MCDB 4101: Manipulating genomes in *Xenopus laevis* using CRISPR-based methods

Instructors:

Prof. Michael Klymkowsky (aka Dr. / Prof. K)
michael.klymkowsky@colorado.edu
office: Porter B425

Dr. Bilge Birsoy
bilge.birsoy@colorado.edu

Who are you
in *Xenopus laevis* using CRISPR-based methods

What is your preferred name, what courses have you taken – expected graduation date, what are you proud about yourself, what are you looking to get our of the course.

course web site: http://virtuallaboratory.colorado.edu/MutatingXenopus/

or you can google: **mutating xenopus boulder**

Our goals:

- Help you become comfortable in a laboratory setting (whether you want to end up there or not).
- How to design, carry out, and justify an experimental project.
- Understanding how various CRISPR-based methods work
- Reinforce your understanding of course molecular biological principles
- Learn how to
  - isolate DNA, do PCR, construct plasmids, synthesize RNA
  - Troubleshoot problems
  - how to read and critically evaluate a research article
  - how to communicate your project (and scientific ideas

Before each 10AM Monday class:

- Read assigned reading BEFORE class using the nota bene system
  - now, check that you can get in…
- first assignment, consider how you would answer the genetics questions (communicate with one another, through NB.
  - http://nb.mit.edu
- Dress code: NO open-toed shoes in the lab.

Background

- What is a model organism, why are they used, what are their limits.
    - how many model organisms can you use, what are they used (for what types of questions).

IN THE LAB

Scientists routinely cure brain disorders in mice but not us. A new study helps explain why

By SHARON BEGLEY @sxbegle / AUGUST 21, 2019

Conserved cell types with divergent features in human versus mouse cortex

Ben-Yang Liao and Jianzhi Zhang. 2008. **Null mutations in human and mouse orthologs frequently result in different phenotypes**. PNAS

One-to-one orthologous genes of relatively closely related species are widely assumed to have similar functions and cause similar phenotypes when deleted from the genome. Although this assumption is the foundation of comparative genomics and the basis for the use of model organisms to study human biology and disease, its validity is known only from anecdotes rather than from systematic examination. Comparing documented phenotypes of null mutations in humans and mice, we find that >20% of human essential genes have nonessential mouse orthologs. These changes of gene essentiality appear to be associated with adaptive evolution at the protein-sequence, but not gene-expression, level. Proteins localized to the vacuole, a cellular compartment for waste management, are highly enriched among essentiality-changing genes. It is probable that the evolution of the prolonged life history in humans required enhanced waste management for proper cellular function until the time of reproduction, which rendered these vacuole proteins essential and generated selective pressures for their improvement. If our gene sample represents the entire genome, our results would mean frequent changes of phenotypic effects of one-to-one orthologous genes even between relatively closely related species, a possibility that should be considered in comparative genomic studies and in making cross-species inferences of gene function and phenotypic effect.

What we, that is you, will be doing…

- Learn how to inject fertilized Xenopus eggs with CRISPR-Cas9 targeted against tyrosinase - as both a positive and negative control.
  - Q: what is a negative control experiment
  - Q: what is a positive control experiment
- Design and clone oligos to generate sgRNA for CRISPR-Cas9 mutagenesis and gene inhibition
- Synthesize sgRNA and inject with Cas9 enzyme to mutate your gene of interest
- Carry out genotype and phenotype analysis
- Present your rationale and your results

CRISPR-based methods

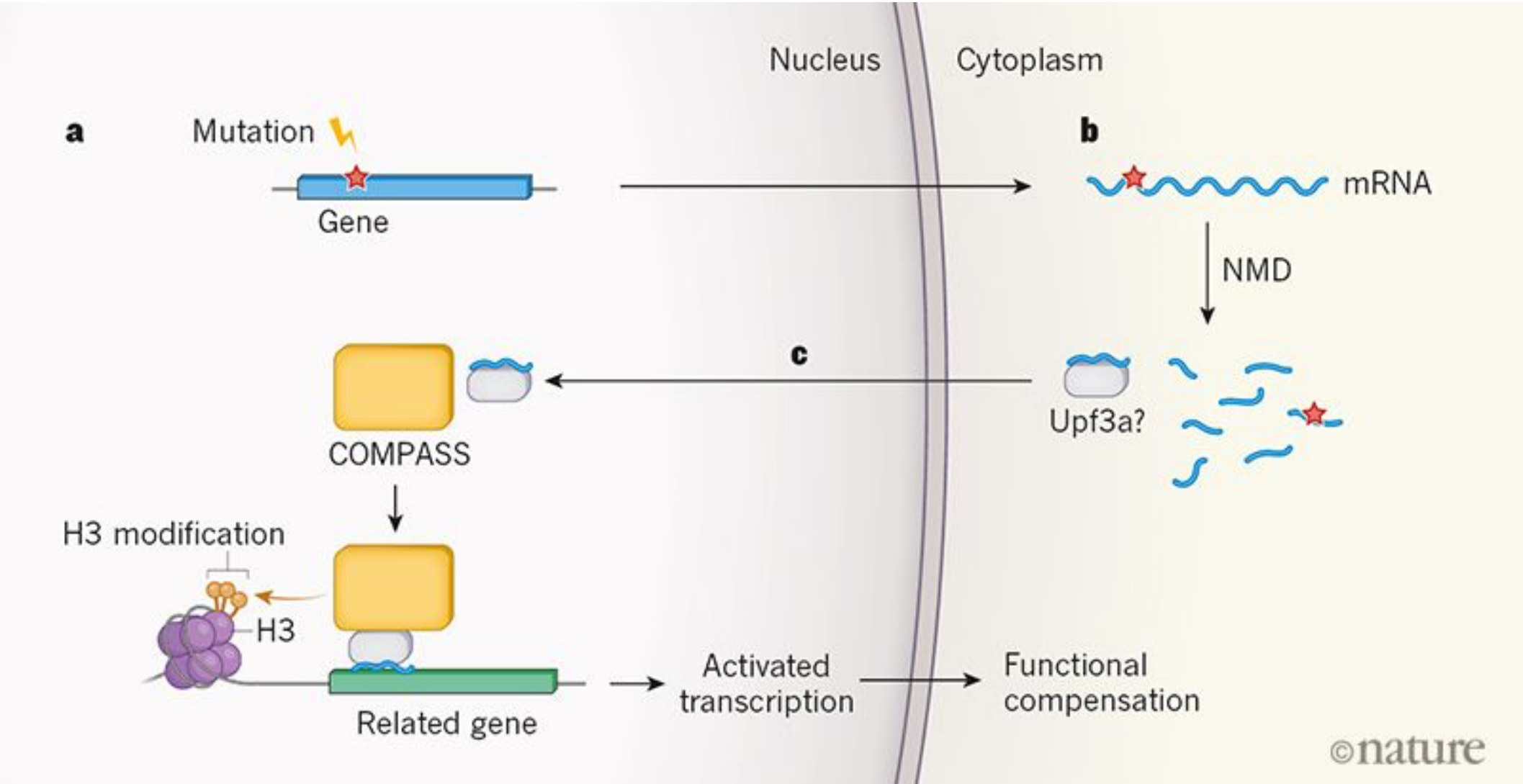
- CRISPR-Cas9 directed mutagenesis
- dCas9-KRAB targeted inhibition of transcription
- CRISPR-Cas9 directed mutagenesis + homologous insertion.
- and even weirder (have your learned about non-sense mediated decay?)

NEWS AND VIEWS · 03 APRIL 2019

Genetic paradox explained by nonsense

Gene mutations that truncate the encoded protein can trigger the expression of related genes. The discovery of this compensatory response changes how we think about genetic studies in humans and model organisms.

Miles F. Wilkinson



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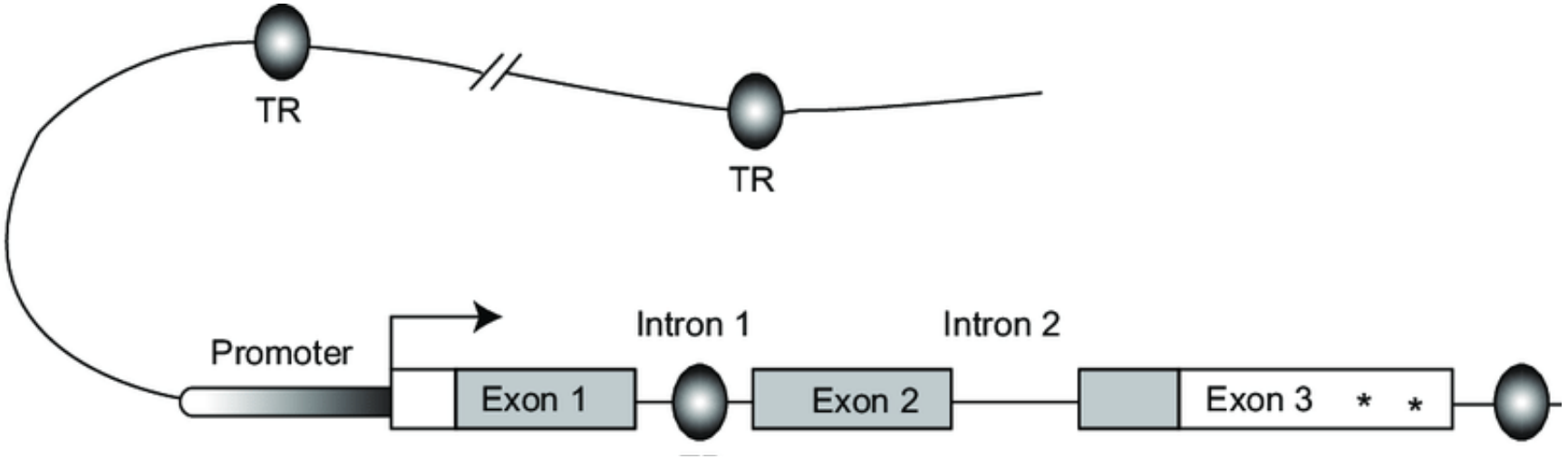
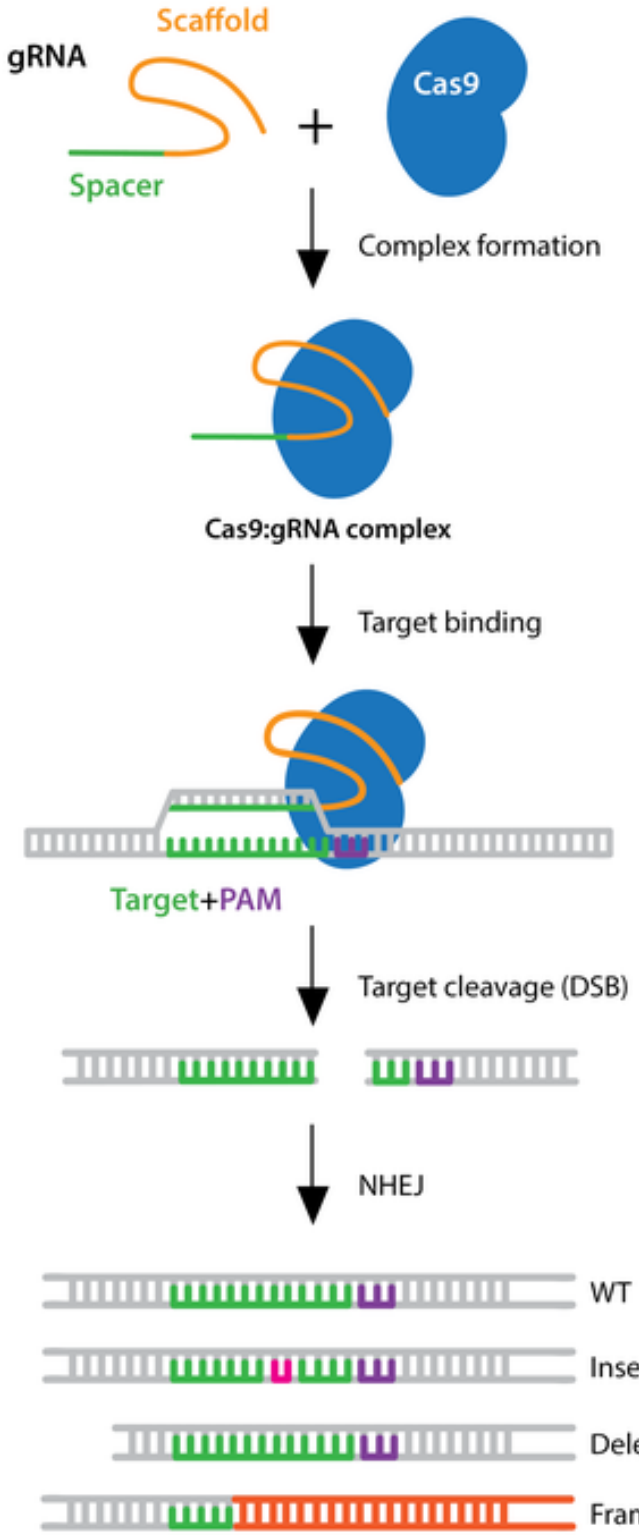
What is a gene?

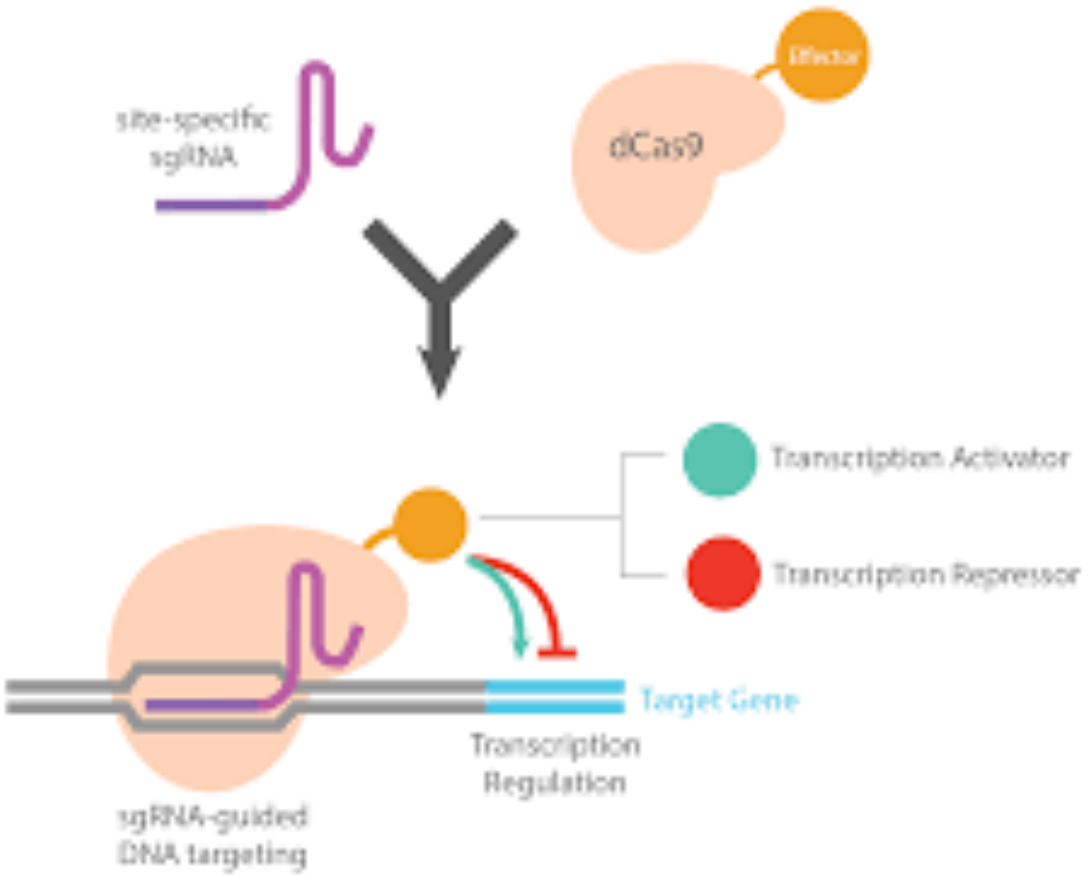


Genetics questions

- **Q1:** You identify an allele of a gene that produces a dominant disease phenotype. Suggest two distinct mechanisms that could produce this outcome. □ no idea how to answer the question.
- **Q2:** You discover a dominant allele of a gene that produces a severe disease phenotype. Later you identify a small subset of people who have the disease-causing allele, but do not have the disease. Provide a plausible explanation for this situation?
- **Q3:** You identify a recessive allele that is present a high frequency in the population. When homozygous, the presence of this allele leads to early childhood death. Suggest a plausible mechanisms that could lead to this situation.
- **Q4:** You identify an allele of a gene that produces a recessive disease phenotype. Suggest two distinct mechanisms that could produce this situation.
- **Q5**: You compare the genomes of humans and other mammals. You discover that one set of sequences, conserved in other mammals, have either been deleted or changed in humans. What process(es) could explain this observation?

What makes a gene interesting (worth) studying?

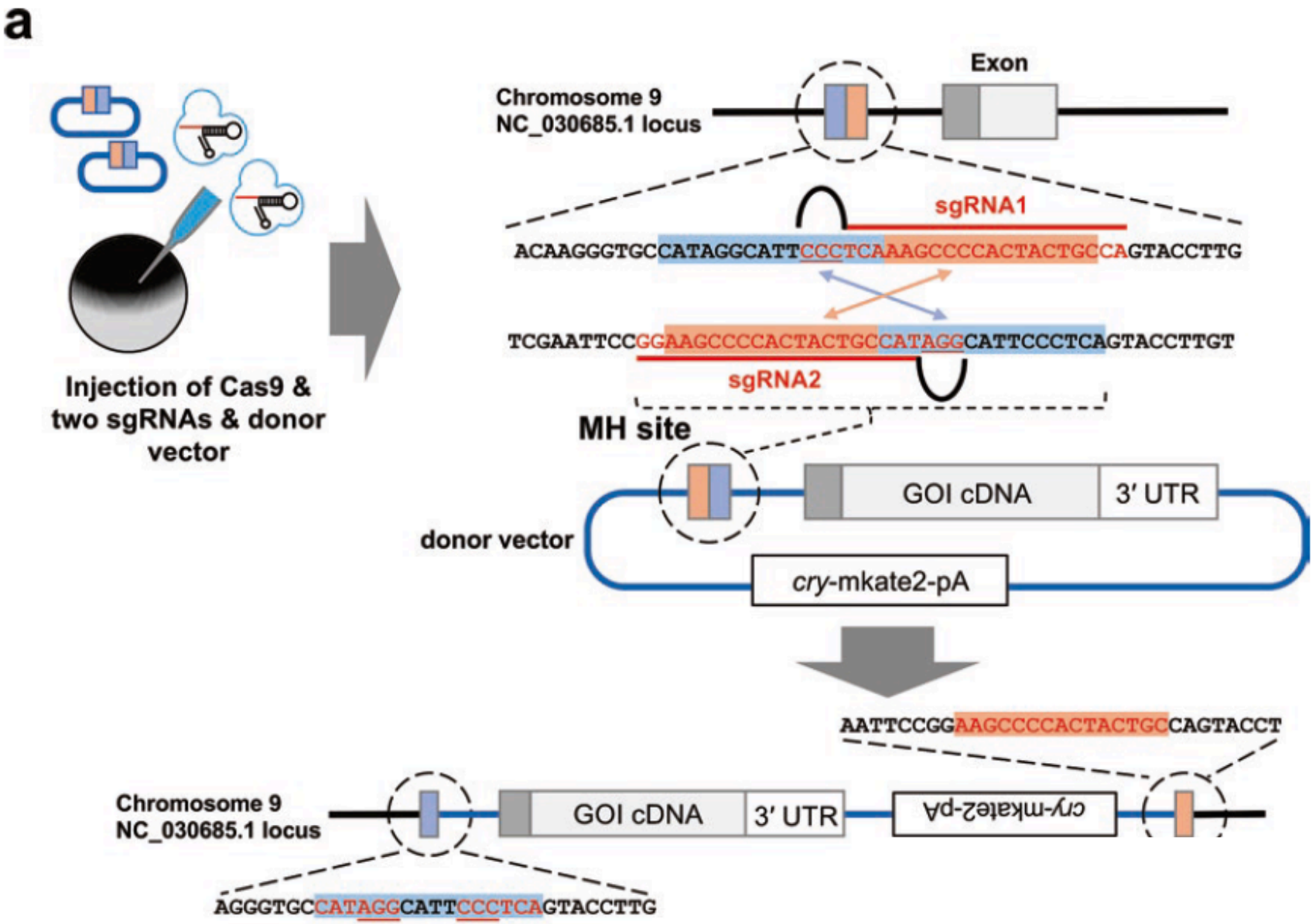




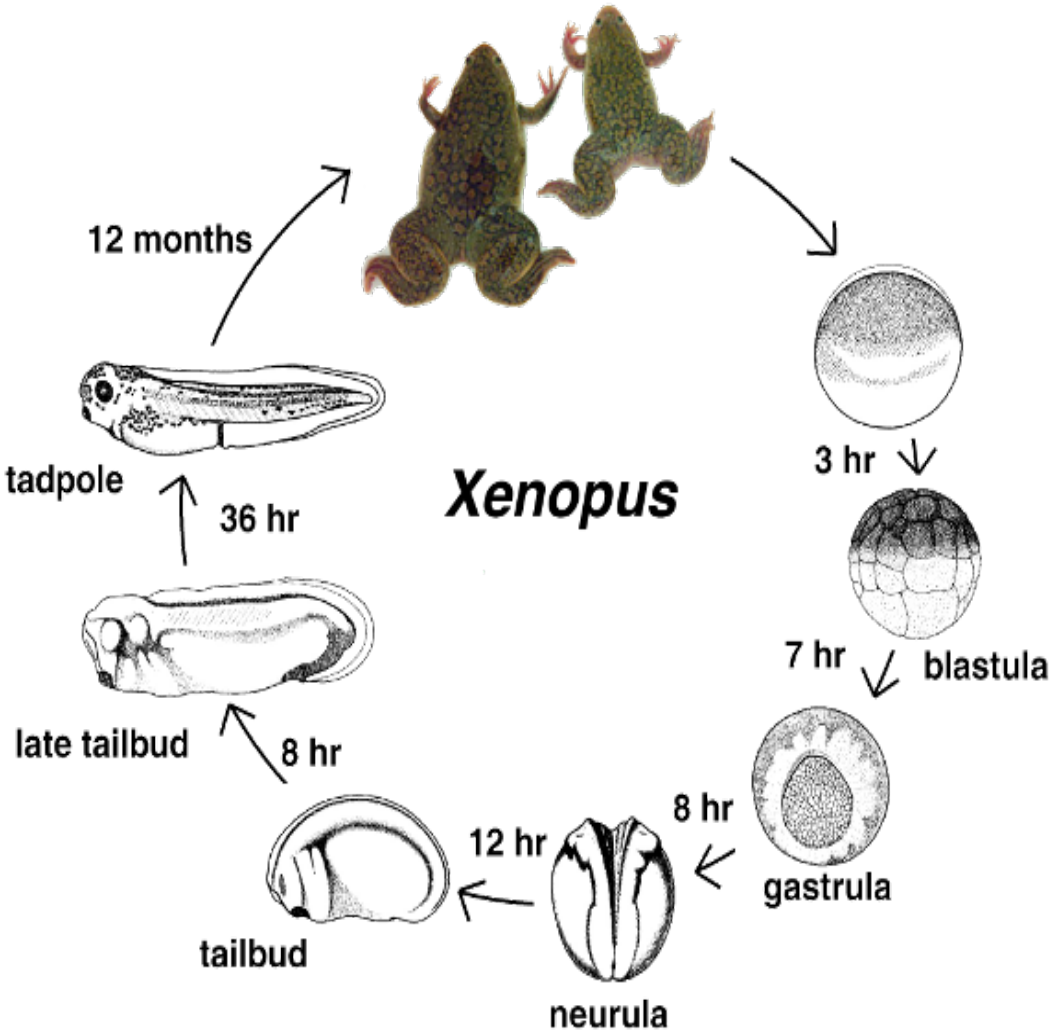
Relative advantages / disadvantages?

A Simple Knock-In System for *Xenopus* via Microhomology Mediated End Joining Repair

Ken-ich T. Suzuki, Yuto Sakane, Miyuki Suzuki, and Takashi Yamamoto



Xenopus laevis (versus tropicalis)



- longer time to sexual maturity
- larger
- allotetraploid
- hardier
- true transgenics not reasonable
- both can use morpholinos

Next: Dr. Birsoy