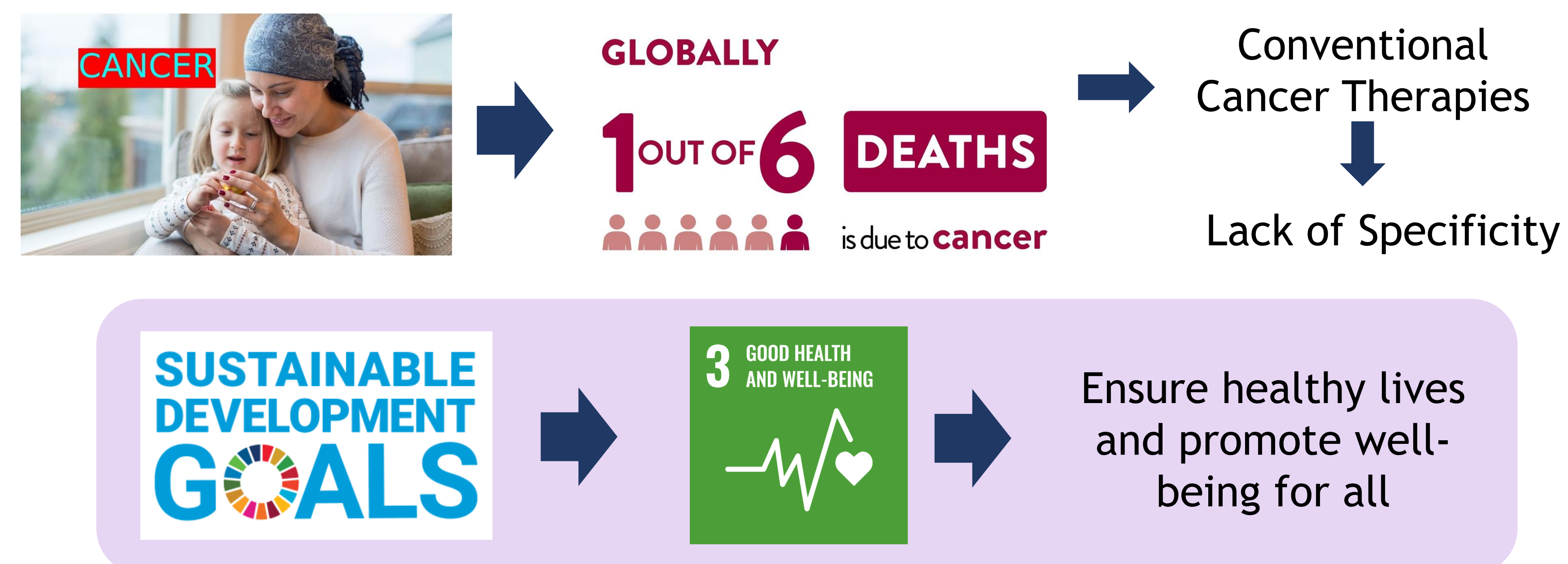


CRISPR/Cas9-mediated silencing of PFK-1 allosteric activation as a novel therapeutic approach for cancer

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Research Problem



Main Objective

CRISPR/Cas9-mediated silencing of PFK-1 allosteric activation using two independent approaches,

- 1) Knocking out the regulatory domain of PFK-1
- 2) Knocking out the PFK-2 by disrupting the PFKFB3 gene to decrease the rate of glycolysis and proliferation of MCF-7 breast cancer cells, for designing a therapeutic tool kit to treat breast cancer

Warburg effect of cancer cells

Cancer cells consume high glucose level compared to normal cells through “Aerobic glycolysis” even in the presence of high oxygen level and functioning Mitochondria

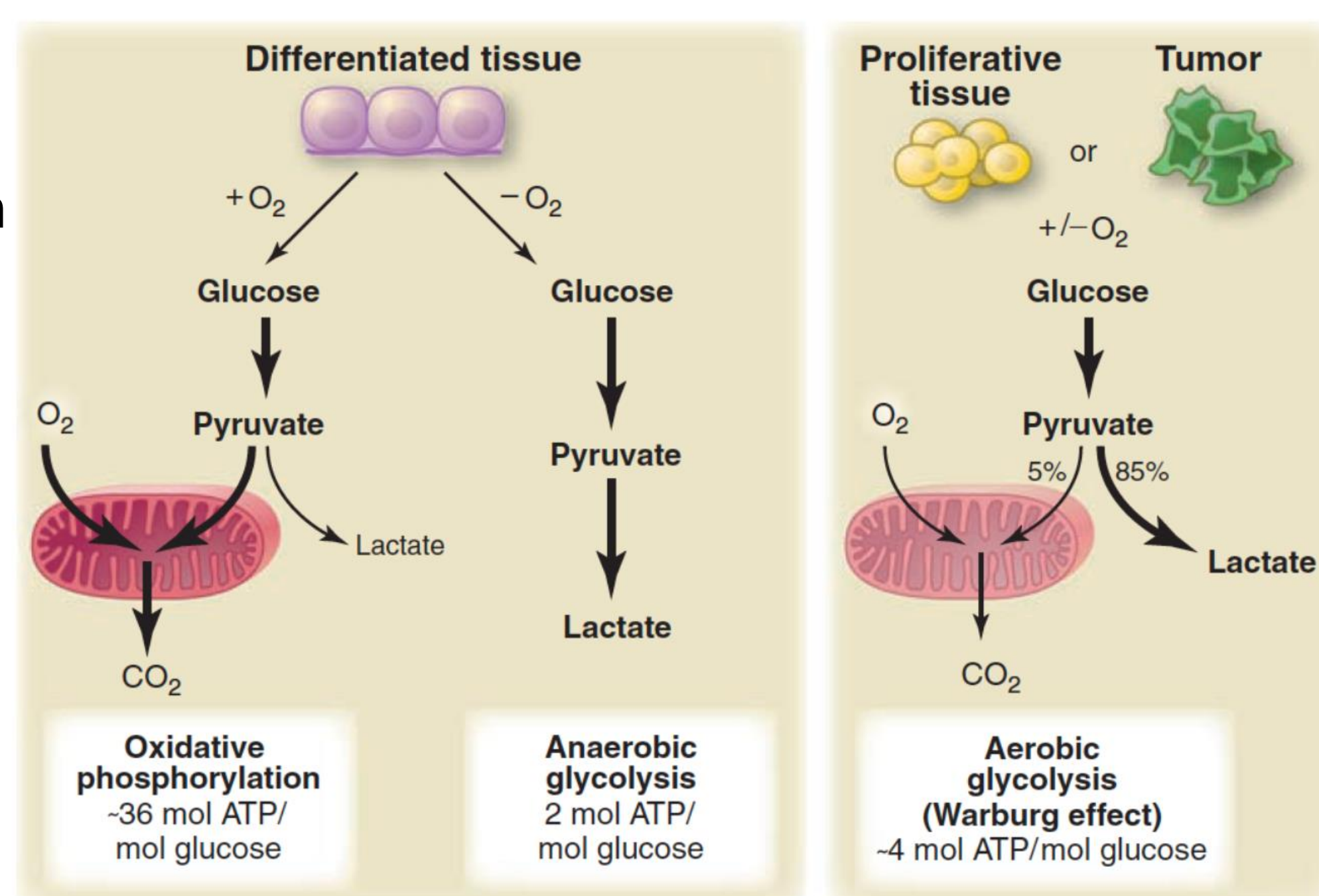


Fig 1. The difference in glucose metabolism between a normal differentiated tissue and a proliferative tissue

Rate limiting step of glycolysis

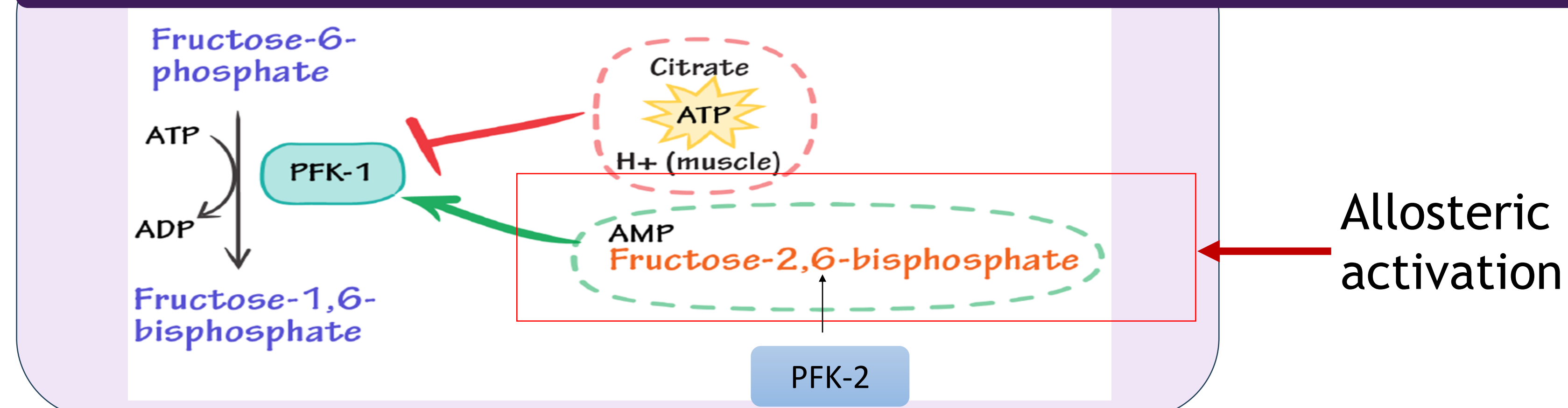


Fig 2. Illustration of the rate limiting step of glycolysis

Two CRISPR/Cas9 based approaches to inhibit allosteric activation of PFK-1

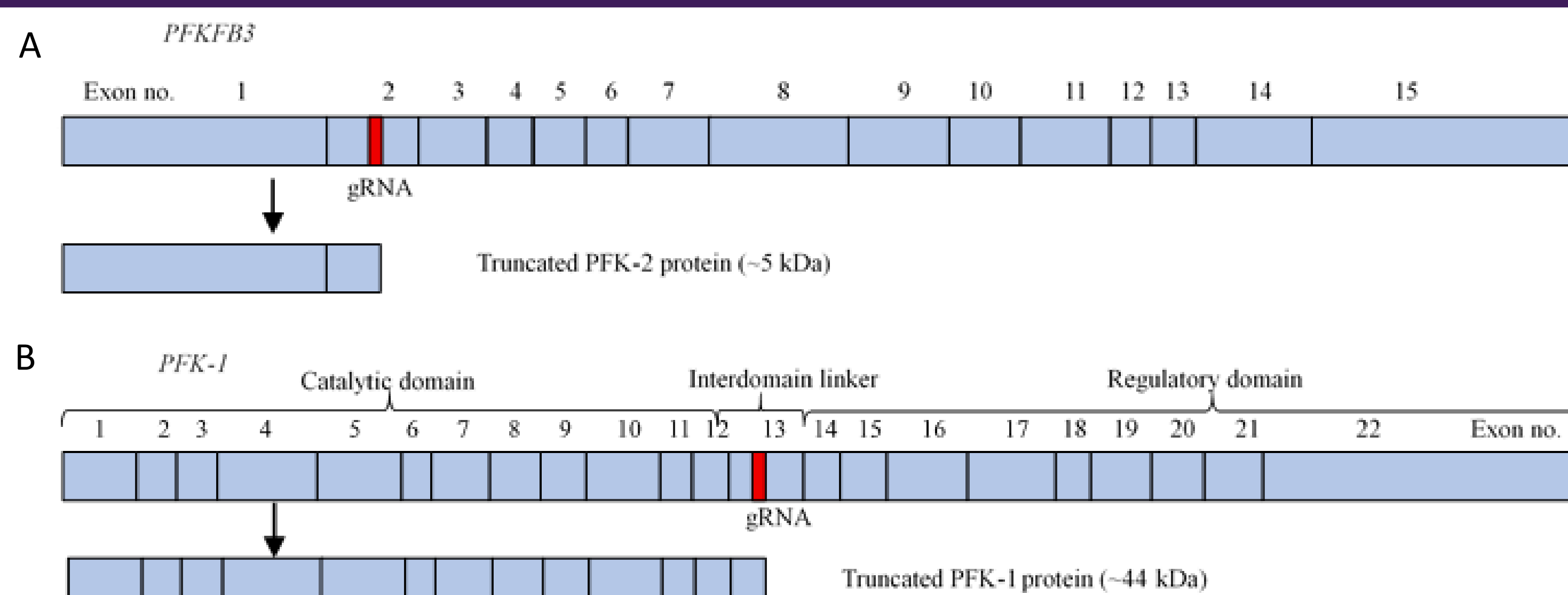


Fig 3. Location of designed gRNAs targeting (A) PFKFB3 (exon 2) and (B) PFK-1 (exon 13)

Colony PCR to confirm the cloning of gRNA

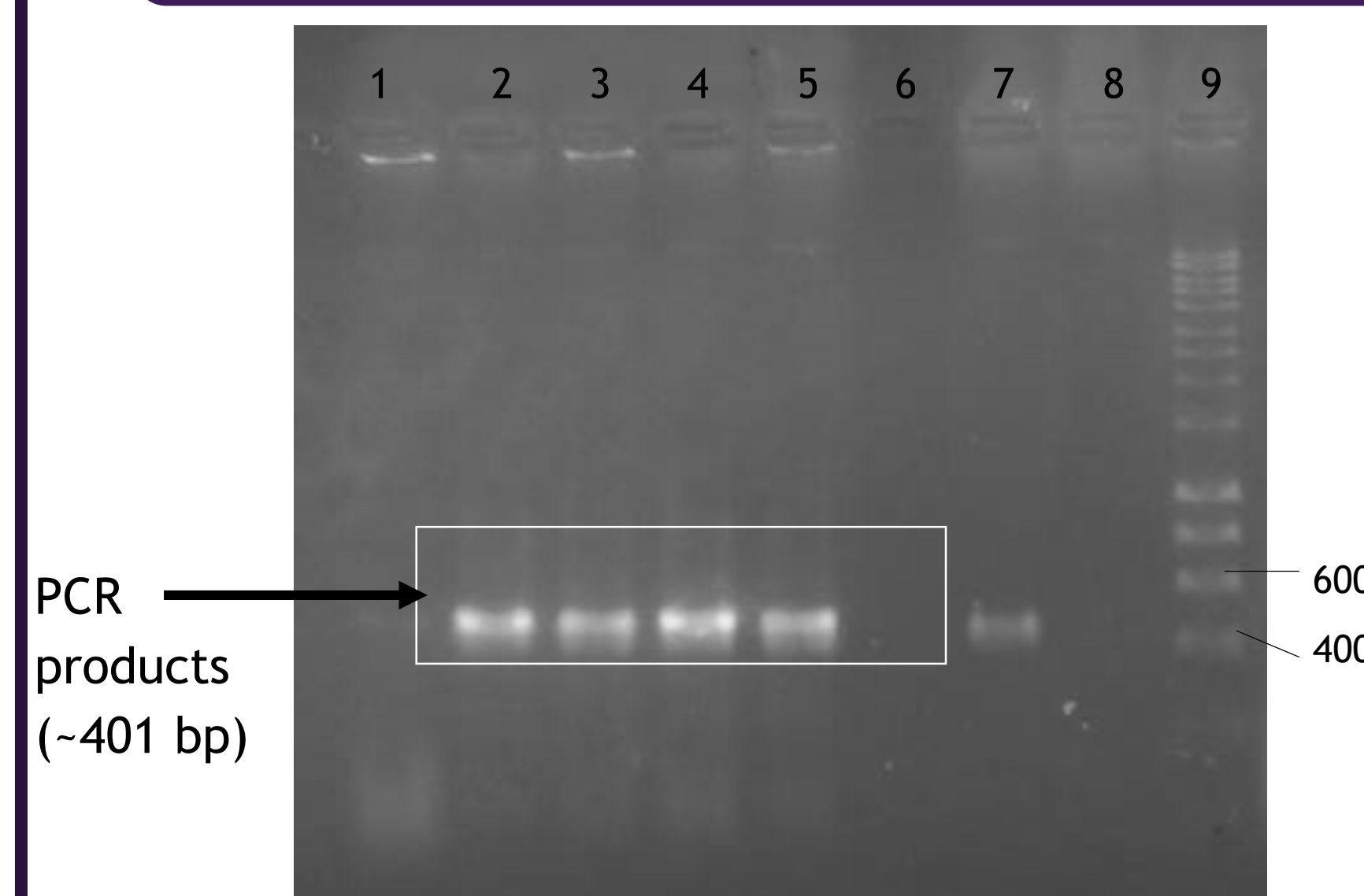


Fig 4. Polyacrylamide gel electrophoresis of Colony PCR products

PEI mediated transfection

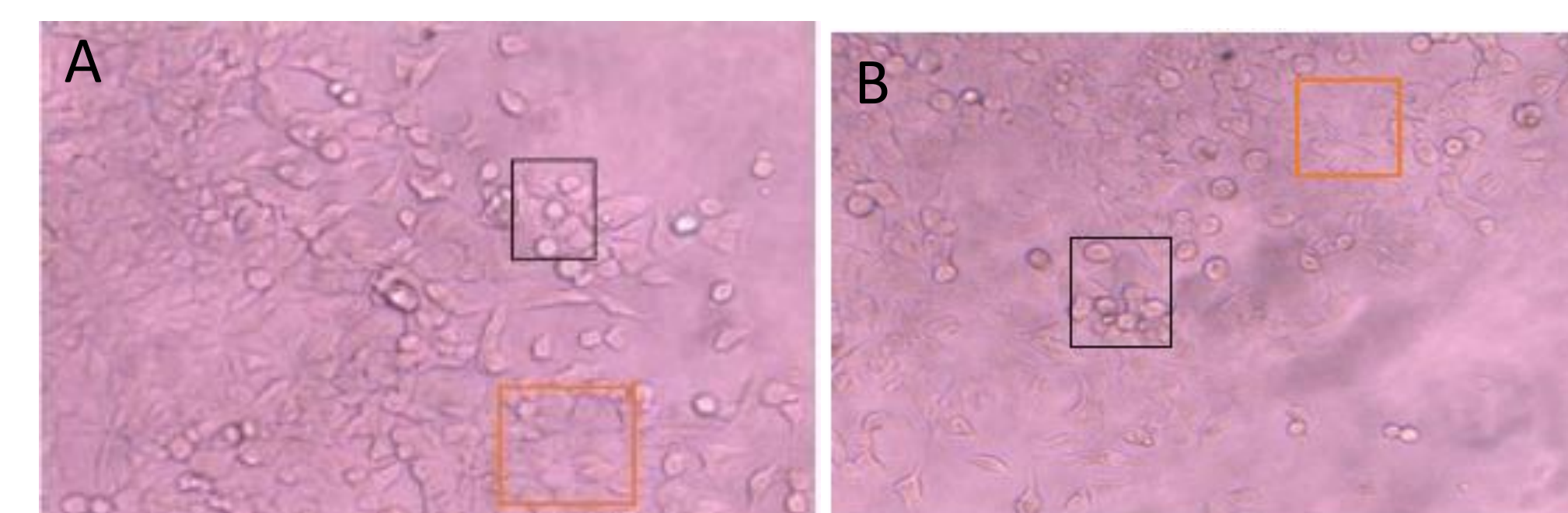


Fig 5. Morphology of MCF-7 cells after PEI transfection (×100). (A) MCF-7 cells treated with PEI: DNA = 1:2. (B) Untreated MCF-7 cells

Conclusion

This study aimed to disrupt the allosteric regulation of PFK-1 using two independent approaches- disruption of the *PFKFB3* gene by targeting exon 2 and disruption of regulatory domain of the PFK-1 enzyme by targeting exon 13 using specifically designed sgRNA- as they would allow healthy cells to maintain sufficient levels of ATP via the basal function of PFK-1 while significantly impacting energy-centric metabolism in cancer cells.

Future work

- Detection of truncated protein
- Sequencing to verify the precise genome modification
- Proliferation assay and downstream analysis to investigate the effect of modified gene

Acknowledgement

Undergraduate research funds, Department of Chemistry, University of Colombo

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