

Data analysis procedure for SMURF-seq reads

Contents

1	Introduction	2
2	Mapping SMURF-seq reads	2
2.1	Prerequisites	2
2.2	Mapping SMURF-seq reads to the reference genome	2
2.2.1	Mapping SMURF-seq reads with BWA-MEM	2
2.2.2	Mapping SMURF-seq reads with Minimap2	3
2.2.3	Mapping SMURF-seq reads with LAST	3
2.3	Generating mapping statistics	4
2.4	Test data	4
3	Generation of copy-number profiles	4
3.1	Prerequisites	4
3.2	Generating CNV profiles	4
3.3	Test data	4
4	Miscellaneous analysis of sequenced reads and mapped fragments	4
4.1	Read length distribution	4
4.2	Fragment length distribution	4
4.3	Closeness to RE sites	4

1 Introduction

SMURF-seq is a protocol to efficiently sequence short DNA molecules on a long-read sequencer by randomly ligating them to form long molecules. The SMURF-seq protocol involves cleaving the genomic DNA into short fragments. These fragmented molecules are then randomly ligated back together to form artificial, long DNA molecules. The long re-ligated molecules are sequenced following the standard MinION library preparation protocol. After (or possibly concurrent with) sequencing, the SMURF-seq reads are mapped to the reference genome in a way that simultaneously splits them into their constituent fragments, each aligning to a distinct location in the genome (for most fragments).

This manual explains how to map SMURF-seq reads, generate copy-number profiles from the mapped fragments, and perform additional optional analysis of the sequenced read or the mapped fragments.

2 Mapping SMURF-seq reads

At this point, we assume that the reads generated from a SMURF-seq experiment are base-called and are in a fastq or fasta file.

The reads sequenced using SMURF-seq protocol needs to be mapped to the reference genome to identify the fragment locations. The reads can be aligned leveraging long-read mapping tools that are designed for split-read alignment.

We present the option of aligning SMURF-seq reads using BWA-MEM [1], Minimap2 [2], and LAST [3], and we present several parameter recommendations for each tool.

2.1 Prerequisites

Reference genome: The CNV analysis procedure described in section 3 makes use of the human reference genome build hg19, and thus this build has to be used to generate CNV profiles using the procedure described here. However, other reference genomes can be utilized if the user does not use the procedure in 3

Software required:

1. Mapping tool: One of BWA, Minimap2, or LAST.
2. samtools [4] (version: 1.9)

Environment variables: Required only when using the provided scripts for mapping SMURF-seq reads. The variable MAPPER can be set using:

```
$ export MAPPER=<path to mapping tool>
```

2.2 Mapping SMURF-seq reads to the reference genome

A user has an option of using either of the tools listed above following the procedure in section 2.2.1, 2.2.2, or 2.2.3 respectively. We recommend using BWA-MEM as this produced higher fragment counts.

2.2.1 Mapping SMURF-seq reads with BWA-MEM

The download and install instructions for BWA can be found at: <https://github.com/lh3/bwa>

Reference genome index creation: The reference genome index is created using the command:

```
$ bwa index <genome file>
```

The index occupies approximately xxx GB for hg19.

Parameter recommendations: The default parameter to map nanopore reads using BWA-MEM is `-x ont2d`. SMURF-seq reads can be aligned using just this option, however, the fragment lengths were longer than optimal. We recommend using the parameters `-A 1 -B 2 -O 0 -E 2` in addition to `-x ont2d`. These parameters constrain the growth of a fragment and their lengths were close to optimal. To further increase the number of fragments obtained, at the expense of a longer runtime, the minimum seed length (*k*) and the minimum chain weight (*W*) can be lowered.

Aligning SMURF-seq reads: SMURF-seq reads are mapped to the reference genome using fast mapping:

```
$ bwa mem -x ont2d -A 1 -B 2 -O 0 -E 2 -t <threads> \
    <index> <reads>
```

or using to obtain a higher fragment count:

```
$ bwa mem -x ont2d -k 11 -W 5 -A 1 -B 2 -O 0 -E 2 -t <threads> \
    <index> <reads>
```

The above commands can also be run using the scripts:

```
$ ./map/smurfseq_BWA_fast.sh <index> <reads>
```

or

```
$ ./map/smurfseq_BWA_fragments.sh <index> <reads>
```

respectively. These scripts require environment variable `MAPPER` set to the location of BWA.

2.2.2 Mapping SMURF-seq reads with Minimap2

Reference genome index creation (Optional)

Aligning SMURF-seq reads

Parameter recommendations

2.2.3 Mapping SMURF-seq reads with LAST

Reference genome index creation

Aligning SMURF-seq reads

Parameter recommendations

2.3 Generating mapping statistics

2.4 Test data

3 Generation of copy-number profiles

3.1 Prerequisites

Software required:

3.2 Generating CNV profiles

Generating higher-resolution CNV profiles

3.3 Test data

4 Miscellaneous analysis of sequenced reads and mapped fragments

4.1 Read length distribution

4.2 Fragment length distribution

4.3 Closeness to RE sites

References

- [1] Heng Li. Aligning sequence reads, clone sequences and assembly contigs with bwa-mem. *arXiv preprint arXiv:1303.3997*, 2013.
- [2] Heng Li. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics*, 1:7, 2018.
- [3] Szymon M Kielbasa, Raymond Wan, Kengo Sato, Paul Horton, and Martin Frith. Adaptive seeds tame genomic sequence comparison. *Genome research*, pages gr-113985, 2011.
- [4] Heng Li, Bob Handsaker, Alec Wysoker, Tim Fennell, Jue Ruan, Nils Homer, Gabor Marth, Goncalo Abecasis, and Richard Durbin. The sequence alignment/map format and samtools. *Bioinformatics*, 25(16):2078–2079, 2009.