CRISPR: New techniques and best practices

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Best practices

- sgRNA design
- selection vs no-selection
- speed vs. cost for implementation
- slides and those of other willing panelists: http://www.jordandward.com/meeting-slides.html

sgRNA

- two main U6 promoters used (one from Calarco paper, other from Dickinson paper)
- F+E variant shown to have higher activity
- prediction tools: UCSC genome browser, Guidescan, SapTrap Builder (high activity/low off-target)

DNA quality is important

- Invitrogen PureLink miniprep works for many presenters
    - Add a wash step for improved results: 4 M GDN-HCl + 40% isopropanol solution (just before the ethanol wash)
- Qiagen midis work for some of us
- Adding a DNA precipitation step with Qiagen mini-preps can help
- Avoid DEPC water

Selection-free methods

- Mello lab protocol: fast, cloning free RNP-based method
- hybrid PCR products with ssDNA arms boosts knock-in efficiency (FP-sized cassettes now can be knocked-in)
- can do co-conversion, or use co-injection markers
- DSB should be near editing site

Selection-based system

- stable Cas9 lines from Matt Schwartz have been amazing for Ward lab
- *unc-119* needs mutant background and potentially excising of selection gene but can use short homology arms so no PCR needed
- SEC can be done in any background, but needs longer arms (500-700 bp homology arms); RoI marker useful in crosses
- Gibson cloning a bit more efficient, SapTrap outstanding for making new vectors

Cost vs speed

- fastest: have pure Cas9, order crRNA and trcRNA [or synthego complexed sgRNA], have a purified repair template (oligo or dsDNA). 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

Important recent work

- Farboud et al., 2018: orientation of PAM can affect edits (asymmetric repair); double cut strategy to insert 10 kb sequences (no selection); ben-1 co-conversion marker
- Paix et al. 2016: template switching, recombineering

Transgenes

- Farboud/Meyer: double-cut method
- SKI-LODGE
- SEC-based plasmids that target standard Mos insertion loci (Dan Dickinson) - chrI and II insertion sites available and work well (we should make more!)

What's missing?

- dCas9-based approaches to regulate gene expression, mark protein localization
- inducible somatic editing

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