Rapid genomic screening of embryos using nanopore sequencing

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Forman EJ & Scott RT Jr *Contemporary OB/GYN* ((2014)
Euploid single-embryo transfer: the new IVF paradigm?
What is a nanopore?

• Protein nanopores occur naturally in cells and can embed themselves in membranes
• Nanopores create tiny holes or channels a few nanometers in diameter
• Molecules can pass from one side of a membrane to the other
How does nanopore sequencing work?

Axopatch 200B - single channel patch clamp amplifier

Nanopore translocation
• Open current
• Red = current change 1
• Purple = current change 2
How does nanopore sequencing work?

RNA ‘squiggle’

Nanopore translocation
• Open current
• Red = current change 1
• Purple = current change 2

open pore current

adapter
polyA tail
1,500 ~nt transcript

open pore current

Time (sec)

Current (pA)

0 5 10 15 20 25
50 100 150 200 250

~1,500 nt transcript adapter polyA tail
How does nanopore sequencing work?

Sensor chip: what's inside:

- Biological nanopore
- Sensing channel
- Array of channels
- Electronic chip

Motor
Nanopore
Membrane

Consumable flow cell
Sample port
USB powers device and passes data to PC
Connector flow cell connects with MinION

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How does nanopore sequencing work?

1. Long reads: tens to hundreds of kilobases
2. Portability
3. Low cost
4. Data generation and analysis in real time
   A. Run-until (no sample batching necessary)
   B. Read-until
Detecting aneuploidy with high-throughput sequencing

Day 1 | Day 3 | Day 5 | Biopsy of 1–3 cells | gDNA extraction | Library preparation and sequencing

Coverage normalised to haploid reference

Euploid male

Male with trisomy 13
Why do PGS / PGD with nanopores?

Programme start: diagnostic tests, medical visits
4–3 weeks

Hormone stimulation
9–7 days

Egg collection

Blastocyst biopsy
After 5 days

Embryo freezing/vitrification
14–days

PGS test

Embryo thawing

Embryo transfer

Medical supervision over implantation

Patient’s next cycle

Medical supervision over implantation

Patient’s next cycle
Why do PGS / PGD with nanopores?

1. Results faster
2. Lower cost per sample
3. Control over whole process
4. Lower startup cost
- Placental anticoagulant
- M2 haplotype associated with recurrent miscarriage
- M2 parent → expectant mother given heparin daily
- Testing embryo would stop unnecessary treatment

- PCR and nanopore sequencing identifies M2 haplotype
- Results confirmed by capillary sequencing
Combined aneuploidy screen and ANXA5 haplotyping

- Biopsy of 1–3 cells
- gDNA extraction
- Whole genome amplification
- Effective but inconvenient

- WGA sample divided into 2
- One half used for ANXA5 haplotyping assay
- One half used for aneuploidy screen

Biopsy of 1–3 cells → gDNA extraction → Whole genome amplification

- Adapter and tether attachment
- PCR with specific primers
- End-prep of WGA DNA
- End-prep of ANXA5 amplicon
- Adapter and tether attachment
- Sequencing

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Combined aneuploidy screen and ANXA5 haplotyping

- WGA sample divided into 2
- One half used for ANXA5 haplotyping assay
- One half used for aneuploidy screen
- Effective but inconvenient
- Combined assay uses limited number of PCR cycles
- Sufficient WGA DNA is still present at the end of PCR
- All DNA is prepared for sequencing
- Low-coverage, whole-genome data and higher-coverage amplicon data are generated together
- Easily adapted to other genes (e.g. Huntingtin)
Combined aneuploidy screen and ANXA5 haplotyping

- All ANXA5 haplotypes confirmed by capillary
- All ploidy levels confirmed by array-CGH
- Whole genome at low coverage for ploidy

Sample: ONT34
<table>
<thead>
<tr>
<th>Ploidy level</th>
<th>ANXA5 diplotype</th>
<th>GATG (WT), homozygote</th>
</tr>
</thead>
</table>

Sample: ONT39
<table>
<thead>
<tr>
<th>Ploidy level</th>
<th>ANXA5 diplotype</th>
<th>GATG (WT), ACMA (M2) heterozygote</th>
</tr>
</thead>
</table>

- Specific region / regions at higher coverage for SNPs
- Long reads would allow amplicons of several kb
Coverage - how low can we go?

- Ability to call aneuploidies robustly from low coverage: 50,000 reads, 500 nt in length required per sample = ~ 0.01x
- Can either multiplex to get low cost per sample, or use ‘run-until’ to get results very quickly
- Cheaper 128-channel flowcells will be available imminently
Next steps

- No significant increase in coverage required to detect sub-chromosomal changes
- Microdeletions and duplications are 1–5 Mb and can arise by de novo NAHR events during meiosis
- Consequences can be devastating
Acknowledgements

Care Fertility
Simon Fishel

Oxford Nanopore Technologies
Sissel Juul
Eoghan Harrington
Applications team
All ONT staff

Buy the MinION Starter Pack
The starter pack includes everything that is needed to explore the technology and investigate the potential of real-time analysis for DNA or RNA.

Starter packs include:
- MinION
- MinION Flow Cells
- Nanopore Sequencing Kit(s)
- Access to the Nanopore Community

Interested in high throughput?
Find out more about GridION and PromethION, our larger benchtop sequencing systems.

Buy the DNA Starter Pack
Buy the RNA Starter Pack

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