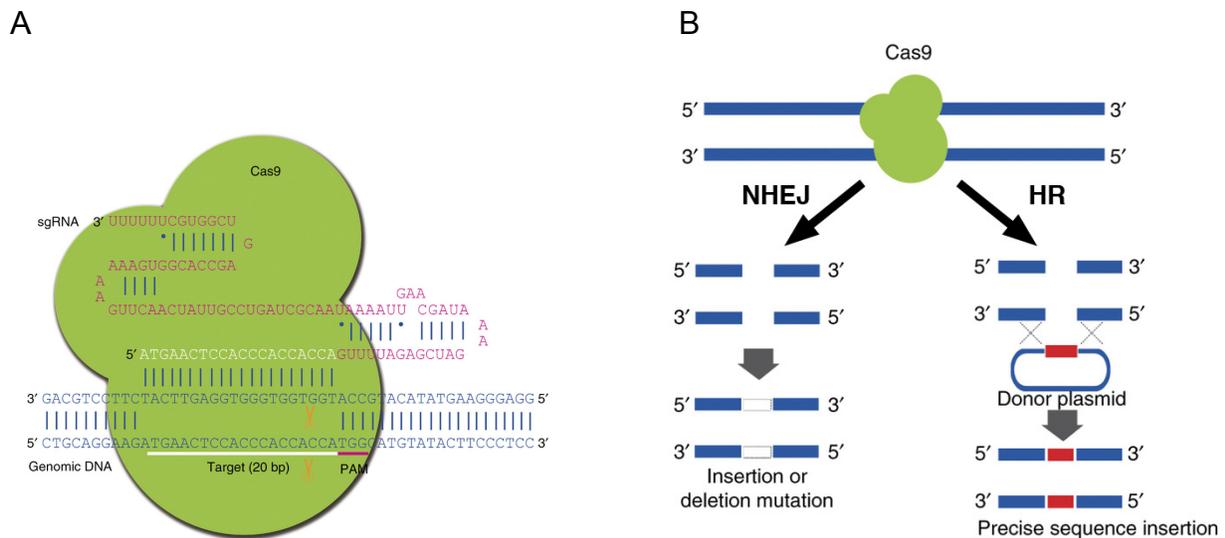


CRISPR/Cas9 genome editing: testing gene function in non-canonical model species

Round Table led by Nipam Patel and Arnaud Martin (UC Berkeley), who have recent experience with CRISPR/Cas9 in an amphipod crustacean and in several lepidopteran species, and Jacob Corn, Director of the Innovative Genomics Initiative, which was established at UC Berkeley to foster the proliferation of genome editing technology.

CRISPR/Cas9 genome editing (abbr. **CRISPR**) is emerging as a technological revolution in the field of functional biology due to its efficiency, universality, and ease-of-use. We also expect the technique to have a deeply transformative impact on the future of evo-devo by allowing experiments in non-conventional species that were unimaginable only a few years ago. This round table will be a forum to share our experiences with CRISPR in a variety of organisms. People interested in adapting the technique to their own needs are encouraged to join, share experiences, and ask for advice from each other.

We will discuss strategies for gene knock-out and gene knock-in while also covering a maximum of technical details that matter at the bench to optimize experiments.

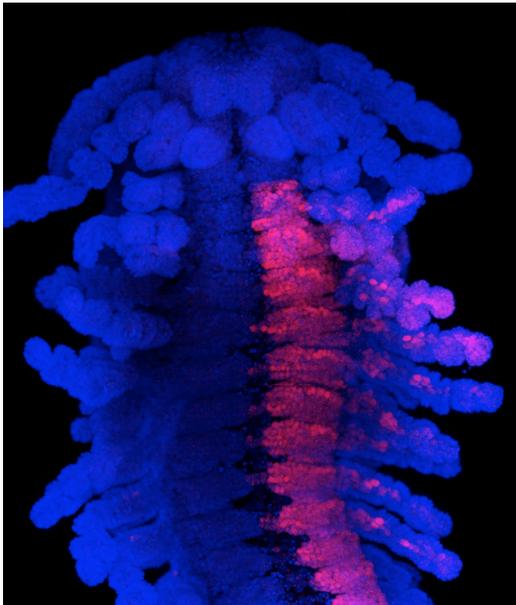


A. The CRISPR/Cas system is composed of a Cas9 nuclease (green) and a synthetic single-guide RNA (sgRNA) that directs the nuclease to the target DNA sequence directly upstream of a requisite adjacent 5'-NGG motif. B. Nuclease-induced double strand breaks (DSBs) can be repaired by two major pathways: nonhomologous end joining (NHEJ) and homologous recombination (HR). NHEJ promotes direct ligation of the DSB ends in an error-prone manner, usually leading to indels (small insertions and deletions). HR requires a homologous sequence, either from a plasmid or from single-stranded oligonucleotides, to serve as a donor template for repair of both broken strands in a high-fidelity manner. Adapted from *Nature Protocols* **9**, 2493–2512 (2014).

1) Gene Knock-Outs

- a) General technical considerations: single guide RNA (sgRNA) design, injections. Is the injection of recombinant Cas9 protein the best choice?
- b) Can **mosaic G₀ CRISPR** (phenotypes from injected embryos) replace RNAi and morpholinos? What are the pros and cons of mosaic CRISPR knockout vs. gene knock-downs?
- c) **How to genotype** injected embryos and screen for mutations? What are the strategies for establishing germline-transmission of mutant alleles?
- d) **targeting non-coding DNA**: how efficient? Possibility to excise hundreds of base pairs (eg. regulatory element) with a pair of sgRNAs?
- e) **tissue-specific** knock-outs: promoter-activated expression of Cas9 or of the guide RNA?
- f) **Controls**. Off target effects. Interpretation of phenotypes, especially in G₀ animals.

A



B



Example of G₀ phenotypes. A. Knockdown of protein expression on one side of a *Parhyale* embryo. B. Hox phenotype on one side of the *Parhyale* embryo. (Martin, Jarvis, and Patel, unpublished)

2) Gene Knock-Ins

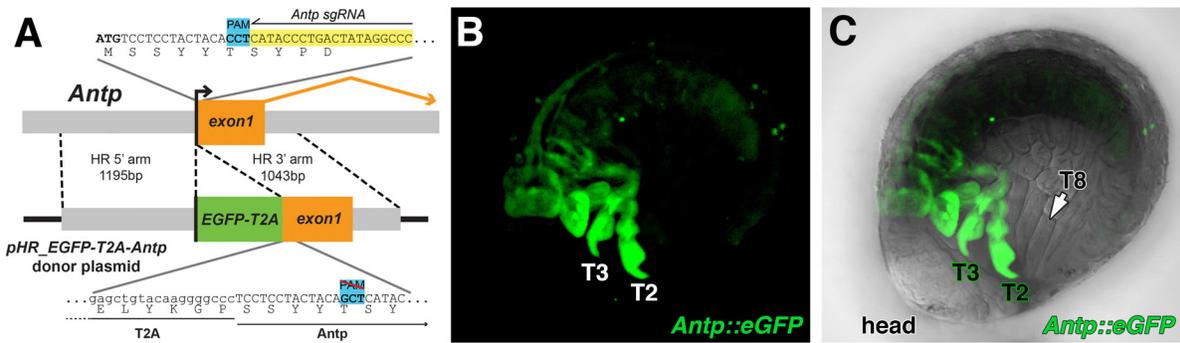
a) **How to bias repair towards HDR?** Several attempts have been made to suppress the NHEJ pathway, but no clear consensus has emerged from the literature.

b) **HR-based Knock-ins:** how short can you go with homology recombination arms?

Paix et al. 2015 report the high-efficiency of HR in the gonads of *C. elegans* using 35bp homologous arms. Does this work in other contexts?

Paix A et al. High Efficiency, Homology-Directed Genome Editing in *Caenorhabditis elegans* Using CRISPR/Cas9 Ribonucleoprotein Complexes. *Genetics*. 2015 Jul 17.

c) HR-based Knock-ins for **fluorescent tagging of proteins**. We illustrate this principle below with the CRISPR-based tagging of the Antennapedia protein, revealing expression in the thoracic claws of *Parhyale hawaiiensis* (Martin and Patel, unpublished). Which fluorescent proteins to use? Is a ribosomal skipping site (2A-type) useful? How to generate donor plasmids efficiently?



d) How many guide RNAs should you inject?

e) HR-based knock-ins: **allele replacement, regulatory element analysis** etc... what are the limits, and the new possibilities opened by CRISPR to address new questions in EvoDevo?

f) **Homology Independent** knock-ins: an interesting route to explore? Auer et al. 2014 shows promising data for the high-efficiency integration of transgenes when precision is not essential

Auer TO et al. Highly efficient CRISPR/Cas9-mediated knock-in in zebrafish by homology-independent DNA repair. *Genome Res*. 2014 Jan;24(1):142-53

