Aneuploidy

Whole chromosome aneuploidy has been shown to affect all chromosomes in IVF embryos. Aneuploidy is a significant cause of IVF failure, especially in women of advanced maternal age.

Pre-implantation Genetic Screening (PGS)

Initial attempts to detect aneuploidy in IVF embryos used FISH screening for a limited subset of chromosomes (5-12 chromosomes only). Clinical data from these first attempts showed no benefit to IVF success rates.

This has changed dramatically since the introduction of advanced 24 chromosome pre-implantation genetic screening (PGS). PGS now assesses the loss or gain of any whole chromosomes.

Selecting euploid embryos for transfer has been demonstrated to:

- reduce the time to pregnancy;
- reduce the incidence of miscarriage;
- achieve comparable single embryo transfer clinical pregnancy rates to unscreened multiple embryo transfer;
- allow the selection of unaffected embryos for vitrification (freezing) avoiding the storage of aneuploid embryos; and
- overcome the maternal age impact on IVF success.

PGS has been shown to increase the clinical pregnancy rate by around 50%.

96 percent of aneuploid embryos fail to implant.
Typically only a few or even a single cell are used for pre-implantation genetic screening (PGS). A single human cell contains approximately 6 picograms (6x10^{-12} grams) of DNA that needs to be reliably amplified (copied) millions of times to obtain enough DNA for screening. This process, which is termed whole genome amplification (WGA), is performed using a specialised type of polymerase chain reaction (PCR). The robustness and fidelity of the WGA is very important, as any errors introduced by this process may affect the accuracy of the results. RHS have used advanced PCR polymerases to optimise the WGA in EmbryoCellect™.

**HOW EMBRYOCELLECT™ WORKS**

EmbryoCellect™ has been designed to specifically screen for **whole chromosome aneuploidy**. It uses array Comparative Genomic Hybridisation (aCGH) to compare the number of chromosomes from a sample cell to a known reference sample. The samples are labelled and the relative fluorescence is measured for each chromosome by hybridisation to the EmbryoCellect™ microarray.

A sample is placed into a PCR tube and enzymatically lysed. The EmbryoCellect™ DOP-PCR whole genome amplification (WGA) then robustly amplifies the genome millions of times.

Typically only a few or even a single cell are used for pre-implantation genetic screening (PGS). A single human cell contains approximately 6 picograms (6x10^{-12} grams) of DNA that needs to be reliably amplified (copied) millions of times to obtain enough DNA for screening. This process, which is termed whole genome amplification (WGA), is performed using a specialised type of polymerase chain reaction (PCR). The robustness and fidelity of the WGA is very important, as any errors introduced by this process may affect the accuracy of the results. RHS have used advanced PCR polymerases to optimise the WGA in EmbryoCellect™.

**HOW EMBRYOCELLECT™ WORKS**

EmbryoCellect™ has been designed to specifically screen for **whole chromosome aneuploidy**. It uses array Comparative Genomic Hybridisation (aCGH) to compare the number of chromosomes from a sample cell to a known reference sample. The samples are labelled and the relative fluorescence is measured for each chromosome by hybridisation to the EmbryoCellect™ microarray.

A sample is placed into a PCR tube and enzymatically lysed. The EmbryoCellect™ DOP-PCR whole genome amplification (WGA) then robustly amplifies the genome millions of times.

Typically only a few or even a single cell are used for pre-implantation genetic screening (PGS). A single human cell contains approximately 6 picograms (6x10^{-12} grams) of DNA that needs to be reliably amplified (copied) millions of times to obtain enough DNA for screening. This process, which is termed whole genome amplification (WGA), is performed using a specialised type of polymerase chain reaction (PCR). The robustness and fidelity of the WGA is very important, as any errors introduced by this process may affect the accuracy of the results. RHS have used advanced PCR polymerases to optimise the WGA in EmbryoCellect™.

**HOW EMBRYOCELLECT™ WORKS**

EmbryoCellect™ has been designed to specifically screen for **whole chromosome aneuploidy**. It uses array Comparative Genomic Hybridisation (aCGH) to compare the number of chromosomes from a sample cell to a known reference sample. The samples are labelled and the relative fluorescence is measured for each chromosome by hybridisation to the EmbryoCellect™ microarray.

A sample is placed into a PCR tube and enzymatically lysed. The EmbryoCellect™ DOP-PCR whole genome amplification (WGA) then robustly amplifies the genome millions of times.
WHAT IS PRINTED ON THE EMBRYOCELLECT™ MICROARRAY?

- Multiple copies of single human metaphase chromosomes are laser captured
- The chromosomes are whole genome amplified using RHS DOP-PCR then repeat depleted
- Whole chromosome paints containing on average 1.2 million sequences per chromosome are printed on microarrays in replicates of 8 per microarray. There are 4 microarrays per slide.

The EmbryoCellect™ microarray is fundamentally different to other microarrays.

Unlike BAC or oligonucleotide arrays, where each probe contains a single DNA target ranging in size from approximately 60-150 basepairs, each feature (spot) on the EmbryoCellect™ array contains a whole chromosome library. This provides on average 1.2 million unique chromosome-specific target fragments of DNA ranging in size from approximately 200 to 4,000 basepairs.

This approach (single cell microarray to detect aneuploidy in embryos) was first described by RHS scientists from the Department of Obstetrics and Gynaecology, The University of Adelaide in 2004 1 and 2007 2 and is exclusively licensed to RHS.

This patented approach allows the EmbryoCellectTM microarray to collect test and reference signal from an entire chromosome in a single result providing a clear indication of whole chromosome count.

PRODUCT ATTRIBUTES

**What is printed on the EmbryoCellect™ microarray?**
Chromosome-specific DOP-amplified and repeat deleted PCR products ranging in size from 120bp – 4kb

**How are the microarrays printed?**
Spot printed as a pooled library of sequences specific to each chromosome

**Number of targets on each microarray**
Over 35 million sequences per array with, on average, 1.2 million chromosome specific sequences per spot.

**Replicate targets on each microarray**
One spot for each of the 24 human chromosomes; Eight replicates per array

**How many microarrays per slide?**
Four allowing the testing of as few as four samples at a time in a standard test versus reference hybridisation. One operator can manage 4 slides in a batch if required

**Ease of analysis**
There is a single spot per chromosome so there is no need to calculate a consensus across the chromosome to detect aneuploidy. The results are very clear and specific for the detection of whole chromosome aneuploidy.

EmbryoCellect™ has been specifically developed to screen for whole chromosome aneuploidy in single or small numbers of cells.
Why EmbryoCellect™?
- The test is simple and robust;
- The results are easy to interpret;
- The raw scanner data is available; and
- The test has been validated for accuracy

Samples can be processed in batches as small as four

The EmbryoCellect™ kit contains:
- Cell lysis buffer and enzyme for test and reference
- Reference male gDNA
- Whole Genome Amplification (WGA) reagents
- Fluorescent labelling PCR reagents
- Five patented EmbryoCellect™ microarray slides with 4 microarrays per slide
- Sufficient reagents to test 20 samples

EmbryoCellect™ has been validated using single cells from a range of euploid and aneuploid cell lines and tested on trophectoderm biopsies. The microarray data has been further validated by Next Generation Sequencing on both the MiSeq and Ion Torrent sequencing platforms.

MiSeq reads for 48,XY,+2,+21 single cells normalized to 46,XX single cells
## The EmbryoCellect™ workflow

<table>
<thead>
<tr>
<th>Protocol Step</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell lysis</strong></td>
<td>Following biopsy, a gentle but effective enzyme-based lysis procedure ensures robust cell lysis and a readily accessible DNA template for whole genome amplification.</td>
</tr>
<tr>
<td>Cell lysis 15 mins</td>
<td></td>
</tr>
<tr>
<td><strong>Whole genome amplification</strong></td>
<td>Whole genome amplification is performed using RHS’s DOP-PCR, which has been optimised for the RHS microarray. DOP-PCR uses degenerate primers to initiate DNA amplification, binding across a broad range of different sequences scattered genome wide.</td>
</tr>
<tr>
<td>Whole genome amplification 2.5 hrs</td>
<td></td>
</tr>
<tr>
<td><strong>Agarose gel assessment</strong></td>
<td>Following amplification, the use of agarose gel electrophoresis is recommended to ensure that cell amplification has been successful.</td>
</tr>
<tr>
<td>Agarose gel assessment 30 mins</td>
<td></td>
</tr>
<tr>
<td><strong>Labelling PCR</strong></td>
<td>Successfully amplified samples are fluorescently labelled by a second DOP-PCR. The test is labelled with a Cy3 equivalent dye and the reference with a Cy5 equivalent dye.</td>
</tr>
<tr>
<td>Labelling PCR 45 mins</td>
<td></td>
</tr>
<tr>
<td><strong>Clean-up and nanodrop</strong></td>
<td>Once purified, these labelled amplicons are again assessed using agarose gel electrophoresis and spectrophotometry to ensure adequate amplification and dye incorporation has occurred.</td>
</tr>
<tr>
<td>Clean-up and nanodrop 30 mins</td>
<td></td>
</tr>
<tr>
<td>Clean-up and nanodrop Agarose gel assessment 30 mins</td>
<td></td>
</tr>
<tr>
<td><strong>Hybridisation</strong></td>
<td>Samples are competitively hybridized to the RHS microarray.</td>
</tr>
<tr>
<td>Hybridisation 3 hrs to overnight</td>
<td></td>
</tr>
<tr>
<td><strong>Microarray washing</strong></td>
<td>After incubation, the microarray is washed and scanned. The ratio of test to reference dye intensity after normalization is determined using RHS proprietary software, providing the ploidy status of each chromosome in each test sample.</td>
</tr>
<tr>
<td>Microarray washing 30 mins</td>
<td></td>
</tr>
<tr>
<td>Microarray scanning and analysis 30 mins</td>
<td></td>
</tr>
</tbody>
</table>
Further background reading


24-chromosome copy number analysis: a comparison of available technologies. Handyside A, Fertility and Sterility 2013:100:595-602


Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. Scott RT Jr, Upham KM, Forman EJ, Zhao T, Treff NR. Fertility and Sterility 2013 Sep;100(3):624-30

Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. Scott RT Jr, Upham KM, Forman EJ, Hong KH, Scott KL, Taylor D, Tao X, Treff NR. Fertility and Sterility 2013 Sep;100(3):697-703

In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. Forman EJ, Hong KH, Ferry KM, Tao X, Taylor D, Levy B, Treff NR, Scott RT Jr. Fertility and Sterility July 2013 100-7


Relevant RHS inventor publications


