Combined PGS and PGD for Thalassemia

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Conflict of Interest
MB, BH, KW and MJ are employees of RHS Ltd

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Why combine PGD with PGS?

Preimplantation Genetic Diagnosis (PGD) and Preimplantation Genetic Screening (PGS) are not routinely combined

PGD unaffected embryos can be aneuploid
  70% of PGD unaffected embryos that failed to implant were aneuploid (Mark Hughes, Genesis Genetics ACMG 2017)

So what are the options to combine PGD and PGS?
Strategies for PGD + PGS

Direct PCR

The region of interest may be amplified during WGA

ADO rate higher than PGD

WGA for PGS

No PGS

WGA + PGD

1 PCR; 2 results

ADO rate equivalent to PGD
DOPlify + PGD

DOPlify WGA
- Proven approach
- Latest generation reagents suited to high resolution NGS
- Optimised and validated for PGS

+ Targeted Sequence Enrichment (TSE)
- RHS patented approach
- Compatible with DOPlify WGA only
- Optimized addition of individual and multiplex primer sets for the specific amplification of single or multiple target regions during WGA
To demonstrate the application of a novel sequence enrichment protocol during WGA using DOPlify for combined PGD and PGS by NGS for β-thalassemia
Common HBB mutations

PCR primer targets

Exon 1 & 2

Exon 3

http://perspectivesinmedicine.cshlp.org/content/3/5/a011700.full
DOPlify standard WGA protocol

Lysis

- Add lysis buffer & enzyme mix
  - *cells only

- Incubate 10 minutes

Amplify 2 hours

WGA

- Add PCR mastermix
DOPlify WGA + TSE protocol

Compatible with routine PGD methods:
- PCR, electrophoresis, Sanger sequencing, NGS
DOPlify PGD + PGS strategies

WGA only

WGA + TSE with primers for Exon 1&2 and Exon 3

Pool WGA + TSE & amplicons 1/20

Exons 1& 2

Exon 3

MiSeq™

40 sample multiplex 2x75bp reads
Ideal outcomes

High throughput MiSeq sample run (48)

• same library prep and NGS workflow for PGS and PGD + PGS

• cost per sample remains competitive

• scalable

Aneuploidy results uncompromised following TSE

Breath of coverage: 100%

Depth of coverage: >30x
Breadth and depth of coverage

Breadth = the proportion of bases in the target sequence that are represented

Depth = the number of times each base is represented
PGS by NGS results

5-cell aliquots
WGA + TSE + Amplicons 1:20
Average 600,000 PGS reads per sample
Amplicon reads were downsampled
Comparison of 3 strategies

40 sample multiplex MiSeq 2x75bp reads

- **WGA only**
  - Avg < 1 reads

- **WGA + TSE**
  - Avg 120 reads

- **WGA + TSE & amplicon pool**
  - Avg 1600 reads
Breadth of Coverage 100%

DNA sequence

-Proportion of amplicon sequenced (%)

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<th>Exon 1&amp;2</th>
<th>Exon 3</th>
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WGA: Whole Genome Amplification
TSE: Targeted Enrichment
Amplicon: A specific region of the genome that is amplified for sequencing.
Depth of Coverage >200x

DNA sequence

30x-60x

Depth of Coverage (x)

Mean ± STDEV

HBB

WGA + TSE

WGA + TSE + 1:20 Amplicon
The novel RHS DOPlify PGS + PGD protocol

- Uses a standard low pass PGS NGS protocol
- Multiplexes 48 samples per run and has achieved:
  - Accurate PGS results
  - 100% breadth of coverage of the most common β thalassemia mutations
  - >60x depth of coverage across exons 1, 2 and 3

**Advantages:**

1. Biopsy
2. WGA
3. 1 NGS sample index per sample

Scalable and economical PGS + PGD