Exorcism of Brunello.

This tutorial will guide you through the reannotation of the Brunello library and CRISPR-Cas9 screen experiments that used the Brunello library using exorcise.

Contents:

* Install exorcise.
* Exorcise Brunello library.
* Exorcise Brunello read counts.

Install exorcise.

This section assumes that you have [Anaconda](https://www.anaconda.com/download) and [git](https://git-scm.com/book/en/v2/Getting-Started-Installing-Git) installed. If not, install those first using the appropriate guide for your system.

Aims:

* Download exorcise.
* Install exorcise binaries.
* Install exorcise conda environment.

1. Navigate to where you’d like to install exorcise (change the parts in yellow):

cd /path/to/install/

1. Then clone the repo:

git clone <https://github.com/SimonLammmm/exorcise.git>

1. Make the binaries executable:

chmod 755 -R exorcise/bin/\*

1. Add the binaries folder to your PATH and install to your zshrc (as appropriate):

echo "PATH=$PATH:/path/to/install/exorcise/bin" >> ~/.zshrc

1. Reload your zshrc:

source ~/.zshrc

1. Install the exorcise conda environment:

conda env create -f env/exorcise.yaml

1. Activate your new environment and verify that exorcise is installed:

conda activate exorcise

exorcise --help

Exorcise Brunello library.

In this section, we will obtain the Brunello library guides and use exorcise to verify them against our specified genome and exome.

Aims:

* Obtain Brunello library.
* Obtain GRCh38 genome assembly.
* Obtain GRCh38 RefSeq exome.
* Obtain GRCh38 feature priorities.
* Exorcise Brunello library.

1. Download Brunello library broadgpp-brunello-library-contents.txt from [Addgene](https://www.addgene.org/pooled-library/broadgpp-human-knockout-brunello/). Scroll down to Depositor Data to find the download link.
2. Download genome hg38.2bit from [UCSC Genome Browser Downloads](https://hgdownload.soe.ucsc.edu/downloads.html). Under "Human genomes", "Dec. 2013 (GRCh38/hg38)", click "Standard genome sequence files and select annotations (2bit, GTF, GC-content, etc)" and then scroll down and click to download the file in the directory listing that shows.
3. Download exome hsa.grch38.refseqall.gz from [UCSC Table Browser](https://genome.ucsc.edu/cgi-bin/hgTables). In the Table Browser, specify the settings:

|  |  |
| --- | --- |
| clade | Mammal |
| genome | Human |
| assembly | Dec. 2013 (GRCh38/hg38) |
| group | Genes and Gene Predictions |
| track | NCBI RefSeq |
| table | RefSeq All (ncbiRefSeq) |
| region | genome |
| identifiers (names/accessions) | Leave default |
| filter | Leave default |
| subtrack merge | Leave default |
| intersection | Leave default |
| output format | selected fields and primary and related tables |
| output filename | hsa.grch38.refseqall |
| output field separator | tsv (tab-separated) |
| file type returned | gzip compressed |

Click "get output". In the page that appears, select the following fields by clicking the checkboxes:

* chrom
* strand
* exonStarts
* exonEnds
* name2

And then click "get output".

1. Find the feature priorities list hg38.priorities.csv.gz in the exorcise repo at exorcise/data.
2. Run exorcise.

exorcise -i broadgpp-brunello-library-contents.txt -g 7 -n 2 -z NGG -c ﻿Non-Targeting -v hg38.2bit -w hsa.grch38.refseqall.gz -y hg38.priorities.csv.gz -o brunello-library

1. Be patient as this command takes a while to run.

Exorcise Brunello read counts.

In this section, we will reannotate read counts from a CRISPR screen using the reannotated Brunello library we made in the previous section.

1. Download 41467\_2020\_14620\_MOESM4\_ESM.xlsx from Supplementary Data 1 in [DeWeirdt et al, 2020](https://www.nature.com/articles/s41467-020-14620-6#Sec28).
2. Open the file in Excel, navigate to the sheet "Brunello", combine the four header rows into a single header row in any way you like. Then, export only the "Brunello" sheet as deweirdt-brunello.txt in tab-delimited text (.txt) format. Ignore all the warnings.
3. Run exorcise.

exorcise -i deweirdt-brunello.txt -g 1 -l brunello-library/exorcise.tsv -o brunello-counts