**Description:**

This protocol aims to describe the steps involved in designing/generating a novel library.

**Materials:**

1. List of gene IDs to be designed for
2. Species of interest
3. Type of gRNAs to be designed
4. Number of gRNAs per gene

**Methods:**

1. Open SRM2, Navigate to CRISPR Library services tab in LIMS.
2. Select the SRM submission you plan to work on, and check in to the worklog template as specified in the “Project Scope”.
3. Fill out a new row in Completed Projects (CAGE).xlsx file to reflect the new library.
4. Create a subfolder in the path below to reflect the lib name and research group.
   1. Z:\ResearchHome\Groups\millergrp\projects\CrisprDesigns\common\screens
   2. i.e. “lib90\_mulligrp”
5. Create a subfolder in your library folder called “design”
6. Connect to the cluster and enter an interactive node by running “hpcf\_interactive”
7. Navigate to the design folder you just created.
8. Run the following command:
   1. cp -r ~/.libProgs/generate\_a\_library/offinder\_design/\* ./
9. Prepare the gene IDs for submission to CRISPick.
   1. Check the submitted list of gene IDs.
      1. Make sure there is a gene ID for every requested gene.
      2. If there is not a gene ID for some genes, copy the gene symbol over into gene ID column.
      3. Make sure there is no “h” or “m” before the gene name (species identifier)
   2. Make a new file and copy the gene ID column over.
   3. Ensure there is no column header.
   4. Remove duplicates (“Data” tab in Excel). Should only be a few.
      1. If there are many duplicates removed, double-check that the new list is okay with requester before continuing.
   5. Save the new file as a TSV (Text, Tab-Separated).
      1. If there is more than 500 IDs, split into multiple files with a maximum of 500 in each file.
10. Open CRISPick: <https://portals.broadinstitute.org/gppx/crispick/public>
    1. Open one tab for each file created in step 5.
11. Choose a Reference Genome (NCBI RefSeq) according to the species requested on SRM2.
12. Choose a Mechanism according to what was requested on SRM2.
13. Choose an Enzyme according to the SRM2 request.
    1. If using SpCas9, choose the “Hsu (2013)” tracrRNA option.
14. Select the “Upload file” option under “Target(s).”
15. Drag/Drop or Select the appropriate TSV file from Step 5.
16. Check ‘Library Mode’
    1. Enter the number of No-Site Controls (NTCs)
    2. Enter 0 for intergenic controls if non are requested
    3. Enter 10 for positive controls if library is human
17. Change the CRISPick Quota to 30 and ensure “Report unpicked sequences?” is UNCHECKED.
18. Click “Validate.” This will take you to a landing page for gRNA generation.
    1. Hit Submit
    2. This will move to a page with the Job ID and Status
    3. Files should end in “-sgrna-designs.txt”
19. Download the ‘sgnra-design.txt’ files generated by CRISPick into the “broad” folder on the cluster.
20. Move to the design folder(In terminal)
21. If multiple files were created in Step 9:
    1. \*\*\*\*finish up functionality in design script
22. If only one file was created in Step 5:
    1. Run the following command:
       1. cp \*-sgrna-designs.txt ../sgrna-designs.txt
23. Move to the design directory (cd ..)
24. Run the following commands:
    1. module load cas-offinder/2.4.1-rhel8
    2. The following are options for the design\_library script
       1. -i name of input file
       2. -l name of library
       3. -design enter ‘crispick’ or ‘guidelist’ depending on workflow
       4. -s species ‘h’ or ‘m’
       5. -q guides per gene quota
       6. -cas Cas type 9 or 12
    3. Enter the following command for a Crispick library design
       1. bash create\_library.sh -design crispick -i sgrna-designs.txt -l lib1234 -s h -q 6 -cas 9
       2. Change -i, -l, -s, -q and -cas accordingly
    4. Enter the following command for an end user specified guide list
       1. Python design\_library.pay -design guidelist -I guidelist.txt -l testlib -s h -q 6 -cas 9
       2. Change -i, -l, -s, -q and -cas accordingly
25. This will create two .xlsx files. An all\_guides and a draft.
    1. The all\_guides file will have all the guides generated by Crispick
    2. The draft file will have the guides with the best OTA scores for each gene. The number of guides depends on the -q parameter from the above step
26. Open the “…\_draft.xlsx” file and highlight any long\_0 scores above 1.
27. Send an email to the requester to ask for approval of the draft.
    1. Attach the “[LIB]\_draft.xlsx” file
28. Await approval from the requester.
29. Make any changes requested.
    1. Delete rows for any gRNAs to be removed.
    2. If any additional genes were requested:
       1. For Crispick designs
          1. Generate another sgrna-designs.txt file from Crispick as described above and rerun the library\_design.py script
       2. Create a new input guide template file and rerun the library\_design.py script
30. Open the following script:
    1. Z:\ResearchHome\Groups\millergrp\projects\CrisprDesigns\common\screens\programs\generate\_a\_library\tools\add\_cloning\_extensions\_offinder.ipynb
31. Change the LIB\_NAME and GRP\_NAME variables.
32. Change the DESIRED\_PRIMER to reflect which primers you wish to use for cloning.
    1. This is based on:
       1. The “primer\_set\_name” column
       2. From the “extension\_for\_TWIST” sheet
       3. In the “lib\_vectors\_and\_primers.xlsx” spreadsheet
       4. Found at:
          1. Z:\ResearchHome\Groups\millergrp\home\common\Screens
33. Click “Run All” at the top.
34. Scroll through to make sure no errors occurred.
35. Send an email to the requester stating that the draft is complete and ready to be ordered from Twist and that they should receive “a discounted quote shortly.”
36. Go to Twist’s website: <https://www.twistbioscience.com>
37. Log in with [shondra.miller@stjude.org](mailto:shondra.miller@stjude.org)
38. Click “Oligo Pools” and enter the library name to name the order draft.
39. Click “STEP 1 Upload your sequences”
40. Drag/Drop or Choose your “…\_twist.tsv” file.
41. Wait for it to upload, then click the arrow on the left side.
    1. Ensure all oligos are the same length (probably 77bp)
42. Click Continue in the bottom-right.
43. Click “Request Quote” on the right side.
44. Add the following emails to the “SHARED WITH” in the middle-right:
    1. Requester’s email
    2. PI’s email
    3. Patrick’s email
    4. Your email
    5. (Plus anyone else you’ve been corresponding with about this library)
45. Ensure the default address is checked.
46. Click “Generate Quote” in the bottom-right