FAST J Slides
MicroHybridization Kit Protocol

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I. Introduction

FAST™ Slides provide excellent reproducibility and sensitivity for microarray assays. They are intended for researchers who design and build their own microarrays and are interested in reliability and reproducibility. The hybridization protocol described here is based on well-established membrane hybridization techniques. Highly efficient mixing as well as the high binding capacity of FAST Slides permits the use of probe concentrations that are substantially lower than with other microarray hybridization methods.

This protocol has been optimized for fluorescent detection, but it can be adapted for virtually any detection method, including isotopic and chemiluminescent. For best results with fluorescent detection we recommend FAST Slides MicroHybridization PreHyb/Wash Buffer and FAST Slides MicroHybridization Hyb Buffer from Schleicher & Schuell, Inc. Ordering information for these products can be found in the Appendix of this protocol. These buffers have been formulated to provide efficient hybridization while eliminating non-specific binding and reducing background fluorescence. Other buffer systems may be compatible with FAST Slides. Although we recommend the S&S buffers, in the Appendix we provide the recipe for an alternative system. Be aware that alternative systems may not provide the sensitivity or low background that the Schleicher & Schuell system does.

Because expression profiling is a common use for microarrays, we have included a section in this protocol dedicated to expression profiling on FAST Slides. However, FAST Slides can be used with virtually any microarray application and are not limited to expression profiling.

For hybridization of one FAST Slide you will need:

-1 slide, arrayed with unmodified DNA
-350 ìl of FAST MicroHybridization Hyb Buffer
-Approximately 20 ml of 10X FAST MicroHybridization PreHyb/Wash Buffer
-1 Hybridization Chamber
-1 Hybridization Clip
-1 Hybridization Chamber Cover
-4 Port Covers

Processing of FAST Slides is extremely simple and fast. Unlike processing other types of slides, FAST Slides do not require chemical fixation, tedious blocking steps and DNA modifications. The net result is a fast, easy-to-use method that produces reliable and reproducible microarray data.

II. Slide Arraying

DNA that is arrayed must be denatured to allow for proper hybridization. Denaturation can be performed either before (pre-array) or after (post-array) it is arrayed onto FAST Slides. Our research scientists have produced excellent results using both techniques. When choosing which method is best for your application, it is important to remember that DNA samples which have been denatured by the pre-array method are not suitable for long-term storage in solution. See Hints & Tips for suggestions for extending the usefulness of pre-array denatured DNA.

A. Pre-Array Denaturation

1. Add 5X Denaturation Solution (see Appendix) to purified amplicon/DNA such that the final concentration is 1X Denaturation Solution. The final DNA concentration should be 100-200 ìg/ml.
2. Array samples onto a dry FAST Slide using an established protocol from a robotic arrayer or manual arraying device. The denatured DNA should be arrayed within 24 hours and subsequently discarded (see Hints & Tips).

Note: *Arraying onto a wet slide causes the spots to diffuse.*

Note: *Attention to environmental conditions is critical to accurate spotting. Temperature and humidity must be controlled to avoid evaporation of liquid during the array process.*

3. After arraying is complete, immobilize the DNA by baking the slides for 30 minutes at 80°C.
4. Briefly wash the slides in sterile water.
5. Incubate slides in Neutralization Buffer (see Appendix) at room temperature for 5 minutes. Use enough Neutralization Buffer to completely cover all of the slides being processed.

6. Place slides in sterile water at room temperature for 5 minutes.
7. Dry slides and store in a dust-free, desiccated environment at room temperature. The slides are now ready for hybridization.

Note: *If using fluorescent detection with FAST Slides, do not label slides with colored inks or permanent markers. The inks may dissolve into the wash solution and interfere with fluorescent imaging.*

B. Post-Array Denaturation

1. Array samples onto a dry FAST Slide using an established protocol from a robotic arrayer or manual arraying device. DNA should be arrayed from a source plate concentration of 250-400 ìg/ml in 10X SSC.

Note: *Arraying onto a wet slide causes the spots to diffuse.*

Note: *Attention to environmental conditions is critical to accurate spotting. Temperature and humidity must be controlled to avoid evaporation of liquid during the array process.*

2. After arraying is complete, immobilize the DNA by baking the slides for 30 minutes at 80°C.
3. Dry slides and store in a dust-free, desiccated environment at room temperature.
4. Immediately prior to prehybridization, denature the DNA by incubating slides in boiling water for 2 minutes.

Note: *If using fluorescent detection with FAST Slides, do not label slides with colored inks or permanent markers. The inks may dissolve into the wash solution and interfere with fluorescent imaging.*

III. Prehybridization

1. Prepare 350 ìl of 1X FAST Slides MicroHybridization PreHyb/Wash Buffer for each slide by diluting 35 ìl of 10X PreHyb/Wash Buffer into 315 ìl of sterile water. Warm the 1X solution to the chosen hybridization temperature.

Note: *Prehybridization and hybridization temperatures must be determined by each researcher based on probe length, base composition, and degree of mismatch with the target DNA. The Schleicher & Schuell FAST Slides MicroHybridization Hyb Buffer contains formamide, allowing most hybridizations to be carried out at 42°C.*

Note: *If you are performing expression profiling using cDNA from total or poly (A) RNA, the prehybridization buffer should contain 0.5 ìg/ml Cot-1 DNA and 0.5*

ìg/ml oligo (dA).

Note: *If post-array denaturation (Section II.B) was used for arraying, the slide must be denatured immediately prior to prehybridization.*

2. Attach a Hybridization Chamber to the slide.
   a. Peel off the clear liner on the bottom of the chamber, exposing the adhesive.
   b. Place chamber, adhesive side up, on a flat surface.
   c. Press an inverted, arrayed FAST Slide onto the chamber gasket, aligning the membrane of the slide within the gasket's interior.
   d. Press gently but firmly over the gasket area to form a tight seal.

3. Add 350 ìl of pre-warmed 1X PreHyb/Wash Buffer via one of the access ports. Wipe any excess solution from the port, ensuring that the top of the chamber is dry.

Note: *Ports can also be wiped with an isopropanol-wetted tissue to ensure proper drying.*

4. Using forceps, place a Port Seal over each port; lightly apply pressure for 5 seconds to both ports simultaneously.

Note: *Excessive pressure is not necessary to seal ports and may result in chamber leakage.*

5. Place the plastic Hybridization Cover over the chamber, forming a sandwich. Insert sandwich into the hybridization clip.
6. Incubate at the appropriate temperature for 1 hour with gentle agitation on an orbital shaker or platform rocker. Ensure that mixing is occurring. The air pocket inside the chamber should be in constant motion.

IV. Hybridization

A. Genomic Analysis (DNA based)

This is a general hybridization method for use with a solution containing a finite number of probe species. If you are performing expression profiling using cDNA, please proceed to Section IV.B.

1. Pre-warm 350 ìl of FAST Slides MicroHybridization Hyb Buffer to appropriate hybridization temperature.
2. Denature labeled probe by incubating at 95°C for 5 minutes or in a boiling water bath for 2 minutes and immediately placing on ice.
3. Remove the prehybridized slide from Hybridization Clip. Gently peel off the Port Seals using forceps. Remove the prehybridization buffer through

one of the ports with a micropipette and discard.

4. Add the denatured probe to 350 ìl of pre-warmed hybridization buffer.

Note: *The user should determine the exact volume of hybridization buffer used empirically. We recommend a starting volume of 350 ìl, however, it is likely that smaller volumes can be used.*

5. Add probe solution to the chamber via one of the access ports.

Note: *Optimal probe concentration will vary depending on the application, the number of probe species, probe length and probe composition and therefore must be determined empirically. Compared to modified glass, FAST Slides retain substantially more arrayed DNA and provide better environment for hybridization.* ***Thus, probes at concentrations that are 50-100 fold lower than those traditionally used with modified glass will provide similar results.*** *As a starting point, we recommend 10-50 ng/ml for probes with a limited number of species.*

6. Place Port Seals over the access ports as described in Section III.4.
7. Place the plastic Hybridization Cover over the chamber, forming a sandwich. Insert sandwich into the hybridization clip.
8. Incubate the slide in a hybridization oven or incubator for 5-16 hours, rocking gently and/or rotating slowly. The appropriate incubation temperature must be determined empirically. If you are using the FAST Slides MicroHybridization Hyb Buffer, incubate at 42°C. If the probe contains a fluorescent label, protect the slide from light during hybridization.

B. Expression Profiling (RNA/cDNA based)

This is a protocol for hybridization of arrayed FAST Slides with labeled cDNA from poly (A) or total RNA, a method commonly used for expression profiling. The procedure was developed for use with Schleicher & Schuell FAST MicroHybridization Prehyb/Wash Buffer and FAST MicroHybridization Hyb Buffer. Although other buffer systems may be compatible with FAST Slides, they may not provide the sensitivity or low background that the Schleicher & Schuell system provides. Use of this protocol assumes that fluorescently labeled cDNA has been synthesized by the end-user and the slides have been prehybridized as discussed in section III.

1. Prepare probe mix by combining in a 500 ìl tube:
   -8 ìl fluorescently labeled cDNA (from 1 ìg poly (A) RNA or 100 ìg total RNA)
   -2 ìl of blocking DNA (final concentration of10 ìg/ml oligo (dA) (12-18mer), 10 ìg/ml human Cot-1 DNA)

-2.1 ìl 20X SSC
-0.4 ìl 10% SDS (Do not add excess SDS to the mix. Excess SDS can interfere with hybridization.)

Note: *Protect the probe from light during all procedures.*
Note: *The slide should be prehybridized first as covered in section III.*

2. Denature probe and block repetitive elements by placing tube in boiling water bath for 1 minute. Cool slowly to room temperature to allow Cot-1 DNA and oligo (dA) to hybridize with probe.
3. After prehybridizing the slide for 1 hour as described in Section III, remove the slide from the Hybridization Clip. Gently peel off the Port Seals using forceps.
4. Remove particulate matter from probe by spinning in a microfuge for 1 minute.
5. Add 12 ìl of probe to 350 ìl of Hyb buffer. For expression profiling, both the PreHyb/Wash Buffer and 1X MicroHybridization Hyb Buffer should contain 0.5 ìg/ml Cot-1 DNA and 0.5 ìg/ml oligo (dA) (12-18mer).
6. Replace the Prehyb/Wash buffer with the hybridization buffer containing the probe through one of the access ports. Cover ports with Port Seals as described in Section III.4 and place the slide back in the hybridization clip.
7. Incubate slide in a hybridization oven or incubator overnight (14-18 hours) while rocking gently. The appropriate incubation temperature must be determined empirically. If you are using FAST Slides MicroHybridization Hyb Buffer, incubate at 42°C. Protect the slide from light during hybridization.

Note: *Prehybridization and hybridization temperatures must be determined by each researcher based on probe length, base composition, and degree of mismatch with the target DNA. The Schleicher & Schuell FAST Slides MicroHybridization Hyb Buffer contains formamide, allowing most hybridizations to be carried out at 42°C.*

V. Washing

1. Dilute FAST Slides MicroHybridization PreHyb/Wash Buffer (10X) to 1X concentration. It is important that sufficient buffer be used to cover all slides being washed. We recommend 30-50 ml of 1X wash buffer per slide for each wash step. If multiple slides are being processed simultaneously, 30 ml per slide should be sufficient. Cot-1 DNA and oligo (dA) are no longer needed in the buffers for expression arrays.
2. Add 30-50 ml of 1X FAST MicroHybridization PreHyb/Wash Buffer for each slide being washed to a glass or plastic container.
3. Remove slide from the hybridization chamber and place in the wash container.
4. Incubate at room temperature, gently rocking, for 15 minutes. Protect the slide from light to avoid bleaching the fluorochromes.

Note: *The stringency of the wash steps may have to be optimized by the end user for particular applications.*

5. Repeat wash 3 times with fresh wash buffer for each wash step.
6. To remove residual SDS, rinse a few times with 1X SSC until no bubbles form. See Hints & Tips for improving the background.
7. Wash 1 time in 30-50 ml of 1X SSC for each slide for 5 minutes.
8. If the slide is to be imaged using a laser-based confocal imaging system, the slide should be dried. After the final wash, remove excess water droplets gently with a lint-free tissue, a compressed N_2 stream, or a quick spin. House air supplies are generally too dirty for this purpose unless they are filtered. An additional, brief drying step at 50-70°C is optional. Store slides in a dust-free, dark place until imaging.

VI. Fluorescent Imaging

FAST Slides can be detected using a variety of confocal and non-confocal laser scanner systems. Settings for the imaging of FAST Slides contained in this protocol are intended as a guideline and may need to be adjusted to provide optimal results. When imaging FAST Slides, the default imager parameters for glass slides are not suitable for FAST Slide detection.

The FAST Slide barcodes assign unique numbers to the individual slides. The barcodes will also automatically set the laser power, PMT, and focal length adjustments for FAST Slides on properly equipped scanners. These settings are starting recommendations only and should be optimized for the specific experimental conditions.

1. GSI-Lumonics ScanArray[7]

Note: *Each of the settings can be adjusted through the software interface on the ScanArray[7] LITE, 4000, and 5000 instruments.*

Focal length:	The focal length should be adjusted to the top of the membrane surface. The default setting of **-2000** should be changed to **-1970** for this parameter.
Laser power:	The laser power should be lowered to between 80-90% of maximum.
PMT:	The photomultiplier tube voltage should be reduced to 40-50% of maximum.

2. Affymetrix[7] 418 Scanner

Note: *The laser power and PMT settings can be adjusted through the software interface on the instrument.*

Focal length:	The focal length may not need to be adjusted. **Consult with instrument manufacturer for details.**
Laser power:	The laser power should be lowered to between 90-100% of maximum.
PMT:	The photomultiplier tube voltage should be reduced to 25% of maximum.

3. Virtek ChipReader **J**

Note: *The laser power and PMT settings can be adjusted through the software interface on the instrument.*

Focal length:	The focal length should be adjusted to the top of the membrane surface.
Laser power:	The laser power should be lowered to between 20-70% of maximum.
PMT:	The photomultiplier tube voltage should be reduced to 400-900V.

4. Axon Instruments GenePix™ 4000

Note: *Only the PMT settings can be adjusted through the software interface on the instrument.*

Focal length:	The focal length needs no adjusting for a good image. Minor adjustments may improve the image slightly.
Laser power:	The laser power cannot be adjusted on this model.
PMT:	The photomultiplier tube voltage should be adjusted (see Hints & Tips).

VII. Appendix- Recommended Buffers

Recommended Hybridization/Wash Buffers:

FAST MicroHybridization PreHyb/Wash Buffer 10X
Available from Schleicher & Schuell, Inc.
Item # 10 484 856
100 ml bottle, suitable for processing at least 4 FAST Slides

FAST MicroHybridization Hyb Buffer 1X
Available from Schleicher & Schuell, Inc.
Item # 10 484 857
10 ml bottle, suitable for processing at least 4 FAST Slides

To obtain pricing and ordering information on the buffers listed above, call Schleicher & Schuell Technical Service at 1-800-245-4024 or email techserv@s-and-s.com.

Denaturation Buffer (5X)	**Neutralization Buffer**
15X SSC	0.5M Tris-HCl pH 7.0
2.0M NaOH	1.5 M NaCl
50 mM EDTA	
Alternative PreHyb/Hybridization Buffer	**Alternative Wash Buffer**

5X Denhardt=s Solution	2X SSC
5X SSC	0.1% SDS
1% SDS	
50% Formamide	

Note: *The alternative buffers are not equivalent to the recommended Schleicher & Schuell buffers either chemically or in performance.*

VIII. Ordering Information

Description	**Quantity**	**Item #**
FAST Slides	20 slides	10 484 182
FAST MicroHybridization PreHyb/Wash (10X) Buffer	100 ml bottle	10 484 856
FAST MicroHybridization Hyb Buffer	10 ml bottle	10 484 857
FAST MicroHybridization Hyb Chambers	25 Chambers	10 484 867
FAST MicroHybridization Port Seals	120 seals	10 484 868
FAST MicroHybridization Hyb Chamber Clip	1 Clip	10 484 869
FAST MicroHybridization Kit	1 kit	10 484 848
FAST Slides	20 slides	
FAST MicroHybridization Hyb Chambers	25 Chambers	
FAST MicroHybridization Hyb Chamber Clip	1 Clip	
FAST MicroHybridization PreHyb/Wash (10X) Buffer	4 x 100 ml bottle	
FAST MicroHybridization Hyb Buffer	1 x 10 ml bottle	
FAST MicroHybridization Port Seals	120 seals	
FAST MicroHybridization Starter Kit	1 kit	10 484 899
FAST Slides	3 slides	
FAST MicroHybridization Hyb Chambers	4 Chambers	
FAST MicroHybridization Hyb Chamber Clip	1 Clip	
FAST MicroHybridization PreHyb/Wash (10X) Buffer	1 x 100 ml bottle	
FAST MicroHybridization Hyb Buffer	1 x 10 ml bottle	
FAST MicroHybridization Port Seals	20 seals	

Note: *There are several patents not owned or licensed by S&S regarding the use of these materials for certain applications. S&S recommends the purchaser or user check with their counsel if they are not sure of the legal rights surrounding the specific use of these products.*

FAST Slides are produced in collaboration with Grace Biolabs, Bend, OR