

Parts Page (for an ADH Part)

Overview

This protocol covers the creation of a parts page for an ADH part.

Materials

- Benchling
- Dropbox
- APE or Snapgene

Procedures

1. Pick a part in 3G_Parts.xlsx: Dropbox > Files > iGEM 2018 > Inventory > 3G Parts.xlsx. The first column lists WM ID's and the second column lists ADH parts names.
2. Create a blank page document: Benchling > Projects > iGEM 2018 > Inventory (as opposed to Notebook) > 3G Parts. Open the folder that corresponds to the type of part chosen (5' UTR, CDS, Promoters, or Terminators). Click the "+" sign and select "DNA sequence." Enter the WM ID for the part (e.g. WM18_BC_001), then select "linear."
3. Begin on the right side of the screen under "Description." To easily achieve correct format, copy the text from a confirmed parts page and paste it into the new document. Delete all information except for the headers (the WM ID for the part, the ADH part name beneath that, then "Review Log," "Update Log," "Description," "Components," and "Sequence"). Modify the WM ID and ADH part name as needed to match the new part. To achieve correct format manually:
 - a. Write the WM ID for the part in bolded "Header 1" font
 - b. Write the ADH part name beneath that in paragraph font
 - c. Write the rest of the headers in bolded and underlined "paragraph" font
 - d. Beneath review and update logs, create a bullet point list.
4. Leave a blank bullet point beneath "Review Log." Whoever reviews the parts page will enter information here.
5. After the first bullet point beneath "Update Log," write "[date] [initials] created file"
6. Under "Description," write the ADH part name (*not* the well. E.g. "BCD6" instead of "B15") and the type of part (e.g. 5' UTR). If possible, find additional information through other sources (Google).
7. Moving onto "Components," copy the ADH part name from 3G_Parts.xlsx. Search for the part name in "Andy's Plate.xlsx": Dropbox > Files > iGEM 2018 > Inventory > Andy's Plate.xlsx. This document will show (again) the ADH part name, the ADH well it corresponds to, and the key name to find the sequence.
8. Go to the folder "ADH_Plate_Sequences" (Dropbox > Files > iGEM 2018 > Inventory > ADH_Plate_Sequences) then pick the folder for the type of part you have (e.g. CDS, Promoters, etc.) In that folder will be a list of sequences, all keyed. Pick the key name that corresponds to the part are making a page for (for example, the UTR BCD6 was keyed as "U22m"). To open this sequence, you will need either APE or Snapgene.

9. Figure out which primers are used with the part. You can do this by searching task lists for PCR instructions, which often state which primers anneal to which template, but this can be inefficient (and the information may not even be on a task list yet). Ideally, you can figure out which primers anneal to the *type* of part you are researching (for example, P023 and P024 anneal to WM18_BC parts). So, if you cannot find the primers for your specific part, look at a related part on the task lists, or scroll through primer parts pages and read the descriptions (which will state which types of parts they anneal to). Once you have an idea which primers anneal to your part, confirm by copying the annealing bp (the last 15 or so bp under “sequence” on the primer’s parts page, usually typed in different font) and searching for them within the sequence on APE/Snapgene. If you have found the correct two primers, the annealing bp will show up within the sequence. Copy everything on the primers’ parts pages, excluding Biobrick prefix/suffix, and paste under the components header.
10. Afterwards, return to APE/Snapgene and copy the bp between the sticky ends (search the primers’ annealing bp again and copy the bp in between. *Not* the “long part.” Remember that the sequence downloaded from ADH_Plate_Sequences is circular and thus the “end” of the sequence at the bottom feeds into the “start” of the sequence at the top. So, there are two regions that could be considered “between” the sticky ends. Pick the shorter one). Title the bp with your parts name and paste under the components header, between the sticky ends. Character count (all components should have character count written in parentheses).
11. After all components have been pasted, write the completed sequence at the bottom of the page under the header “Sequence.” To do so, copy each component and paste in order, making sure different parts are distinguishable (different highlights or fonts, etc.). Character count, then paste under “sequence map” on the left side of the screen.
12. Click on the bookmark icon at the right side of the screen to annotate the sequence map. Search all libraries > auto-annotate. If a component is not in the libraries, edit feature libraries as “wmigem” (not your Benchling name) and open existing feature library “iGEM 2018” (by Ethan801). Fill in the blanks with your component’s information.