CRISPR: New techniques and best practices

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Best practices

- sgRNA design
- selection vs no-selection
- speed vs. cost for implementation
- huge variability in what works for labs (we still don't understand)
- slides and those of other willing panelists: http://www.jordandward.com/meeting-slides.html

Getting started

- plasmids and RNPs: validate with a good control, like *dpy-10* and *gtbp-1* (RNP) or *klp-12 or dpy-10* (plasmid)

- SapTrap: kit comes with an efficient targeting vector

- many folks grow worms at 25°C, unpublished evidence that Cas9 is more active at 25°C than 20°C

- think hard about what types of experiments you want to perform to choose the best system (number of edits, number of sites, number of genes, lab expertise, etc.)

DNA quality is important

- Invitrogen PureLink miniprep works for many presenters
- Qiagen midis work for some of us
- Adding a DNA precipitation step with Qiagen mini-preps can help
- Avoid DEPC water

sgRNA design

- use both high efficiency/activity and low-off target: SFU sgRNA design site and UCSC genome browser CRISPR track (WS220/Ce10 build)

- for RNPs closer is best (ideally DSB within 10 bp of modification)

- G upstream of PAM is good, GC rich (C upstream is bad?)

- for selection-based approaches DSB can be within a 100 bp window

sgRNA design

- tend to use F+E sgRNA (increased activity)
- a lot of folks use R07E5.16
- *U6* optimal promoter? Open question. Start with robust reagents
- RNPs don’t need U6 promoter or to worry about U6 terminator

To select or not?

- SapTrap very modular system-easy to introduce new fusions, compatible with *unc-119* or SEC-based selection (Dan Dickinson; available soon at AddGene)

- **pre-cut vectors with SapI and purify

- *unc-119* needs mutant background and potentially excising of selection gene but can use short homology arms so no cloning

- SEC can be done in any background, but needs longer (500-700 bp homology arms)

To select or not?

- selection-free method with linear homology donors=cloning-free

- excellent for small insertions, deletions, epitope knocking up to GFP-sized edits (Ward lab has lower efficiency with larger knock-ins)

- used in conjunction with co-conversion (or even fluorescent co-injection markers)

Cost vs speed

- fastest: have pure Cas9, order crRNA and trcRNA, have a purified repair template (oligo or dsDNA) recombineering. Very efficient.

- cost can be offset by making sgRNA, DNA polymerase, and Cas9 in house (happy to share protocols)

- cheaper: plasmids work, are cheap, use techniques common in most molecular biology lab; also good for larger insertions

What's missing?

- dCas9-based approaches to regulate gene expression, mark protein localization
- brighter tags to facilitate visual screening
- develop stable Cas9 lines (good for somatic editing)
- develop high-throughput methods to deliver sgRNAs/RNPs/plasmids
- coordinated optimization from multiple labs?

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Thanks and let’s open it up to the panel for questions!