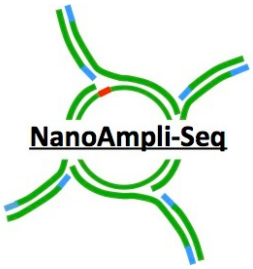
Oct 31, 2018

NanoAmpli-Seq - Bioinformatics Workflow



DOI
dx.doi.org/10.17504/protocols.io.u25eyg6

Szymon T STC Calus[1], Umer Zeeshan Ijaz[2], Ameet Pinto[3]

[1]Ex-Uni of Glasgow/Birmingham/Aberystwyth; [2]University of Glasgow; [3]Northeastern University

Pinto Lab

Szymon T STC Calus
Ex-Uni of Glasgow/Birmingham/Aberystwyth

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.u25eyg6

External link: https://www.biorxiv.org/content/early/2018/07/04/244517

Protocol Citation: Szymon T STC Calus, Umer Zeeshan Ijaz, Ameet Pinto 2018. NanoAmpli-Seq - Bioinformatics Workflow. **protocols.io https://dx.doi.org/10.17504/protocols.io.u25eyg6**

Protocol status: Working
We use this protocol in our group and it is working very well.

Created: October 26, 2018

Last Modified: October 31, 2018

Protocol Integer ID: 17213

Guidelines

Test data is available on the European Nucleotide Archive (ENA) website
https://www.ebi.ac.uk/ena/data/view/PRJEB21005

Safety warnings

The highest accuracy of the data is being achieved when only 1D2 reads are used with INC-Seq, chopSEQ and nanoCLUST algorithms.

We tested 1D data as well with NA-S bioinformatics workflow, however, noticed increase in overall error rates and presence of false positive OTUs - validated on mock samples.

We do not recommend using 1D data for high accuracy profiling i.e. clinical samples. However, 1D reads can be used for research purposes e.g. development of correction algorithms etc.

Before start

Make sure all the necessary programs and dependencies are installed on your PC or server and work correctly.

Download and install all the required software.

1

Software	
Albacore	NAME
Linux	OS
Oxford Nanopore Tech.	DEVELOPER
https://community.nanoporetech.com/downloads	SOURCE LINK

Software	
INC-Seq	NAME
Linux	OS
Genome Institute of Singapore	DEVELOPER
https://github.com/CSB5/INC-Seq	SOURCE LINK

Software	
chopSeq	NAME
Linux	OS
University of Glasgow	DEVELOPER
https://github.com/umerijaz/nanopore	SOURCE LINK

Software

nanoCLUST	NAME
Linux	OS
University of Glasgow	DEVELOPER
https://github.com/umerijaz/nanopore	SOURCE LINK

Basecalling of raw nanopore data with Albacore software.

2

Command

Raw data (HDF5) generated with MinKNOW has to be basecalled with Albacore v2.3.3 (or newer) software. The output of the basecalling should be in FASTA format. Further analysis requires 1D2 data only so, full_1dsq_basecaller.py algorithm must be used.

```
# Program requires input data (-i), version of the flow cell (-f),
# version of the sequencing kit (-k), output file (-o),
# amount of cores used for analysis (-t) and saving directory (-s).

/home/opt/.pyenv/versions/3.5.0/bin/full_1dsq_basecaller.py -i data/ -
f FLO-MIN107 -k SQK-LSK308 -o fasta -t 20 -s .
```

Consensus calling of long 16S rRNA concatemerized reads with use of the INC-Seq algorithm.

3

Command

The INC-Seq software requires basecalled data (e.g. Albacore) from Step 2. Correction of the data with INC-Seq algorithm uses only 1D2 data and is divided into two main steps:
1) Identification of segments made of 16S rRNA genes.
2) Anchor alignment of concatamerised amplicons and consensus calling with PBDAGCon.
Corrected reads have got ~98% accuracy and can be directly used as an input for chopSEQ software. (Linux)

```
# Export all necessary PATH's for the required programs.
# These PATH's are specific to our cluster and may differ
# to yours, depending on where you have installed these programs.

export PYENV_ROOT="/home/opt/.pyenv"
export PATH="$PYENV_ROOT/bin:$PATH"
eval "$(pyenv init -)"
export PYTHONPATH=/home/opt/INC-Seq/utils:$PYTHONPATH
export PATH=/home/opt/pacb/bin:$PATH
export PATH=/home/opt/pbdagcon/src/cpp:$PATH
export PATH=/home/opt/ncbi-blast-2.2.28+/bin:$PATH
export PATH=/home/opt/INC-Seq:$PATH
export PATH=/home/opt/.pyenv/versions/3.4.0/bin:$PATH


# INC-Seq consensus calling requires input data (-i),
# aligner (-a) e.g. poa, output file name (-o),
# minimum number of concatemers (--copy_num_thre) and --iterative.

inc-seq.py -i input.fasta -a poa -o incseq.fasta --iterative --
copy_num_thre 3
```

Correction of wrongly oriented reads and size filtration with a chopSeq algorithm.

4

Command

The chopSeq requires INC-Seq corrected data from Step 3.
Correction of the data is divided into multiple steps:
1) Identification of forward and reverse primers (e.g. 8F and 1387R) with pairwise2 aligner.
2) Re-orientation of incorrectly concatamerised reads and removal of tandem repeats recognised with use of etandem (EMBOSS) and subsequent merging of reads.
3) Size filtration with Biopython.
Now reads are qualified for nanoClust OTU binning and consensus calling. (Linux)

```
# Algorithm requires input data (-i) from previous step,
# forward (-f) and reverse (-r) primer sequence,
# lower (-l) and maximum (-m) size filtration range,
# and new file destination (> new_file.fasta),
# while verbosity (-v) mode is optional.

chopSEQ.py -i incseq.fasta -f "AGRGTTTGATCMTGGCTCAG" -r
"GGGCGGWGTGTACAAGRC" -l 1250 -m 1500 -v > chopseq.fasta
```

Read binning and generation of OTUs with a nanoCLUST algorithm.

5

Command

The nanoCLUST requires chopSEQ corrected data from Step 4.
Correction of the data is divided into multiple steps:
1) Data is partitioned (i.e. 1-450,451-900, 901-1300bp).
2) Reads from each partition are grouped according to 97% similarity with VSEARCH.
3) VSEARCH partition dereplication, singleton removal and binning are performed on split data.
4) Optimal read sections are used for clustering.
5) MAFFT G-INS-i is used for within OTU alignment and consensus calling of data.
6) Consensus sequences are generated (~99.5% accuracy).
7) Abundance table is generated. (Linux)

```
# Export all necessary PATH's for the required programs.
# These PATH's are specific to our cluster and may differ
# to yours, depending on where you have installed these programs.

export PATH=/home/opt/vsearch/bin:$PATH
export PATH=/home/opt/mafft-7.273-without-extensions/core/bin:$PATH
export MAFFT_BINARIES=/home/opt/mafft-7.273-without-
extensions/core/libexec/mafft


# Provide chopSeq corrcted data (-i) and window split
# range (-s) for read partitioning and output folder (-o).

nanoCLUST.py -i chopSEQ.fasta -s 0,450,451,900,901,1300,-1 -o
nanoclust_output/
```