Solving the problem of rare: automated microfluidics for accurate variant detection by PCR-free WGS of very small human samples

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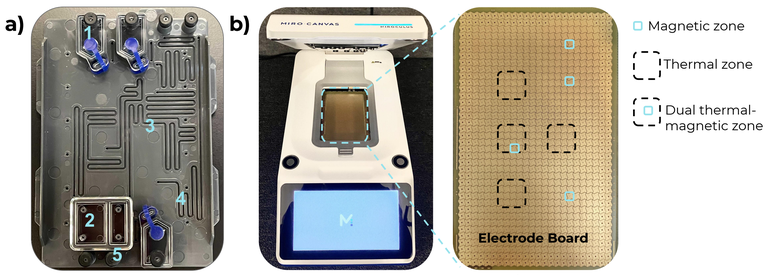
Introduction

- Preimplantation genetic testing (PGT) currently relies on whole genome amplification (WGA) to provide limited information on ploidy. Currently only 30% of genetic etiologies can be identified due to the inability to fully sequence embryos to detect *de novo* mutations and autosomal dominant disorders.
- PCR-free whole genome sequencing (WGS) can provide pristine genomic information to prevent genetic diseases, increase implantation rates and guide clinical care through pregnancy and beyond.
- To enable variant detection in samples with low number of cells, Miro Canvas™ was combined with the Ionic® Purification System to enable the preparation and sequencing of gDNA isolated from as few as 2,500 cells.
- We aliquoted different numbers of GM07339, GM07461, K562 and GM12878 cell lines with known genotypes to test our technology. We report here successful implementation of a microfluidic workflow across different cell input material with concordance to known results.

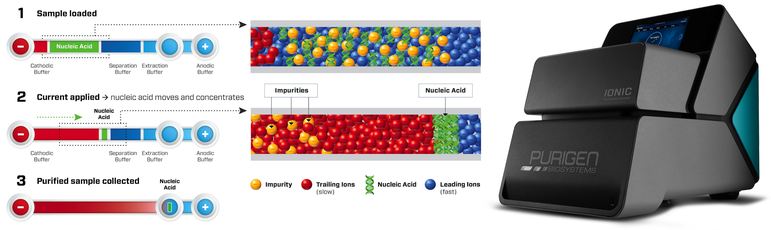
Technology

Miro Technology (Miroculus) consists of: **a)** single-use cartridges, **b)** Miro Canvas, and **c)** software for automated protocol execution (not shown).



Miro Cartridges utilize reservoirs for reagents (1) and waste (2); dispensing/mixing channels (3); reagent inlets (4); and Canvas interface ports (5). Miro Canvas integrates operations to perform many processes: dispense, mix, and merge; multiphase reagent control, isothermal and thermocycle control, and magnetic control.

The Purigen Biosystems Ionic Purification System uses isotachophoresis (ITP) technology, Ionic Fluidic Chips, and Ionic Purification kits to isolate nucleic acids without binding to or stripping from physical surfaces. Samples are gently lysed and added to a chip placed on the system. To enable ITP, an electrical current is applied causing nucleic acids to separate in solution solely based on their inherent electrophoretic mobility.



Experimental workflow

Cell Culture Expansion

Cells were cultured and harvested so that each sample contains a specific number of cells.
- GM07339 & GM07461
  - 10,000 cells
- K562
  - 3,000 cells
- GM12878
  - 1,000 cells
  - 2,500 cells
  - 5,000 cells

DNA Extraction

The Purigen Ionic Purification System was used to extract DNA from these limited samples. Samples containing 2,500 and 5,000 cells yielded approximately 15 and 35 ng of DNA, respectively.

Library Preparation

Library preparation was automated on the Miro Canvas instrument.
- Fragmentation, End Repair & A-Tailing
- Adapter ligation
- Bead clean ups
- Elution

Setup: 20 minutes
Hands-off time: 2 hours

Quantify and Concentrate

The final library elution volume was reduced by 80%, using a SpeedVac Concentrator, to achieve library concentrations compatible with sequencers.

Sequence

Libraries were sequenced on a NovaSeq instrument (Illumina, Inc.)

PCR-free WGS from 10,000 lymphoblast cells reveals known cystic fibrosis genotypes

- We assessed two CTFR pathogenic variants associated to cystic fibrosis.
- F508 deletion mutation [PHE508DEL] in CTFR Chr7:117199644 ATCT>A identified in gDNA purified and prepped from 10,000 GM07339 cells.

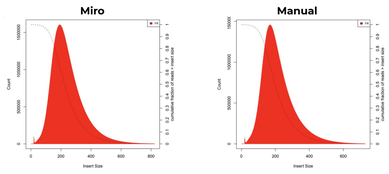
Metrics	GM07339	GM07461
% alignement	99.88	99.89
% Q30 score	92.5	91.4
Mean insert size	192	155
Mean coverage	16X	11X
Median coverage	16X	11X
% at 10X coverage	91.66	66.83

- R553X mutation [ARG553TER] in CFTR Chr7:117227865 C>T identified in gDNA purified and prepped from 10,000 GM07461 cells.

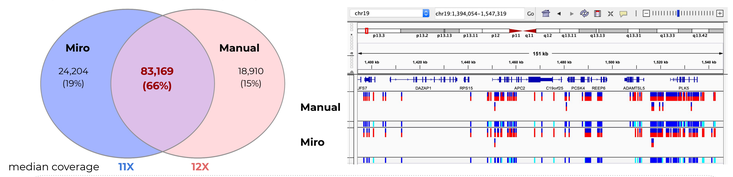
Chr19 SNVs/INDELs from as few as 3,000 K562 cells

- Comparable sequencing metrics for manually and Miro-prepped PCR-free WGS libraries from as few as 3,000 cells.

Metrics	Miro	Manual
% alignment	99.92	99.95
% Q30 score	95.24	95.25
Mean insert size	240	257
Mean coverage	12X	12X
Median coverage	11X	12X
% at 10X coverage	66.3	69.9



- Good overlap in SNVs reported in Miro technology-prepped libraries compared to manual preparations.



- INDELs in Chr-19 are detectable in the same chromosomal regions independent of the library preparation method.

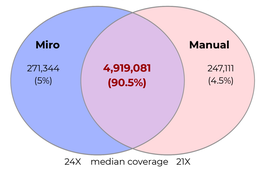
Redefining the lower limits for PCR-free WGS down to 2,500 GM12878 cells with Miro Canvas

- Adequate yields allowing downstream sequencing were generated from PCR-free library preparation of as few as 2,500 cells.
- Miro Canvas runs present superior sequencing metrics than manual when PCR-free assay is challenged with very low input amounts.

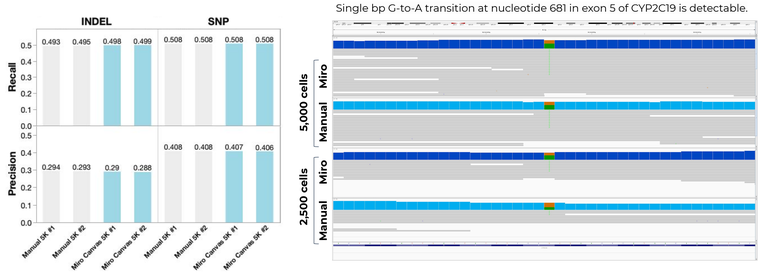
Sample	gDNA Input to Library Prep (ng)	Library Yield (nM)
Manual - 1k rep1	4.28	0.05
Manual - 1k rep2	4.67	0.06
Miro Canvas - 1k rep 1	4.27	0.12
Miro Canvas - 1k rep 2	4.84	0.13
Manual - 2.5k rep 1	14.81	0.09
Manual - 2.5k rep 2	17.29	0.15
Miro Canvas - 2.5k rep 1	12.09	0.46
Miro Canvas - 2.5k rep 2	13.22	0.12
Manual - 5k rep 1	37.76	0.88
Manual - 5k rep 2	34.12	0.7
Miro Canvas - 5k rep 1	36.80	0.87
Miro Canvas - 5k rep 2	36.31	0.7

Sample	Mean insert size	Mean coverage	Median coverage	% 10X	% 15X
Manual - 2.5k	364	6.2	6	0.129	0.009
Manual - 2.5k	392	8.11	8	0.321	0.042
Miro Canvas - 2.5k	350	11.78	12	0.703	0.251
Miro Canvas - 2.5k	392	11.56	11	0.681	0.235
Manual - 5k	370	19.84	20	0.949	0.837
Manual - 5k	373	21.48	22	0.954	0.883
Miro Canvas - 5k	329	23.79	24	0.958	0.917
Miro Canvas - 5k	331	24.78	25	0.959	0.931

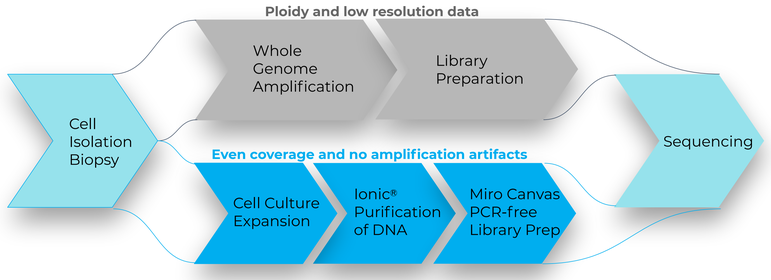
Total variants called with Manual and Miro preparation methods using 5,000 cells input



- Comparison against the high confidence calls generated by GIAB consortium shows concordance between Miro and manual.



Future outlook for PGT



Conclusions

Combining the Miro Canvas with the Ionic Purification System allows for hands-off, PCR-free library preparation for WGS of low cell number samples. The percentage of variants obtained through this method is comparable or better to manual processes. We have successfully detected known pathogenic variants across different cell lines using as few as 2,500 cells.

This innovative workflow combines novel DNA purification and library preparation technologies into a simple solution for PGT applications. Continued efforts to develop novel methods of embryo biopsy culture, and to further drive down the lower limits of detection will enable PCR-free WGS to be an optimal tool for informed, data-guided decisions in PGT.

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