**Design a Library via CRISPick**

1. Create library folder in the ‘screens’ directory
2. Create a folder named in design in the library folder created in step 1
3. Enter the following command:

cp ~/.libProgs/offinder\_design/\* ./

1. Enter the appropriate information into CRISPick.
   1. Use library mode
   2. Enter 0 for no site and intergenic controls.
   3. Enter 10 for positive controls
2. Copy sgrna-designs.txt file to the design folder
   1. If multiple sgrna-designs.txt files created give each a unique name and copy to the ‘merge\_folder’ that was copied into the design folder
   2. Enter the following command and follow prompts

bash create\_library -f merge\_crispick

* 1. Check to be sure a merged sgrna-designs.txt file will be created in the designs folder.

1. Run the following command with your desired parameters. Be sure to include the \ and spaces when pasting into cluster

bash create\_library.sh \

-l {library name} \

-i {sgrna-design file name} \

-d crispick \

-s {species} \

-q {guide quota} \

-c {cas type} \

-n {number of NTCs if percentage add '%' ie 10 percent = 10%; 10 guides = 10, input 0 if no NTCs wanted}

\* If cas-offinder is taking a while, look for errors in the offinder\_errror\_log file in the designs folder

1. The following files will be created:
   1. Library\_name\_all\_guides
   2. Library\_name\_draft
   3. Library\_name\_guide\_counts
   4. Library\_name\_mageck

**Design a library from a list of guides**

1. Create library folder in the ‘screens’ directory
2. Create a folder named in design in the library folder created in step 1
3. Enter the following command:

cp ~/.libProgs/offinder\_design/\* ./

1. Copy guide and sequence information into the guide\_list\_template.xlsx file. Be sure to include the PAM (NGG, TTTV). Leave off any guide number (SHH.g1 🡪 SHH).
2. Run the following command with your desired parameters. Be sure to include the \ and spaces when pasting into cluster

bash create\_library.sh \

-l {library name} \

-i {guide list file name} \

-d list \

-s {species} \

-c {cas type} \

-n {number of NTCs if percentage add '%' ie 10 percent = 10%; 10 guides = 10, input 0 if no NTCs wanted}

\* If cas-offinder is taking a while, look for errors in the offinder\_errror\_log file in the designs folder

1. The following files will be created:
   1. Library\_name\_all\_guides
   2. Library\_name\_draft
   3. Library\_name\_guide\_counts
   4. Library\_name\_mageck

Auxillary commands are on the next page

**Add Twist Adapters**

1. Run the following command and follow the prompts \*\*Will update vector/primer naming:

bash create\_library.sh -f twist

**Merge Library Drafts:**

Use this to combine drafts created from a guide list and CRISPick files.

1. Run the following command and follow prompts:

bash create\_library.sh -f merge\_drafts

1. After merging the drafts you will be prompted to update the mageck file

**Update Mageck File**

1. Run the following command and follow prompts

bash create\_library.sh -f update\_mageck

**Merge CRISpick Design Files:**

Use this to combine multiple CRISPick sgrna-designs.txt files.

1. Move all sgrna-designs.txt files to the ‘merge\_folder’
2. Run the following command and follow prompts:

bash create\_library.sh -f merge\_crispick