COMBATING THE FRESHWATER CRISIS:

In vivo Studies Investigating the Elongation of the Loop of Henle

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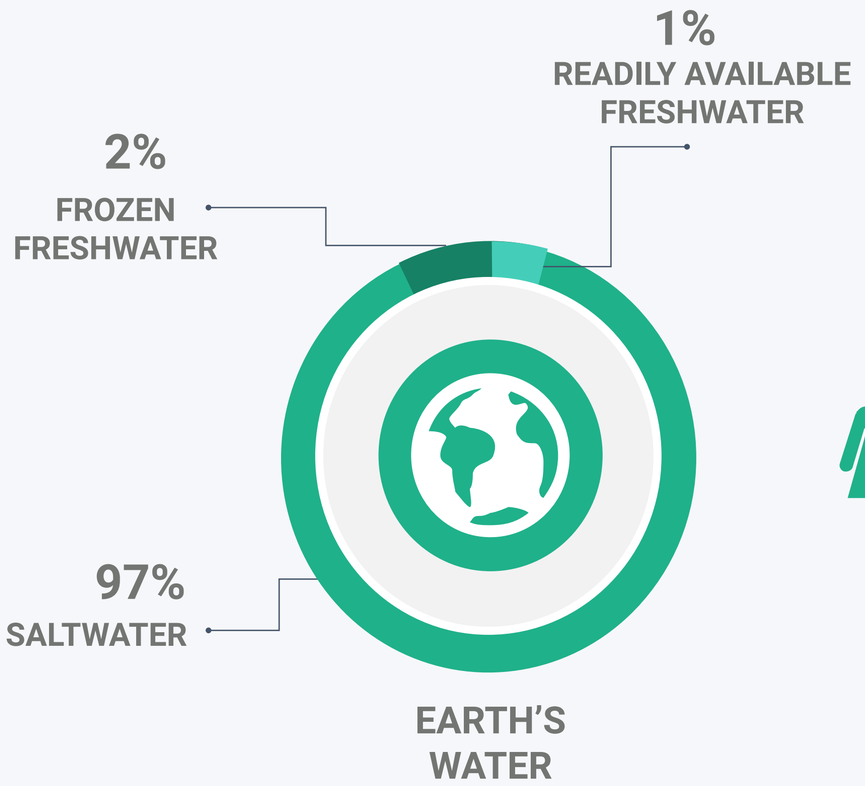
FRESHWATER CRISIS

What's the problem?

1 AVAILABILITY



2 WATER SCARCITY

2.7 billion people

Experience water scarcity at least one month of the year

3 WATER DEMAND

- Human Population
- Global Temperatures
- Water Availability

Water demand is expected to increase by 55% between 2000 and 2050

Background

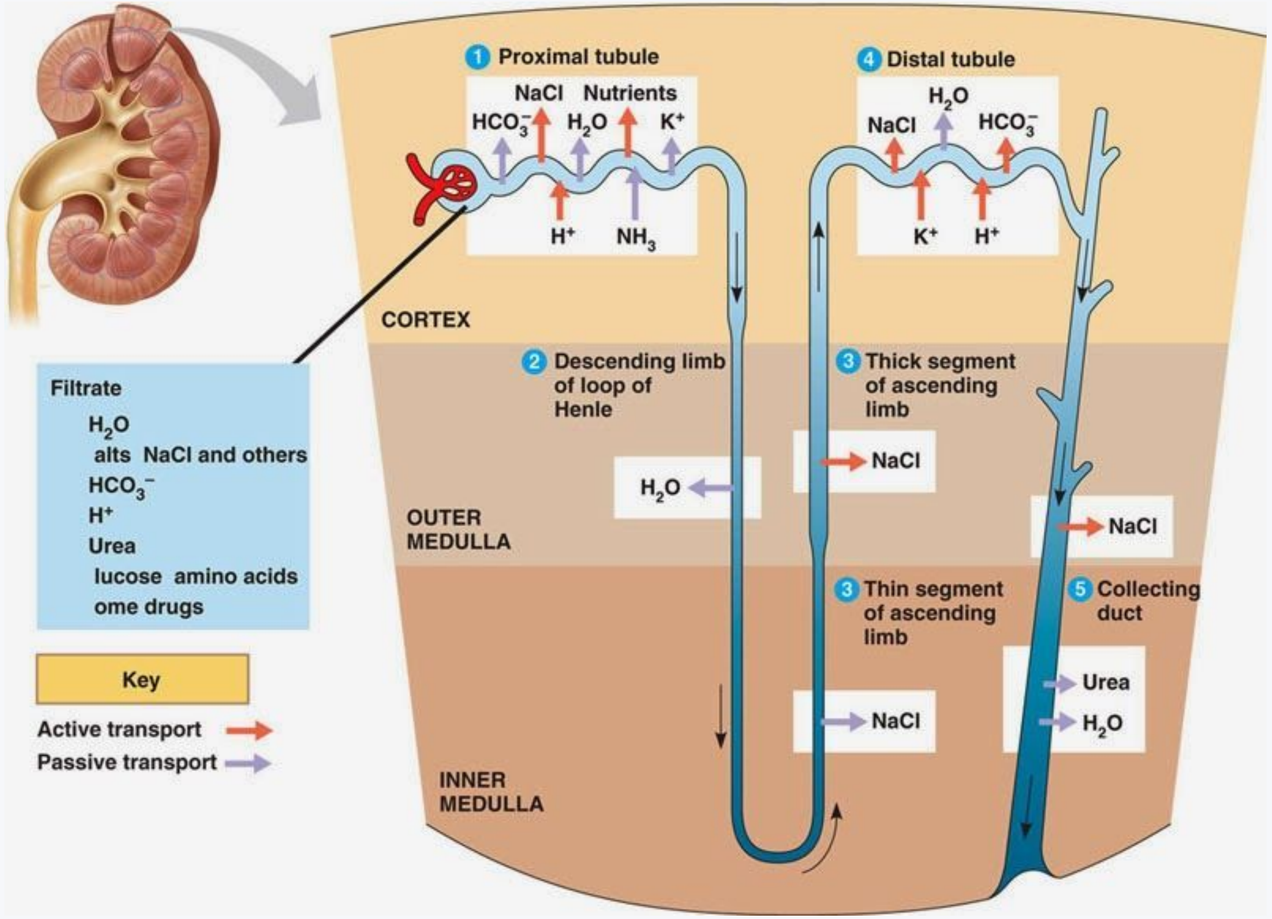
Dipodomys ordii (kangaroo rat)

Production of hyperosmotic urine from elongated loop of Henle

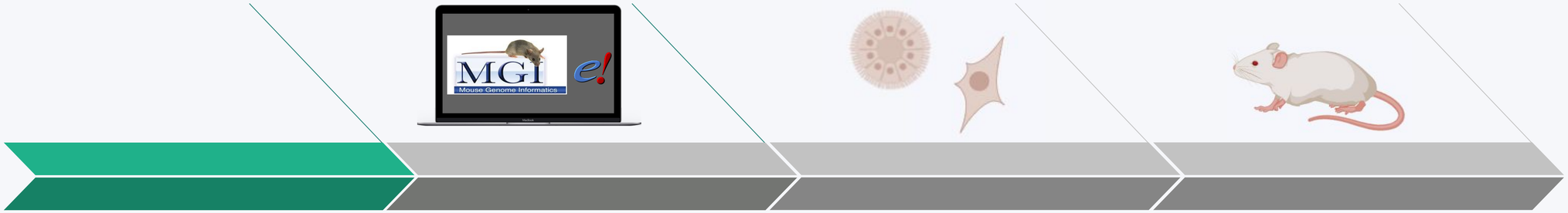
- Kangaroo rats are able to consume seawater while maintaining healthy conditions
- Maximum urine electrolyte concentration reaching ~1200 mN (more than 2x sea water levels!)

Background

- Long hairpin-like structure within each nephron
- Comprised of three sections
  1. Descending limb
  2. Thin ascending limb
  3. Thick ascending limb
- Concentrates urine through osmotic gradients
  - Length determines size of gradient



Experimental Setup



FRESHWATER CRISIS

In silico studies

1

In vitro studies

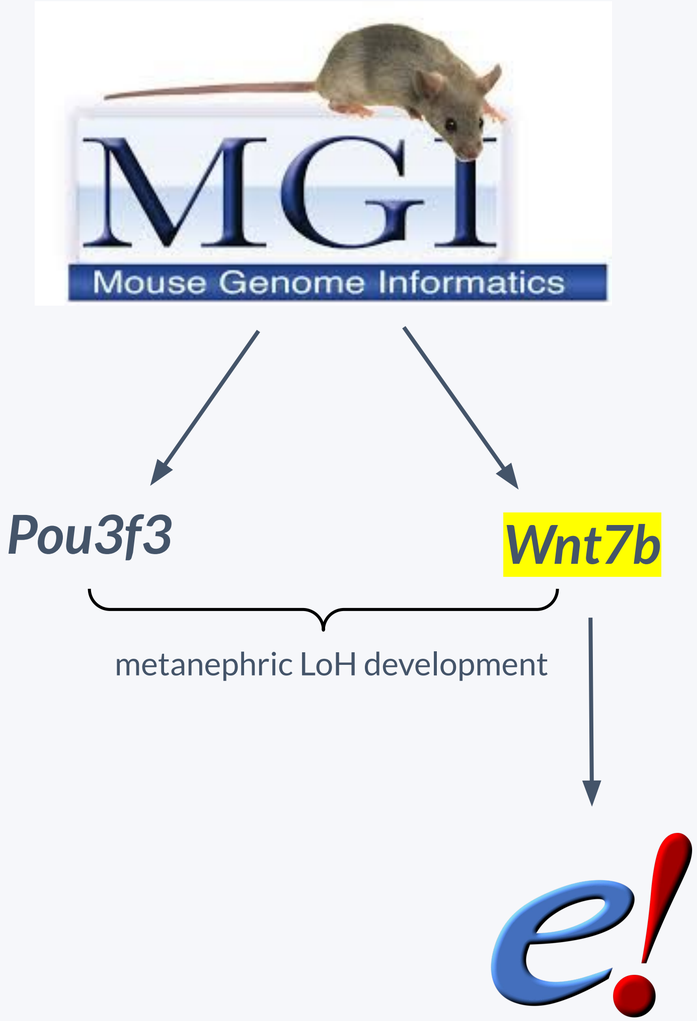
2

In vivo studies

3

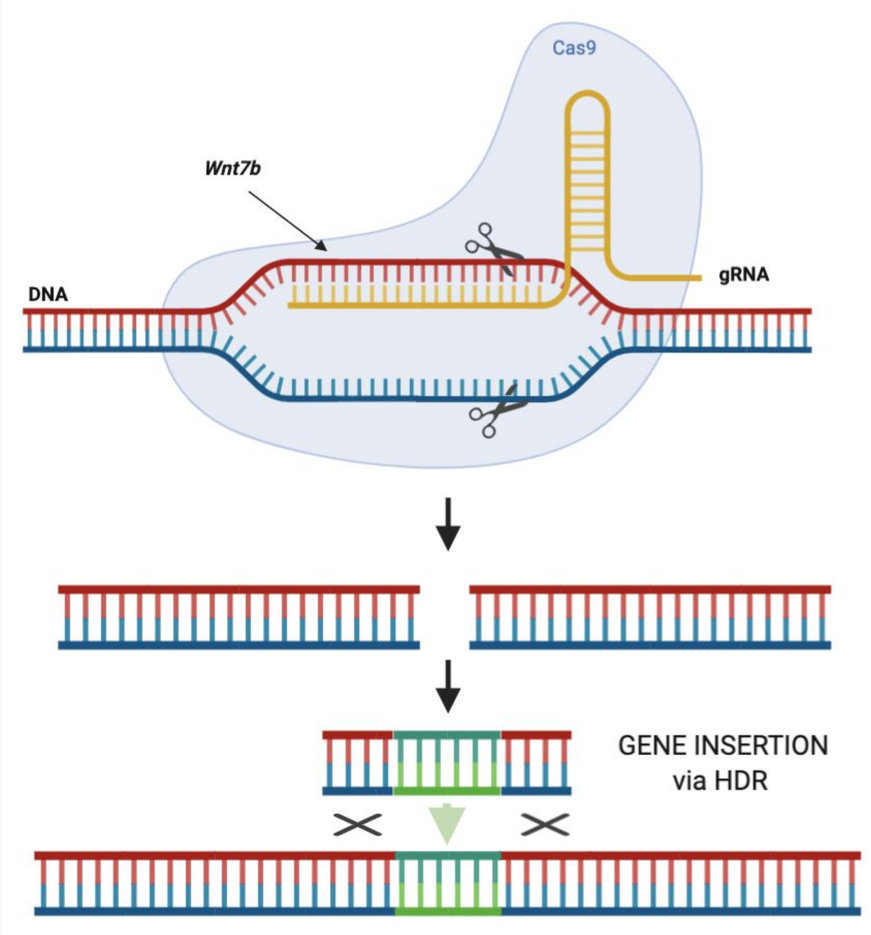
In silico studies

- Search & identification of Loop of Henle development genes using Gene Ontology (GO) in MGI database
- Use of comparative genomics to assess genetic composition of Wnt7b between kangaroo rat *and* laboratory mouse
  - Prior to mutational analyses



Genome Editing: CRISPR-Cas9

- Cas9 – Endonuclease that initiates double-stranded DNA breaks
- gRNA – Complementary to the *Wnt7b* to add/remove genomic regions
- Homology directed repair (HDR)



In vitro studies

- Characterize molecular and cellular consequences
- Human murine-derived renal epithelial and kidney cells of 1-month old M. musculus
    - Cell viability
    - qPCR to determine mutation
- Organ cultures
    - Compare length of Loop of Henle
    - Laminin and Tamm-Horsfall Protein immunostaining

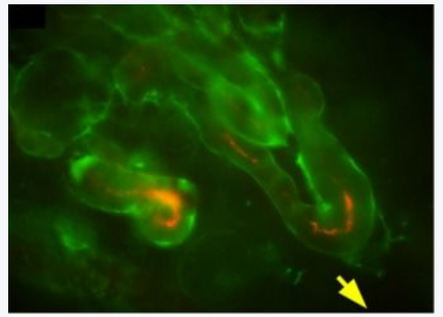


Image of LoH in organ culture immunostained with laminin (green) and Tamm-Horsfall Protein (red)

In vivo studies

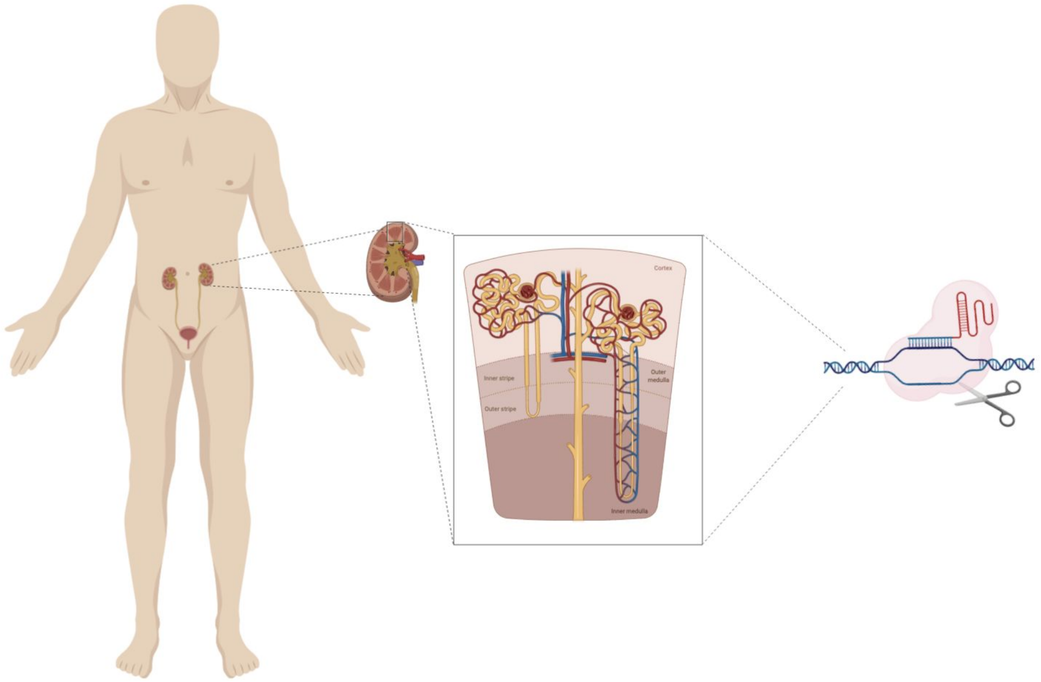
- Murine models containing genetic mutation will be nourished with diluted salt water
- **Primary objective**: Determine if models can consume salt water
- **Secondary objective**: Determine any adverse health effects

Analyze various factors

- Length of loop of Henle: antibodies against AQP1 and CIC-K
- Blood samples: measure serum sodium levels and osmolarity
- Other health impacts: salt poisoning, weight changes

Future Applications

- Advances in genetic engineering
- Saltwater-tolerant generation of humans with elongated Loop of Henle
    - More sustainable than desalination technologies
    - Sustainable lifestyle for future generations



- **All in efforts to conserve the limited amounts of freshwater present on Earth**
- **Drastic measures must be taken <u>now</u> to mitigate unfortunate outcomes**

Thank You for Listening

QUESTIONS?

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