21ST

PGDIS CONFERENCE

PREIMPLANTATION GENETIC DIAGNOSIS INTERNATIONAL SOCIETY

PGDIS

6-8 May 2024
Kuala Lumpur
Malaysia

PGT and BEYOND...

21ST PGDIS CONFERENCE

Sample swap and contamination detection in PGT-A

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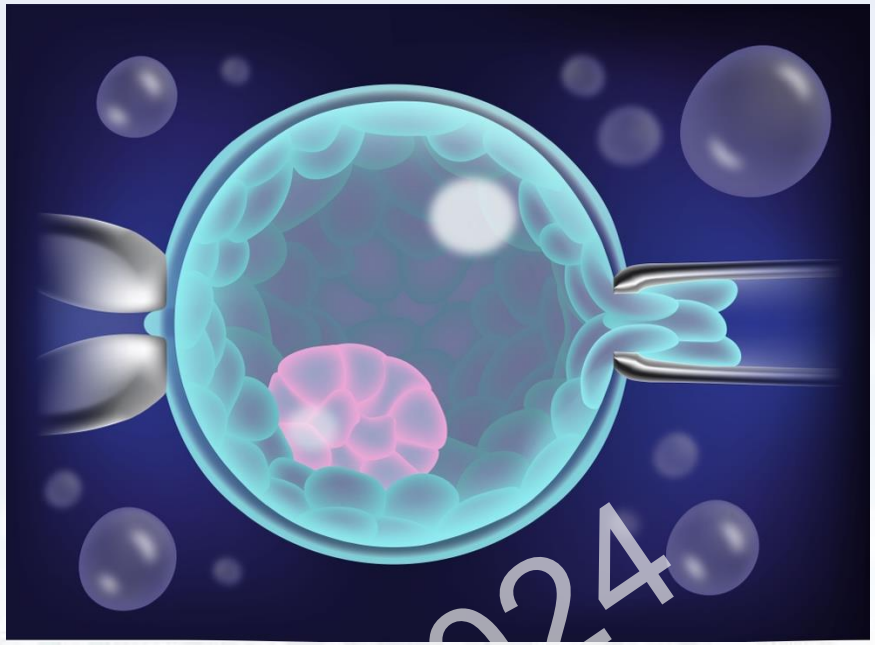
The problem of contamination in PGT-A



PGT-A is a widely used technique during in vitro fertilization procedures, serving as a decision-making tool. Detecting contamination during PGT-A is crucial to prevent misclassification of the embryos.

Implications*

- Discard potentially viable embryos
- Transfer of aneuploid embryos wrongly classified as mosaic, potentially leading to increased rates of implantation failure and miscarriage.

*Clark, G., et al. (2023). *Human Reproduction*, 38(Supplement_1), dead093.337

Lab practices to mitigate contamination risk

1. Negative Controls: Implement always negative controls during sample processing. Limited by the fact that cannot evaluate contamination in the actual tube containing the biopsy.

2. Laboratory Practices: Adhere to strict laboratory practices to minimize the risk of contamination. This includes maintaining a clean environment, proper gowning procedures, and sterilization of equipment.

3. Barrier Technology: Utilize barrier technology to prevent cross-contamination. This can involve physical barriers such as laminar flow hoods or biological safety cabinets.

4. Personnel Training: Train laboratory personnel thoroughly on contamination control measures. Regular training and awareness programs can help maintain a high level of vigilance.

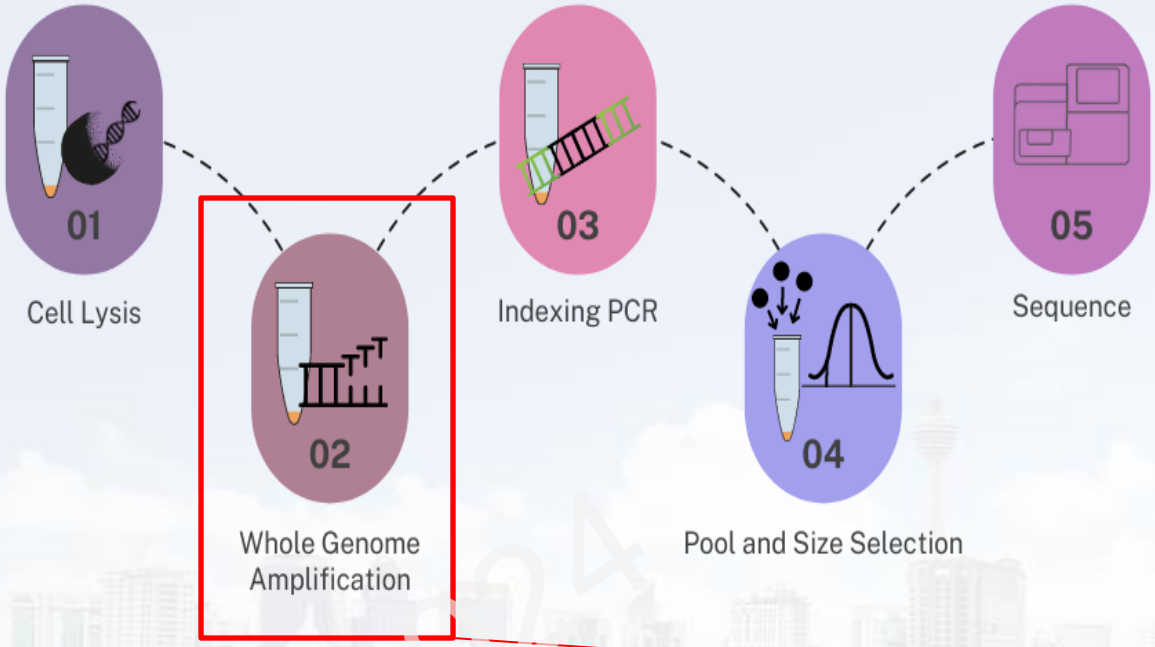
Extent of the problem*

On average 0.4% variable from clinic to clinic

*Clark, G., et al. (2023). *Human Reproduction*, 38(Supplement_1), dead093.337

Contamination detection with WGA-based method

PG-Seq™ Rapid v2

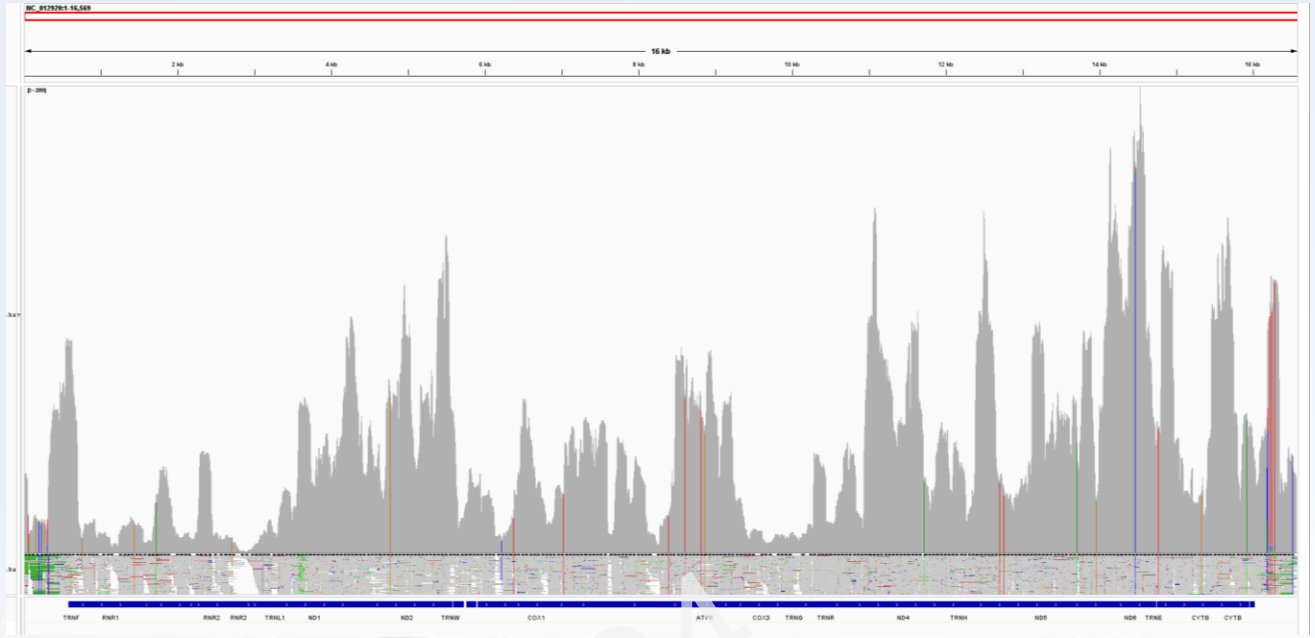


- Ready-mixed reagents
- Minimal tube opening and pipetting steps
- No normalization required

Degenerate Oligo PCR-based method

- Amplifies nuclear gDNA
- **Amplifies mtDNA**

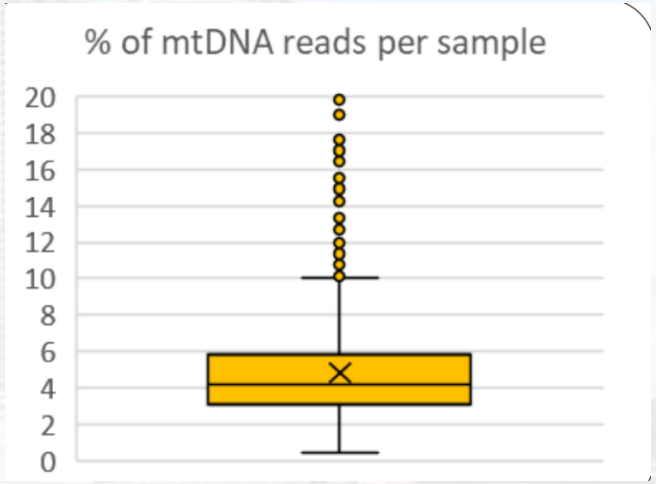
mtDNA amplification



Real D5 embryo sample processed with PG-Seq™ Rapid v2

Alignment visualized with IGV to illustrate coverage

- FastQ generated in the standard PG-Seq Rapid™ v2 workflow can be used to obtain information about mtDNA sequence of the sample
- Percentage of reads aligning to mitochondrial genome range from 0.4% to 20.2%, with average of 4.8% (n = 1140)

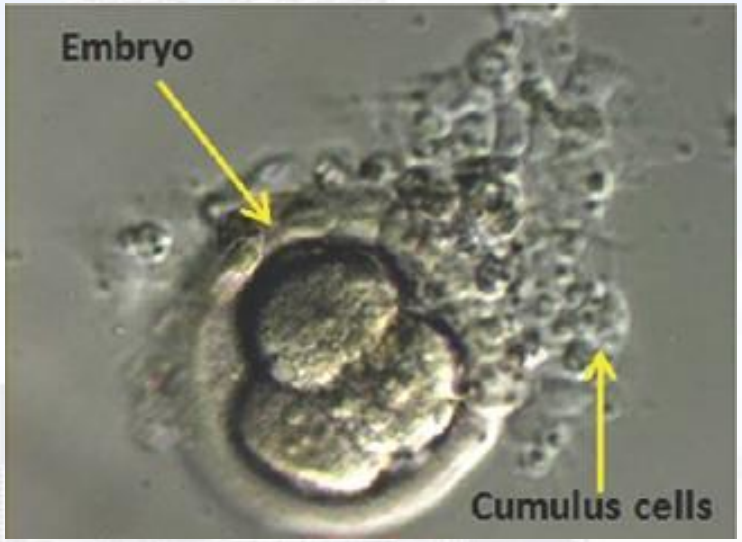


Pros

1. **Unique Inheritance Pattern**:
   - Unlike nuclear DNA, mtDNA is **maternally inherited**.
   - Each embryo inherits its mtDNA exclusively from the mother.
   - By analyzing mtDNA variants, we can trace the maternal lineage and uniquely identify embryos.
2. **Contamination Detection**:
   - External contamination (e.g., from lab personnel or sample handling) can introduce foreign mtDNA.
   - By comparing the embryo's mtDNA profile with expected maternal mtDNA, we can identify any discrepancies.
   - Contaminated samples can be flagged, ensuring accurate results.
3. **Confirmation of Sibling Relationships**:
   - When multiple embryos are tested, mtDNA confirms whether they share the same maternal lineage.
   - It ensures that tested embryos are indeed full genetic siblings.

Cons

1. **Maternal DNA contamination**
   - Maternal and embryonic mtDNA are identical,
   - If contamination from cumulus cells is present in the biopsy, it wont' be detected by this method

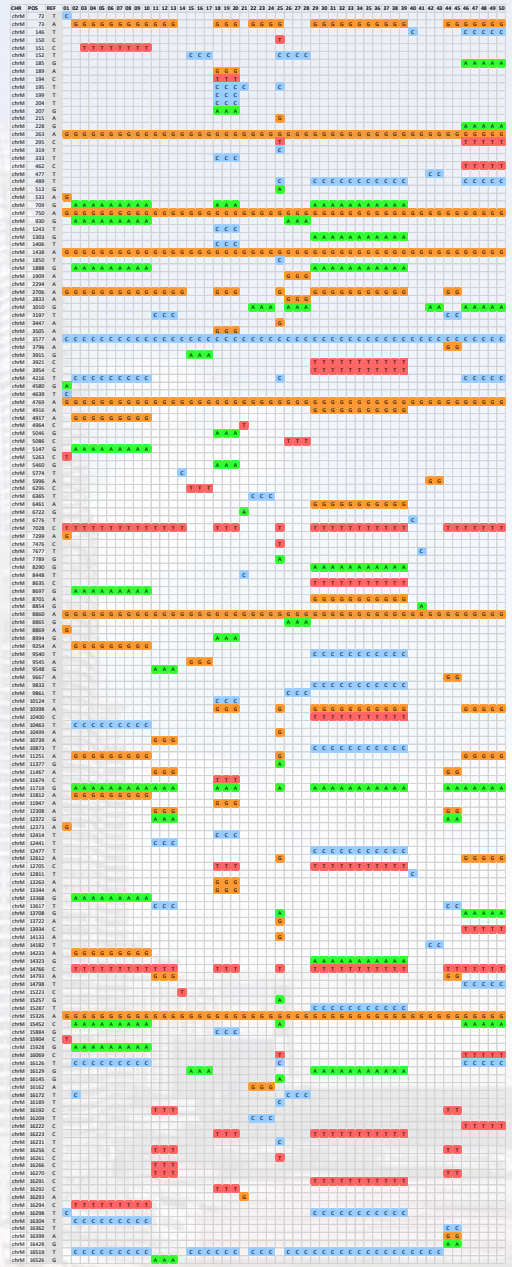


http://www.ijmr.org.in/viewimage.asp?img=IndianJMedRes_2017_146_3_341_223630_f2.jpg

Proof of principle

- Custom bioinformatic analysis was developed that extracts mtDNA SNV then compares similarity between samples.
- Known pathogenic variants and low depth SNV (depth <2) excluded.
- 1140 samples were processed and sequenced, with 5.4M total read on average.
- After downsampling to 500,000 reads, fastq were re-run through the pipeline

	5.4M avg read	0.5M read
Correctly grouped	1137 (99.73%)	1128 (98.94%)
Incorrectly grouped together	1	5
Incorrectly grouped separately	0	2
Not enough mtDNA reads for analysis	2	5



mtDetect™ Web App

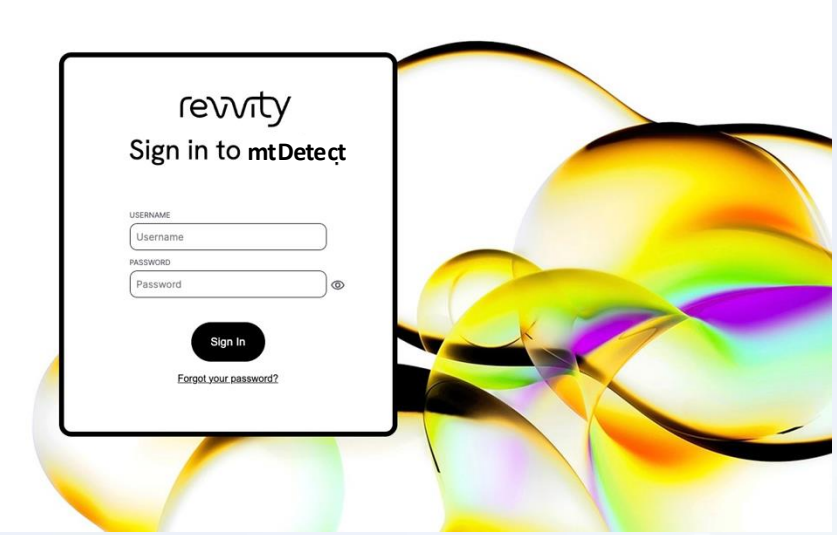
- Cloud-based software is free and to be used as an additional tool along with the PG-find software*.
- Output is an excel containing sample groupings
- Can be used to detect exogenous contamination and sample swaps**.

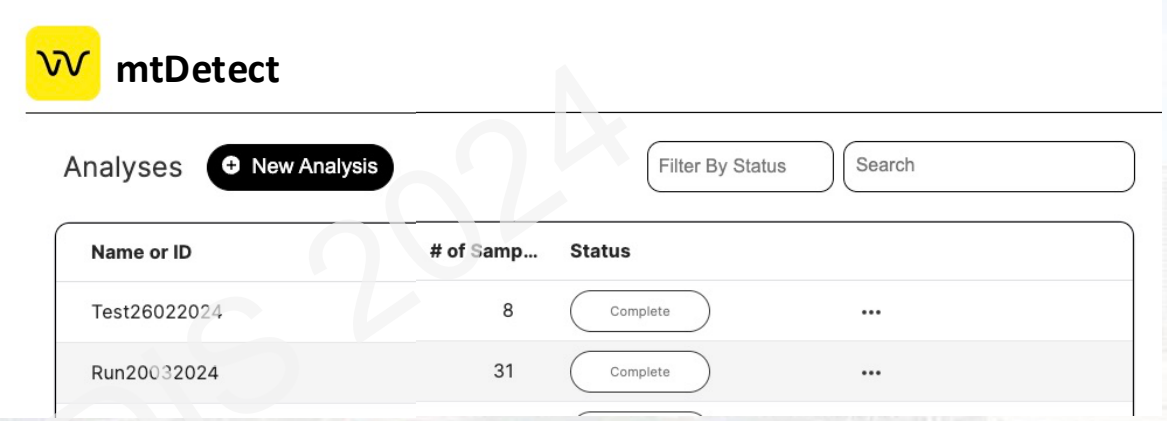
Group	Samples										
G1	01										
G2	02										
G3	03	04	05	06	07	08	09	10			
G4	11	12	13								
G5	14										
G6	15	16	17								
G7	18	19	20								
G8	21										
G9	22	23	24								
G10	25										
G11	26	27	28								
G12	29	30	31	32	33	34	35	36	37	38	39
G13	40										
G14	41										
G15	42	43									
G16	44	45									
G17	46	47	48	49	50						

*Server currently located in USA

**Percentage of sample swapping taking place in current PGT-A labs is unknown to the best of our knowledge, but consequence is same as contamination: misclassification

mtDetect™ Web App





Register on the platform

↓

Upload your data

↓

Run analysis

↓

Get results

THANK YOU