Clinical Use of SNP Arrays for Preimplantation Genetic Screening

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Overview

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- Current state of PGS
- Hope for new tests (testing all 24 chromosomes)
- Materials and Methods
- Results
- Discussion

Current State of PGS

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- Most clinical applications of PGS today still utilize FISH-based methods testing for 8-12 chromosomes
- Biopsy is performed on Day-3 of embryo development followed by fresh transfer on Day-5

Current State of PGS

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- Chromosomes chosen for testing based on observation of aneuploidy in live-borns and aborted specimens
- FISH-based methods suffer from:
  - mistakes due to spreading issues (overlapping signals)
  - mistakes due to hybridization issues (split signals)
  - limited availability of unique labels requiring multiple rounds of FISH

Early Embryos and Mosaicism

- It is a well-known fact that 30% of early embryos are chromosomally mosaic, although most mosaics are 100% abnormal
- The estimated error rate produced by mosaics is 5-7%
- Mosaicism can lead to incorrect diagnosis of embryo status depending on which cell is removed and tested

Munne, 2007; H. Ballsackz, 2006

Current State of PGS

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- Early PGS studies supported the hypothesis that removing aneuploid embryos from IVF cohorts would increase pregnancy rates
- PGS has been under fire recently due to the highly publicized RCT by Mastenbroek et al showing very poor pregnancy rates following PGS

Current State of PGS

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- A great debate followed the publication of the Mastenbroek paper which centered on the technical skill of the laboratory performing the study and some of the decisions made during the trial
- The debate continues today as other RCTs have concluded with similar results to Mastenbroek

Hope for new tests

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- Preliminary data from a few groups testing for 24 chromosomes clinically has been encouraging
- These studies have shown high ongoing pregnancy rates, even in poor prognosis patients
- More clinical data is needed!

Materials & Methods

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- All samples lysed using NaOH/DTT or KOH/DTT
- Lysis carried out at 65˚C followed by cooling on ice until all samples collected
- Fibroblasts were isolated by hand and picked up as single cells, or groups of 5-10 cells for analysis

Materials & Methods

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- Neutralization of the lysis mix was done along with addition of the MDA mix
- MDA was performed at 30˚C, followed by a brief incubation at 65˚C

Materials & Methods

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- All samples were cleaned using the QiaAmp MiniDNA kit with slight modifications
- Real-time PCR was carried out with primers and probes for the amelogenin gene
- Only samples with a C_t of less than 35 were considered for analysis on the array

Materials & Methods

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- All samples were run on the new Illumina HumanCytoSNP-12 chip
- Analysis was done using Illumina's Karyostudio software package

Materials & Methods

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- All samples were run on the array as per manufacturer's instructions with no modifications
- The microarray tech and the person analyzing the data were blinded to the sample make-up

Results

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- Baseline experiment
  - 4 cell lines (Monosomy X, Trisomy 13, Trisomy 18, Trisomy 21)
  - Compared NaOH lysis to KOH lysis
  - 24 different samples run on array
  - Some of the above 24 samples were run in triplicate to assess reproducibility among amplified samples

Results

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- Baseline experiment
  - 6 single cells out of 80 isolated cells (7.5 %) did not pass the real-time PCR test
  - 2 out of the 6 were completely negative for product most likely indicating no cell delivered to the tube
  - Therefore, 4 out of 78 cells (5.1 %) delivered to the tube did not pass the real-time test

Results

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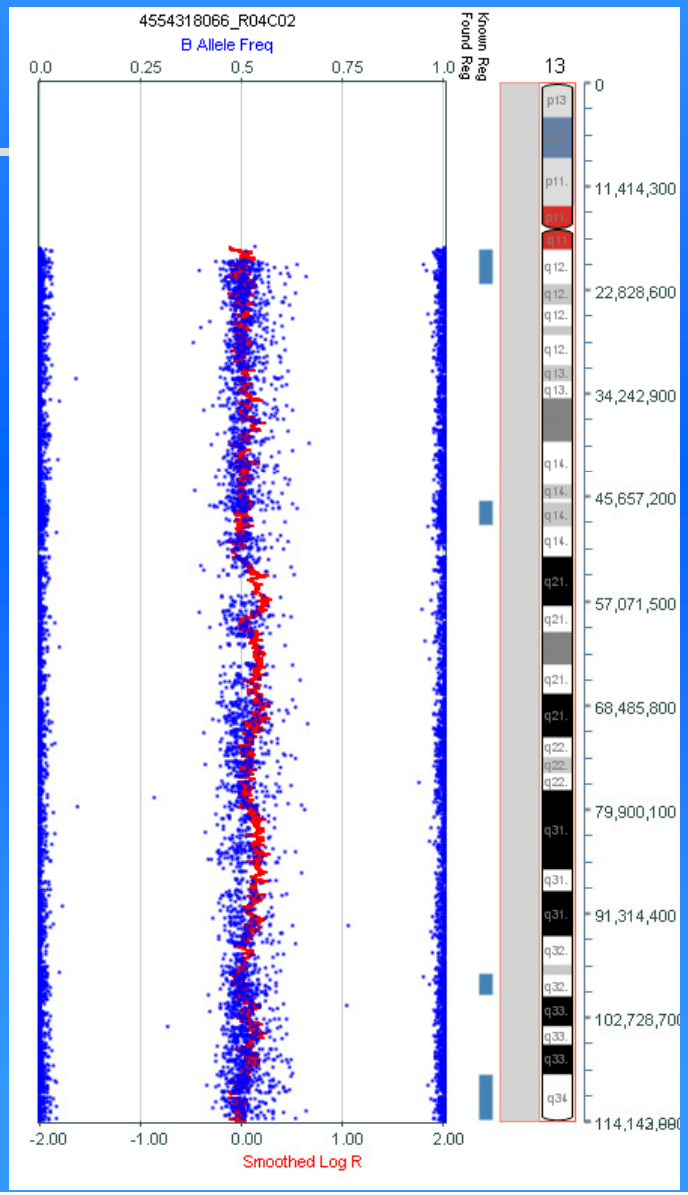
- Baseline experiment
  - 23 out of the 24 single cells run were correctly identified by the scorer (blindly)
  - 1 sample was ambiguous (trisomy 18 cell line)
  - In addition to the known aneuploidy, some samples had false positive partial aneuploidies for smaller chromosomes including 16, 17, 18, 19, 21 and 22

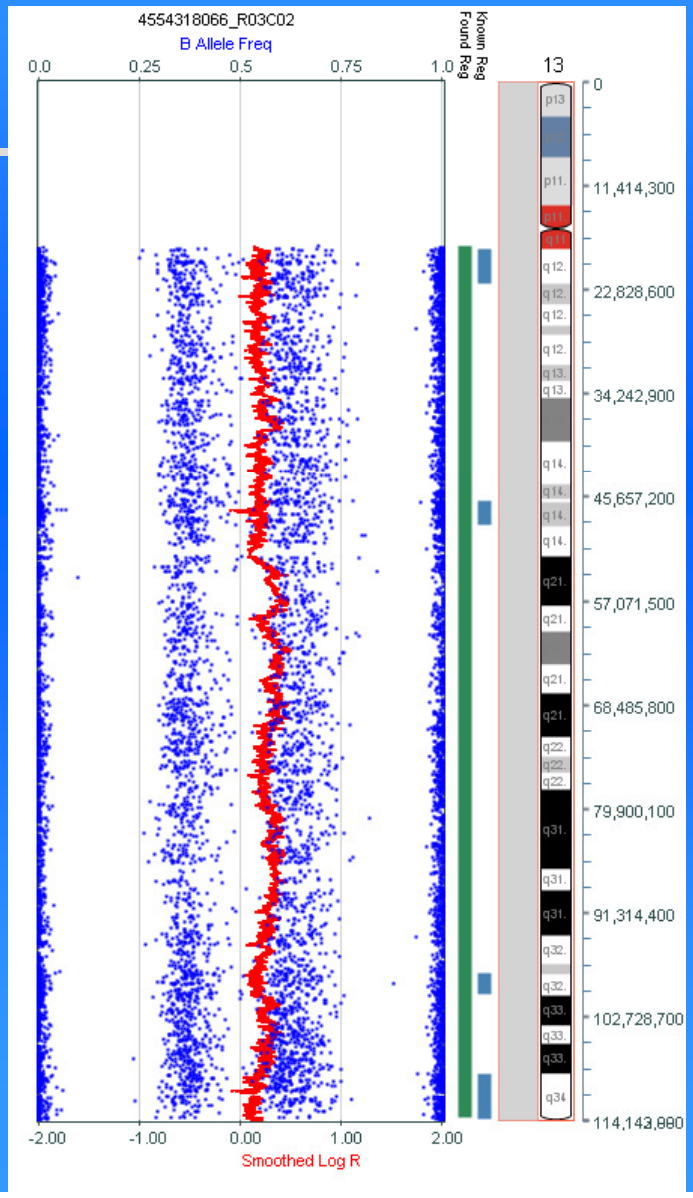
Results

- Template = DNA
- Normal cell line



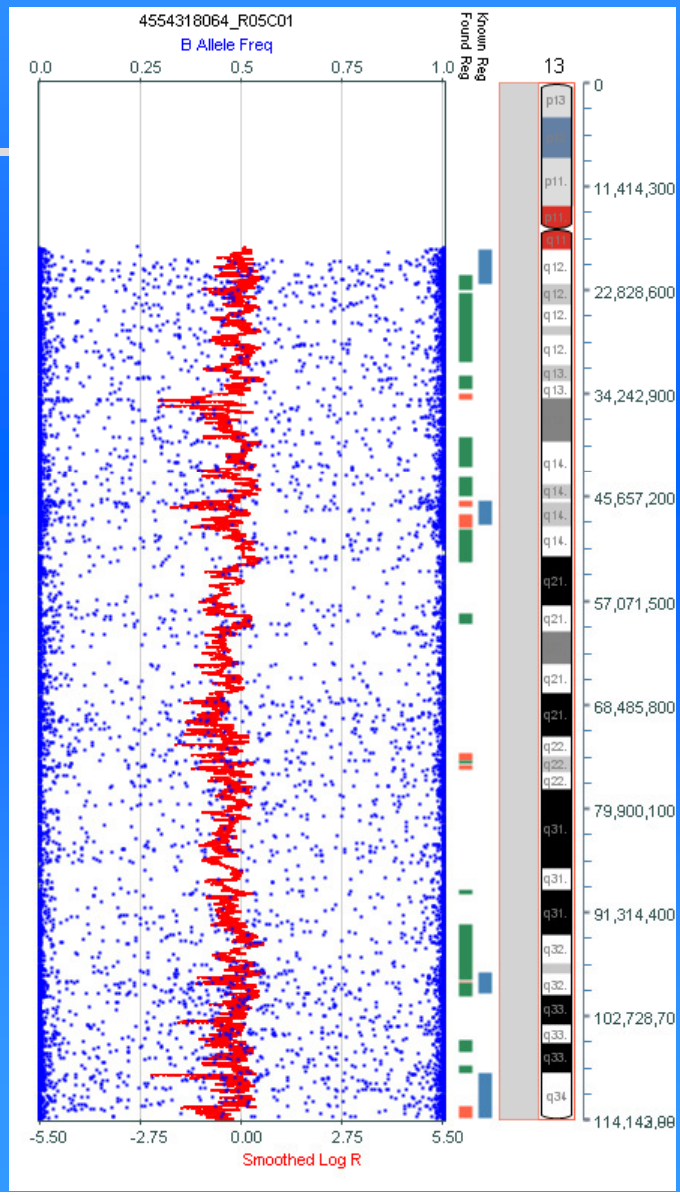
Results

- Template = DNA
- Trisomy 13 cell line



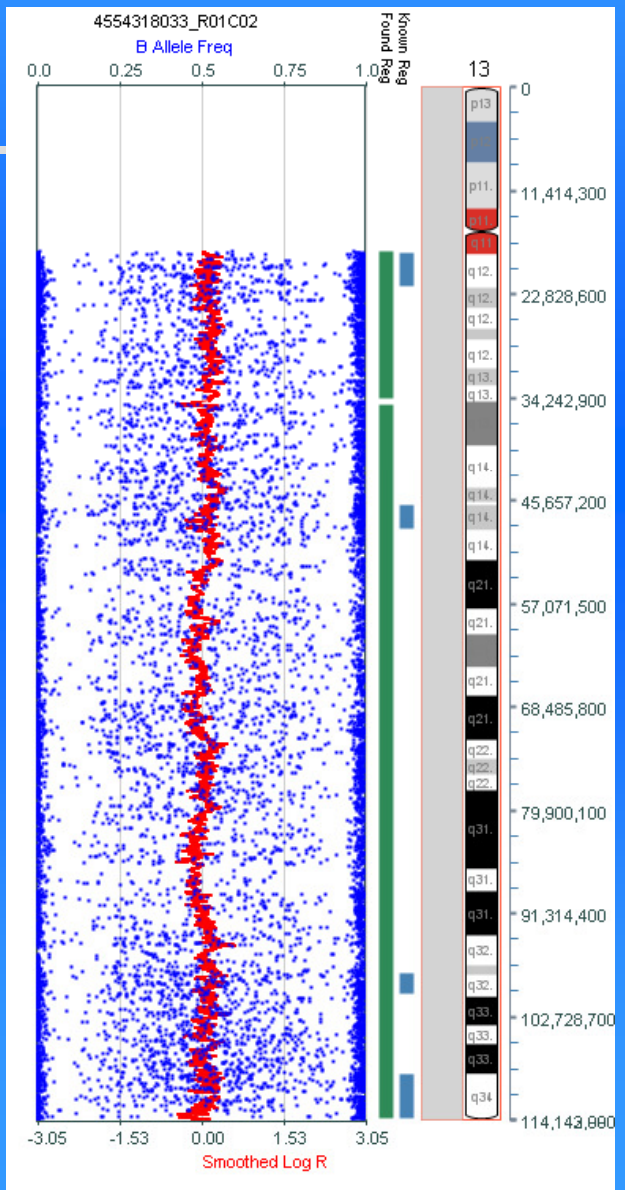
Results

- Template = single cell
- Diploid cell line



Results

- Template = single cell
- Trisomy 13 cell line



Results

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- Preamplification experiment
  - 4 different protocols used to prepare cells for analysis
  - Changes included reduced MDA time, MDA without modification from manufacturer, titration of alkaline lysis mix and no pre-treatment
  - No improvement seen with any of the changes

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Results

- Fetal cell experiments
  - Single amniocytes from discarded specimen isolated and subjected to MDA
  - Same clean-up and real time PCR analysis
  - One single cell from each amnio sample chosen to analyze on array
  - Comparison of results from chip analysis to karyotype

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Results

- Fetal cell experiments
  - Each single cell was run on the chip in triplicate (3 different samples from one MDA product)
  - Thus far, 30 single amniocytes have been run and analyzed
  - 21/30 amniocytes analyzed have had acceptable call rates for single cell analysis

Results

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- Fetal cell experiments
  - When sorted by call rates, each triplicate "bunched" together almost perfectly in all samples
  - Same false-positive issues seen in these samples as those in the fibroblast samples

Results

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- Fetal cell experiments
  - At least 50 more single amniocytes have been isolated and amplified and are waiting to be run on chips
  - These samples have not been compared to the karyotype for confirmation of results-ongoing

Discussion

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- Single cells can be reliably amplified using a modified MDA protocol for analysis on a SNP array
- Illumina's new HumanCytoSNP-12 chip coupled with Karyostudio software can detect aneuploidy from single cells

Discussion

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- Future directions of this work include:
  - Reduction in time necessary to perform analysis to allow for Day-3 biopsy and Day-5 fresh embryo transfer
  - Combined testing of monogenic disease and aneuploidy screening in a single test

Discussion

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- Future directions of this work include:
  - Use of similar techniques on multiple cell samples from blastocyst biopsy samples
  - Genetics & IVF Institute plans to offer a clinical trial using this system in late 2009

Genetics & IVF Institute

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