zhoujj2013 / **Tag-seq**

Data analysis pipeline for Tag-seq.

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bin	v1.1	11 hours ago
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global.png	v1.1	11 hours ago
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python.package.requirement.txt	v1.1	11 hours ago
sites.png	v1.1	11 hours ago
stat.txt	v1.1	11 hours ago

README.md

Tag-seq

Data analysis pipeline for Tag-seq.

System requirements

Tag-seq runs under the Linux (i.e., Centos, see also https://www.centos.org/ for further details) on a 64-bit machine with at least 32 GB RAM.

Tag-seq requires PERL v5, R, Python 2.7, pip and several python packages listed in python.package.requirement.txt.;

Tag-seq also requires some third-party packages:

STAR aligner
FASTQC
AdapterRemoval
BEDTOOLS
SAMTOOLS
PICARD
umi_tools
bedops
water in EMBOSS
RIdeogram

Tag-seq have been tested in CentOS release 7.4 (Linux OS 64 bit).

Installation

Get Tag-seq pipeline

```
git clone https://github.com/zhoujj2013/Tag-seq.git --depth 1
```

Preparation

Download reference genome and build index.

```
# download genome
mkdir hg19
cd hg19
wget http://hgdownload.soe.ucsc.edu/goldenPath/hg19/bigZips/hg19.fa.gz
wget http://hgdownload.cse.ucsc.edu/goldenpath/hg19/bigZips/hg19.chrom.sizes
gunzip hg19.fa.gz

# build genome index
/path_to/STAR  --runMode genomeGenerate --genomeDir ./ --genomeFastaFiles ./hg19.fa --runThreadN 32
```

Run test

If you have obtained the reference genome, STAR index, you can run test to examine whether the package works well (the test dataset is placed in ./test directory within Tag-seq).

Tag-seq requires a configure file containing paths of input files, sgRNA, Tag primers and genome etc. (See config.TEST.txt for more details.)

```
cd test

# run
sh work.sh

# around 30 mins.
# you can check the report in out.XXX/find.target/.
```

Result

1. QC statistics

you can check stat.txt.

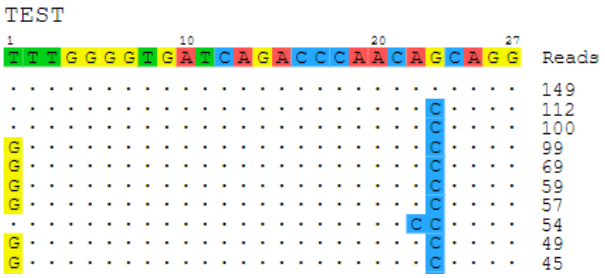
2. Information of potential targets in bed format

```
chr1    10111   10112   AAVS1.E_minus_minus_2_9,AAVS1.E_plus_minus_1_13 0       29      0       12
chr1    55903742        55903743        AAVS1.E_minus_minus_3669_8,AAVS1.E_plus_minus_5324_6    0       11      0
17
chr1    68164302        68164303        AAVS1.E_minus_minus_4802_6,AAVS1.E_plus_plus_6944_6     8       0       0
7
chr1    111700139       111700140       AAVS1.E_minus_minus_7763_6,AAVS1.E_plus_plus_11377_6    9       0       0
5
chr1    121478642       121478643       AAVS1.E_minus_plus_8420_6,AAVS1.E_plus_plus_12435_6     9       0       7
0
```

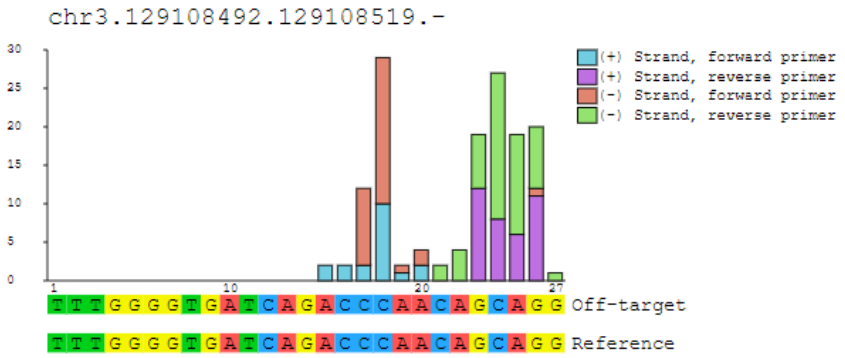
Column 1: chromosome
Column 2: start
Column 3: end
Column 4: id
Column 5: read count for plus strand in plus library
Column 6: read count for minus strand in plus library
Column 7: read count for plus strand in minus library
Column 8: read count for minus strand in minus library

3. Potential off-targets

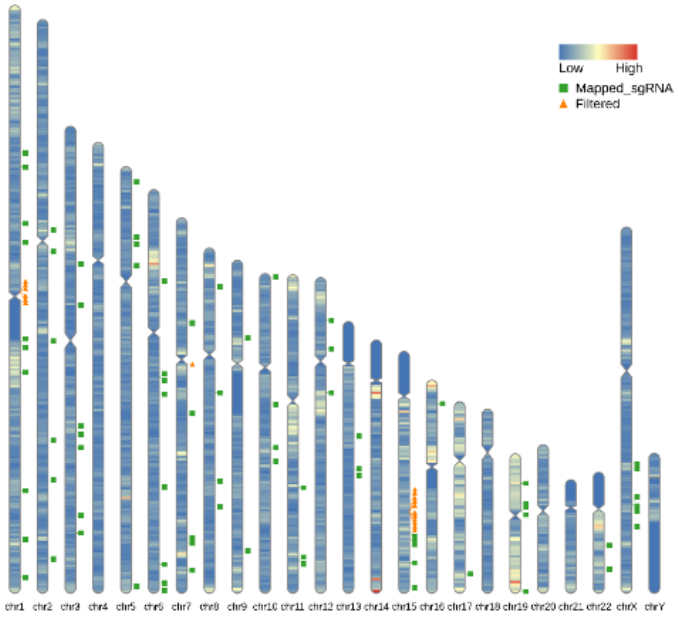
Illustrate of off-targets sites and read count.



4. Read counts across sgRNA in target and off-target sites



5. Global view of target and off-target sites



Tag-seq Runtime

The running time of Tag-seq depends on the size of sequencing depth (For 30M flagments, it takes 30mins).

Please cite

1. xxxx Tag-seq (underreview)