pRESTO: a toolkit for processing high-throughput sequencing raw reads of lymphocyte receptor repertoires

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ABSTRACT

Summary: Driven by dramatic technological improvements, large-scale characterization of lymphocyte receptor repertoires via high-throughput sequencing is now feasible. Although promising, the high germline and somatic diversity, especially of B-cell immunoglobulin receptors, presents challenges for analysis requiring the development of specialized computational pipelines. We developed the REpertoire Sequencing TOolkit (pRESTO) for processing reads from high-throughput lymphocyte receptor studies. pRESTO processes raw sequences to produce error-corrected, sorted and annotated sequence sets, along with a wealth of metrics at each step. The toolkit supports multiplexed primer pools, single- or paired-end reads and emerging technologies that use single-molecule identifiers. pRESTO has been tested on data generated from Roche and Illumina platforms. It has a built-in capacity to parallelize the work between available processors and is able to efficiently process millions of sequences generated by typical high-throughput projects.

Availability and implementation: pRESTO is freely available for academic use. The software package and detailed tutorials may be downloaded from http://clip.med.yale.edu/presto.

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Supplementary information: Supplementary data are available at Bioinformatics online.

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1 INTRODUCTION

High-throughput sequencing technologies now enable large-scale characterization of lymphocyte receptor repertoires (Rep-Seq) (Benichou et al., 2012). Rep-Seq studies have used a variety of next-generation sequencing platforms, including Roche’s 454 and Illumina’s Genome Analyzer. Researchers are now beginning to take advantage of newer platforms, such as the MiSeq offered by Illumina, which can generate >10 million paired-end 300 base-pair reads in a single run. Many experimental protocols take advantage of these high read volumes by tagging reads with sample-specific barcode sequences and multiplexing sample groups. More recently, protocols to barcode single mRNA molecules with unique identifiers (UID) before PCR amplification have emerged (Shiroguchi et al., 2012; Vollmers et al., 2013), allowing PCR amplification effects to be resolved and offering the potential to dramatically reduce sequencing-dependent error rates using single-molecule consensus reads.

We have developed a suite of utilities, the REpertoire Sequencing TOolkit (pRESTO), that provides an integrated framework to handle all stages of sequence processing prior to germline segment assignment, which may then be handled by other available software such as IMGT/HighV-QUEST (Alamyar et al., 2012). pRESTO is designed to handle either single- or paired-end reads, has been tested on data from both the Roche 454 and Illumina MiSeq platforms and includes a wide range of features designed to meet the needs of various Rep-Seq protocols; see Supplementary Material Section 1.1 and Supplementary Tables S1 and S2.

2 FEATURES

2.1 Overview and implementation

The pRESTO software package is provided as a set of command-line utilities, all of which are implemented as platform-independent Python modules. pRESTO is designed to allow for maximum flexibility in workflow organization to meet the unique needs of different sequencing projects. Particular emphasis is placed on providing support for multiplexed primers, multiplexed sample pools and emerging technologies that use UID barcoding. However, pRESTO is equally suitable for experimental protocols that do not use complex mixtures of molecular tags. Each tool accepts sequences in the form of FASTA or FASTQ (with Phred scoring scheme) files. The more computationally expensive tools in the pRESTO suite are natively parallelized, allowing users to take advantage of multicore systems by specifying the number of subprocesses to execute. Additionally, pRESTO allows users to easily integrate

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10 million paired-end

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can convert both sequence-embedded and logged annotations into data tables suitable for automated analysis and plotting (Supplementary Material Sections 2.11 and 2.12).

2.3 Quality control and error profiling
pRESTO provides comprehensive quality control tools to filter reads based on sequence properties such as Phred quality scores (Supplementary Material Section 2.2), valid barcode labeling (Supplementary Material Section 2.3), primer identity (Supplementary Material Section 2.3) and abundance of duplicate reads (Supplementary Material Section 2.9). pRESTO also provides tools to measure the diversity and error profiles of sets of annotated reads (Supplementary Material Sections 2.5 and 2.8); such information may be used to estimate sequencing error rates and remove highly variable UID read groups from the analysis.

2.4 UID barcoding and paired-end reads
Recent advances in Rep-Seq protocols are allowing researchers to improve both the sequencing and quantification accuracy of repertoire data by labeling each starting nucleic acid sequence with a unique single-molecule identifier (UID) before amplification (Shiroguchi et al., 2012; Vollmers et al., 2013). The pRESTO suite includes special operations tailored for UID barcoding technologies, including tools to multiple align UID read groups (Supplementary Material Section 2.5) and generate consensus sequences from UID read groups (Supplementary Material Section 2.6).
	pRESTO also provides support for de novo assembly of overlapping paired-end reads (Supplementary Material Section 2.7). pRESTO does not require maintenance of file ordering across pair-end read files, facilitating independent filtering of separate paired-end read files. However, the sequence sampling and sorting tools may operate in a paired-end mode, allowing users to create uniformly ordered paired-end files (Supplementary Material Section 2.10). pRESTO allows users to propagate annotations between paired-end records (Supplementary Material Section 2.4), which is required for protocols where the sample barcode or UID are found on only one read of the mate pair.

3 CONCLUSIONS
We have developed a flexible toolkit, pRESTO, for rapid processing of high-throughput lymphocyte receptor sequencing data originating from either mRNA or DNA templates. pRESTO is compatible with the latest molecular barcoding and sequencing technologies and provides a means to generate high-fidelity repertoire datasets. This output can be used to drive subsequent analysis, such as (i) identifying V(D)J gene segments (Alamyar et al., 2012; Gaëta et al., 2007; Thomas et al., 2013; Volpe et al., 2006; Ye et al., 2013), (ii) clustering clonally related sequences (Chen et al., 2010), (iii) inferring individual genotypes (Kidd et al., 2012), (iv) creating lineage trees (Barak et al., 2008), (v) quantifying affinity-dependent selection (Yaari et al., 2012) and (vi) analysis of somatic hypermutation patterns (Yaari et al., 2013). The software package, detailed example workflows and sample datasets are available online at http://clip.med.yale.edu/presto.
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Conflict of Interest: none declared.

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