7.003 Spring 2022
Day 7 In-Lab Questions

1) Why are you transforming your mutant strains with the pRSQ2-URA3 plasmid today?

2) Draw a diagram of what the insertion site will look like after the BamHI-digested pRSQ2-URA3 plasmid integrates into the yeast genome of your α-factor-resistant mutants.

3) For this question, you will use SnapGene to visualize how the pRSQ2-URA3 plasmid will integrate into the mTn3 transposon by finding the homologous sequences (i.e. where the pRSQ2-URA3 plasmid will homologously recombine with the mTn3 transposon in the yeast genome of your mutants). You can download the free version of SnapGene in the Lab Resources section.

Download the mTn3.dna file and the pRSQ2-URA3 plasmid.dna file from Lab Day 7 and open both files in SnapGene. The “Map” and “Sequence” tabs at the bottom of the SnapGene screen will let you toggle between viewing an overall diagram of each DNA construct and a close-up view of the actual base-pair sequence of that DNA construct. Key features or elements will be highlighted.

To view how the *lacZ* gene portions on pRSQ2 match up with the *lacZ* gene in the mTn3 transposon, you can use SnapGene to perform a sequence alignment. On the Sequence view of the pRSQ2-URA3 Plasmid, highlight the first 619 bp of the plasmid – this selection should include the BamHI site and the entire beginning portion of the *lacZ* gene (black arrow feature) on the plasmid. Copy this sequence selection (e.g. Ctrl+C, etc).

On the Map view of the mTn3 Transposon, click the “Show alignments”  button in the lefthand navigation panel. Choose the “Align copied sequence” option and click “Align.” The Map view diagram should now show an arrow corresponding to the sequence you copied from pRSQ2 and indicating where it aligns with the mTn3 transposon sequence.

Go back to the Sequence view of the pRSQ2-URA3 Plasmid, and now highlight the last 623 bp of the plasmid – this selection should include just the entire end portion of the *lacZ* gene (gray arrow feature) on the plasmid. Copy this sequence selection.

Go to the Map view of the mTn3 Transposon again, and click the “Aligned sequences” menu (near the bottom of the screen). Go to “Align copied sequence…” again. You should now see a second arrow alignment on the Map view diagram corresponding to the new sequence you just copied from pRSQ2.

Go to the Sequence view of the mTn3 file to see the actual basepair alignments between the mTn3 sequence and the two different pRSQ2 *lacZ* sequences. (Note: you can click each pRSQ2 sequence listed in the peach box in the bottom alignment panel to show the sequence details.) Make a note of the basepair positions within the mTn3 transposon sequence that match up to where the pRSQ2 *lacZ* sequences align.

Create a SnapGene DNA construct file that represents the mTn3 transposon after the BamHI-linearized pRSQ2 plasmid has been integrated. One way to do this is to use the mTn3 file as a starting template and replace the desired bases from mTn3 with the pRSQ2 plasmid sequence (you can use the “Edit” menu at the top of the screen to delete or insert bases). Another way is to create an entirely new DNA construct from scratch (go to “Design synthetic construct…” under the “Tools” menu at the top of the screen) and copy together the desired DNA sequence fragments in the correct order (use the “Insert Bases” button) to generate the final construct. Save your final mTn3 + pRSQ2 construct under a new filename (e.g. “mTn3 plus pRSQ2”).

A) How many basepairs long is the entire mTn3 + pRSQ2 construct?

The integration of pRSQ2 into mTn3 may require you to alter the annotations of either the beginning *lacZ* gene sequence (black arrow feature) and/or the end *lacZ* gene sequence (gray arrow feature) in the file. You can select a feature on either the Map or Sequence view and right-click to go to “Edit feature...” In the Edit Feature window, you can adjust the basepair locations corresponding to that feature to shorten or lengthen it. If you change the basepair locations of one of the *lacZ* features, you should also adjust the corresponding gene sequence information for that feature – in the drop-down menu of the Edit Feature window, select “/note” and update the matching *lacZ* gene coordinates accordingly.

B) In the final mTn3 + pRSQ2 construct, how many basepairs long is the entire beginning portion of the *lacZ* gene sequence? What basepair positions of the full-length *lacZ* gene does this fragment correspond to?

C) In the final mTn3 + pRSQ2 construct, how many basepairs long is the entire end portion of the *lacZ* gene sequence? What basepair positions of the full-length *lacZ* gene does this fragment correspond to?

Make sure you save your final mTn3 + pRSQ2 construct – you will use this SnapGene .dna file for future ILQs!

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