Hybridization to a scanning array made on glass

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Equipment and reagents

- Scanning array
- Hybridization solution: 1 M NaCl, 10 mM Tris-HCl, 1 mM EDTA, 0.01% SDS
- 50–100 fmol radiolabelled transcript (see Preparation of target RNA for hybridization to arrays by *in vitro* transcription using T3, T7 or SP6 RNA polymerase)
- A glass plate of the same size as the array plate
- A moist chamber (a large plastic or glass lidded box containing wetted paper towels)
- Incubator
- Storage phosphor screen (Fuji or Kodak)
- PhosphorImager or STORM (Molecular Dynamics)

Method

1. Clean the glass plate (non-array) with acetone and ethanol and siliconize it by treatment with dimethyl dichlorosilane solution (Merck) and place in lidded hybridization chamber.
2. Place moist towel papers into the lidded box.
3. Dilute the radiolabelled transcript in an appropriate volume of the hybridization buffer (for an array of 250 mm × 50 mm use 500 μl).
4. Place items 1–3, the scanning array, and enough hybridization buffer to wash the array into the hybridization chamber in the incubator set at desired temperature (4–37 °C). Incubate for 30 min.
5. Pipette the hybridization mix (from step 3) in a line evenly along the length of the non-array plate such that no air bubbles form.
6. Starting at one end, carefully lay the scanning array plate face down on top of the hybridization mix. The mix will spread out and form a thin film of liquid between the scanning array and the non-array plate.
7. Hybridize for 2-4 h.
8. Separate the two plates from each other and wash the array plate with hybridization buffer to remove the unbound hybridization mix.[a]

9 Drain the plate and allow to air dry.[b]

10 Cover with cling film and expose on a storage phosphor screen for 16–20 h.

11 Scan the screen on a PhosphorImager [c] and analyse the image using *xvseq*.[d]

Notes

a The hybridization intensity is not affected by a longer wash time.

b For hybridizations below 37 °C, care must be taken not to touch the plates because this can lead to melting of short duplexes. For hybridizations below room temperature, the cling film and PhosphorImager screen must be cooled to the hybridization temperature and exposed at the same temperature.

c Use the smallest pixel size (50 µm on the STORM) to achieve the greatest accuracy for analysis. For a diamond array with a displacement of 2.5 mm this gives 50 pixels per width of each cell. An image generated from an array of 115 bases, with a mask size of 42.5 mm and displacement of 2.5 mm generates a file of around 15 megabytes.

d Computer-aided image analysis is needed to obtain quantitative information about hybridisation intensities and the oligonucleotide sequences that generated them. We have developed a software package called *xvseq* to analyse scanning array images. The SUN Solaris binary version of the software is available by anonymous ftp (`ftp://bioch.ox.ac.uk/pub/xvseq.tar.gz`). The program reads and displays images generated by a Molecular Dynamics or a Fuji PhosphorImager and can perform standard image manipulation operations such as clipping, scaling, rotation, and colourmap control. Its main purpose however is to calculate and display integrated intensities of array oligonucleotides, each of which corresponds to an image cell formed by the intersection of overlapping array templates. The user can specify the template size, shape and location, step size between successive templates, as well as the sequence that generated the array pattern. The program superimposes the template grid on the array image, and the template parameters can be adjusted interactively so that the grid can be registered correctly with the hybridisation pattern. Registration can be aided by the use of fixed reference points on an array such as those mentioned in the section on the mounting polypropylene on a glass plate.