst the techniques involved in preparing labeled cDNA for hybridization to CpG island microarrays are very different from traditional gene expression assays, CpG island microarrays are, in effect, simply cDNA microarrays. As such, once the labeled probe material has been prepared, CpG island microarrays can be treated in much the same manner as any other cDNA for hybridization on the SlideBooster instrument.