Aminosilane

Protocol for DNA

Introduction

The decision to use aminosilane and poly-L-lysine is frequently made by what you are interested in spotting and how you choose to attach to a surface. Nucleic acids and proteins will attach to aminosilane and poly-L-lysine slides via the material's 5' end or by the phosphate backbone. These attachment strategies yield printed slides that perform very well.

Whichever chemistry you require on a microarray slide (aminosilane, poly-L-lysine), Thermo Fisher Scientific now offers the same attachment chemistries on a new **es** (enhanced) surface. **es** can be described as microscopic mountains and valleys with uniformly coated functional groups. Arrayer spot-size is often controlled by the surface energy of the coating— hydrophobic coatings give smaller spot sizes, while hydrophilic coatings give larger spot sizes. The benefit of **es** is more uniform spot size without altering your chemistry. The **es** surface will not affect the focusing or use of microarray spotters and scanners because it is microscopic.

The principles behind aminosilane slides

The aminosilane and poly-L-lysine slide surface provides available amine groups for initial ionic attachment of the negatively charged phosphate groups in the DNA backbone. The DNA can subsequently be attached covalently to the slide by either baking or by UV irradiation. Typical PCR products are double stranded DNA molecules; however, they can be used for hybridization experiments provided they are first denatured. This is achieved by immersing the slides in boiling water. This method yields very robust slides with high signal to noise ratios.

Important Notes:

1. Several processing steps call for drying slides by centrifuging, or blowing with clean dry air. If you choose to blow the slides with air, please note that standard compressed air may contain oils that will contaminate the chemistry. Ensure that your air source is "bottled air," or of such a quality that it does not deposit background fluorescence onto the slide. A simple method of testing air quality is to scan a clean slide before and after blowing with air. If the second scan has increased background the air may need additional filtering.

2. The mBox™ rack can be removed from the base and cover to provide a convenient slide holder through post-hyb processing steps. Additional accessories include the mHandle to hold and transfer the rack of slides, and the mTub™ to contain liquids for slide immersion. The mTub™ features an open well area under the rack where a stir bar can be employed for liquid agitation.

1. Array Printing

Recommended Spotting Buffer: In-house testing has shown the use of 3X SSC or 150mM Sodium Phosphate (pH8.5) result in the best spot morphology and signal intensity. Other buffers such as 50% DMSO or 3X SSC+0.75M betaine may be used if a low evaporation buffer is desired.

Recommended relative humidity during printing is 45 – 55%.

Scribe slides with a diamond scriber. Blow off debris with clean air source prior to loading into the spotter.

1. Re-suspend samples in printing buffer to final target concentration and load appropriate volumes (usually 10-25µl per well) of the samples into at 96 or 384-well plate. Target concentration for cDNA PCR product is generally up to 0.5uM. If using a pre-loaded plate, follow manufacturer’s direction for reconstituting sample.
2. Print slides according to arrayer manufacturer’s recommendations.

Note: Exercise care to maintain the cleanliness of the slides prior to, and during the printing process. Airborne contamination in the lab and in the atmosphere of the arrayer can compete on the slide surface with the biology being spotted.

2. DNA Immobilization

Incubate for at least 2 hrs at room temperature after printing, then UV crosslink at 400mJoules. If using a low evaporation buffer (i.e.50% DMSO or 3X SSC+0.75M betaine spotting buffer) incubation time should be increased.

Note: Slides may be stored after immobilization for future post-processing. Store in a closed container, such as the mBox™, at room temperature. One method of maintaining low background is to prevent airborne organic contamination from settling on the slides over time.

3. Blocking

The blocking procedure blocks the remaining reactive groups on the slide surface surrounding the spotted DNA. This step is necessary to prevent unspecific binding of the labeled target, thereby reducing the background of the final microarray.

The BSA Blocking step is normally preferred for all Thermo Fisher Scientific microarray slides.

BSA Blocking – Application of Bulk DNA

Reagents Required:

- Fraction V BSA
- 20X SSC
- 10% SDS

Stock BSA Solution:

1. Dissolve 10g of BSA into 700ml of Ultra pure water on a stir plate at room temperature. Allow BSA to dissolve completely.
2. Add: 250ml 20X SSC
10ml 10% SDS
3. Bring volume to 1000ml with Ultra pure water and filter solution. BSA stock solution can be stored at 4ºC for up to 3 months.

Procedure:

1. Bring appropriate volume of stock BSA solution to room temperature (enough to completely immerse slides).
2. Place the slides in the solution and leave on an orbital mixer (or stir plate) for at least 30 minutes.
3. Pour off solution into appropriate waste container, add ultra pure water and dip 15 – 20 times.
4. Rinse slides vigorously in 4 additional changes of ultra pure water.
5. Proceed to DNA Denaturization step below. For best results, transfer wet slides directly from last rinse to first denaturing step.

4. DNA Denaturization

This step separates double stranded PCR product into single stranded DNA to enable the hybridization reaction. The baking/cross linking process used in the DNA Immobilization step has opened the double stranded DNA to some extent; however, proper denaturing by boiling will complete the separation and result in higher signal after hybridization.

1. Transfer arrays to boiling ultra pure water bath for 2 minutes.
2. Quickly dry the slides using clean dry air or by centrifugation.

Slides are ready for hybridization

5. Hybridization

Note: This protocol includes the use of **Thermo Scientific LifterSlips** as a substitute for standard coverslips to cover and contain the hybridization fluid. LifterSlips™ were developed to eliminate the problem of glass-to-glass contact between the coverslip and the array surface with the addition of rails along the outer edges. The rails lift the coverslip to create a uniform cavity for liquid injection and hybridization over the array surface.

LifterSlip Preparation

1. Clean LifterSlips with clean water followed by 100% EtOH rinse.
2. Dry LifterSlips with clean air

Hybridization Solution Preparation

Reagents Required:

- 4X SSC
- 10% SDS
- Labeled Nucleic Acid
    - Suggested starting concentration for nucleic acid is approximately 25 ng/µl

1. Calculate and prepare sufficient hybridization solution for the number of slides you are processing. Mix 99µl of 4X SSC for each 1µl of 25ng/µl labeled nucleic acid. The total volume needed will vary depending on the size of your LifterSlip (volumes typically vary between 30 µl – 70 µl).
2. After combining the 4X SSC and nucleic acid, heat the solution to 95°C for 6 – 8 minutes to denature the nucleic acid and allow to cool slightly. If desired add 1 µl of 10% SDS per 100 µl of hybridization solution.

Hybridization

1. Place a cleaned LifterSlip over the spotted area with white rails facing down.
2. Slowly pipette at least half of the solution onto the slide, just adjacent to one corner of the LifterSlip. Pipette the remaining solution under the opposite corners. Capillary action will allow the solution to 'wick' under the LifterSlip, yielding uniform coverage of the microarray. Pipetting warm solution often helps filling and avoiding trapped bubbles.
3. Place the slide into a commercially available hybridization device or into the bottom of a light-blocking container with a tight fitting lid.
   Note: Exposure of the Cy-labeled nucleic acid to light during all proc-essing steps must be minimized to prevent photo destruction of the dyes.
4. Add an appropriate amount of blank hybridization buffer inside the hybridization device to insure 100% humidity throughout the incubation

step. The amount used will depend on the device you are using. Please follow manufacturer's directions.

5. Incubate overnight at 55°C. Protect from light during this step.

6. Post-hybridization Washing

Note: To avoid cross-contamination, do not wash slides that have been hybridized with different nucleic acid in the same wash container. Do not allow the slides to dry between wash and rinse steps.

1. Carefully place slide in a container filled with 1X SSC, 0.01% SDS letting the LifterSlip float away from the slide surface. Take care to prevent white rails of the LifterSlip from dragging across the array area during removal.
2. Transfer slide to a clean container (or series of containers) and perform the following wash steps:

   - 2 minutes in 1X SSC, 0.01%SDS
   - 30 seconds in 1X SSC (Do this wash step twice)
   - 2 minutes in 1X SSC
   - 30 seconds in 0.5X TE buffer*
   - Immerse in 5% EtOH solution

*1X TE stock: mix 10mM Tris and 1mM EDTA, bring pH to 8.0 for DNA; dilute as needed

3. Dry slides using a centrifuge or by blowing with clean dry air.

Slides are ready for scanning. For best results, scanning should be performed within several hours after the final wash. Minimize exposure to light or high temperature during this period.

For Assistance or Technical questions, please call: 1-888-374-3724

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