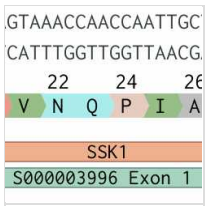

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Designing Knockout Oligonucleotides (Instructor Protocol)

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Yeast ORFans CURE

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ABSTRACT

This is the instructor protocol for *Designing Knockout Oligonucleotides*, linked below.

Designing Knockout Oligonucleotides
by Brian Teague,
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PREVIEW RUN

PROTOCOL CITATION

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Lab Setup

1 This is a "dry" lab - no pipetting involved. Each group of students will need a computer or a laptop.

2 Students will need accounts on Benchling before they dive in to this protocol. I use Benchling to run my entire lab section -- that's where protocols live and student notebooks are kept. I set up Benchling for each semester using this guide:

How to use Benchling for your teaching lab!

3 In particular, it is important that students share their individual projects with the instructor. This is so after they finish this lab, the instructor can find the oligos they created. (I also require my students to keep their notebooks on Benchling, so this facilitates my grading their notebooks as well. Alternately, you can ask them to copy the oligos into a shared folder.)

4 If there are any genes that students should not choose, prepare this list in advance. In our case, students cannot pick any genes that have already been successfully knocked out. (Because many students don't move off of the first search results page, this includes most of those genes.)

5 After the students have completed the lab, coallate all of the oligos (I use Benchling's Search function -- or, as above, ask students to copy into a shared folder.) If the section is large, it's difficult to completely double-check all of the oligos -- but I usually will check the following:

- The "Guide" oligos are 26 bases long and start with "gacttt"
- The "Guide RC" oligos are 26 bases long, start with "aaac" and end with "tt".
- That the variable sequences of the guide and RC oligos are different.
- The URA3 PCR F oligo is 59 bases long and ends with "tagtc"
- The URA3 PCR R oligo is 57 bases long and ends with "attgg"
- The KO PCR oligos are about 22 bp long and have a melting temperature of about 60.

6 Don't forget to order the oligos! If you get them from IDT, they'll be ready to use within a week.

We have labs Tuesday and Thursday - if the oligos are ordered by the end of the business

day on Thursday, they'll usually be in-hand on Tuesday and ready to use.

Instructor Tips and Common Student Errors

7 Instructor Tips

- It helps to be familiar with Benchling -- there is a learning curve!
- This works better if both students have a laptop and both are out -- one open to Benchling, and the other open to the instructions.
- When Benchling has a split workspace, you can "drag" a tab from one side to the other.

8 Common Student Errors

- Not including the 1000 bp upstream and downstream of the gene in the import.
- Forgetting to copy the reverse complement of the guide oligo (and instead copying the forward sequence.)
- Not opening a split window for the CRISPR target selection.

You need a split window open, with the sequence view on one side and the CRISPR tool on the other, for the target "flags" to show up.