

Technical Instructions for ALDEHYDE microarrays

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I. INTRODUCTION

Aldehyde Microarray Slides are ideally suited for covalent and directed immobilization of amino modified nucleic acids (molecules with free amino-groups, e.g. synthetically fabricated oligonucleotides and/or PCR-products and/or cDNA's) or small protein fragments such as peptides. The aldehyde- group will reversibly react with the accessible primary amino-groups of the biomolecules to form a Schiff's Base. Following the reduction with sodium borohydride there is an irreversible immobilization of the probes and deactivation of any remaining un-reacted aldehyde-groups. In combination with the spotting solution **Qlspot** (2x) from **QInstruments** nucleic acid molecules that are dispensed with the various spotting procedures bind covalently to the glass surface.

II. GENERAL PRECAUTIONS

The protocols contained in this document are meant to be general guidelines only and some optimization may be required depending on the application and sample being used.

1. Refer to manufacturer supplied Material Safety and Data Sheets (MSDS) for proper handling and disposal of the slides and all chemicals.
2. QInstruments buffers and solutions are for research use only, not for in vitro diagnostic use.

III. REAGENTS REQUIRED

For the spotting and binding of DNA-probes onto aldehyde surface coating Slides we recommend the optimized QInstruments spotting solution **Qlspot** (2x).

For majority of hybridization applications and wash steps we recommend our hybridization buffer **Qlhyb** and wash buffers **Qlwash I, II & III** (10x).

1. Deionized water (dH₂O) - at least 18.2 Megohms-cm resistance is recommended
2. 2x spotting solution **Qlspot** or alternatively 3x SSC or alternatively 1.5 M betaine in 3x SSC
3. **Aldehyde Blocking Solution:** Sodium borohydride + PBS + ethanol (dilute 1.0 g NaBH₄ in 300 ml 1x PBS + 100 ml 99% ethanol; use ethanol to reduce bubbling)
4. Hybridization buffer **Qlhyb** or alternatively 3-5x SSC + 0.1 % SDS
5. Washing buffer **Qlwash I, II & III** (10x) or alternatively:
 - a) Saline Sodium Citrate (20x SSC) – Ambion #9673
 - b) Sodium Dodecyl Sulfate (SDS) – Fisher #BP166-500 or alternatively 10 % SDS solution for washing (10 g dodecyl sulfate sodium salt in 100 ml dH₂O, dissolve at room temperature)

IV. EQUIPMENT REQUIRED

1. QInstruments incubation chamber **IHC1** for 12 slides or 3 microtiter plates
2. QInstruments hybridization system **HC4-S** or alternatively heat block - capable of heating to 95°C
3. Centrifuge with slide holders or compressed nitrogen gas for drying slides
4. Coplin jars (VWR #25457-006) or slide dish and rack combo (Fisher #900200) for washing slides

V. ARRAY PRINTING

The probes should be dissolved in water and diluted 1:1 with 2x **Qlspot** spotting solution to yield a 1x spotting solution. Therefore, the DNA-molecules have to be adjusted to the **double** of the final spotting concentration according to the recommended values in the following table. For quantitative validations, e.g. gene expression profiles, the DNA-molecules to be immobilized must always be available in excess.

1. Add detergents to **Qlspot** (2x) spotting solution to improve the performance:

Recommended solutions for different applications	
Characteristics	Add detergents to improve the performance
Create relatively small spots for majority of applications	-----
Good results using the Ring-And-Pin Technology	0.02 % Hexadecyltrimethylammonium bromide (C ₁₉ H ₄₂ BrN) <i>Merck, #814119</i>
Create bigger spots with pin and pipetting spotting systems	0.02 % Triton X-100 <i>Merck, #108603</i>

2. Resuspend cDNAs at 0.20-1.0 µg/µl and oligonucleotides at 20-40 µM in distilled H₂O.
3. Transfer 5.0 µl of each resuspended DNA sample into a 96- or 384-well microtiter plate.
4. Add 5.0 µl per well of **Qlspot** (2x) to obtain a recommended final probe concentration according to the following table:

Recommended final spotting concentrations		
Surface coating	Oligonucleotides	PCR products, cDNA
Aldehyde	10 to 20 µM	0.1 to 0.5 µM (0.2 to 1 µg/µl)

5. Mix the samples thoroughly by pipetting up and down 10 times and centrifuge the plate for 5 min.
6. Setup the arrayer according to the manufacturer's recommendations.
7. Print the DNA samples onto Microarray substrate (optimal environment is 20 °C and 40-50 % relative humidity)

Notes:

- The results always depend on your equipment, your probes and your experimental conditions.
- Do not use any spotting solution containing primary amino-groups like Tris!
- The spot morphology and fluorescence uniformity can be modified by the chemical composition and concentration of the spotting solution. The optimal concentration mainly depends on the spotting technology and always needs to be adjusted to the experimental setup.
- **Qlspot** spotting solution is recommended for majority of applications.
- For Ring-And-Pin systems and for pipetting systems based on the capillary principle we also recommend trying out lower concentrations of **Qlspot** spotting solution.
- When using PCR-products containing amino-functional primers, the primer should be separated prior to spotting (e.g. spin columns, microplates for primer removal).
- Use of the 2x **Qlspot** spotting solution is advantageous especially when spotting oligonucleotides up to a length of 50 bases.
- Alternatively 3X SSC or 3X SSC containing 1.5 M betaine can be used as spotting buffers.
- DNA-probes in **Qlspot** spotting solution can be stored at -20°C until spotting. If the probe solution shows a white precipitation prior to spotting, heat the probes to 50 – 80°C for 2 min and avoid any change of concentration by condensation.
- If you were previously using slides that were thicker than 1.0 mm, for optimal spotting you may need to re-calibrate the distance between the slide surface and the spotting pins.
- If you use a diamond scribe to mark the boundaries of the array, this produces small glass fragments, which may get trapped under the coverslip and damage parts of the array.

VI. DNA IMMOBILIZATION

1. For completion of the covalent binding of DNA-probes on the slide surface after spotting it is necessary to incubate the slides:
 - a) for a period of min. 15 min in humid chamber consisting of dH₂O at room temperature (e.g. in QInstruments incubation chamber **IHC1** for 12 slides)
 - and b) for a period of 60 - 90 min at a temperature of 120 °C.
2. Proceed to Washing

Notes:

- After spotting and immobilization, the arrays can be used immediately or stored under dry and dark conditions at room temperature.
- The washing steps after immobilization should not be carried out until immediately prior to hybridization.

VII. WASHING & BLOCKING

After spotting it is important to remove unbound DNA-molecules and buffer substances from the slides by extensive washing to avoid any interference with subsequent hybridization experiments. Subsequently, we recommend to treat the exposed aldehyde groups with sodium borohydride (*NaBH₄*) thereby reducing them to hydroxyl groups.

To get 1x Aldehyde Blocking Solution for approximately 5 slides:	
Surface coating	
Aldehyde	Dilute 1.0 g NaBH ₄ in 300 ml 1x PBS + 100 ml 99% ethanol (<i>use ethanol to reduce bubbling</i>)

1. Rinse the Aldehyde Microarray Slides:
 - a) 2 x 2 min in 0.2 % SDS at room temperature (*dilute 5 ml 10 % SDS + 245 ml dH₂O*),
 - b) 2 x 2 min in dH₂O at room temperature,
 - c) (*Denaturing step for arrays spotted with PCR-probes*)
1 x 3 min in boiling dH₂O (95 - 100°C),
 - d) 1 x 1 minute in dH₂O at room temperature,
 - e) 1 x 15 min in Aldehyde Blocking Solution at room temperature,
 - f) 2 x 2 min in 0.2 % SDS at room temperature and
 - g) 2 x 2 min in dH₂O at room temperature
2. Dry the slides in an oil-free air or nitrogen stream or centrifuge (2 min at 150 to 200x g) to avoid any water stains on the slide surface.
3. Proceed to hybridization.

Notes:

- If you have spotted PCR probes, an additional denaturing step (step c) is essential. Boil slides 1 x 3 min in dH₂O at 95 - 100°C.
- The volume of washing solution should be at least 250 ml for 5 slides.
- The volume of Aldehyde Blocking Solution should be at least min. 100 ml for 5 slides.
- Make sure that slides do not dry between washing steps and between washing and blocking.

VIII. HYBRIDIZATION

The hybridization of microarray slides can be done with 'cover slips' or 'gene frames' in **QInstruments hybridization chamber HC4** or in other commercially available hybridization chambers.

1. Labeling probe and Purify using Purification Kit.
2. Re-suspend the dried, fluorescent labeled target that will be applied to the array in **Qlhyb** hybridization buffer. In case the target is already dissolved in a different buffer or in water, the sample can also be diluted in **Qlhyb** to get at least 90% (v/v) in the final hybridization solution (mixture ratio sample:buffer = 1:9).

Recommended final sample concentrations	
Sample	
cDNA / Oligonucleotide and other DNA molecules	Dilute sample in Qlhyb to get at least 90% (v/v) in the final hybridization solution, mixture ratio sample:buffer = 1:9

Notes:

- The amount of buffer depends on the desired target concentration and the size of hybridization chamber used.
 - As an alternative to the QInstruments hybridization buffer, a buffer with 3-5x SSC + 0.1 % SDS can be used.
3. Denature the suspended target by heating at 95 °C for 3 min in a water-filled well of a heat block, perform a quick spin in a micro-centrifuge.
- Note:**
- If the sample cannot be applied immediately after denaturation, then place it in a 42°C water-filled well of a heat block (e.g. QInstruments **HC4-S**).
4. Pipette the appropriate volume onto the array surface of a blocked slide under the cover slip and inside a **QInstruments hybridization chamber HC4**.
 5. Hybridize the probe to the microarray under the appropriate conditions. QInstruments hybridization chamber **HC4** or temperature systems **HC4-S** or **HC4-S2** are recommended.

IX. POST-HYBRIDIZATION WASHING

Wash away unbound labeled probe. QInstruments microarray wash buffers **Qlwash I, II & III** (10x) are recommended.

To get 500 ml of 1x wash buffer:	
Wash buffer	
Qlwash I	Dilute 50 ml 10x Qlwash I with 450 ml dH ₂ O (<i>mix under agitation</i>)
Qlwash II	Dilute 50 ml 10x Qlwash II with 450 ml dH ₂ O (<i>mix under agitation</i>)
Qlwash III	Dilute 50 ml 10x Qlwash III with 450 ml dH ₂ O (<i>mix under agitation</i>)

Cautions:

- Do not allow slides to dry between washes, and protect from light as much as possible.
 - Never wash the slides with dH₂O after hybridization.
 - Make all wash steps with vigorous agitation.
 - The solutions recommended below for washing are a general guideline; your application may require alternative stringency washes.
1. Place the microarray slides (max 10 pcs.) into a slide rack and immerse in a dish containing 500 ml **Qlwash I**. Wash in the above solution 1 x 10 min at room temperature.
 2. Wash 1 x 10 min in 500 ml **Qlwash II** at room temperature.
 3. Wash 1 x 10 min in 500 ml **Qlwash III** at room temperature.

or alternatively Step 1 to 3:

- a) Place the array into a slide rack and immerse in a dish containing 2x SSC and 0.2% SDS. Wash in the above solution 1 x 10 min at room temperature.
- b) Wash 1 x 10 min in 2x SSC.
- c) Wash 1 x 10 min in 0.2x SSC at room temperature.

Notes:

- The volume of the washing solution should be at least 250 ml for 5 Slides.

4. Dry the microarray slides in an oil free air or nitrogen stream or by centrifugation at 2 min at 150 to 200x g to avoid water stains on the slide surface.
5. Protect the array from light, dust and abrasion of the array surface, until ready for scanning.

X. SCANNING

Scan the microarray to acquire a fluorescent image. Ensure that the laser and filter set of the scanner is compatible with the fluorescent labeling of the probe molecules.

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