**Protocol for aligning sequencing results to reference sequence, Ab31375**

Watch this tutorial first: **https://benchling.com/tutorials/29/aligning-to-a-template**

1. Download and import Ab31375 in benchling: **https://benchling.com/tutorials/10/importing-dna**
   1. Click the '+' button on the blue panel on the far left.
   2. Choose DNA sequence then 'New DNA seq'
   3. Choose the first tab called 'Convert files' in the pop-up window.
   4. Drag and drop or choose Ab31375 file.
2. Download sequencing results from the class data tab and import to benchling:
   1. On the far-right panel click the alignment icon (looks like 4 stacked lines.)
   2. Choose file(s)
   3. Choose .ab1 files for one clone (either 2 or 5.)
3. Find gaps or mismatches:
   1. Use the right arrow next to 'Find Mismatches' to scan to the first inconsistency.
   2. You can also use the highlight function in the panel at the bottom of the screen with the representation of your DNA alignment. This panel is the easiest way to zoom in and zoom out of a particular sequence area.
4. Evaluate the sequencing result:
   1. Using the sequence or trace file from our sequencing result decide whether a mismatch or gap is a mutation or an unreliable sequencing result.
   2. Toggle sequence and trace file views using the dropdown menu next to the sequence file name. The icon is a grey arrow.
      1. Remember distinguishable sequence readout typically begins about 40-50 bases downstream of the primer binding site. It's common to have N included where sequencing results cannot clearly identify a base.
      2. To determine if the mismatch you see is an unidentified base or mutation use your best judgment comparing the trace profiles of both the forward and reverse sequence.
5. Note the location of all gaps or mismatches in the sequence in your benchling notebook.
   1. Relevant location information includes the bp number in the original sequence (not sequencing result) and the gene annotation associated with the DNA sequence, example Heavy chain.
6. Determine if DNA sequence change(s) result in protein sequence change.
   1. Create a translation of your region of interest. Make sure you start the translation in frame! The easiest way to do this is to start the translation at the start of an annotated gene, example heavy chain.
   2. Drag the highlighted region between the black lines to encompass the region you would like to translate.
   3. Right click the highlighted region and choose 'create translation' then 'forward.'
   4. Repeat step c for the template and sequencing results if necessary.
7. If mutation in the DNA sequence result in changes to the amino acid sequence, jump to set #12 in Part 2 on the wiki.