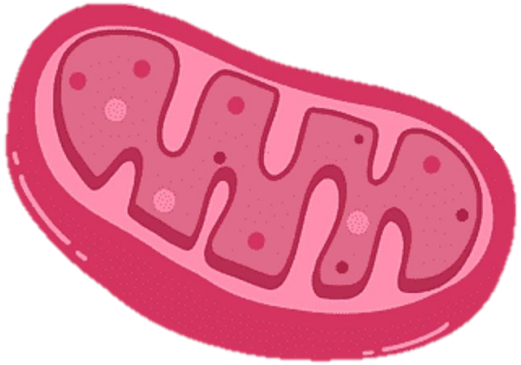
Mitochondrial Genome Sequencing for variants identification in cancer



Group 2 - Pink

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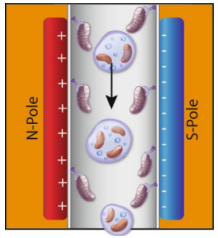
Genomics Technologies Course - 15th December 2023

OUR PROPOSAL

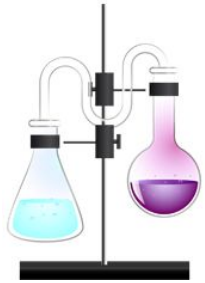
- Efficient **organelle isolation**
- **mtDNA extraction** and recovery
- **Quality Control** (size, amount and purity)
- **PacBio Sequel IIe NNGS** (mtDNA denaturation + custom-made primer set)

No library? – No problem!



- **Fractionated Mitochondria Magnetic Separation (FMMS)**

- **Lysis Buffer**
- **Phenol-Chloroform Extraction**
- **RNases and Proteases Treatment**



- **Quality Control (low-voltage agarose gel electrophoresis + Qubit + Nanodrop)**

- **Sequencing on PacBio Sequel IIe platform - Circular Consensus Mode (CCS)**

DNA extraction

mtDNA amplification

Quality control

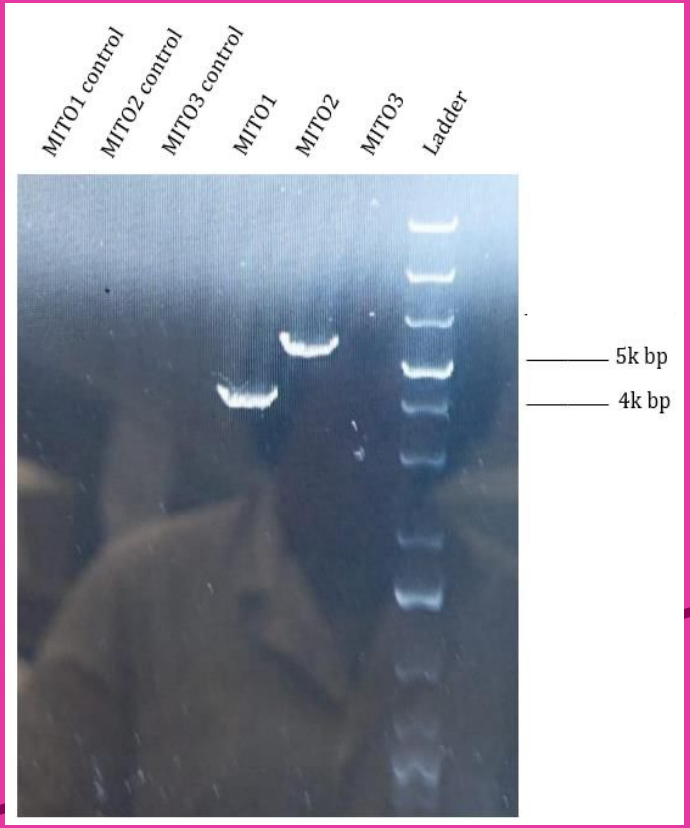
DNA library preparation

Library purification and quality control

MiSeq Illumina sequencing

WetLab Workflow

1. **DNA extraction** (both nuclear and mitochondrial)
2. **mtDNA amplification**
    a. MITO amplicons
        i. gel electrophoresis
    b. ROI amplicons
        i. purification via Agencourt AMPure XP
        ii. capillary electrophoresis via LabChip GX
3. **Library preparation**
    a. via Nextera XT kit (MITO)
    b. addition of adapters via PCR (ROI)
4. **Library purification and quality control**
    a. purification via Agencourt AMPure XP
    b. Qubit
    c. capillary electrophoresis via LabChip GX
5. **NGS via Illumina MiSeq**



Gel electrophoresis of MITO amplicons. The MITO3 band is not visible.

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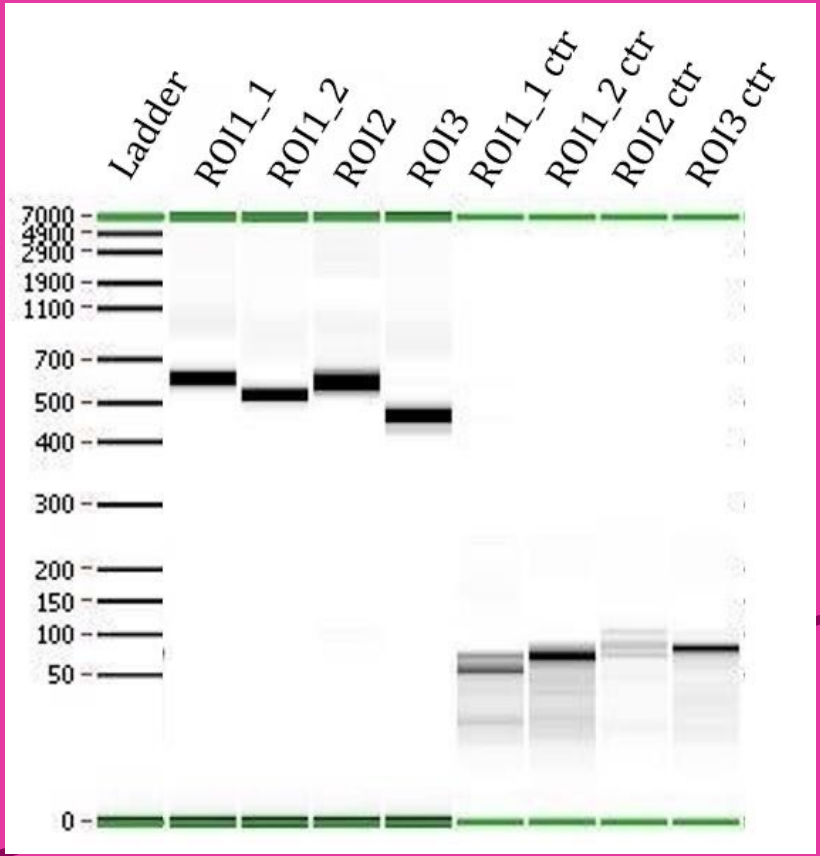

Results of capillary electrophoresis of the ROI amplicons. The control bands (without DNA template) are shorter in length and not purified.

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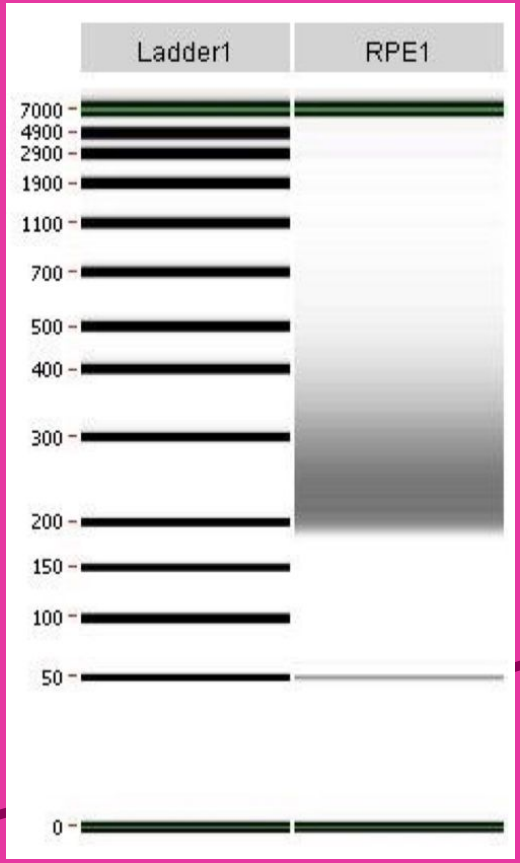

Results of capillary electrophoresis on Revvity LabChip GX of the MITO library.

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5. **NGS via Illumina MiSeq**

Computational Analysis Workflow

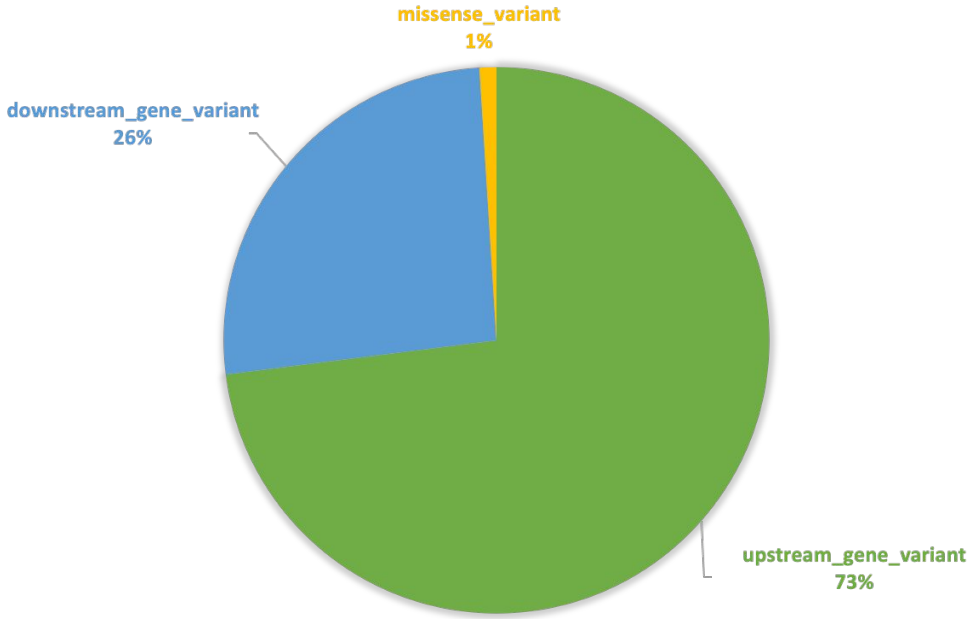
1. **Reads Quality Control**
    a. before and after adapter removal (*FastQC*)
    b. Adapters removal (*Trimmomatic*)
2. **Alignment** of the reads with a reference genome (*BWA*)
3. **Variant calling** (*Mutect2*)
    a. Filtering of variants (*FilterMutectCalls*)
4. **Variant functional annotation**
5. **Comparison of samples** (*PCA*)

Results
VEP RPE-ROI

Variant Effector Predictor - *Ensembl*

- Processed variants: 6
- Novel variants: 1
- Existing variants: 5

100% missense variants (coding)



Results - VEP RPE-ROI

- VEP (Variant Effector Predictor) - *Ensembl*
- Considered predictors:
  - ➔ **SIFT**: based on sequence homology and the physical properties of amino acids
  - ➔ **Polyphen**: based on phylogenetic considerations

Location	Consequence	Symbol and Gene	Existing variant	APPRIS	SIFT	PolyPhen	Clinical Significance
M:3739-3739	Missense variant	MT-ND1 ENSG00000198888	-	P1	0.23	0.119	-

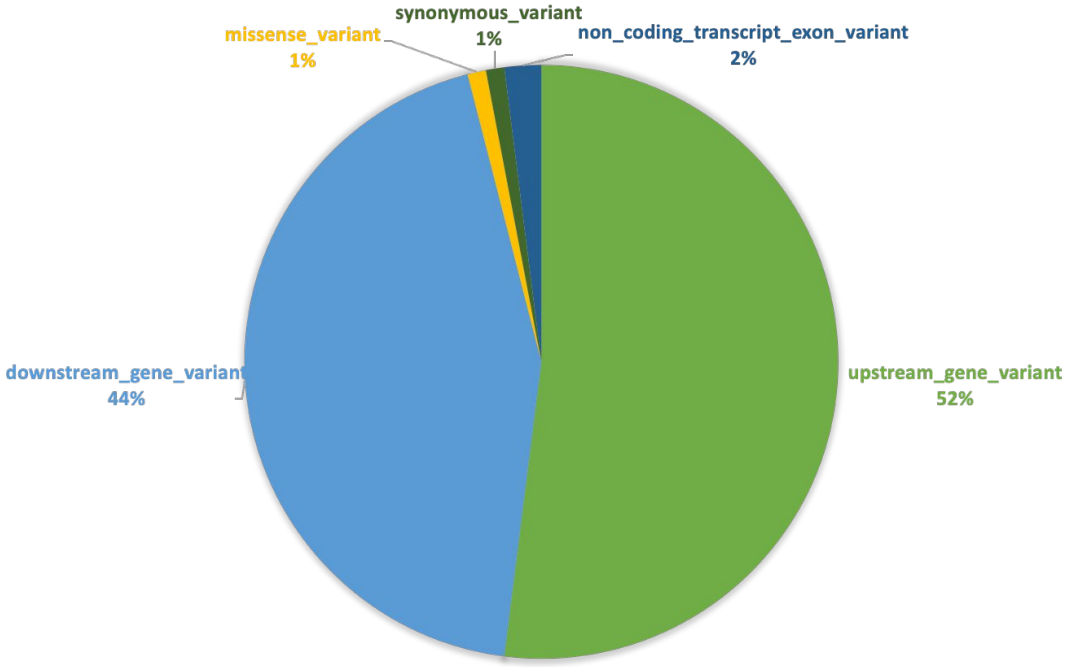
Results
VEP RPE-MITO

Variant Effector Predictor - *Ensembl*

- Processed variants: 21
- Novel variants: 1
- Existing variants: 20

50% missense variants

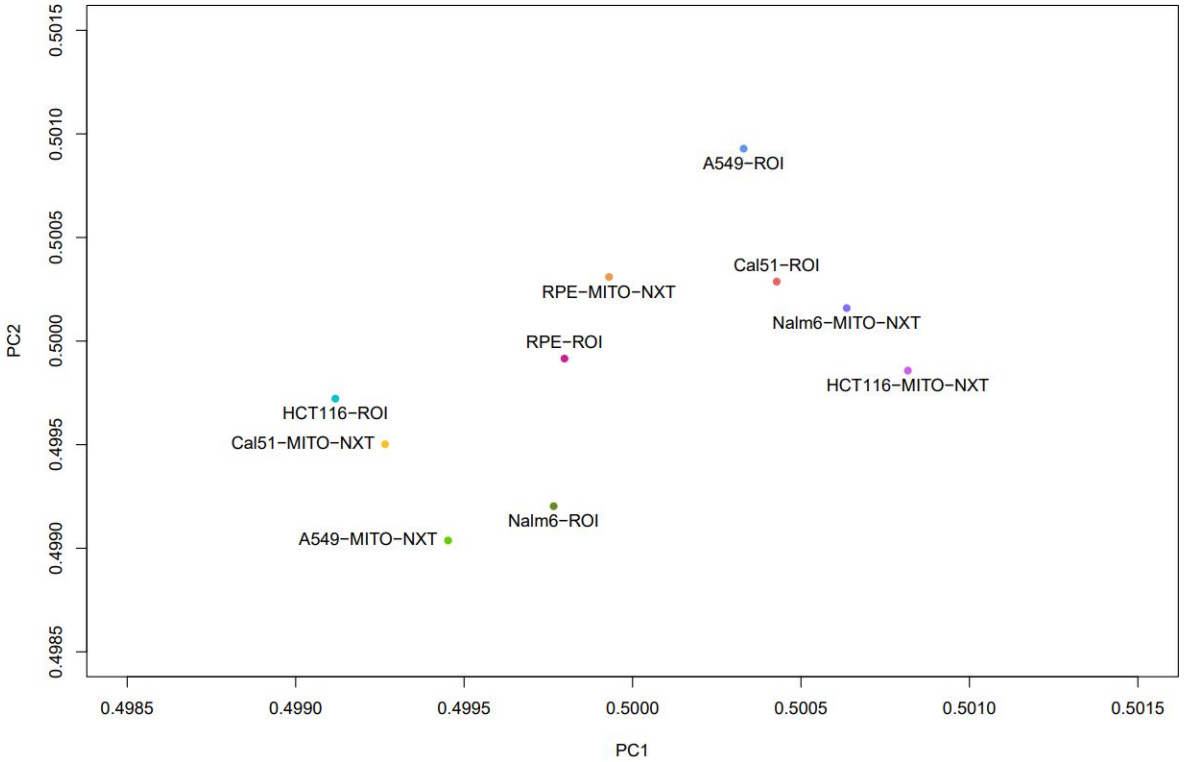
50% synonymous variants



Results - VEP RPE-MITO

Location	Consequence	Symbol and Gene	Existing variant	APPRIS	SIFT	PolyPhen	Clinical Significance
M:13145-13145	Missense variant	MT-ND5 ENSG00000198786	rs386829175	P1	1	0.003	benign
M:6419-6419	Missense variant	MT-CO1 ENSG00000198804	rs1603220461	P1	0	0.999	-
M:9912-9912	Missense variant	MT-CO3 ENSG00000198938	rs28580363	P1	0	0.957	-
M:15326-15326	Missense variant	MT-CYB ENSG00000198727	rs2853508	P1	0.21	0.009	benign, likely pathogenic
M:8860-8860	Missense variant	MT-ATP6 ENSG00000198899	rs2001031	P1	0.43	0.003	benign

Results - PCA



Expectations

- ★ Healthy and cancer cell lines are separated
- ★ MITO and ROI cluster together

Limitations

- ★ Overlapping points
- ★ Random distribution (Jitter function)

Explanations

- ★ Similar data
- ★ Problems in the PCA

VCF file analysis

Table S1 | Mutational burden of cancer vs healthy cell lines. The variations have been counted.

	A549	Cal51	HCT116	Nalm6	RPE
MITO	36	26	34	57	27
ROI	8	10	7	21	8
MITO ∩ ROI	39	32	38	67	30

Conclusions

- ★ Mutational burden higher in cancer cell lines
- ★ ROIs were valuable to identify variants

VCF file analysis

Only one variant is present in all tumor cell lines but not in RPE cells

MT-NDR2 gene

chrM:2.465

Total count: 5024
A : 760 (15%, 33+, 727-)
C : 4 (0%, 4+, 0-)
G : 19 (0%, 7+, 12-)
T : 4241 (84%, 2949+, 1292-)
N : 0

DEL: 8
INS: 1

Table S2 | Position and type of the SNPs found in RPE samples but not in tumor cells.

Position in ChrM	Gene	Type	RPE MITO	RPE ROI
152	-	Upstream/downstream gene variant	yes	yes
1959	MT-RNR2	Non-coding transcript exon variant	yes	no
3363	MT-ND1	-	no	yes
5318	MT-ND2	Synonymous variant	yes	no
5691	MT-TN	-	no	yes
9912	MT-CO3	Missense variant	yes	no
9950	MT-CO3	Synonymous variant	yes	no
13145	MT-ND5	Missense variant	yes	no
15466	MT-CYB	Synonymous variant	yes	no
15721	MT-CYB	Synonymous variant	yes	no
16192	-	Upstream/downstream gene variant	yes	no

OUR PROPOSAL VS THE HARSH REALITY

Main Divergences

Distinct sequencing technologies:

- *Illumina* MiSeq
- *PacBio Sequel IIe*

Distinct extraction processes:

- *Nuclear/mitochondrial DNA extraction + enrichment*
- *Organelle isolation + mtDNA extraction*

Distinct quality control approaches and timing

Library preparation

Some Commonalities

Quality control techniques:

- *Qubit fluorometer*
- *Gel electrophoresis*

CRITICALITIES AND PROBLEMS

(THE DAILY BREAD OF ANY RESPECTABLE SCIENTIST)

Notable Mentions

- ***Unsuccessful MITO3 amplification***
- ***Unsatisfactory library size and purity***
- ***Irrelevant PCA plot***

THANK YOU FOR YOUR ATTENTION!

TEAM

PINK