

## Horizon scanning- new methods of genetic screening

### Background

1. 'New methods for genetic screening' was identified as one of the issues that could have an impact on assisted reproductive technologies in the future by the horizon scanning panel. The issues raised by panel members were presented to SCAG at the last meeting and it was suggested that the work was picked up as part of the emerging issues in PGD information gathering work. The purpose of this paper is to provide a brief description of three techniques; whole genome amplification, comparative genome hybridisation and microarray analysis.

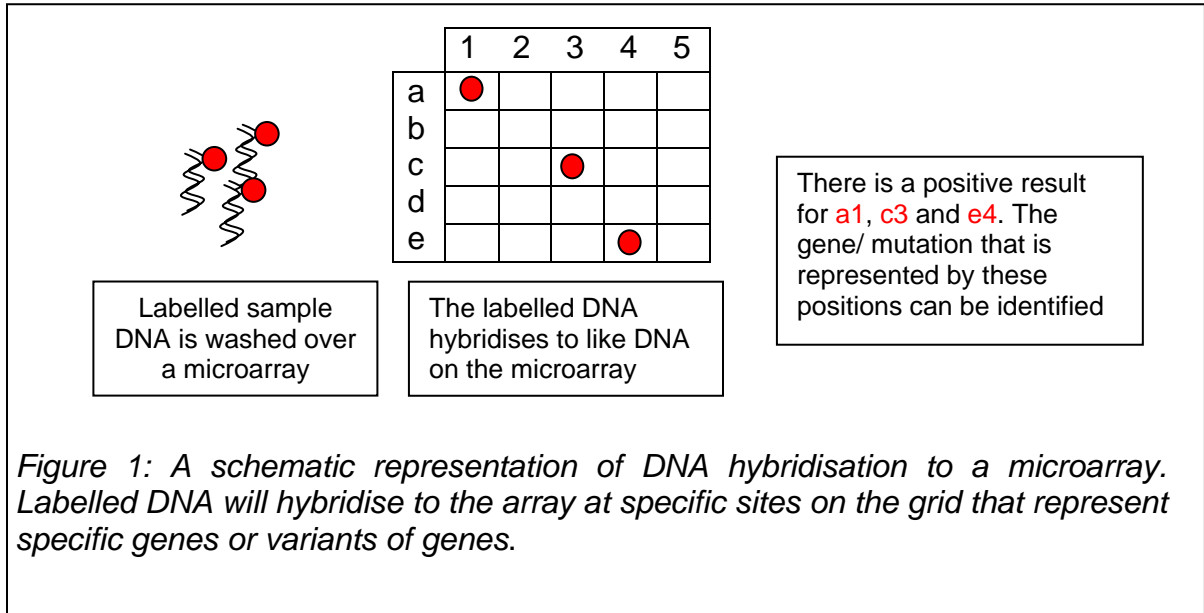
### Whole genome amplification

2. Whole genome amplification (WGA) is a variation on polymerase chain reaction (PCR). Instead of copying one specific region of DNA the whole genome is copied. This means that many copies of the whole genome will be produced increasing the total amount of DNA. This is significant in preimplantation genetic diagnosis because of the limited amount of DNA normally available from one individual blastocyst cell. When one or two cells are removed from an embryo there is a limited amount of DNA available meaning that there is only sufficient DNA to carry out limited number of genetic tests. Using WGA would mean that the initial amplification would be the same for all patients and if used in conjunction with a microarray (see below) or comparative genome hybridisation (see below) genetic testing can be carried out as a more standardised, potentially automated way.

### Microarray

3. Microarrays or gene chips consist of a silicon or glass surface onto which thousands of specific DNA sequences are attached in a grid-like formation. The DNA that is attached to the microarray can represent individual genes or small sections of DNA that represent variations in a given gene. Sample DNA can then be tested against the DNA on the chip to analyse the relative amount or specific gene variant that is present in the sample. The technique is dependent on a characteristic of DNA that allows it to bind to identical sequences. This means that where there is an exact match of base pairs between the DNA in the sample and the DNA on the chip the two DNA molecules will bind to each other, this is known as hybridisation. The DNA sample is fluorescently labelled so the position

(and therefore the DNA) to which the sample bound can be identified. This is usually done by a computer and software designed to read microarrays.



4. Microarrays could be used in several ways in the ART field. They could be used to detect specific mutations in PGD; this is described in more detail below. Microarrays could also be used to detect chromosomal deletions or duplications working in a similar way to comparative genome hybridisation (see below); however, this technique would not be useful for detecting translocations. The final way that microarrays could be used would be in the testing of the viability of an embryo. A single cell could be removed from the embryo and tested for the expression of a set of 'viability genes'. Those embryos that were expressing all the viability genes could be selectively put back into the woman. Currently embryologists rely on a morphological analysis of the embryo to decide which embryo is most likely to implant.

5. A simplified microarray has been produced for some of the mutations that cause cystic fibrosis (Savaldo *et al.*, *Reproductive BioMedicine Online*, **8(1)** 107-114). The group showed that the basic microarray was able to correctly identify the version of the cystic fibrosis gene present in affected, carrier and unaffected embryos. They tried the technique with three different variations in the cystic fibrosis gene that are known to cause the disease.

6. Using this technique it would be possible to produce a microarray that had spots representing the DNA of all the known variants of many different common genetic disorders. This would represent a universal platform for the diagnosis of any single gene disorder.

7. Using such a microarray could have advantages when compared to the current technique. It would reduce the 'work-up' time that is currently required for PGD. Currently, specific sections of DNA are amplified and analysed for affected copies of the gene using polymerase chain reaction (PCR) and specific primers. The reaction has to be optimised so that it will work with the very small amount of DNA that is available from a single biopsied cell. Using the microarray could eliminate this work-up period because all the gene variants that can cause a disease would be represented on the gene chip. Using the microarray, DNA from the embryo (and the parents) could be directly applied to the chip and the mutation present in that family could be detected.

8. The success of this technique is dependent on the further development of whole genome amplification (see above) and a microarray that allows testing for genes of interest to be produced. Microarrays are expensive to produce and the relative usefulness of any array is dependent on the DNA that it represents. Many microarrays have been produced that represent the entire genome of different species (human, mouse and zebrafish). To date, there has not been a microarray produced for use with PGD.

### **Comparative Genome hybridisation (CGH)**

9. Comparative genome hybridisation (CGH) is a molecular cytogenetic technique that can be used to screen embryos for abnormalities in chromosome number. A significant proportion of spontaneous miscarriages are caused by numerical chromosome imbalance or aneuploidy. Fluorescent *in situ* hybridisation (FISH) is currently used to screen for aneuploidy but this only allows the analysis of some of the chromosomes. Consequently only the most common aneuploidies are screened for, however recent work has shown that other aneuploidies are also often found. The advantage of using CGH is that you are able to analyse every single chromosome and also regions along the chromosome allowing the detection of imbalance in chromosome segments.

10. In CGH, sample DNA is extracted from the embryo, whole genome amplification (as described above) is carried out to ensure sufficient sample DNA. The sample DNA is then labelled with a red fluorochrome and normal, reference DNA is labelled with a green fluorochrome (see Figure 2). Both

samples are applied to a slide which contains a full set of chromosomes affixed to it. Both the sample and the reference DNA will hybridise to the chromosomes on the slide. The relative amount of chromosomes present in the sample can be worked out by analysing the colour of the chromosomes on the slide. Where there is an equal amount of a given chromosome in the sample as there is in the reference DNA the chromosome will appear yellow on the slide (the combination of the red and green fluorochrome results in yellow). Where there is more of a given chromosome in the sample than there is of a reference chromosome the chromosome on the slide will appear red. This would mean that there is likely to be trisomy of the chromosome in question in the embryo from which the sample was taken. Where the chromosome on the slide appears to be green, it would suggest that there is less of that specific chromosome in the sample compared to the reference DNA and that the sample and therefore the embryo will be monosomic (only have one copy) for that chromosome.

11. The disadvantage of CGH is that the hybridisation process takes 2-3 days. Consequently, the biopsied embryos need to be cryopreserved until the result of the CGH is available. It is thought that the success rate of frozen biopsied embryos is lower than that of normal frozen embryos. One way to overcome the slow process of hybridisation is to use microarray for the target DNA. The same principle would apply (sample DNA labelled in red and reference DNA labelled in green) but these would then be applied to a microarray which had DNA sequences specific to human chromosomes on it. Microarray CGH has been carried out to detect aneuploidy from single cells but further refinement is needed to improve the accuracy of the results.

12. CGH could be used to screen embryos for abnormal chromosomal number as an alternative to PCR techniques that are currently used for PGS.

See over for figure 2

