

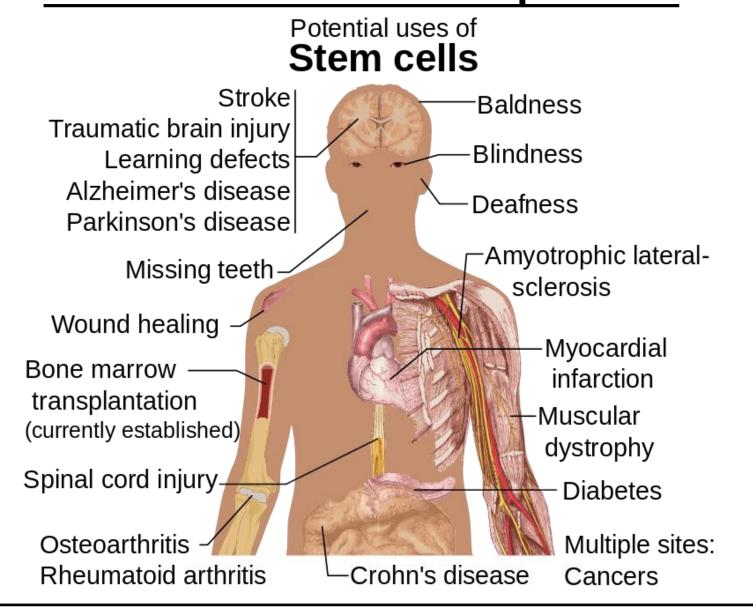
Investigation of SOX-2 Protein from E Coli. Bacteria

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1. What are stem cells & how are they used?

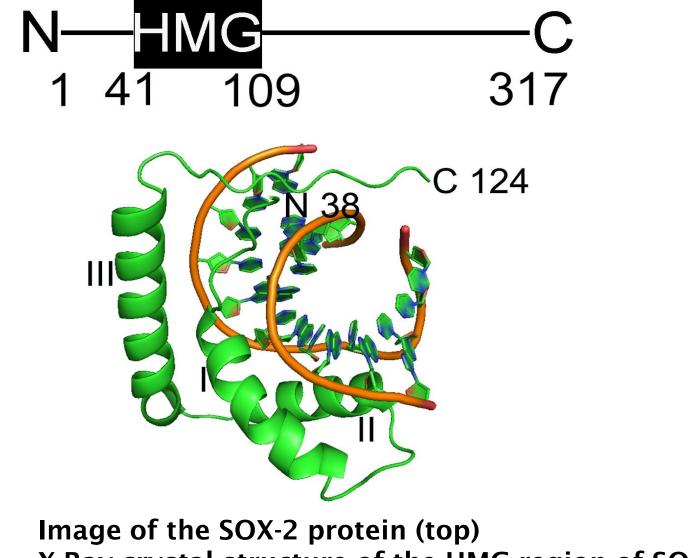
Embryonic (ESCs) vs Induced Pluripotent (iPSCs) Blastocyst iPSCs can then be made into supportive cell types (e.g., support Generation of cells for neurons, etc.) Inner cell mass Pluripotent stem cells

Stem cell treatment for patients



2. What are we studying specifically?

- SOX-2 binds to and bends DNA to make modifications to amino acids which are the building blocks of protein (important for iPSCs creation)
- Lysine (K) 73 gives cells the ability to change into any cell type & causes SOX-2 to leave the nucleus
- Threonine (T) also gives cells the ability to change into any cell type & increases gene creation for the cell



X-Ray crystal structure of the HMG region of SOX-2 bound to DNA (Bottom)

3. How are we studying this?

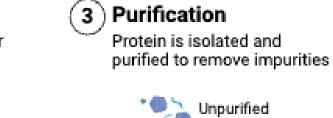
Protein Expression

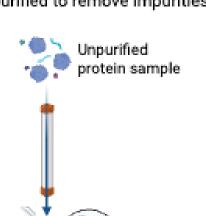
Somatic cells

(1) Cloning

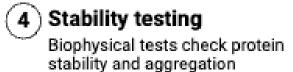


2 Expression

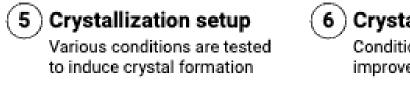


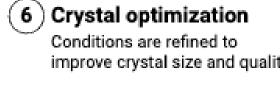


Purified protein







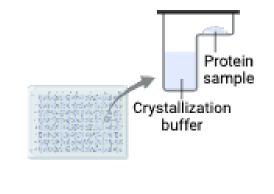




Crystals are mounted and

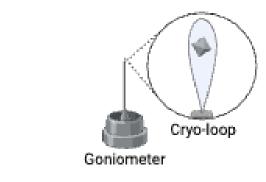
8 X-ray diffraction Crystals are exposed to X-rays



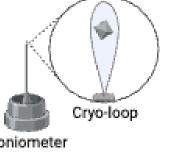


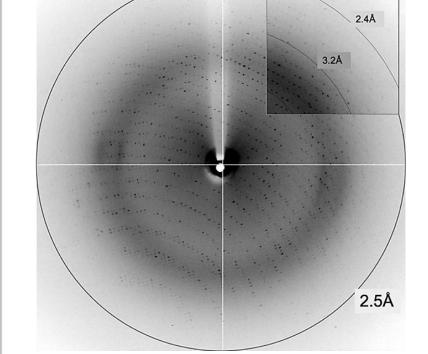
Crystallized





7 Crystal harvesting



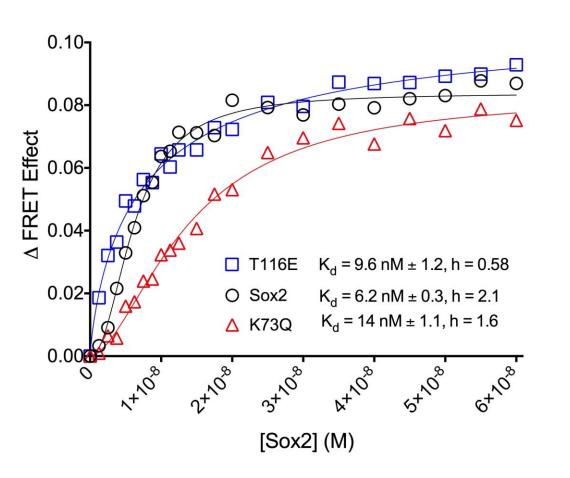


X-Ray Diffraction Pattern

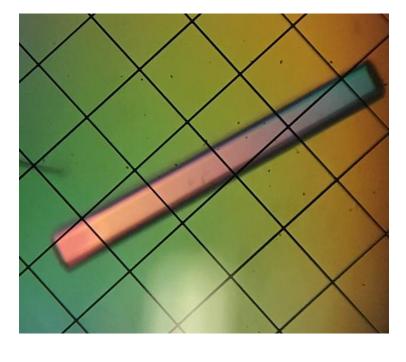
Fluorescence Resonance **Energy Transfer (FRET)**

Used to measure DNA binding and bending ability of SOX-2 and our 2 target proteins.

FRET DNA Binding and Bending



X-Ray Crystallography



SOX-2/DNA crystalized in 20% PEG 3350 Diffracted X-rays to 2.8 Å

SOX-2/DNA Electron Density Map

- SOX-2 binds to DNA with strong connections
- T116 makes DNA very non-adhesive, but helps in DNA bending
- K73 leads to low DNA binding, low cooperativity, & decreased bending

*Future work to continue x-ray imaging on both SOX-2 targets

5. Why is this important for Colorado?

- Understanding how to more efficiently generate iPSCs
- Could lead to better access for Colorado Blood Cancer Institute patients