# Hybridization to scanning arrays made on glass or polypropylene[a]

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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)
- A piece of silicone rubber (0.5 mm thick) cut into a 'U' shape to seal the plates on 3 sides
- Binder clips
- Syringe and needle

## Method

1. Assemble the array and the non-array glass plate by inserting the silicone rubber between them and clamping them together with binder clips.
2. Dilute the radiolabelled transcript in the appropriate volume of hybridization buffer (5–10 ml).
3. Place 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. Apply the hybridization mix to the space between the two plates using a needle and syringe.
4. Make sure the array is horizontal.
5. Hybridize for 2-4 h.
6. Separate the two plates from each other and wash the array plate with hybridization buffer to remove the unbound hybridization mix.[b]
7. Drain the plate and allow to air dry.[c]

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

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

## Notes

a This is an alternative method to Hybridization to a scanning array made on glass and Hybridization to a scanning array made on polypropylene.

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

c 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.

d 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

e 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.