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. Download gene annotations from [NCBI Datasets](https://www.ncbi.nlm.nih.gov/datasets/gene/). Type Human in the Taxon field and leave the Gene symbol(s) field blank. Hit Search. In Select columns, tick Symbol and Gene type, then Apply. Select all the genes, click Download, and then Download Table. Save the file as hsa.priorities.tsv. This file will be used as the feature priorities list.
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 hsa.priorities.tsv -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