21ST

PGDIS CONFERENCE

PREIMPLANTATION GENETIC DIAGNOSIS INTERNATIONAL SOCIETY

PGDIS

6-8 May 2024

Kuala Lumpur

Malaysia

PGT and BEYOND...

21ST PGDIS CONFERENCE

Rapid, efficient, decentralized PGT-A with Oxford Nanopore Sequencing

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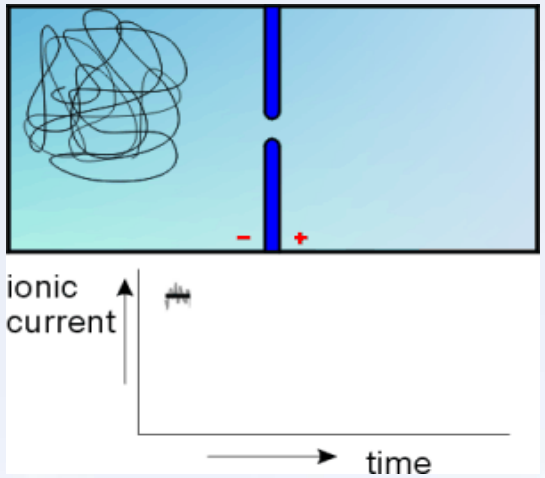
8th, May, 2024

Disclosures

Full-time employee, R&D director of Yikon Genomics

Nanopore Sequencing: principle and technical advantages



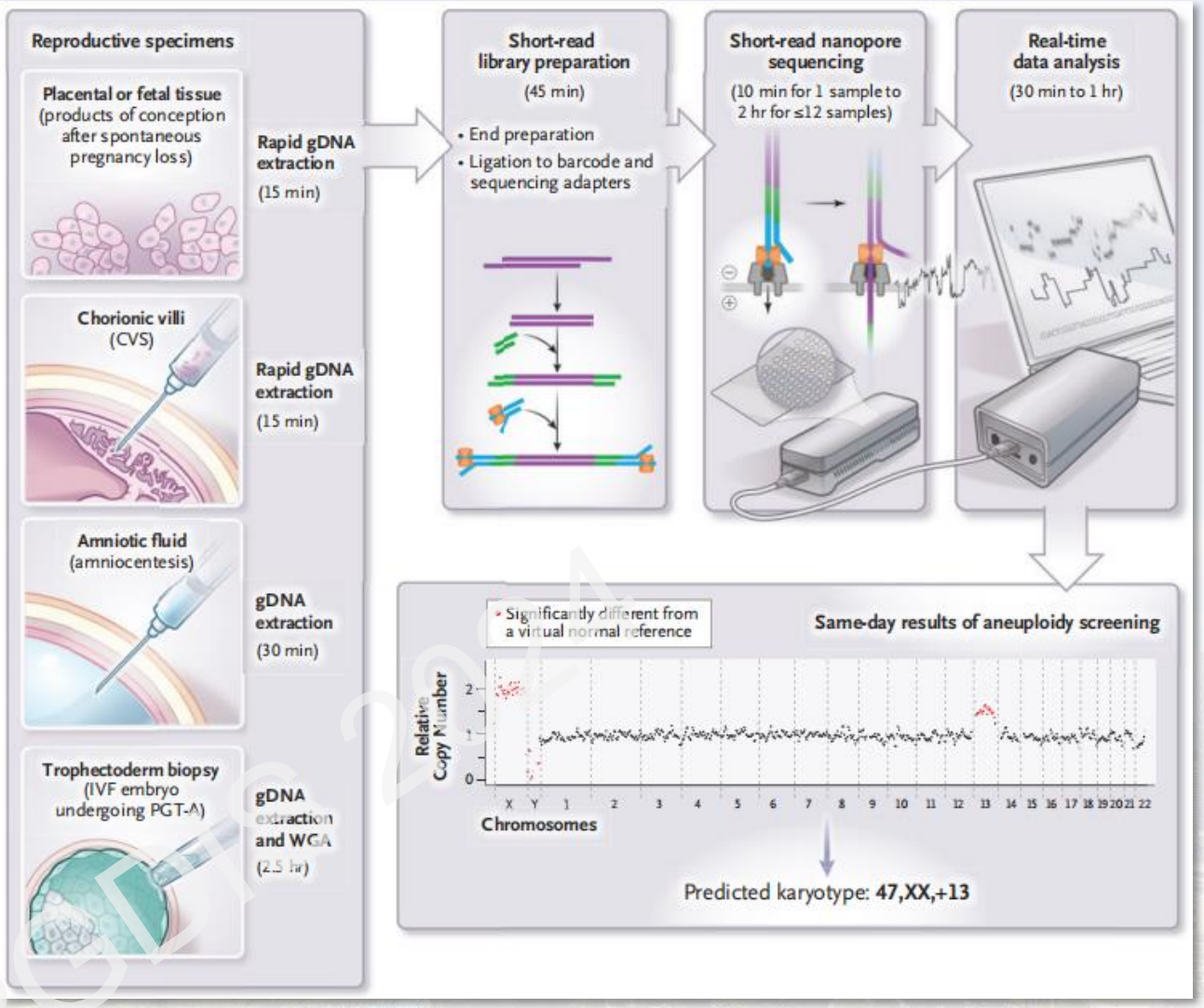
Principle

- Coulter counter
- Base recognition based on current resistive pulse signal

Technical characteristics

- **Long-read sequencing**, realizing the detection of various mutations and direct construction of haplotypes, without the need for probands
- **Real-time basecalling**. The unique **adaptive sequencing** enables flexible customization of panels without the need for primers. Significantly reduce sequencing costs through sample mixing
- **Single-molecule sequencing**. No PCR required
- **Portable, and cheap equipment**

Technical workflow



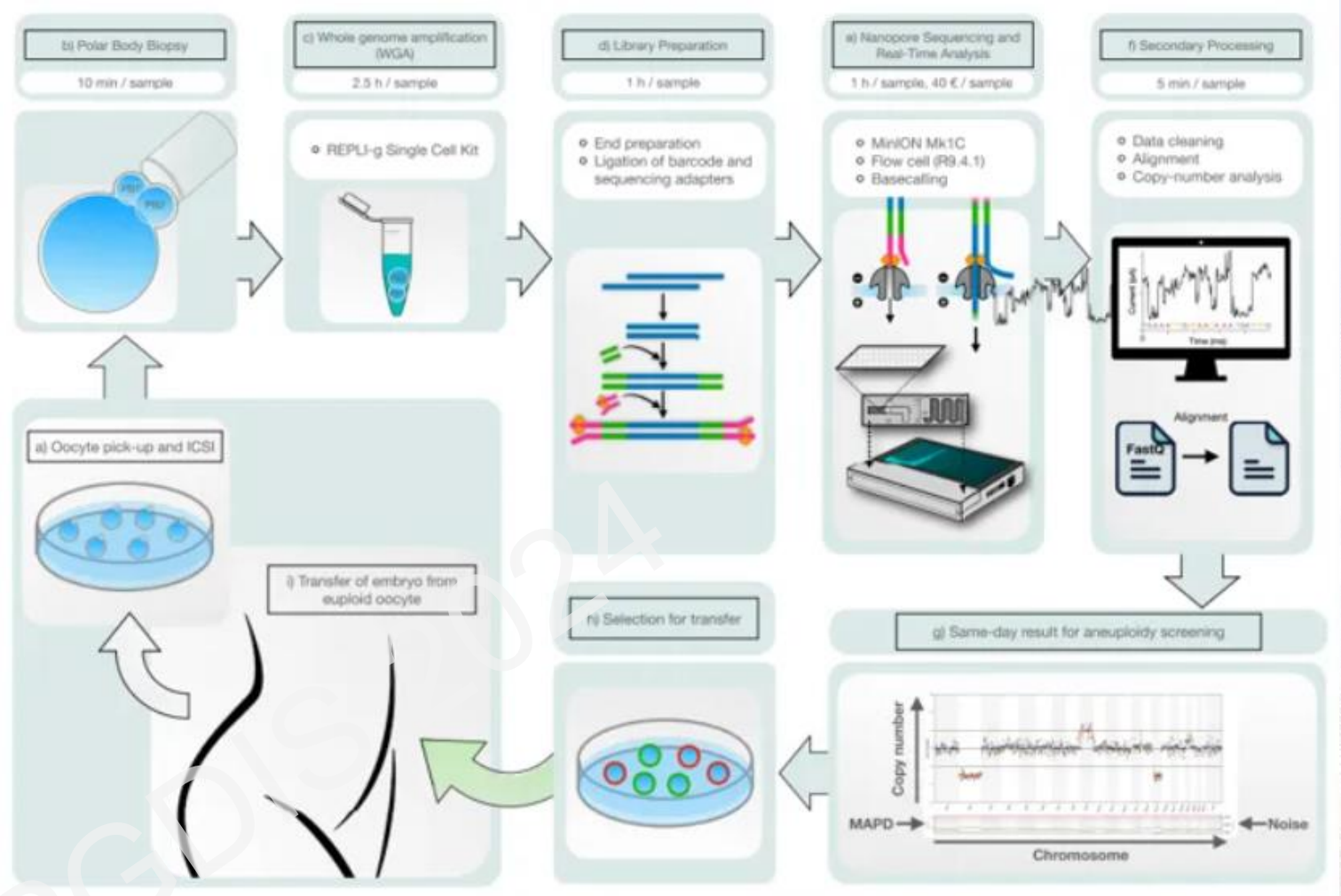
Advantages

- **Ultrafast** (Fresh embryo transfer on the same day)
- **Easy operation**
- **Small and cheap equipment**
- **Flexible and expandable** (Resolution selectable, suitable for MaReCs, PGT-M, and niPGT-A)

45+ Publications

Wei S, Djandji A, et al. N Engl J Med 2022; 387:658-660

Comparative experimental study



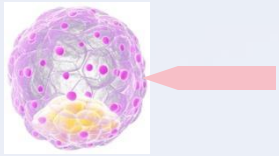
Polar Bodies PGT-A with ONT platform

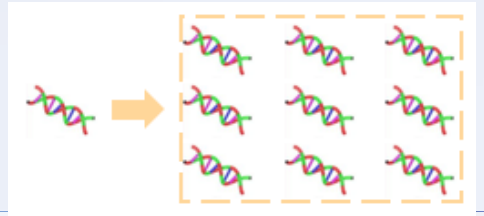
Informative rate (QC pass)	97.1% (99/102)
Concordance (vs aCGH)	97.0% (96/99)
Sensitivity	0.957
Specificity	1
PPV	1
NPV	0.906

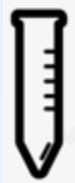
PPV: positive predictive value
NPV: negative predictive value

Oberle A, Hanzer F, et al. Clin Chem. 2024;70(5):747-758.

Ultra-fast PGT-A with ONT: 12 samples in 5 hours







Embryo biopsy

Biopsy TE cells from blastocyst

WGA

Whole-genome amplification based on **ChromInst**

3hr

Library construction

Prepare library with the **Ligation Sequencing Kit V14**

0.5hr

Nanopore Sequencing

Perform sequencing with **P2 solo and R10 flow cell**

1hr

Data analysis

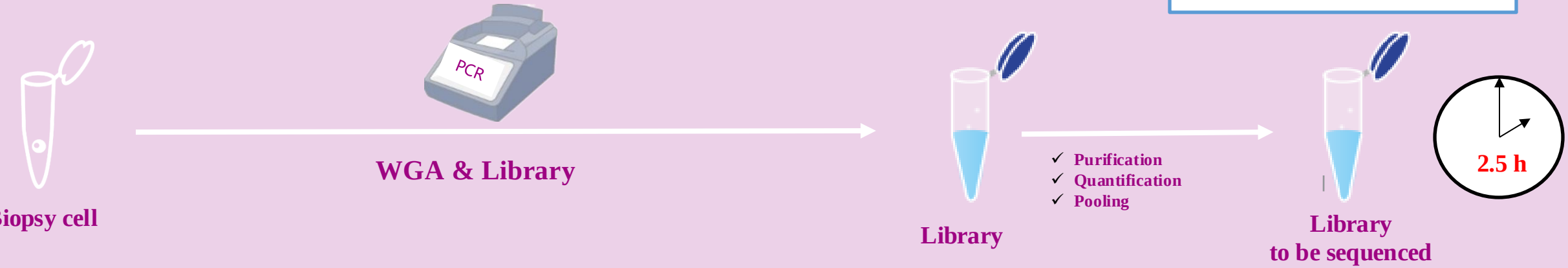
Analyze data and output report using **ChromGo**

0.5hr

5hr in total

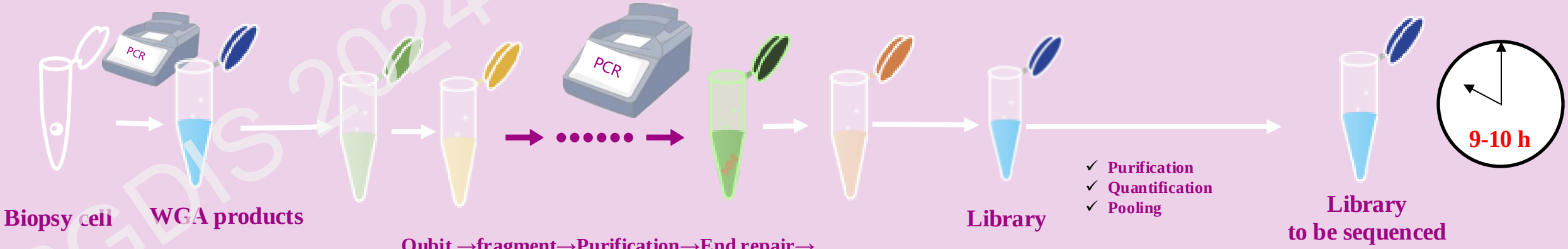
ChromInst® PGT-A solution

Open cap：4 time
Change tube：1 time



Traditional Solution

Open cap：13 time
Change tube：9 time



P2 Solo: Flexible, high-yield nanopore sequencing for every lab

Choose PromethION2 Solo (P2 Solo) devices for flexible, high-yield sequencing, in a compact, accessible design. Utilising flow cells that generate hundreds of gigabases, enable PromethION-scale benefits in labs. Suitable for users with various sample processing requirements, up to **~250 flow cells per year.**

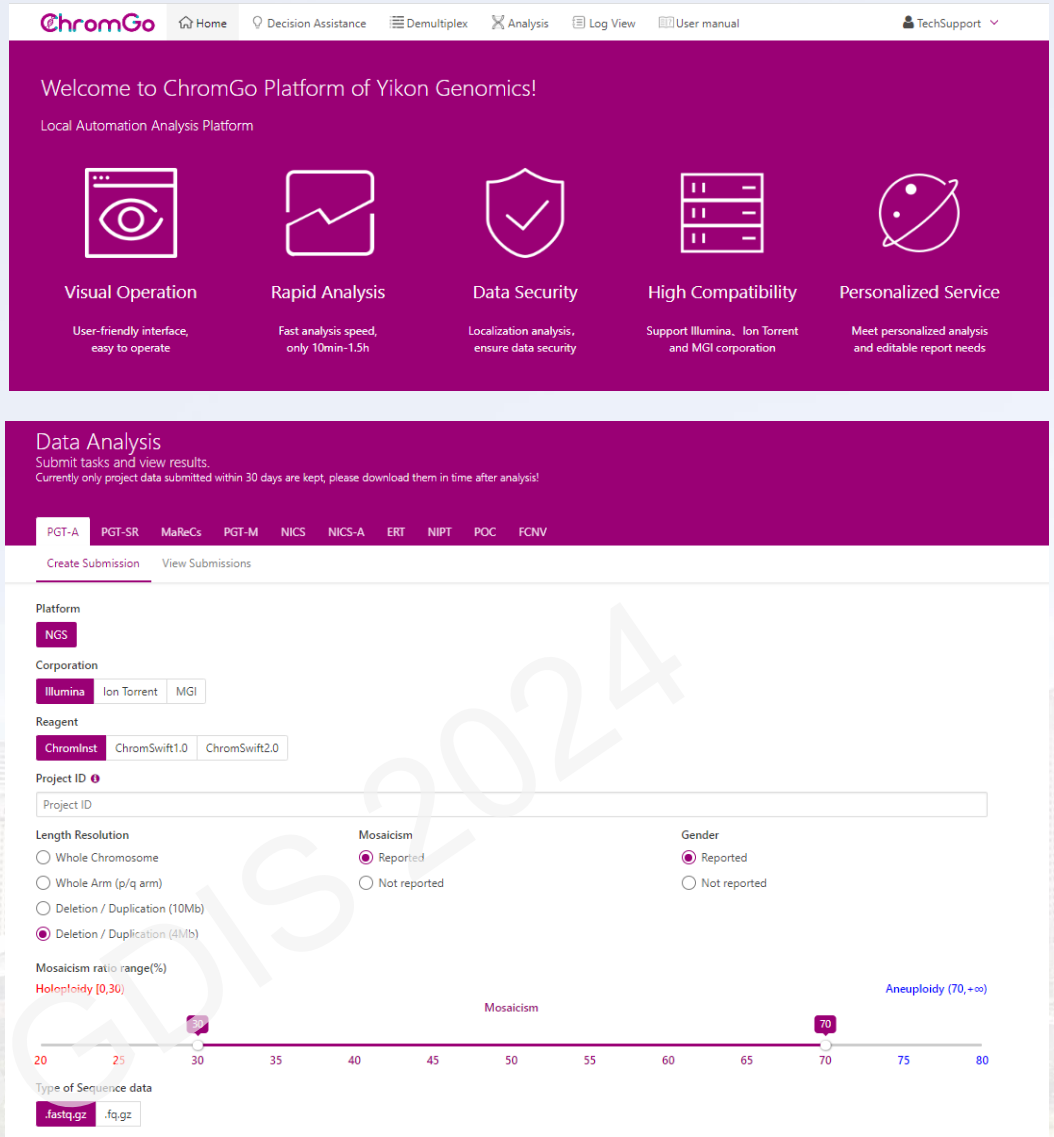
PromethION 2 Solo (P2 Solo) Specifications

- **Two high-yield flow cells**, delivering **up to 400 Gb of data**
- Flexible, on-demand sequencing. **Run flow cells individually or together**

Flow cell Specifications

- **100-200 Gb native gDNA reads**
(read N50 ~20 kb)
- **>100 million cDNA reads** for isoform-level analysis
- Multiplexed sequencing of **up to 96 samples** with PCR or PCR-free barcoding
- Reusable for up to 7 times.

Date analysis-ChromGo™



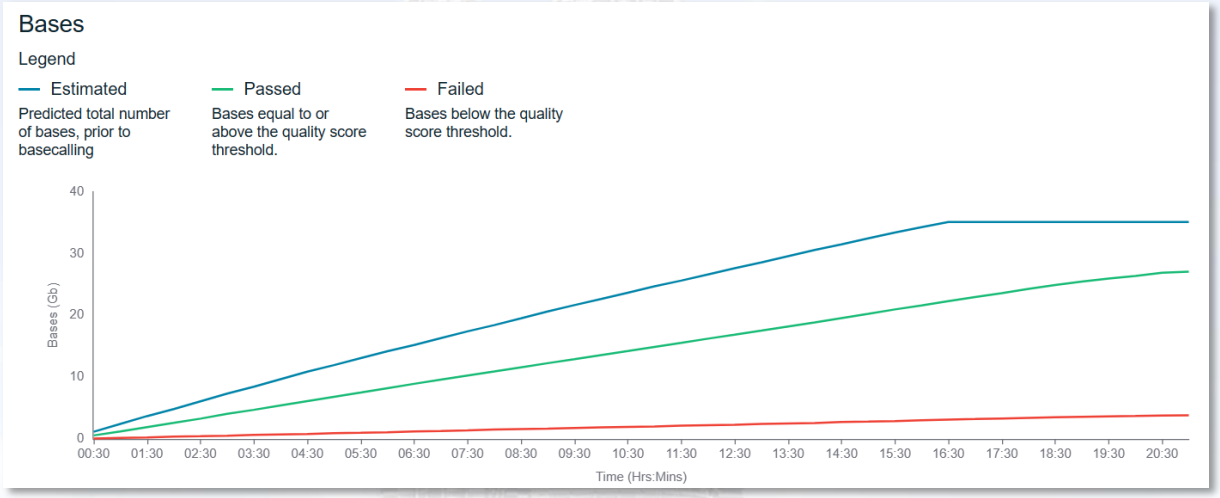
User friendly genome analytical platform

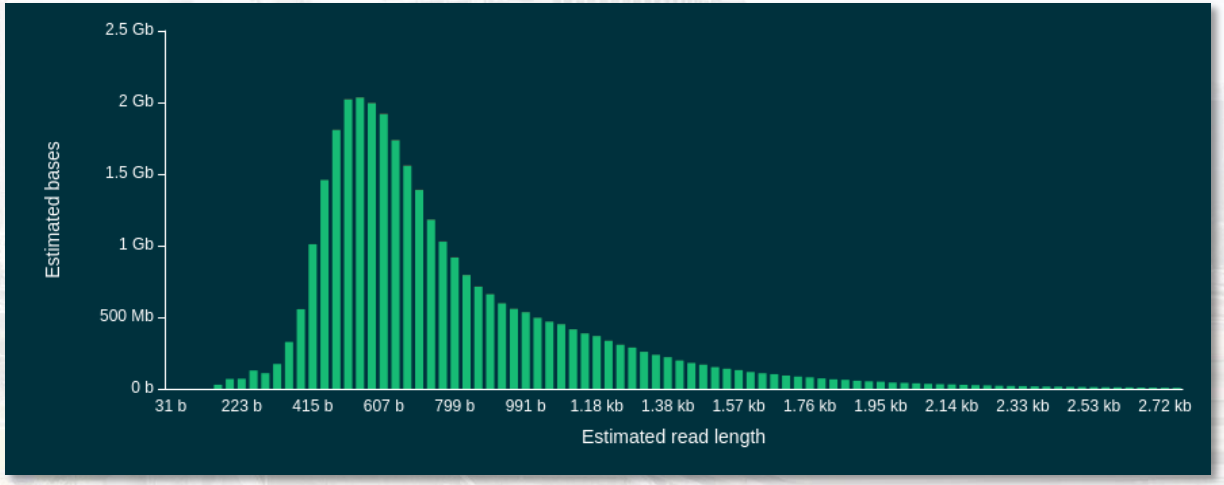
- **Fully Integrated：Analytic platform for PGT-A/-M/-SR/ERT/NICS/POC/FCNV, etc**
- **Sufficient data verification has been completed to ensure the accuracy of the results.**
- **More than 100,000+ samples per year.**

Experimental results

- **Aneuploidy screening is performed using P2 solo with short fragment mode (SFM).**
- **WGA products of 12 embryo samples are mixed and sequenced on the same flow cell.**

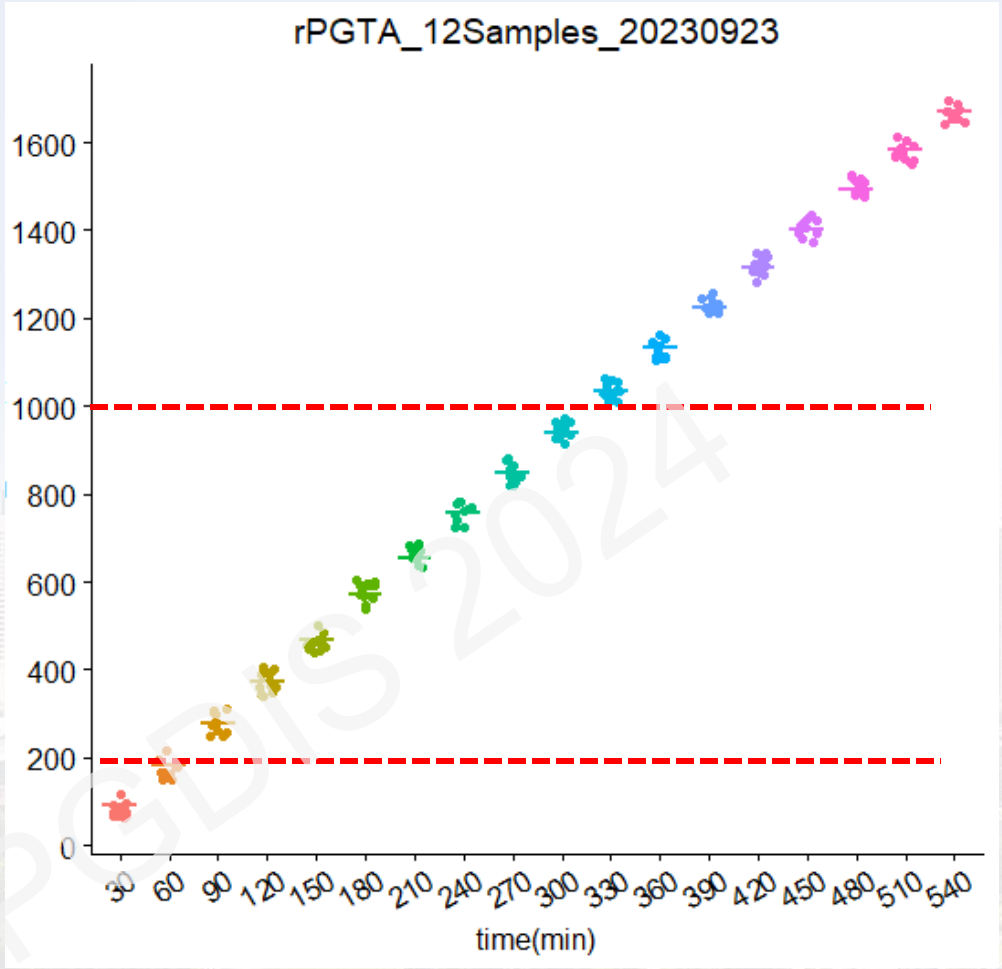
Barcode	CNV information(NGS)	Gender
01	+15	XX
02	dup(3)(p26.3p11.1)(~88.40Mb,~54%)	XY
03	+1(~54%),dup(4)(q31.22q35.2)(~44.00Mb,~51%),-12(~44%),-13(~38%),-18(~45%)	XY
04	46, XN	XY
05	46, XN	XX
06	46, XN	XY
07	+9	XY
08	46, XN	XX
09	+21	XY
10	-21	XY
11	+13,dup(16)(p13.3p11.2)(~32.00Mb),dup(16)(p11.2q24.3)(~58.55Mb,~50%)	XY
12	+18	XY

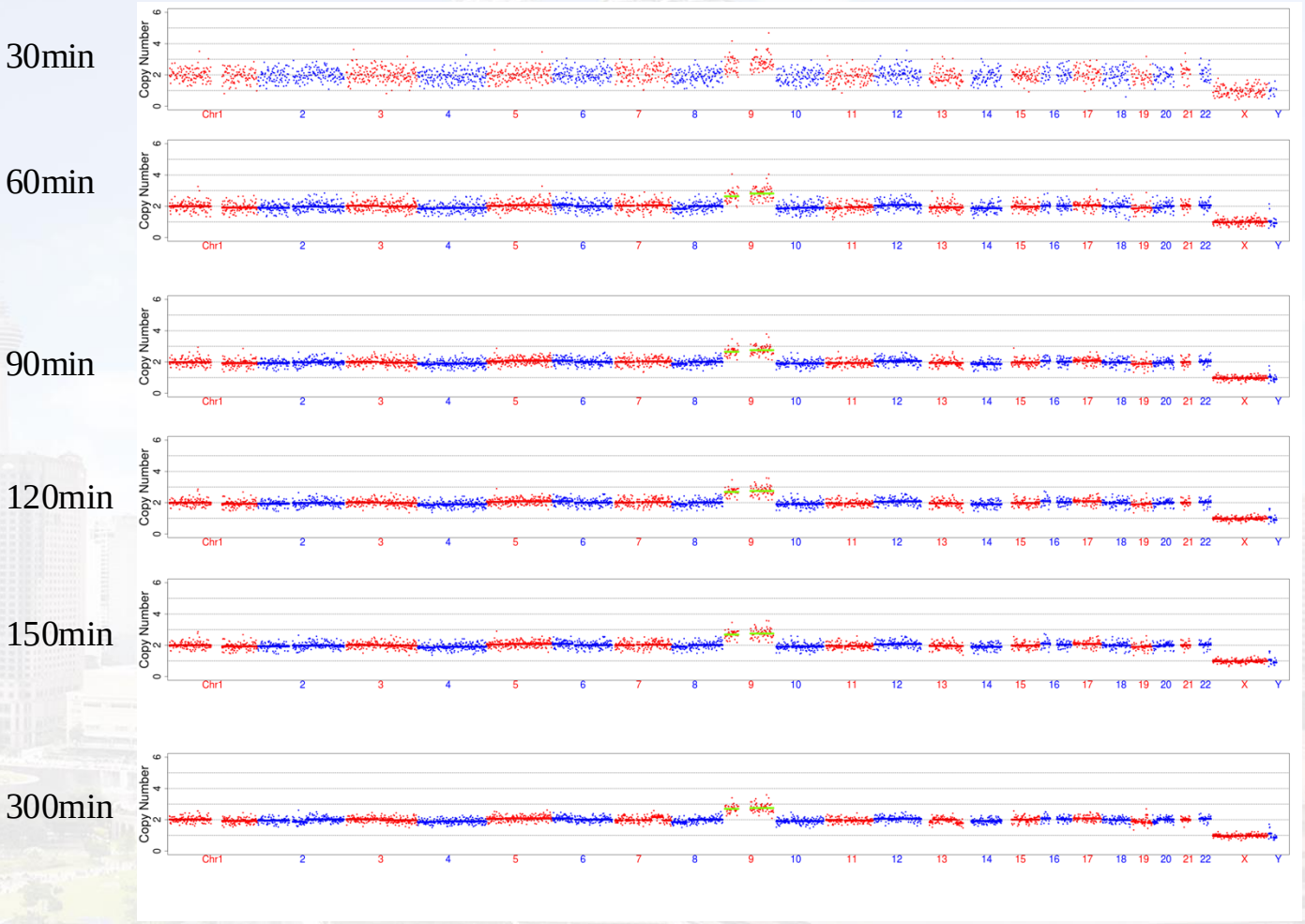




Experimental results

- **The resolution continues to improve as the sequencing time increase.**
- **~200k valid reads (300K raw reads)/1 h meets the demand for 10Mb resolution.**





Validation results

Clinical Samples

Platform	Karyotype	Number	Examples
P2 solo	Euploid	32	46,XN
	Aneuploid	10	+15
	Micro deletion/micro duplication	27	dup(3)(p12.2p12.1)(~5.00Mb)
	Mosaic	17	-2(~34%)
Total		**86**	

Enterprise Reference Samples

Enterprise Reference	Karyotype	Number	Examples
N series	Negative control (Euploid)	5	46,XN
D series	Micro deletion/micro duplication	29	del(2)(q24.2q31.1)(~8.60Mb)
M series	Mosaic	4	del(5)(p15.33p13.3)(~29.80Mb,~65%),dup(5)(p13.3p13.2)(~4.60Mb)
P series	Positive control (Aneuploid)	6	+2
Total		**44**	

Consistency criteria	Data volume	Number of consistent results	Consistency rate	Sensitivity	Specificity
There is a difference in chimerism ratio or a difference in CNV length detected, but the trend of copy number is consistent	0.5M	126	96%	96.3%	98.0%
	0.4M	128	98%	97.5%	100%
	0.3M	**128**	**98%**	**97.5%**	**100%**
	0.2M	126	96%	95.2%	100%
	0.1M	125	95%	94.0%	100%

(n=130)

Features and benefits

- **CNV resolution**

  300k raw reads for 10Mb

  1M raw reads for 4Mb
- **Mosaic detection**

  30%~70%

  Adjustable according to user needs
- **Detection success rate**

  >98%
- **Turnaround time (TAT)**

  5 hours for 12 samples

  48 hours for 96 samples
- **Equipment investment**

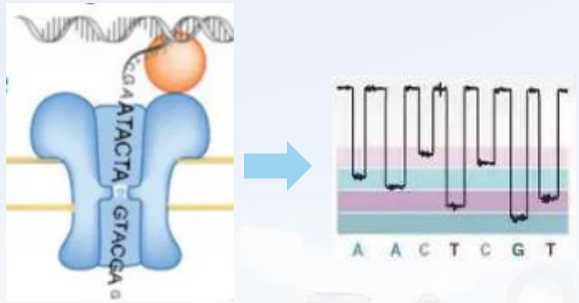
  <1/2 of NGS-based PGT lab

ONT-based PGT: developing plan

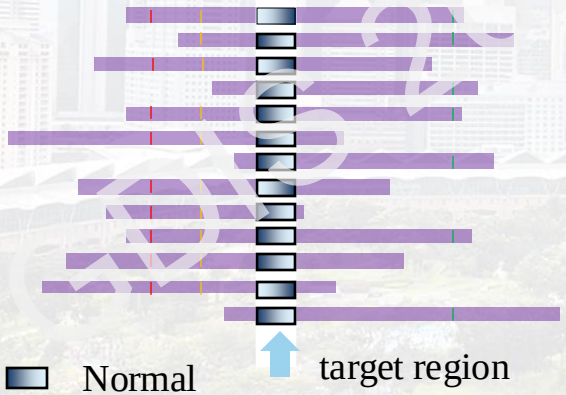
Variants detection

peripheral blood

nanopore sequencing

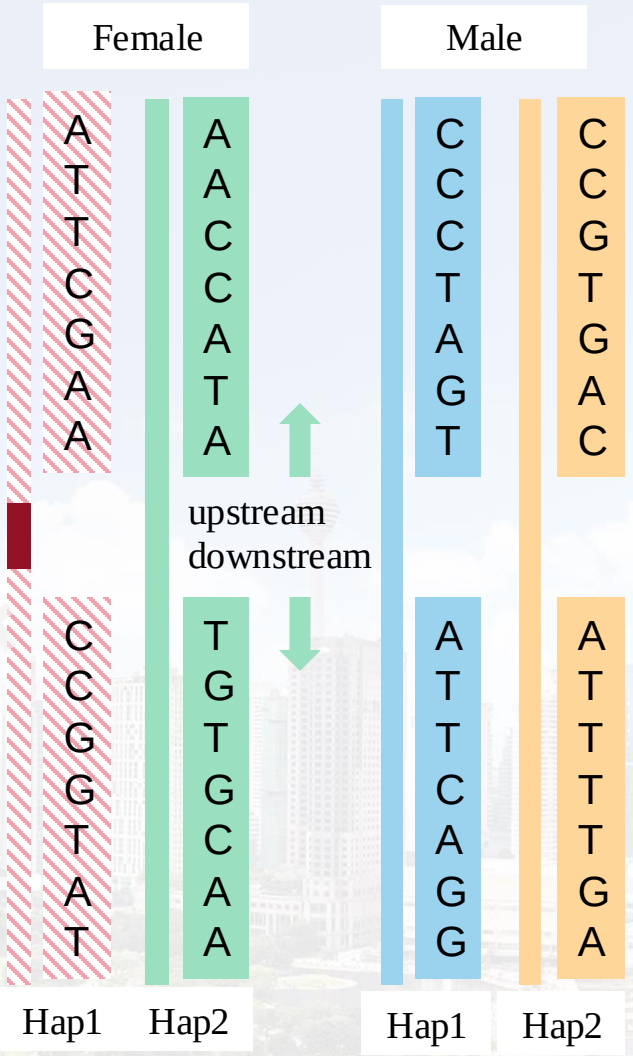


Variants detection



Normal
Inversion for example
target region

Phasing

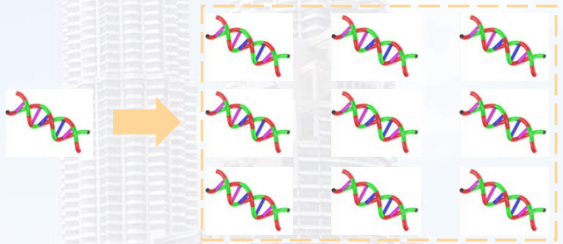


Pathogenic variant
Variant linked haplotype

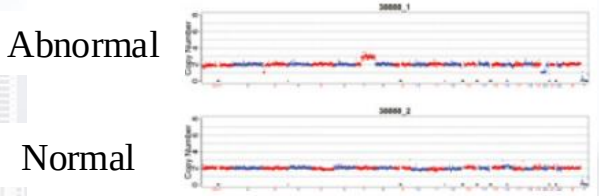
Embryo detection

Blastocyst biopsy

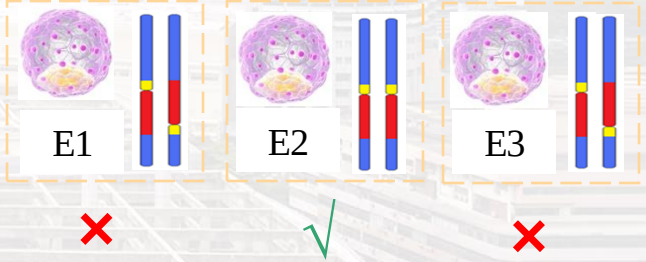
WGA



Chromsome screening



Haplotype linkage analysis

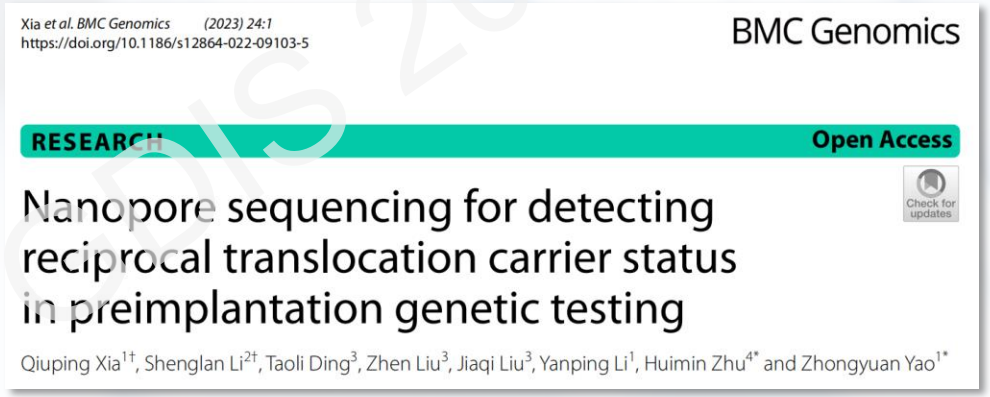


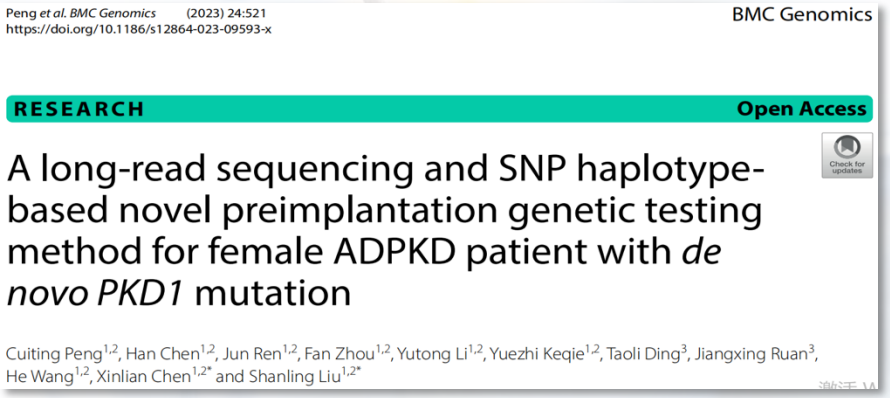
PGT

- PGT-A*
- PGT-SR
- MaReCs
- PGT-M

Yikon Genomics: a TGS service provider

- Starting from January 2022, Yikon Genomics provides ONT based PGT products to the public. Currently, direct haplotyping with ONT for PGT-M has been used in **200+ clinical families**;
- Yikon has established cooperation relationship with **15+ hospitals**, with a total of **25 research projects**. Completed TGS of **67 scientific research samples**;
- **3 articles** have been accepted and **6 articles** are being submitted. Generate **2 cooperative patents**;
- An ONT database containing **over 200 Chinese population** has been set up;
- **Yikon Genomics** is an **official OEM partner** of **Oxford nanopore technology (ONT)**;
- Yikon Genomics is the largest TGS service provider in China and even the world in reproductive health.





Oxford NANOPORE Technologies™

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THANK YOU