

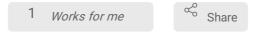


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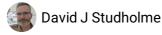
De-novo assembly of Xanthomonas genomes from Illumina NovaSeq reads

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ABSTRACT

This protocol describes the *de-novo* assembly of *Xanthomonas* genome sequences from short-read genomic shotgun sequencing data. It includes quality control of the raw sequence reads, assembly and finally polishing of the assembly based on alignment of reads against the preliminary assembly.

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1 Software pre-requisites.

This protocol assumes that you have already installed fastp, SPAdes, SAMtools, BowTie2 and Pilon. I also assumes that the paired Illumina sequence data comprises two gzipped FASTQ files called *name_r1.fq.gz* and *name_r1.fq.gz*.

Perform quality-based filtering and adapter trimming using fastp.

mkdir name_fastp_out

Creates a directory for the fastp QC report files.

fastp -i name_r1.fq.gz -I name_r2.fq.gz -o name_trimmed_r1.fq.gz -O name_trimmed_r2.fq.gz --unpaired1 name_trimmed_unp.fq.gz --unpaired2 name_trimmed_unp.fq.gz -r --cut_right_window_size 5 --cut_right_mean_quality 20 -c -I 50 -j name_fastp_out/name_fastp_report.json -h name_fastp_out/name_fastp_report.html

Generates trimmed and filtered sequence files and QC reports on the Illumina NovaSeq FASTQ sequence files.

3 Perform *de-novo* assembly using SPAdes.

spades.py -1 name_trimmed_r1.fq.gz -2 name_trimmed_r2.fq.gz -s name_trimmed_unp.fq.gz --careful --cov-cutoff auto -o name_spades_out

Performs the de-novo assembly.

4 Polishing with Pilon

This step assumes that the SPAdes assembly is contained in a file in the current working directory called *name.fasta*. It is assumed that the two trimmed-and-filtered gzipped FASTQ files are also in the current working directory. If these files are located elsewhere, then you can make symbolic links to them in the current working directory.

bowtie2-build name.fasta name

Creates BowTie2 index files with 'name' as the prefix for their filenames.

bowtie2 -x name -1 name_trimmed_r1.fq.gz -2 name_trimmed_r2.fq.gz - S name_vs_name.sam

Performs alignment of the trimmed-and-filtered reads against the genome assembly to generate an alignment in SAM format.

samtools view -b -T name.fasta name_vs_name.sam -o name_vs_name.sam.bam

Converts the SAM-formatted file into BAM format.

samtools sort --reference name.fasta name_vs_name.sam.bam -o name vs name.sam.bam.sorted.bam

Sorts the BAM file.

samtools index name_vs_name.sam.bam.sorted.bam

Indexes the sorted BAM file.



rm name_vs_name.sam.bam \$name_vs_\$name.sam

Removes the intermediate files to save disk space.

pilon --genome name.fasta --frags name_vs_name.sam.bam.sorted.bam --output name.pilon --outdir name_pilon_out

Generates a modified genome assembly based on reconciling discrepancies between assembly and aligned reads.



The polished genome assembly in FASTA format can be found in the Pilon output directory: ./name_pilon_out/name.pilon.fasta

This file can now be subjected to further quality control and/or submitted to public repositories.

5 Bibliography

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