Reproducible, portable, and efficient ancient genome reconstruction with nf-core/eager

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Abstract

The broadening utilization of ancient DNA (aDNA) to address anthropological, palaeontological and biological questions is resulting in a rising diversity in the size of laboratories and scale of analyses being performed. In the context of this heterogeneous landscape, we present nf-core/eager, an advanced and entirely redesigned pipeline for the analysis of genomic ancient DNA data. nf-core/eager builds on existing ideas and concepts introduced in the original EAGER pipeline 1, and improves various aspects of the analysis procedure by building on computational frameworks such as Nextflow 2 and nf-core 3. The pipeline aims to address three main points: adaptability to different computing configurations, reproducibility to ensure robust analytical standards in the field, and bring the pipeline up-to-date with the latest routine ancient genomic practises. This new version of EAGER has been developed within the nf-core initiative, to ensure high quality software development and maintenance support; contributing to a long-term lifecycle for the pipeline. nf-core/eager will assist in ensuring that ancient DNA can be utilised by a diverse range of research groups and fields.

Introduction

Ancient DNA has become a widely accepted source of biological data, helping to provide new perspective for range of fields including archaeology, ecology cultural heritage, and palaeontology. The utilisation of next-generation-sequencing has allowed the recovery of ancient DNA from a wide variety of sources, including but not limited to, the skeletal remains of animals (4, 5, 6, [???]), modern and archaic humans (7, doi:0.1038/s41586-018-0455-x), bacteria (8, 9, 10), Viruses (11, 12), plants (13, 14), but also coprolites (15), dental calculus (16, 17), sediments (18, 19), medical slides (20) parchment (21), and most recently ancient 'chewing gum' (22, 23). Improvement in laboratory protocols to increase yields of otherwise trace amount of DNA has at the same time led to studies that can range from one to thousands of ancient individuals (24, [???]), spanning single (25) to thousands of organisms (16). These differences of disciplines have led to a heterogeneous landscape in terms of types of analyses thus types of computing resources required by different labs - particularly in regions of the world where ancient DNA is still emerging.

Two previously published and commonly used pipelines in the field are PALEOMIX (26) and EAGER (1). These two pipelines take a similar approach to link together standard tools used for Illumina NGS data processing (quality control, adapter removal/and or merging, mapping, genotyping etc.), but with a specific focus on tools that are designed for or well-suited for ancient DNA (such as bwa aln (27) for ultra-short reads and mapDamage (28) for aDNA characteristic evaluation). Yet, neither have had major updates to bring in line with current routinely carried out aDNA analyses. In particular, metagenomic screening of off-target genomic reads for pathogens or microbiomes (16, 17) has becoming particularly common, given it's role in revealing widespread infectious disease and possible epidemics that had previously been undetected in the archaeological record (29, 30, 11, 12). Without easy access to the latest field-established analytical routines, aDNA studies from groups new to the field risk being published without the necessary quality controls checks to ensure authenticity of their data and without yielding the full range of possibilities from the data.

To address these short comings, we have completely re-implemented the latest version of the EAGER pipeline in Nextflow, a domain-specific-language (DSL) designed for the construction of 'omic analysis pipelines (2). In addition, the newly named pipeline - nf-core/eager - has been developed in the context of the nf-core community framework (3). nf-core enforces strict guidelines for best-practises in software development to ensure robust, long-term maintenance and high quality pipelines.

Results and Discussion

Scalability, Portability, and Efficiency

The reimplementation of EAGER into Nextflow offers a range of benefits over the original custom pipeline framework.

Firstly, the new framework provides immediate integration of nf-core/eager into various schedulers in POSIX High-Performance-Cluster (HPC) environments as well as local computers. This portability allows both small and big labs to run nf-core/eager regardless of the type of computer or cluster cluster sizes with minimal effort or configuration, facilitating reproducibility and therefore maintenance of standards within the field. This is further assisted by the in-built compatibility with software environments and contains such as conda, docker and singularity. This ensures exact versions of software are used by a user, regardless of the set-up of the cluster environment. Another major change with nf-core/eager is that the GUI input is now replaced with a command-line-interface as the primary user interaction mode. This is more compatible and portable with most HPCs (that may not offer X11 forwarding) and is in line with the vast majority of bioinformatic tools. We therefore believe this will not be a hindrance to new researchers from outside computational biology, however the nf-core initiative is in the processing in the near future of offering multiple alternatives including a command-line wizard, and a web-based input GUI.

Reproducibility is further made easier through the use of 'profiles' that can define configuration parameters for both the computing environment (schedulers, cache locations, maximum resource etc.), which can be centrally managed to ensure all users of a group use the same settings. Pipeline level profiles, specifying default parameters for nf-core/eager itself is therefore more portable between different groups through separation of the parameters from input data. Compared to the original EAGER that utilised per-FASTQ XML files with hardcoded filepaths, researchers can publish alongside their publications the specific profile used in their nf-core/eager runs to ensure other groups can generate the same results. Using of profiles also reduces mistakes caused by insufficient 'prose' based reporting of program settings, which often occurs in papers written by researchers unfamiliar with informatics. The default nf-core/eager profile uses parameters evaluated in different aDNA specific contexts (e.g. [31]), and will be updated in each new release as new studies are published.

nf-core/eager provides improved efficiency over the original EAGER pipeline by replacing the sample-by-sample sequential processing with Nextflow's asynchronous parallelisation. This combined with nf-core/eager-defined per-process customisation of resource parameters reduces unnecessary resource allocation that can occur with new users to each step of an NGS data processing pipeline. This is particularly pertinent given the increasing use of centralised HPCs or cloud computing, which often use per-hour cost calculations.

Latest aDNA practices

nf-core/eager follows a similar structural foundation with the original EAGER. Given Illumina short-read FASTQ and/or BAM files, and a reference FASTA file.

This can be split into four main stages:

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1. Preprocessing
    1. Sequencing quality control (FastQC)
    2. Sequencing artefact clean-up (AdapterRemoval2)
    3. Preprocessing statistics generation

2. Mapping and post- processing
    1. Alignment against reference genome (BWA, CircularMapper)
    2. Mapping quality filtering (Samtools)
    3. PCR duplicate removal (DeDup, Samtools MarkDuplicates)
    4. Mapping statistics generation (PreSeq, Qualimap)
3. aDNA Evaluation and Modification
    1. Damage profiling (DamageProfiler)
    2. aDNA reads selection (PMDTools)
    3. Damage removal (Bamutils)
    4. (Human) contamination estimation (ANGSD)
4. Genotyping and Consensus Sequencing (GATK, VCF2Genome)

In nf-core/eager, all tools also originally used in EAGER have been updated to latest compatible versions, as available on conda to ensure widespread accessibility and stability. **Modules that have not been carried over are Schmutzi and MapDamage because ALEX TO WRITE SOMETHING NICE HERE**. Support for Bowtie will be added in the near future, after consultation with the nf-core user community. New tools to the basic workflow include fastp ([32](#)) for the removal of poly-G sequencing artefacts that are common in 2-colour Illumina sequencing machines (such as the increasingly popular NextSeq and NovaSeq platforms). We have also included the FreeBayes genotyper as an alternative to the human-focused GATK tools. We have also maintained the possibility of using the now unsupported GATK UnifiedGenotyper, as the GATK HaplotypeCaller performs *de novo* assembly around possible variants, which may not be suitable for low-coverage aDNA data.

We have further extended functionality of the pipeline, primarily focusing on allowing for ancient metagenomic analysis to be run alongside genomic of the reference genome. We have added the ability to screen all off-target reads from mapping to the supplied reference FASTA with two metagenomic profiles: MALT ([33](#), [34](#)) and Kraken2 ([35](#)). Characterisation of properties of authentic ancient DNA ([**???**]:) from MALT alignments is carried out with the HOPS pipeline ([36](#)). The pipeline also integrates a SNP alignment generation tool, MultiVCFAnalyzer (doi:10.1038/nature13591), which allows assessment of level of cross-mapping to a reference genome from different related taxa - a common challenge in ancient bacteria genome reconstruction ([37](#)). Simple coverage statistics of particular annotations (e.g. genes) of an input reference is offered by bedtools ([38](#))

New tools supplied with the pipeline include a dedicated 'endogenous DNA' calculator (endorS.py, by A.A.V), to give approximate level of on-target DNA yield within a sample. Secondly, ancient metagenomic studies may include comparative samples from living day individuals ([39](#)). To support open data, whilst respecting data privacy, nf-core/eager includes a 'strip_fastq' script (by M.B.) which creates raw FASTQ files but with reference-genome mapped reads removed. This then allows safe upload of sequencing data to public repositories with identifiable data removed. When using a human reference genome, nf-core/eager also can give estimates of the biological sex of a given individual with Sex.DetEERRmine ([40](#)).

Complex sequencing strategies is also now accounted for with nf-core/eager. Given the large amount of sequencing often required to yield sufficient genome coverage from ancient DNA data, palaeogeneticists can sometimes use multiple (differently treated) libraries or sequencing runs. As an alternative to direct paths, the pipeline can also accept a TSV file which includes file paths and additional metadata such as sample name, library name, sequencing lane, colour chemistry and UDG treatment. This allows simultaneous processing and appropriate merging of heterogeneous data of multiple sequencing runs and/or libraries types.

Finally, the original EAGER tabular report format has been replaced with MultiQC ([41](#)). The original EAGER pipeline required users to look through many independent output directories and files to make full assessment of their sequencing data. Aggregation of all log files into a single interactive

report will assist users in making fuller assessment of their sequencing and analysis runs. Most tools within nf-core/eager have a corresponding MultiQC module to ensure as complete evaluation as possible.

Accessibility

Alongside the portable new pipeline report, we have written extensive documentation on all parts of running and interpreting the output of the pipeline. Given that a large fraction of aDNA researchers come from fields such as social sciences that have limited computational training (such as J.A.F.Y), we have written documentation that also gives guidance on how to interpret each section of the report, specifically in the context of NGS and aDNA. This includes schematic images (by Z.F.) of best practices or expected output, published under CC-BY licenses to allow for use in other training material. This open-access resource will make the aDNA discipline more accessible to researchers new to the field, by providing practical and 'applied' knowledge to how aDNA characteristics translate to downstream analyses.

The development of nf-core/eager in nextflow and the nf-core initiative will also improve open-source community contributions to the pipeline. While nextflow is written primarily in the language groovy, the nextflow DSL simplifies a lot of concepts to a level intermediate level bioinformaticians without Java/Groovy experience can easily access (regardless of own programming language experience). Furthermore, Nextflow places ubiquitous command-line interfaces such as bash in a prominent position within the code, rather than custom java code and classes. This is particularly relevant for researchers ancient DNA, who often come from social sciences and do not have backgrounds or training in computational biology. We hope this will motivate further bug fixes and feature contributions to keep the updated pipeline with standard practises during a longer life-cycle of this version of the pipeline. This will also be supported by the active and welcoming nf-core community who provide general guidance and advice on developing nextflow and nf-core pipelines.

Conclusion

nf-core/eager is an efficient, portable, and accessible pipeline for processing ancient DNA genomic data. The re-implementation with nextflow and nf-core will improve reproducibility and inclusion of rapidly increasing ancient DNA datasets, for both large and small laboratories. Extensive documentation also allow newcomers to the field get a practical understanding on how to interpret ancient DNA in the context of NGS data processing. Finally nf-core/eager brings in the latest tools and routine screening analysis commonly used in the field, and sets up the pipeline staying at the forefront of palaeogenetic analysis.

Methods

Installation

nf-core/eager requires java, nextflow and either a environment/container system.

Running

Further customisation can be made using profiles

Basic input is FASTQ or BAMs, FASTA reference. Complex and/or heterogeneous input can be supplied with a TSV file.

Runs can be monitored via terminal console or tower.nf.

Example data is included.

Output includes both files for close inspection and downstream analysis, as well as the emailable MultiQC report.

Competing Interests

No competing interests are declared.

Data and software availability

All code is available on github at https://github.com/nf-core/eager and archived with Zenodo under the DOI 10.5281/zenodo.1465061. All test data is from the ENA public repository.

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