# Delving into Northern White Rhino Stem Cells

## Metabolic and Transcriptomic Analysis

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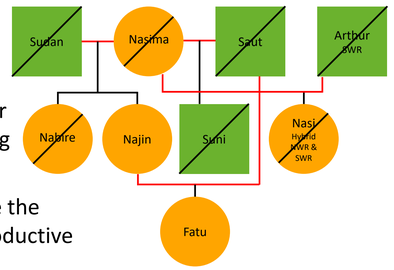
## Northern White Rhino (NWR) Project

In the 1970s, there were around 500 Northern White Rhinos (NWR). Since then, poaching has reduced them to only 2 individuals: mother and daughter Najin and Fatu, thereby rendering them functionally extinct.[1]




The NWR Project hopes to revive the species using stem cell and reproductive technologies.

## Introduction

The NWR induced pluripotent stem cells (iPSCs) that we have generated will be utilized for gamete development and assisted reproduction with the aim of maintaining population viability and genetic diversity. To improve our understanding of NWR iPSCs and their differentiated derivatives, we studied their metabolic and transcriptomic features.

- **Metabolism**: uncover some of NWR iPSCs energy processes
- **Transcriptome**: uncover up and down-regulated genes using RNA transcripts

## Experimental Design

**Focus cell lines:**
- Fibroblasts (a common connective tissue)
- NWR induced pluripotent stem cells (iPSCs)
- NWR embryoid bodies (EBs) induced from iPSCs

### Transcriptome: Drylab

**Analyze RNAseq data for enriched biological processes:**
Used R to create an analysis pipeline that extracts upregulated and downregulated genes for gene ontology analysis

**Compare cell type transcriptomes:**
Used R to generate principal component analysis (PCA) graphs and conduct unsupervised hierarchical clustering

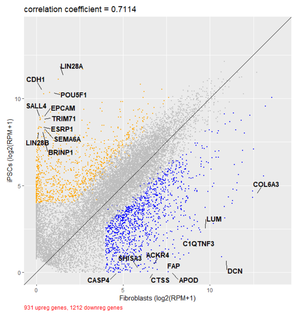
### Metabolism: Wetlab

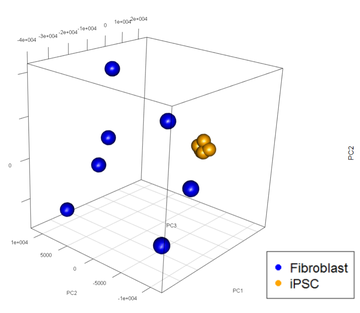
**Identify active metabolic pathways**
Used assay kits to quantify glycolysis activity and ATP content

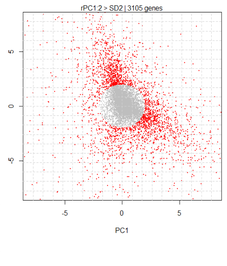
**Flow Cytometry (FACs)**
Used fluorescent antibodies to detect functional mitochondria and essential pluripotent gene expression

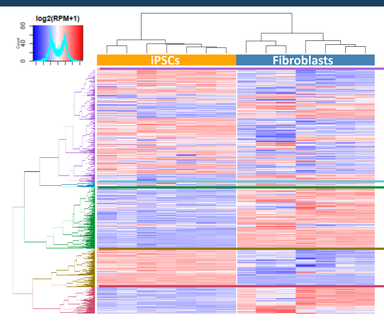
## RNAseq Analysis Pipeline[2]

### NWR Fibroblasts & iPSCs




**Cluster 1: Translational initiation, rRNA processing, translation**
*DDX21, NHP2, LARP1, RPL12, EIF1, FAU*

**Cluster 2: Cytoskeleton organization, mRNA**
*CTNNB1, ACTG1, CALR, DYNC1H1, FLNA, HSP90B1, MYH9, NPM1, PDIA3, TMSB4X, TUBB*

**Cluster 3: Cell motion regulation, blood and bone development**
*ACVRL1, ADAM17, CDH13, EGFR, FURIN, HDAC6, IGF1R, LAMA2, PTEN, PDGFRA, VEGFA*

**Cluster 4: Mitosis, nuclear division**
*BCCIP, TP53, BRCA1, SPAG5, MCM2, LIG1, KIF15, INCENP, CCNB1, CDC6, POLA1*

**Cluster 5: Negative regulation of transcription, blood vessel development**
*FOXO3, HMOX1, LIFSTAT2, PKIA, NFIB, BCL7A, WDTC1, ANKRD1*

### Pairwise Comparison of Gene Expression




### PCA Graph




### Radial Plot of PCA Loading Scores




Gene loading scores with SD > 2 selected using radial plot

### Heatmap of significant PCA genes




### Gene Cluster Ontology: Biological Process [3]

**Cluster 1: Mitosis, nuclear division, RNA splicing**
*DHCR24, CD2AP, DAXX, SKA2, KIF11, MDC1, TERF1, TP53, TUBB2A*

**Cluster 2: RNA Processing, chromatin and chromosome modification**
*DNMT3B, CDH1, SOX2, NASP, NPM1,*

**Cluster 3: Cell migration, neuron differentiation, epidermis, ectoderm, and epithelial cell differentiation**
*WNT1, TGFB1, NOG, JAK2, POU3F2, BMP4, KLF4, TFAP2A, TGFB1I1, IRF6, BNC1*

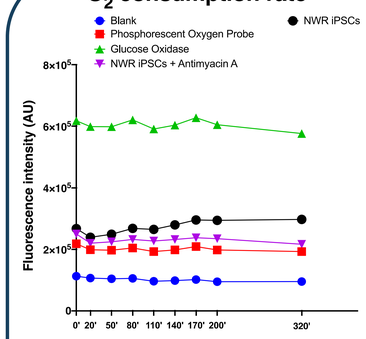
**Cluster 4: Sensory organ development, embryonic organ development, ear development**
*SOX1, WNT1, WNT4, RAX, MITF, HAND1, HES3, BDNF*

**Cluster 5: Ion transport, regulation of cell death, positive regulation of immune system process**
*MCF2, DGKK, ADORA2B, LCK, ACTN3*

### NWR iPSCs & EBs

## Metabolism Analysis

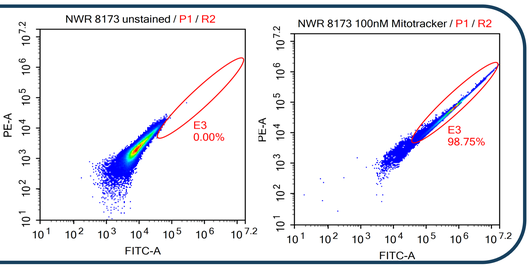
### $O_2$ consumption rate



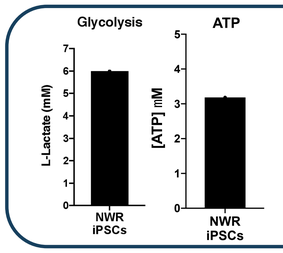

Negative control (purple) and NWR iPSCs (black) are similar, which contrasts with the positive control (green). These results indicate little to no active oxidative phosphorylation in iPSCs.

### Viable Mitochondria

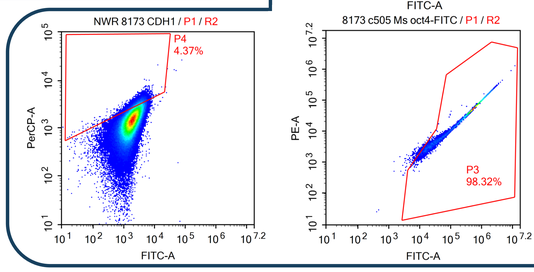
Flow cytometry stained 98.79% of cells as opposed to 0% in unstained control NWR iPSCs. This indicates functional mitochondria in the majority of cells.

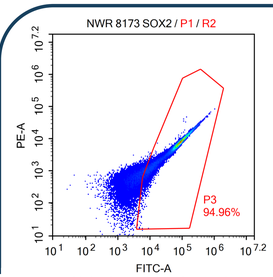



### Glycolysis & ATP




According to our glycolysis and ATP experiments, the NWR iPSC energy production is mainly derived from the break down of glucose.




### Pluripotency Markers

Flow cytometry showed that the majority of iPSCs expressed the Yamanaka factors SOX2 and OCT4, essential regulators for pluripotency in humans and mice.
CDH1 has been reported to have low expression in human iPSCs, which resembles our data for NWR iPSCs

**Citations**

**[1]** International Rhino Foundation. 2002. Rhino Information – Northern White Rhino. 19 September 2006
**[2]** Yamashiro, et al. Generation of human oogonia from induced pluripotent stem cells in vitro. Science. 19 October 2019: 356-360
**[3]** Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources. *Nature Protoc. 2009;4(1):44-57*
Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res. 2009;37(1):1-13.*

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