EmbryoCellect™

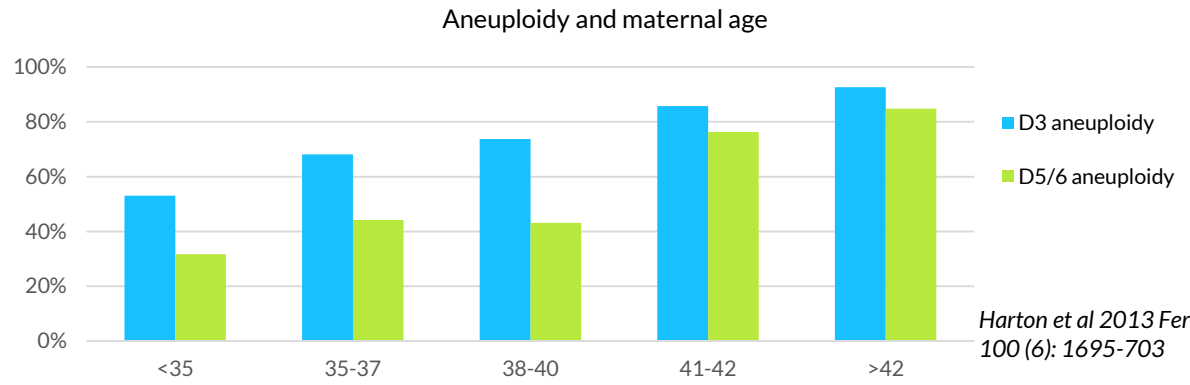
Pre-implantation Genetic Screening Kit

TECHNICAL INFORMATION

RHS
Reproductive Health Science

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.



Harton et al 2013 Fertil Steril Dec 100 (6): 1695-703

96 percent of aneuploid embryos fail to implant

Scott et al 2012 Fert Steril Apr 97 (4): 870-5

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 embyros 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%

Yang et al. 2012 Mol Cytogenet., 5: 24

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

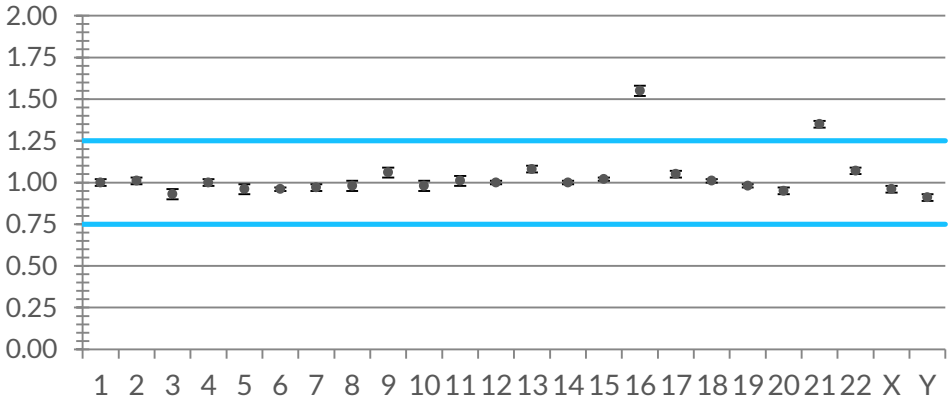
Typically only a few or even a single cell are used for pre-implantation genetic screening (PGS). A single human cell contains approximately 6 picograms ($6x10^{-12}$ grams) of DNA that needs to be reliably amplified (copied) millions of times to obtain enough DNA for screening. This process, which is 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™.

The reference sample is labelled with red fluorescent dye

The test sample is labelled with green fluorescent dye

The test and reference are combined and they compete for binding positions on the EmbryoCellectTM microarray

After microarray scanning and rapid data analysis, the relative fluorescence signals of the test and reference are compared



Outliers indicating extra copies (trisomy) of chromosomes 16 and 21

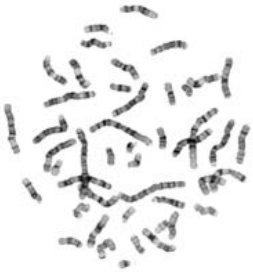
Equal number of chromosomes to the male reference

This EmbryoCellect™ result was generated from a single fibroblast from a male cell line with trisomy in chromosomes 16 and 21 (arr(16)x3,(21)x3)

EmbryoCellect™ is for research use only and is not for use in diagnostic procedures.

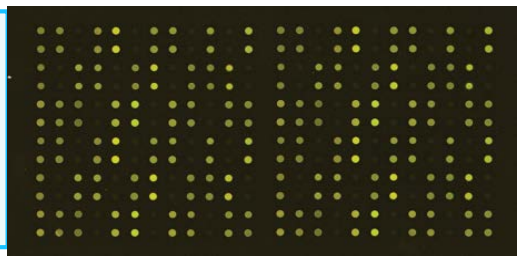
WHAT IS PRINTED ON THE EMBRYOCELLECT™ MICROARRAY?

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 1.2million 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 1and 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.

1. Aneuploidy detection in single cells using DNA array-based comparative genomic hybridization. Hu, D. G., Webb, G., & Hussey, N. Molecular Human Reproduction 2004, 10(4), 283–9.
2. Gender determination and detection of aneuploidy in single cells using DNA array-based comparative genomic hybridization. Hu, D. G., Guan, X. Y., & Hussey, N. Methods in Molecular Medicine 2007, 132, 135–151.

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™?

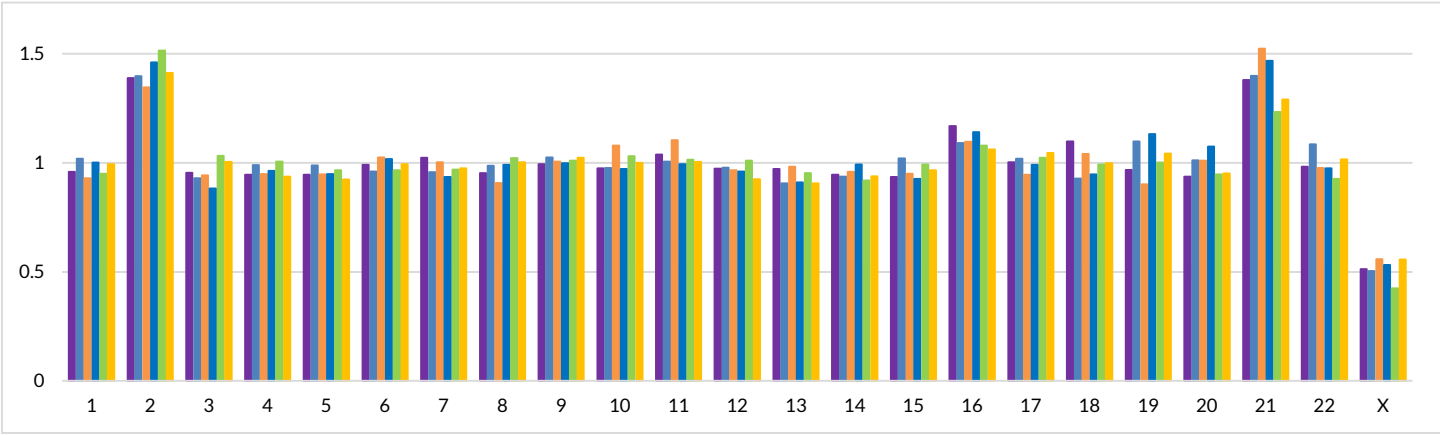
- The test is simple and robust;
- The results are easy to interpret;
- The raw scanner data is available; and
- The test has been validated for accuracy

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

Protocol Step	Explanation
Cell lysis 15 mins	Following biopsy, a gentle but effective enzyme-based lysis procedure ensures robust cell lysis and a readily accessible DNA template for whole genome amplification.
Whole genome amplification 2.5 hrs	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.
Agarose gel assessment 30 mins	Following amplification, the use of agarose gel electrophoresis is recommended to ensure that cell amplification has been successful.
Labelling PCR 45 mins	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.
Clean-up and nanodrop 30 mins **Agarose gel assessment** 30 mins	Once purified, these labelled amplicons are again assessed using agarose gel electrophoresis and spectrophotometry to ensure adequate amplification and dye incorporation has occurred.
Hybridisation 3 hrs to overnight	Samples are competitively hybridized to the RHS microarray.
Microarray washing 30 mins **Microarray scanning and analysis** 30 mins	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.

About Reproductive Health Science Ltd

Reproductive Health Science Ltd is a developer of advanced single cell genomic technologies with a focus on improving health and research outcomes.

Further background reading

Comparative genomic hybridization selection of blastocysts for repeated implantation failure treatment: a pilot study. Greco E, Bono S, Ruberti A, Lobascio AM, Greco P, Biricik A, Spizzichino L, Greco A, Tesarik J, Minasi MG, Fiorentino F. BioMed Research International 2014:article 457913

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

Diminished effect of maternal age on implantation after preimplantation genetic diagnosis with array comparative genomic hybridization. Harton GL, Munné S, Surrey M, Grifo J, Kaplan B, McCulloh DH, Griffin DK, Wells D; PGD Practitioners Group. Fertility and Sterility 2013 Dec;100(6):1695-703

Preimplantation genetic screening (PGS) with Comparative genomic hybridization (CGH) following day 3 single cell blastomere biopsy markedly improves IVF outcomes while lowering multiple pregnancies and miscarriages. Keltz MD, Vega M, Sirota I, Lederman M, Moshier EL, Gonzales E, Stein D. Journal of Assisted Reproduction and Genetics 2013 Oct;30(10):1339-9

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

Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. Yang Z, Liu J, Collins GS, Salem SA, Liu X, Lyle SS, Peck AC, Sills ES, Salem RD. Molecular Cytogenetics 2012 May 2;5(1):24

Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study. Scott RT Jr, Ferry K, Su J, Tao X, Scott K, Treff NR. Fertility and Sterility 2012 Apr:97(4):870-5

Relevant RHS inventor publications

Gender determination and detection of aneuploidy in single cells using DNA array-based comparative genomic hybridization. Hu DG, Guan XY, Hussey N. Methods in Molecular Medicine 2007;132:135-51

Singleton births after routine preimplantation genetic diagnosis using exclusion testing (D4S43 and D4S126) for Huntington's disease. Jasper MJ, Hu DG, Liebelt J, Sherrin D, Watson R, Tremellen KP, Hussey ND. Fertility and Sterility 2006 Mar;85(3):597-602

Preimplantation genetic diagnosis for BRCA1 exon 13 duplication mutation using linked polymorphic markers resulting in a live birth Jasper MJ, Liebelt J, Hussey ND. Prenatal Diagnosis 2008: Apr 28(4): 292-8

Aneuploidy detection in single cells using DNA array-based comparative genomic hybridization. Hu DG, Webb G, Hussey N. Molecular Human Reproduction. 2004 Apr;10(4):283-6

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