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target-methylseq-qc: a lightweight pipeline for collecting metrics from targeted sequence mapping files.

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Summary

Next-generation targeted genome sequencing allows the analysis of regions of interest within a genome. While it is possible to incorporate targeted sequencing into whole-genome sequencing (WGS) bioinformatics pipelines, there remains a gap in accurately converting WGS metrics into precise target sequencing metrics and filtering the raw BED files into the targeted regions. Here, we introduce the target-methylseq-qc pipeline (https://doi.org/10.5281/zenodo.13147688), designed to (i) collect metrics from alignment files generated in targeted-methylation sequence analysis using the `picard_profiler` mode and (ii) filtering bedGraph for features overlapping with the reference BED file using the `bed_filter` mode, both of these modes are subworkflows written using the Nextflow (Di Tommaso et al., 2017) workflow language.

The target-methylseq-qc pipeline, when used in the `picard_profiler` mode accepts inputs in various alignment formats, including SAM, BAM and CRAM files (*HTS Format Specifications*, 2023). Additionally, to refine the metrics to the target regions the inclusion of a FASTA reference file and BED intervals file is required. Upon completion of the analysis, a MultiQC report (Philip Ewels et al., 2016) will be generated, encompassing the updated sequencing coverage data for the targeted regions with some extras. The `picard_profiler` mode of the pipeline integrates Picard metrics from GATK picard tools (McKenna et al., 2010; *Picard Toolkit*, 2019), using two specific metrics: (i) collecthsmetrics (*CollectHsMetrics (Picard)*, 2019), which relies upon the hybrid-selection technique to capture exon sequences for targeted sequencing experiments; and (ii) collectmultiplemetrics (*CollectMultipleMetrics (Picard)*, 2021), which captures closely related metrics such as alignment summary, insert size, and quality score.

On the other hand, `bed_filter` mode of the pipeline is designed to filter the bedGraph files outcome from nf-core/methylseq (Phil Ewels et al., 2024) using the reference bed panel, in this case the Twist Human Methylome panel (https://www.twistbioscience.com/resources/data-files/twist-human-methylome-panel-target-bed-file) and best practices *Twist Methylome* (2016b) using bedtools (Quinlan & Hall, 2010) filter command. Filtering raw BED files with the targeted regions is crucial because it ensures that the analysis focuses on specific genomic targets accurately and efficiently. This step minimizes the inclusion of off-target sequences and reduces the potential for including sequencing artifacts, which can be

introduced during capture-based targeted sequencing processes. Downstream analyses from the filtered BED files will enable the calculation of CpG ratios and the testing for differentially methylated cytosines (DMCs) or regions (DMRs).

Regardless of the usage mode of the pipeline, the final MultiQC report automatically collates the relevant reports from FastQC (Andrews, 2010), Bedtool and Picard tools in an HTML document, which could be shared with collaborators or added as supplementary material in publications.

target-methylseq-qc is a portable pipeline compatible with multiple platforms, such as local laptop or workstation machines, high-performance computing environments and cloud infrastructure. Although target-methylseq-qc was originally created for calculating sequencing coverage in target sequencing as a follow-up step to the `nf-core/methylseq` pipeline (Phil Ewels et al., 2024), within the Airway Epithelium Respiratory Illnesses and Allergy (AERIAL) paediatric cohort study (Kicic-Starcevich et al., 2023); its versatility allows for extending its application to other sequencing panels from various next-generation methods.

Statement of need

The target-methylseq-qc pipeline is designed to streamline the quality control process for target methylation sequencing data. Researchers and bioinformaticians working with methylation sequencing data often face challenges in ensuring data quality and consistency across different samples and experiments. This pipeline addresses these challenges by providing a standardized and automated workflow for quality control, leveraging the capabilities of the nf-core framework. Key features of the target-methylseq-qc pipeline include (i) Standardized Input Format: The pipeline expects a CSV samplesheet with specific fields tailored to different modes (picard_profiler and bed_filter), ensuring consistency and ease of use (ii) Flexible Execution Modes: Users can choose between different subworkflows (picard_profiler and bed_filter) based on their specific needs, enabling tailored quality control processes (iii) Comprehensive Parameter Control: Users can fine-tune the pipeline's behavior through a wide range of parameters, covering execution modes, input/output options, reference genome options, and infrastructural configuration. By automating and standardizing the quality control process, the target-methylseq-qc pipeline helps researchers save time, reduce errors, and ensure high-quality data for downstream analysis and clinically applicable insights.

Design principles and capabilities

The target-methylseq-qc pipeline builds upon the standardised pipeline template maintained by the nf-core community (P. A. Ewels et al., 2020) for Nextflow pipelines as well as makes use of the nf-core/modules project to install modules for FastQC, MultiQC (Philip Ewels et al., 2016), Bedtools, Picard as well as Samtools (Danecek et al., 2021) within the pipeline Figure 1.

The use of the nf-core template facilitates keeping the design of the pipeline generic and portable across different execution platforms, therefore the target-methylseq-qc pipeline can be used on local machines, HPC orchestrators (e.g. SLURM, PBS), and cloud computing systems such as AWS Batch, Azure Batch, Google Batch, in addition to the more generic Kubernetes distribution.

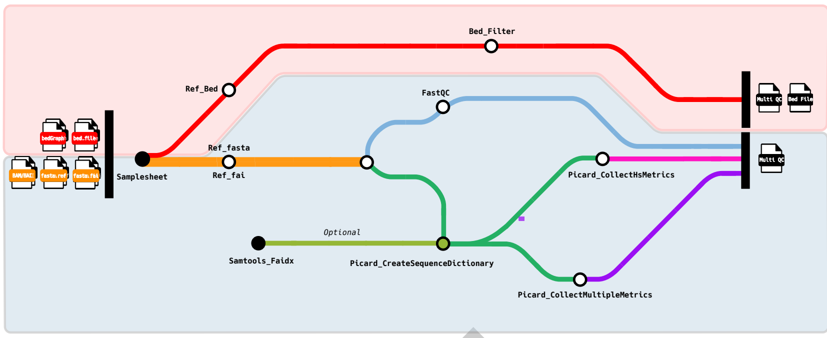

Figure 1: Subway map for various steps in the target-methylseq-qc pipeline.

In addition to the base workflow as mentioned in Figure 1, the pipeline also includes optional picard/createsequencedictionary (*CreateSequenceDictionary (Picard)*, 2022) and Samtools modules to aid users in automatically generating the required genome dictionary (DICT) file, in case they have only the reference FASTA and BED files but intend to use the pipeline. Furthermore, depending on the quality check requirements of the users, we have enabled the metrics collection for 10x, 20x, 30x and 50x coverage.

Tutorials and documentation

The steps needed to configure the pipeline inputs and configuration for the relevant infrastructure are available in the documentation within the GitHub repository as well as a dedicated documentation website (*Target-Methylseq-Qc Website*, 2024).

Pre-requisites

To ensure proper operation of the target-methylseq-qc pipeline, three dependencies must be available in the execution environment: `Java` (LTS > 11), `Nextflow` (> 24.04), and a package manager such as `conda` (Gruning et al., 2018) or a container system such as `docker` or `singularity` (Veiga Leprevost et al., 2017).

Getting started with the pipeline setup is straightforward given that (i) `Java` (LTS > 11) (ii) `Nextflow` (> 24.04) and (iii) a package manager (e.g. `conda`) or a container system (e.g. `docker` or `singularity`) are available in the execution environment. The in-built test profile from the pipeline can then be used to execute the profile on the relevant infrastructure with some test dataset.

Pipeline installation

target-methylseq-qc pipeline can be downloaded from the GitHub code repository using the `git` command line tool or directly through using the `Nextflow` command line tool using the following commands

```
# Git based download
$ git clone https://github.com/wal-yan/target-methylseq-qc
```

```
# Nextflow based download
$ nextflow pull https://github.com/wal-yan/target-methylseq-qc
```

Test profiles

Two built-in test profiles are available in target-methylseq-qc pipeline for each mode of execution. These profiles can be used to run tests on the relevant infrastructure using the bundled test datasets (Agudelo-Romero, 2024), helping users to identify and resolve any infrastructural issue before the analysis stage.

```
# picard_profiler mode
$ nextflow run wal-yan/target-methylseq-qc \
  -profile docker,test_picard_profiler
```

```
# bed_filter mode
$ nextflow run wal-yan/target-methylseq-qc \
  -profile docker,test_bed_filter
```

Input

Following the convention for standard input in the Nextflow pipelines, target-methylseq-qc expects a CSV samplesheet as an input with the following fields. An example of a samplesheet Table 1 for target-methylseq-qc in `picard-profiler` mode containing three columns, capturing the (i) name of the sample (ii) path to BAM file and (iii) path to the BAM index (BAI) file.

Table 1: Samplesheet structure for `picard_profiler` mode .

sample	bam	bai
sample-01	/path/to/sample-01.bam	/path/to/sample-01.bai
sample-02	/path/to/sample-02.bam	/path/to/sample-02.bai

Whereas the `bed_filter` mode requires a different set of columns in the input samplesheet CSV file, as shown in Table 2.

Table 2: Samplesheet structure for `bed_filter` mode .

sample	bedGraph
sample-01	/path/to/sample-01.bedGraph
sample-02	/path/to/sample-02.bedGraph

Execution

The pipeline initialization step, as per the best practices of the nf-core template, checks the validity of the file paths specified to be either a POSIX-compliant file system or a cloud object storage path for files storaged in AWS S3, Azure Blob Storage or Google Cloud Storage buckets.

The behaviour of the pipeline can be controlled through the pipeline parameters which are divided into different groups such as (i) Execution Mode, (ii) Input/Output Options (iii) Reference Genome Options in addition to the generic parameters inherited from the nf-core template such as (i) Max job request options (ii) Generic options and (iii) Institutional config options. A complete list of the parameters specific to target-methylseq-qc pipeline is summarised in Table 3

Table 3: Summary of pipeline-specific parameters for target-methylseq-qc pipeline .

Parameter Name	Description
picard_profiler	Enable this boolean option to use the picard_profiler subworkflow
bed_filter	Enable this boolean option to use the bed_filter subworkflow
input	Path to comma-separated file containing information about the samples in the experiment.
outdir	The output directory where the results will be saved.
ref_fasta	Path to FASTA genome file.
ref_fai	Path to the FASTA index file.
ref_bed	Path to the BED file for the reference panel.

Output

Upon completion, the two subworkflows generate different outputs which are presented together in the MultiQC file. For picard_profile mode, a MultiQC file is produced, providing the relevant results related to the coverage metrics Figure 2. For the bed_filter mode, a BED file is generated with the methylation positions filtered based on the BED intervals file from the targeted methylation profile Figure 3.

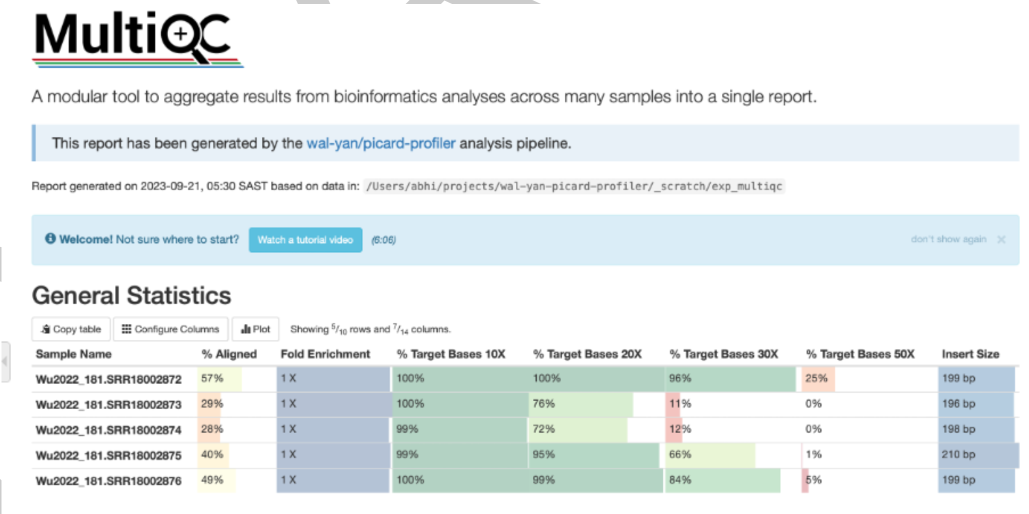

Figure 2: MultiQC report generated for target-methylseq-qc, in `picard-profiler` highlighting the refine metrics from targeted sequencing at 10X, 20X, 30X and 50X coverage.

chr1	10524	10525	100	1	0
chr1	10541	10542	50	1	1
chr1	10562	10563	66	2	1
chr1	10570	10571	75	3	1
chr1	10576	10577	50	2	2
chr1	10578	10579	75	3	1
chr1	10794	10795	100	1	0
chr1	10810	10811	0	0	1
chr1	29359	29360	0	0	2
chr1	29367	29368	0	0	2

Figure 3: Filtered bedGraph file generated using the `bed_filter` mode of target-methylseq-qc.

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