



**Genorama<sup>®</sup> Microarray Slides  
and Spotting Solutions  
User Guide**

## 1. GENERAL INFORMATION

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Genorama's microarray slides are developed and tested primarily for DNA-based applications. However, RNA and proteins can also be immobilized.

The activated glass slides are produced under rigorous quality control and tested for low background and good immobilization characteristics. As a result, the slides have a very clean and uniform surface, highly suitable for printing top quality microarrays. The microarray slides have a double-sided coating with a printable area of 23 x 73 mm.

Coated slides are packed in boxes of 25 and sealed in an alufoil bag to protect the activated surface from light and maintain a stable storage environment.

The protocols of the stringent production procedures have been developed in Asper Biotech Ltd. and the process is certified to be in accordance with ISO9001 standards.

### Two types of surface coatings are available:

Type	Coating	For binding	Molecule length	Type of binding
<b>SAL</b>	enhanced aminosilane	short aminated DNA	8-75-mer oligonucleotides	Covalent bond
<b>SA</b>	aminosilane	unmodified DNA, long aminated DNA	Up to 1 kb	Non-covalent bond

The slides are produced in two formats. Standard size slides 25 x 75 x 1 mm and thin slides 24 x 60 x 0.15 mm (one specially refined edge to ensure maximum uniformity of light distribution via total internal reflection fluorescence excitation in the Genorama® QuattroImager Imaging System).

Genorama also provides bar-coded slides upon request.

Genorama strongly recommends the use of Genorama® Spotting Solutions together with the SAL and SA slides in order to ensure proper quality.

### Two Spotting Solutions for the slides are available:

Name	For use with
<b>Genorama® Spotting Solution Type I</b>	SAL slides
<b>Genorama® Spotting Solution Type II</b>	SA slides

The 2x stock solution is delivered in bottles of 50 ml and 100 ml.

## 2. INSTRUCTIONS FOR USING THE GENORAMA<sup>®</sup> MICROARRAY SLIDES

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### Storage of coated slides and spotting solutions

- Store the slides in a closed package at room temperature.
- Coated glass-slides can be stored for 6 months in an unopened original package.
- After opening the foil package store the box of slides at in a clean environment in the dark.
- After removing the slides from +4°C environment, allow a few minutes for the temperatures to harmonize inside and outside the container.
- Store spotting solutions at +4°C.

### Tips for using slides

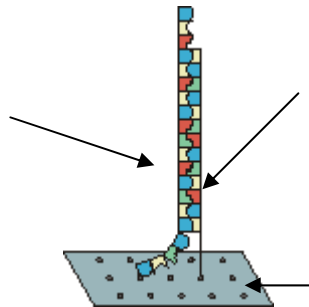
- Use the slides in a dust-free environment. Particles on the slide surface may cause defects in the probe binding and cause uneven background. Avoid fingerprints and mechanical damage.
- For probe immobilization use microarrayers, which allow a quick and precise deposition of oligonucleotides on the activated slide surface.
- After printing, loose DNA and buffer residues must be removed from slide surface by extensive washing and free reactive groups of the slide coating blocked.

### Storage of printed slides

- After printing, store the slides in a closed box in the dark, preferably at +4°C. Storage of the slides in bench-top desiccators at room temperature is also recommended.

### Terms in protocols

**Target** – unidentified mixture of nucleic acids (here – your sample to be analysed).



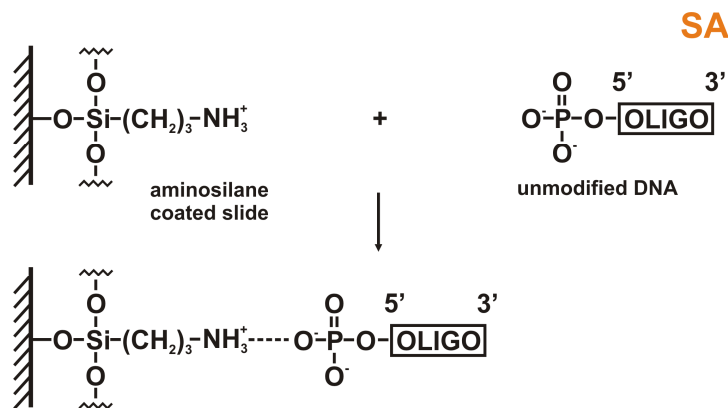
**Probe** - a known DNA or RNA fragment or a collection of different known fragments (here – the substance that binds to the glass surface).

**Slide**

### 3. SA SLIDES

**For binding:** unmodified DNA

**Coating:** aminosilane (3-Aminopropyltrimethoxysilane)



**Probe attachment:**

Unmodified oligos bind to the aminosilane coated slide electrostatically. Oligos can be attached either at their 5' termini or via internal phosphate groups (backbone).

**For printing to SA-slides we strongly recommend:**

1. Dissolve oligonucleotides to a final concentration of 50 µM in Genorama® Spotting Solution Type II
2. Dissolve PCR products in Genorama® Spotting Solution Type II. DNA concentrations between 0,2 – 0,5 µg/µl are recommended to ensure attachment of a sufficient amount of the probe and obtain uniform spot morphology
3. Spot DNA on glass slides at 20-23°C and 70% relative humidity
4. For DNA immobilization and blocking free reactive groups on the surface of the slide:
  - 1) Fill the bottom of a humid chamber with ~50-100 ml 1x SSC and place the printed arrays DNA side down over the 1x SSC
  - 2) Let the DNA rehydrate approximately 5 minutes (light vapor is observed across the slide and spots will glisten). Note! If you are using a minimum spot to spot distance do not let the spots merge
  - 3) Snap-dry the slide (DNA side up) on a 80°C heat block for 3 seconds
  - 4) UV crosslink DNA to glass at 65 mJ

Dissolve 6 g succinic anhydride in 350 mL 1-methyl-2-pyrrolidinone. Immediately after the reagent has dissolved add 15 mL sodium borate (1M, pH 8) (see appendix). Place the slides into the solution and shake for 15 minutes

- 5) Wash the slides in hot ddH<sub>2</sub>O for 2 minutes
- 6) Briefly plunge the slides 5 times in 95% ethanol, do not soak
- 7) Spin-dry the slides in a centrifuge

**Alternative protocol for printing to SA-slides:**

Please see Appendix.

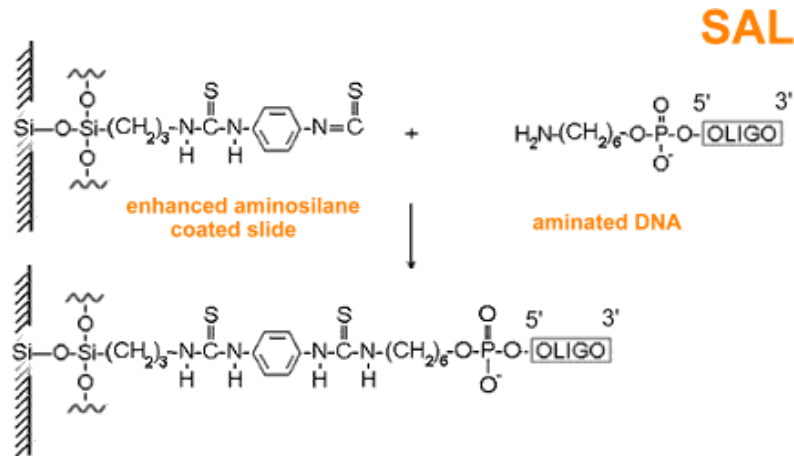
**An alternative protocol for DNA immobilization and blocking:**

1. Bake the printed array slides at 80°C for 2 – 4 hours or UV crosslink DNA to the slide at 150 – 300 mJ
2. Wash the slides with hot water (ddH<sub>2</sub>O) for 2 minutes
3. Plunge slides 5 times in 95% ethanol, do not soak
4. Spin-dry the slides in a centrifuge

## 4. SAL SLIDES

**For binding:** aminated DNA

**Coating:** aminosilane with linker  
(3-Aminopropyltrimethoxysilane + 1,4-Phenylenediisothiocyanate)



**Probe attachment:**

Aminated DNA attaches via 5' termini to 3-Aminopropyltrimethoxysilane + 1,4-Phenylenediisothiocyanate coated glass surface by formation of a covalent bond.

**SAL advantages compared to epoxy slides:**

1. SAL slides have at least 2 times better binding capacity of aminated DNA than epoxy coated slides.
2. As SAL slides are more reactive, the probe immobilization time to SAL is much shorter; (only 2 hours for SAL coating, 24 hours for epoxy slides)
3. Lower pH of the printing solution. (epoxy – pH over 11.0 required to activate the surface. SAL – pH 9.0)
5. Due to the linker molecule binding occurs further from the surface, theoretically leaving more space for target hybridization.

**For printing to SAL-slides we strongly recommend the following procedure:**

1. Dissolve amino modified oligonucleotides to a final concentration of 50  $\mu\text{M}$  in Genorama<sup>®</sup> Spotting Solution Type I.
2. Dissolve amine-PCR products in Genorama<sup>®</sup> Spotting Solution Type II. DNA concentrations between 0,2 – 0,5  $\mu\text{g}/\mu\text{l}$  are recommended to ensure attachment of a sufficient amount of the material and obtain uniform spot morphology
3. Spot DNA onto glass slides at 20-23°C and 70% relative humidity
4. For DNA immobilization and blocking free active groups on the surface of the slide:
  - 1) incubate the slides in a humid chamber at 37° C for 1 hour
  - 2) soak the slides in 1% ammonia solution for 10 minutes or, alternatively keep the slides in ammonia vapors for 1 hour
  - 3) wash the slides 2 x with ddH<sub>2</sub>O (room temperature).

**Alternative protocol for printing to SAL-slides:**

Please see Appendix

#### **4.1 SAL Ultra slides**

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We also have a SAL Ultra available, which uses the exact same chemistry, as the SAL, only the slide substrate is of higher quality level with optically flat surface.

## 5. HYBRIDISATION PROTOCOLS

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A variety of different protocols can be used for hybridisation of labelled targets. We will include protocols which have been tested with Asper Biotech slides. We would highly appreciate feedback and suggestions on improvement or modification of the protocols.

### Target hybridisation with formamide:

1. Dissolve the target in a hybridisation buffer containing 50% formamide, 5×SSC (see appendix), 0,1% SDS. The volume depends on the area to be covered and the size of the coverslip. Add 20 µg of Cot1 DNA and 20 µg of poly(A)-DNA to block non-specific hybridisation.
2. Denature the target at 95°C for 2 minutes.
3. Cool the target on ice (microcentrifuging for collecting the target is recommended) and pipet it onto the array. Cover the array with the appropriate coverslip and place the slide in the hybridisation chamber.
4. Hybridise the slides 12-16 hours at 42°C in a waterbath or hybridisation oven.

### Target hybridisation without formamide

1. Dissolve the target in a hybridisation buffer containing 3×SSC, 0,1% SDS. The volume depends on the area to be covered with a coverslip. Add 20 µg of Cot1 DNA and 20 µg of poly(A)-DNA to block non-specific hybridisation.
2. Denature the target at 95°C for 2 minutes.
3. Cool the target on ice (microcentrifuging for collecting the target is recommended) and pipet it onto the array. Cover the array with an appropriate coverslip and place the slide in the hybridisation chamber.
4. Hybridise the slides 12-16 hours at 65°C in a waterbath or hybridisation oven.

### Post-hybridisation washes

1. Prepare washing-solutions:
  - a. 1×SSC, 0,2% SDS
  - b. 0,1×SSC, 0,2% SDS
  - c. 0,1×SSC
2. Rinse the slide in each solution for 5 minutes. NOTE: Make sure the slides are not allowed to dry out between washing steps
3. Dry the slides
4. Scan the slides

## 6. TROUBLESHOOTING

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<b>Problem</b>	<b>Solution</b>
<b>Comet shaped spots</b>	<ol style="list-style-type: none"><li>1. Use a lower concentration of PCR products/oligos</li><li>2. Repeat the blocking step</li></ol>
<b>Irregular spot morphology</b>	<ol style="list-style-type: none"><li>1. Check the printing pins of the microarrayer</li><li>2. Try increasing the viscosity of the printing buffer (by adding for instance approximately 0.01% SDS)</li><li>3. Optimize the rehydration time before cross linking</li><li>4. Check the purity of the components of printing solution.</li></ol>
<b>Donut shaped spots</b>	<ol style="list-style-type: none"><li>1. Increase the humidity during spotting</li><li>2. Use another spotting buffer with lower evaporation rate.</li></ol>
<b>Uneven or high background</b>	<ol style="list-style-type: none"><li>1. Try washing again with more stringent solutions</li><li>2. Make sure that your sample is free of unincorporated labeled nucleotides (Clontech's labelling kit includes a very nice purification kit, using Qiagen PCR columns is also an alternative)</li><li>3. Be sure that the probe has not dried out under the coverslip during incubation (if needed increase the sample volume, increase the humidity in the hybridisation chamber)</li></ol>
<b>Weak signals after hybridization</b>	<ol style="list-style-type: none"><li>1. Check the labelling efficiency.</li><li>2. Extend the hybridisation time</li></ol>
<b>Nonspecific signals</b>	<ol style="list-style-type: none"><li>1. Be sure you have added blocking agents (Cot1 DNA, poly A-DNA) into your hybridisation mixture</li><li>2. Do not wash arrays with different samples in the same solutions (always use new washing solutions)</li></ol>
<b>Bright speckles within DNA spots</b>	<ol style="list-style-type: none"><li>1. Try to decrease the salt concentration within spotting solution by purifying your PCR products more or use another buffer.</li></ol>

## 7. APPENDIX

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**For the best result, Genorama strongly recommends the use of Genorama® Spotting Solution Type I and II.**

### **Alternative protocol for printing to SA-slides:**

1. Dissolve oligonucleotides at a concentration of 50  $\mu\text{M}$  in 3  $\times$  SSC (see appendix) (concentrations ranging from 20-70  $\mu\text{M}$  can also be used)
2. Dissolve PCR products in 3  $\times$  SSC or in 50% DMSO. DNA concentrations between 0,2 – 0,5  $\mu\text{g}/\mu\text{l}$  are recommended to ensure attachment of a sufficient amount of the probe and obtain uniform spot morphology
3. Spot DNA on glass slides at 20-23°C and 40-50% relative humidity

### **Alternative protocol for printing to SAL-slides:**

1. Dissolve amino modified oligonucleotides to a final concentration of 50  $\mu\text{M}$  (concentrations ranging from 20 to 70  $\mu\text{M}$  can also be used) in 100 mM carbonate buffer pH 9,0 (see appendix)
2. Dissolve amine-PCR products in 3  $\times$  SSC (see appendix) or in 50% DMSO. DNA concentrations between 0,2 – 0,5  $\mu\text{g}/\mu\text{l}$  are recommended to ensure attachment of a sufficient amount of the material and obtain uniform spot morphology
3. Spot DNA onto glass slides at 20-23°C and 40-50% relative humidity

### **Printing Solutions:**

**1. 20  $\times$  SSC** Dissolve 175g of NaCl and 88,2 g sodium citrate in 800 ml of H<sub>2</sub>O. Adjust the pH to 7.0 with a few drops of a 14N solution of HCl. Adjust the volume to 1 liter with H<sub>2</sub>O. Dispense into aliquots. Sterilize by autoclaving. The final concentrations of the ingredients are 3.0M NaCl and 0.3 sodium acetate.

**2. Carbonate buffer, pH 9.0** 100 mM Na<sub>2</sub>CO<sub>3</sub>/NaHCO<sub>3</sub> (1:1), adjust pH with concentrated glacial acetic acid.

We recommend to do at first 1M Na<sub>2</sub>CO<sub>3</sub> and 1M NaHCO<sub>3</sub> solution, mix in ratio 1:1, adjust pH with concentrated glacial acetic acid, then dilute to the final concentration.

**3. Sodium Borate (1M, pH8)** Use boric acid and adjust pH with NaOH.

### **Reagents could be obtained for example:**

- Dimethyl-sulfoxide (DMSO) – Sigma Cat # D-8418
- 1-methyl-2-pyrrolidinone – Sigma Cat # M-6762
- Boric acid – Sigma Cat # B-6768
- Bovine serum albumin – Sigma Cat # B-4287
- Cot1 DNA – Gibco BRL LifeTechnologies Cat # 25279-011
- Formamide – Sigma Cat # F-7508
- Sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) – Sigma Cat # S-1641
- Sodium bicarbonate (NaHCO<sub>3</sub>) – Sigma Cat # S-7277
- Poly (A)- DNA – Pharmacia Cat # 27-7836-01
- Sodium dodecyl sulfate (SDS) – Sigma Cat # L-4390
- Sodium hydroxide – Sigma Cat # S-0899
- Succinic anhydride – Sigma Cat # S-7626

## 8. ORDERING INFORMATION

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### **What is the minimum quantity?**

The slides are packed in boxes of 25, which is also the minimum quantity.

### **Direct ordering can be done:**

- by e-mail: [slides@genorama.com](mailto:slides@genorama.com)
- by fax: +372 7 307 298

Please mention type of slide, quantity, and address for delivery, your phone number and name.

### **After you have placed your order**

You should receive an email acknowledgement within one business day to confirm that we are processing your order. When your slides have been shipped out, we will notify you via email and give you a UPS tracking number.

### **Payment Terms**

The full price is payable within 14 days after the date of the invoice.

Genorama accepts:

- Money transfers
- Credit cards - Visa, MasterCard by fax

### **Shipping and Handling**

Separate charges for shipping and handling will be added to the cost of the order. Slides will be shipped via UPS, DHL or airmail, approximate delivery time is 2-3 days.