NanopoReaTA: a user-friendly tool for nanopore-seq real-time transcriptional analysis

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Abstract

Summary: Oxford Nanopore Technologies' (ONT) sequencing platform offers an excellent opportunity to perform real-time analysis during sequencing. This feature allows for early insights into experimental data and accelerates a potential decision-making process for further analysis, which can be particularly relevant in the clinical context. Although some tools for the real-time analysis of DNA-sequencing data already exist, there is currently no application available for Transcriptome data analysis designed for scientists or physicians with limited bioinformatics knowledge. Here we introduce NanopoReaTA, a user-friendly real-time analysis toolbox for RNA sequencing data from ONT. Sequencing results from a running or finished experiment are processed through an R Shiny-based graphical user interface (GUI) with an integrated Nextflow pipeline for whole transcriptome or gene-specific analyses. NanopoReaTA provides visual snapshots of a sequencing run in progress, thus enabling interactive sequencing and rapid decision-making that could also be applied to clinical cases. **Availability:** https://github.com/AnWiercze/NanopoReaTA

1 Introduction

In standard sequencing experiments, practical steps and data analysis are usually performed independently, with the latter initiated by bioinformatics experts once after sequencing is complete. Nowadays, new technologies such as Oxford Nanopore Technologies (ONT) offer a unique opportunity to start downstream analysis while sequencing is still ongoing (Amarasinghe et al., 2020, Wang et al., 2021). Some platforms, such as EPI2ME from ONT (https://epi2me.nanoporetech.com/) or minoTour (https://github.com/minoTour/minoTour, Munro et al. 2022), already provide real-time pipelines for rapid ONT data acquisition integrated into a user interface (UI), and thus accessible to users with limited bioinformatics skills. However, as these platforms' focus mainly lies on the analysis of DNA sequencing data, there is a lack of real-time applications in the field of transcriptomics. Here we introduce NanopoReaTA, an on-demand toolbox for real-time transcriptomic analysis that provides rapid insight on RNA sequencing data from ONT. Users receive transcriptome-wide and gene-specific information directly while sequencing is still running, such as differences between conditions or expression levels of individual genes. In addition, implemented quality control features allow the user to monitor data variability during the ongoing sequencing process. Ultimately, the tool can provide frequent biologically relevant snapshots of the current sequencing run, which in turn can enable interactive fine-tuning of the sequencing run itself, facilitate decisions to abort the ongoing run to save time and material, e.g., when sufficient accuracy is achieved, or even accelerate the resolution of clinical cases with high urgency.

2 Material and methods

2.1 Usage

NanopoReaTA can be launched directly after starting a sequencing run of cDNA or direct RNA via ONT's sequencing software MinKNOW (Figure 1 and Fig. S1). Within NanopoReaTA's UI, the user will be guided through several configuration settings to extract all information required for data processing such as reference sequences, annotation files, output directory defined in MinKNOW (into which sequencing output is written), and more (Supplementary Material, Fig. S2A-C). Preprocessing of basecalled reads from a running or completed experiment is integrated into a nextflow pipeline and can be started via a one-button-click within the UI (Fig. S2D). As soon as sequencing data are generated, the nextflow pipeline automatically updates generated files, including gene counts or mapping files. Based on the output files from preprocessing, downstream analyses can be performed within the following tabs integrated into NanopoReaTA: "Overview", "Gene-wise Analysis" and "Differential Expression Analysis" (Fig. S3-6). The resulting figures can be constantly updated during sequencing (Fig. S7-6).

2.2 Preprocessing via nextflow

The nextflow pipeline takes all fastq files that pass the quality threshold defined in MinKNOW and performs genome and transcriptome alignment using minimap2 (Li, 2018) as well as feature quantification using FeatureCounts (Liao et al., 2014) and Salmon (Patro et al., 2017). In addition, we incorporated a quality control utility extracting sample- and group-wise read length distribution, variability measurements, genome/transcriptome coverage based on RseQC (Wang et al., 2012), and gene count per iteration, enabling the assessment of specific quality metrics over time (Fig. S7).

2.3 Downstream analyses based on R

The subsequent downstream analyses are based on commonly used R packages such as DESeq2 (Love et al., 2014) for principle component analysis and differential gene expression, DEXSeq (Anders et al., 2012) for differential transcript expression, and DRIMSeq (Robinson, & Nowicka 2016) for differential transcript usage (Figure 1E). In addition, gene body coverage and counts per sample and condition can be visualized for a subset of genes of interest (Figure 1E). All tables and figures can be downloaded via button clicks (Fig. S3-6).

2.4 Installation and requirements

The installation of NanopoReaTA is done via Anaconda (Pinheiro et al. 2006) using an environment file containing all package requirements. For installation, requirements, and user manual, please visit https://github.com/AnWiercze/NanopoReaTA.

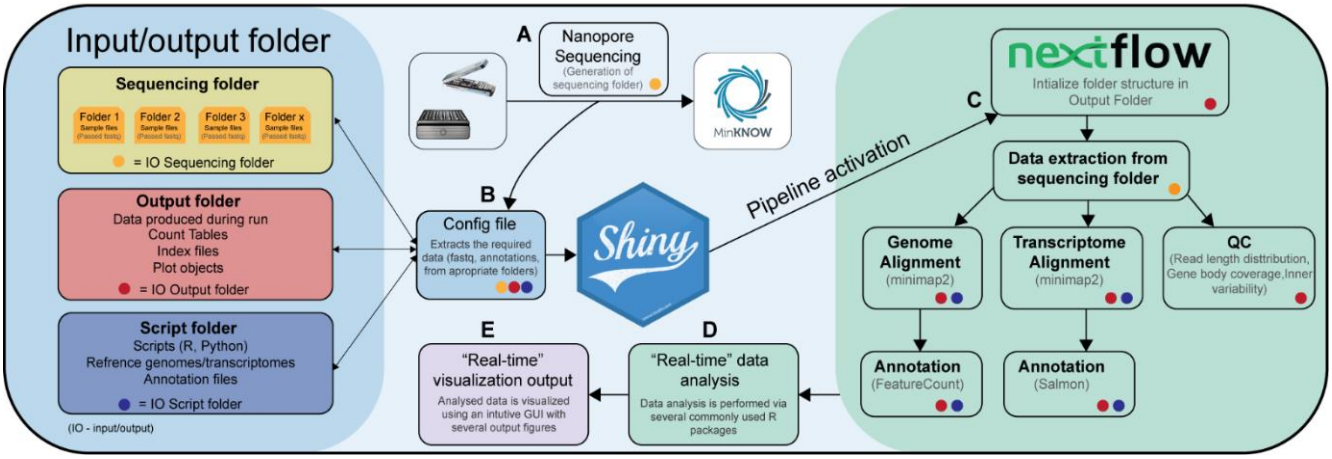

Figure 1. NanopoReaTA workflow visualized as a graphical sketch. Detailed information on the individual modules are given in the Supplementary Material, as well as in the user manual on the Github repository: https://github.com/AnWiercze/NanopoReaTA

Hardware. 64GB RAM, 16 threads, Linux-based operating system. *Software.* Anaconda, python (version ≥3.8), R (version ≥4.1.2).

3 Discussion

NanopoReaTA represents a real-time analysis toolbox that allows users to perform interactive transcriptional analyses of cDNA and direct RNA sequencing data in real-time via a user-friendly and intuitive user interface based on R shiny. We aim to provide a tool that supports users from biological research and clinical diagnostics of transcriptomics by accelerating decision-making processes of future experiments or patient treatment, especially when time and money are limiting factors. From future perspectives, we envision that additional functions such as novel transcript detection, RNA modification detection, and integration of multi-omics levels in real-time can be integrated. Our application is open source to also enable the scientific community to contribute such enhancements

Author contributions

TB and SG conceived and supervised the project. AW and SP designed, implemented, and tested the GUI. TB, AW, and SP wrote the manuscript. SG, MH, and SM edited the manuscript and provided valuable input and feedback in various discussions. All authors read and approved the final manuscript.

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Conflict of Interest: None declared

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