CRISPR/Cas9-Mediated Genome Editing For Fluorescence Single-Cell Microscopy

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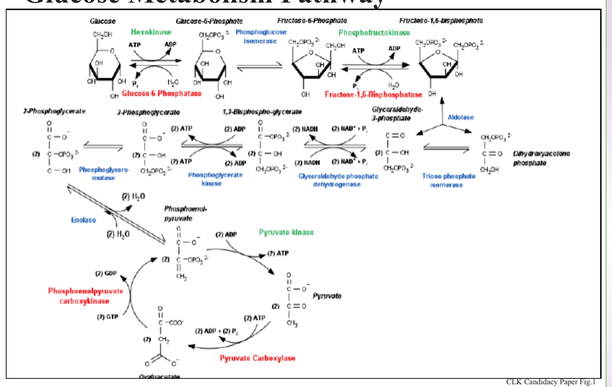
Introduction

Abstract

Dysregulated glucose metabolism is an emerging hallmark of cancers. We have investigated the spatiotemporal role of human liver-type phosphofructokinase (PFKL) in glucose metabolism under fluorescence single-cell microscopy. According to our preliminary data, we hypothesize that PFKL is a scaffold to other enzymes involved in human glucose metabolism. We have aimed to develop a genome-edited cell line expressing endogenously-tagged PFKL by knocking in a gene encoding an enhanced green fluorescent protein (EGFP), using the Clustered Regularly Interspaced Short Palindromic Repeats/Caspase 9 (CRISPR/Cas9) technology. Currently, we are preparing a nuclease plasmid containing a Cas9 nuclease and a guide RNA sequence pinpointing the genomic locus for EGFP insertion, and a donor plasmid containing PFK homology arms and EGFP. We anticipate that these plasmids will be transfected into human breast cancer Hs578T cells to incorporate the EGFP tag into the C-terminal region of the genomic PFKL gene via a homology-directed DNA repair system. Development of this cell line will allow us to investigate the scaffold hypothesis of PFKL and further the importance of its scaffold role in spatiotemporal regulation of glucose metabolism.

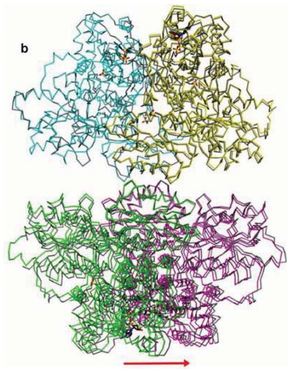
Glucose Metabolism Pathway



CLK Candidacy Paper Fig.1

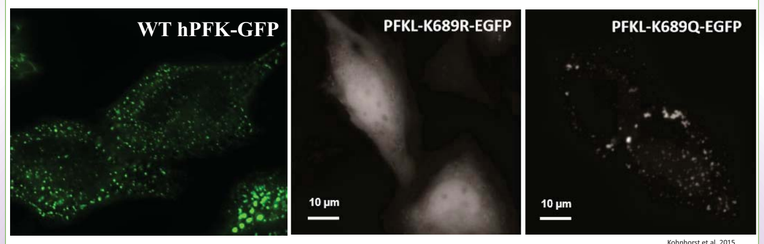
Phosphofructokinase

- Catalyzes 3rd step of glycolysis: fructose-6-phosphate→fructose-1,6-bisphosphate
- Commits molecule to glycolytic pathway
- Most regulated enzyme of glycolytic pathway
  - numerous metabolic activators/inhibitors and methods of hormonal control



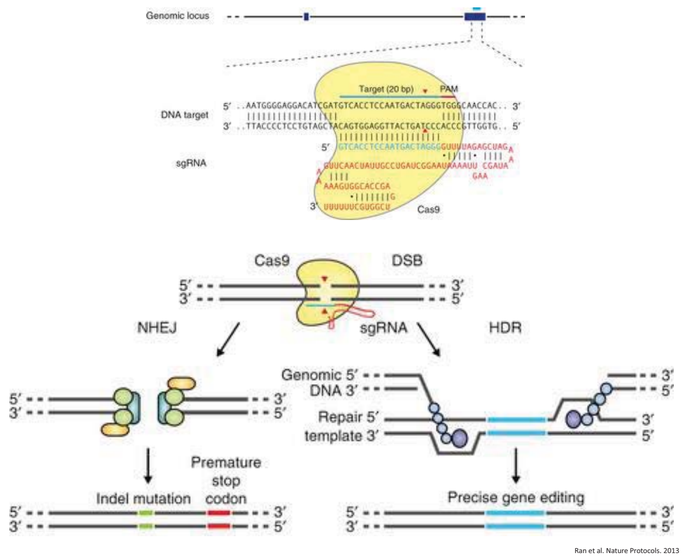
Human PFK-1. Nature. 2015

Previous Work



Kohnhorst et al. 2015

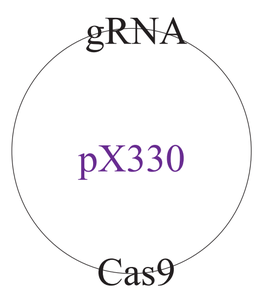
CRISPR Overview



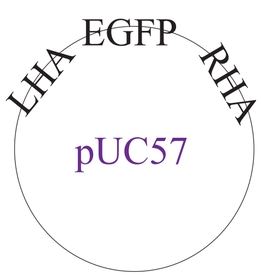
Ran et al. Nature Protocols. 2013

Plasmid Construction

Donor Plasmid



Nuclease Plasmid



Primer Design

PFK Genomic DNA

Exons-highlighted
LHA-green
Stop Codon-red
RHA-purple

Nuclease Plasmid Primers

An-SB-PFKL-FgRNA1
CACCGCGCACCCTGAGCATGGACAA

An-SB-PFKL-BgRNA1
AAACTTGTCCATGCTCAGGGTGCC

An-SB-PFKL-FgRNA2
CACCGAGCATGGACAAGGGCTTCTG

An-SB-PFKL-BgRNA2
AAACCAGAAGCCCTTGTCCATGCTC

Donor Plasmid Primers

Left Homology Arm

Forward Primer (EcoRV)
5' GCAA*GATATC*ggtggcgctccaacccctttgaccggaac 3'

Backward Primer (BglII) – for gRNA 1
5' GCAA*AGATCT*ccatgctcagggtgcggcgggtcacgtgc 3'

Backward Primer (BglII) – for gRNA 2
5' GCAA*AGATCT*aagcccttgtccatgctcagggtgcggcgg 3'

EGFP

Forward Primer (BglII)
5' GCAA*AGATCT*cgggcccgggatccaccggtcgccaccatg 3'

Backward Primer (MluI)
5' GCAA*ACGCGT*ttacttgtacagctcgtccatgccgagag 3'

Right Homology Arm

Forward Primer (MluI) – for gRNA 1
5' GCAA*ACGCGT*caagggcttctgaggccagccatgcccacg 3'

Forward Primer (MluI) – for gRNA 2
5' GCAA*ACGCGT*ctgaggccagccatgcccacgcccctcccc 3'

Backward Primer (EcoRV)
5' GCAA*GATATC*ggctgtgctctccggagaccgatgcacccc 3'

Future Directions

- Develop several other cell lines for PFK expression
  - WT Hs578T (already developed)
  - Hs578T-PFKL-EGFP (current project)
    - Expand to super resolution cell line Hs578T-PFKL-mEOS
  - Hs578T-KO-PFKL
  - Hs578T-PFKL-(Point Mutation)-EGFP
  - Hs578T-PFKL-K689R

Acknowledgements

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