Delving into Northern White Rhino Stem Cells

Metabolic and Transcriptomic Analysis

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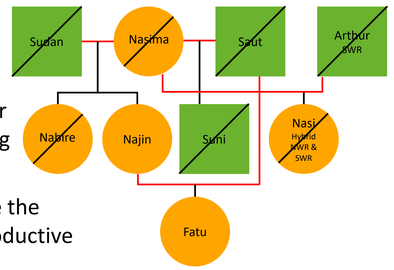
Northern White Rhino (NWR) Project

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

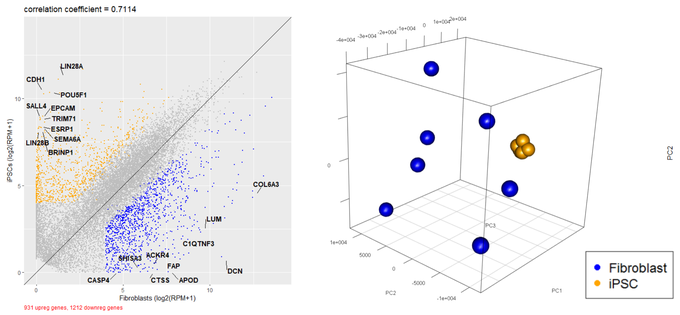
Metabolism: Wetlab

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

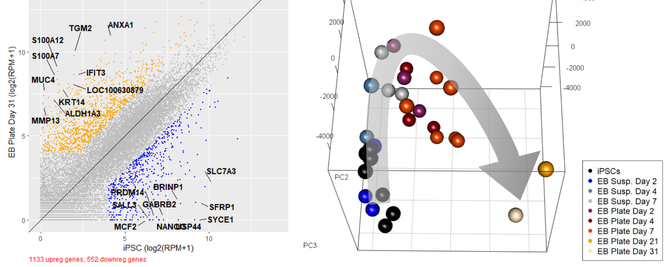
Flow Cytometry (FACs)
Used fluorescent antibodies to detect functional mitochondria and essential pluripotent gene expression

RNAseq Analysis Pipeline[2]

NWR Fibroblasts & iPSCs



Pairwise Comparison of Gene Expression | **PCA Graph** | **Radial Plot of PCA Loading Scores** | **Heatmap of significant PCA genes** | **Gene Cluster Ontology: Biological Process [3]**

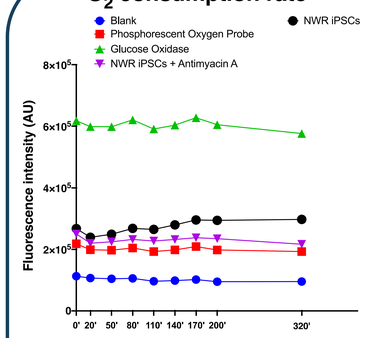


Gene loading scores with SD > 2 selected using radial plot

NWR iPSCs & EBs

Metabolism Analysis

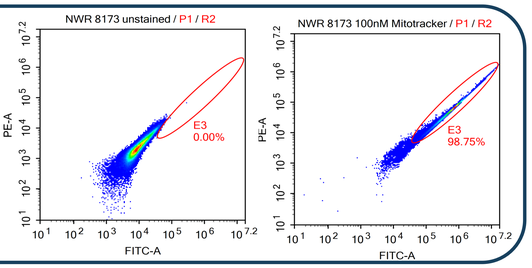
O_2 consumption rate

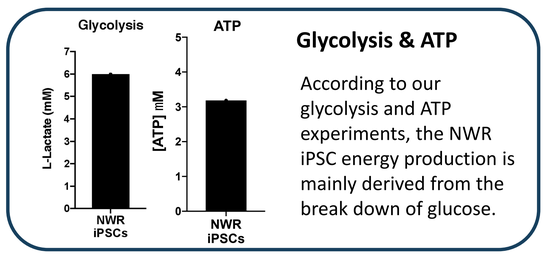


Negative control (purple) and NWR iPSCs (black) are similar, which contrasts with the positive control (green). These results indicate little to no active oxidative phosphorylation in iPSCs.

Viable Mitochondria

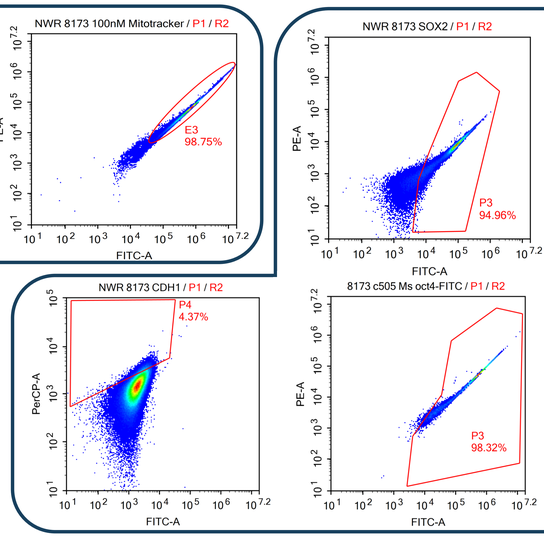
Flow cytometry stained 98.79% of cells as opposed to 0% in unstained control NWR iPSCs. This indicates functional mitochondria in the majority of cells.





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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