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Commentary

An innovative and affordable quantitative assessment of human TCR repertoire

Roberta Amoriello, Clara Ballerini*

Department of Experimental and Clinical Medicine, University of Florence, Florence Italy

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Current techniques available to describe the individual T cell receptor (TCR) repertoire are highly expensive and necessitate specific computational analysis tools in order to describe the repertoires qualitatively and quantitatively and to correct sequencing errors. Montagne et al. [1] in their work propose a new approach to TCR repertoire sequencing investigation. The diversity of the TCR repertoire is the result of multiple processes of DNA stochastic somatic recombination and it is responsible for effective immunity against pathogens. A TCR molecule is a heterodimer composed of alpha and beta chains and in humans of a minor population of delta and gamma chains. These chains are very diverse, because of the imprecise rearrangements of either V and J segments (alpha chain) or V, D and J segments (beta chain), that determine the non-template addition or removal of DNA base pairs, resulting in a huge variety of complementary determining regions (CDR3) and in an extraordinary genomic diversity, similar only to the ones we find in the antibodies [2]. For many years it was a challenge to investigate this hypervariable molecule and a complete description of the TCR repertoire has never been achieved. Recent advances in high-throughput sequencing (HTS) techniques paved the way to explore the TCR repertoire dynamics bridging cell-based methods and computational ones into qualitative and quantitative receptor description [3] and antigen discovery [4]. Of note, HTS is not as accurate as Sanger sequencing and introduces errors during library amplification and/or during sequencing [5], therefore, several expensive strategies and different algorithms have been developed to attenuate the impact of errors on the TCR repertoire analysis and on the consequent biological data interpretation [6,7]. In their work, Montagne et al. developed an original experimental pipeline to sequence TCR and analyze CDR3 repertoire, dropping the cost (from a mean of \$500–\$1000 to \$20 per sample) for primers and for unique molecular identifiers (UMIs) to correct amplification bias. The proposed Framework Region 3 AmplifiKation sequencing (FR3AK-seq), is a simplified multiplex PCR-based involving a minimal primer set targeting the upstream sequence of the complementarity determining region 3 (CDR3), the framework region 3 (FR3). Authors use the TCRB FR3 regions, collected from the Immunogenetics reference directory (IMGT/GENE-DB) [8] for primers design. They individuate 47 distinct 3′-sequences of 20 nucleotides (20-mers) that represent the 128 functional TCRBV alleles in IMGT; using these sequences as target allows scientists to significantly minimize CDR3 amplicon length. Therefore, from the selected 47 TCRB FR3 3′ 20-mers, Montagne et al. obtained three sets of primers: 1MM set (34 primers), Compact set (20 primers) and Full set (47 primers) required to multiply CDR3 region of TCR beta chains. The combinations of this experimental pipeline with an algorithm that they have personally developed, Inferring Sequences via Efficiency Projection and Primer Incorporation (ISEPPI), permitted the determination of linkage between CDR3 and corresponding V fragment of the TCR beta chain, as further verification of FR3AK-seq performance. The accuracy of ISEPPI was found to be maximal for the Full primer set, ranging between 67.7 and 72.4% depending on CDR3s clone counts (≥10 - ≥100). To verify the reliability of FR3AK-seq technology, the authors compared their method to two sensitive and quantitative existing commercial methods specialized in TCR sequencing from adaptive Biotechnologies and ArcherDX's and subsequently found no differences in the performance of FR3AK-seq in terms of CDR3 quantification and amplification bias. In order to apply this methodology to a pathology, Montagne et al. analyzed TCR repertoire on a large cohort of patients with idiopathic inflammatory myopathies (IIMs), an inflammatory heterogeneous disease in which the pathogenic role of T cells is still unknown [9], and compared them with healthy donors. TCR repertoire was successfully described in peripheral blood lymphocytes and in muscle infiltrating cells, demonstrating that a cluster of closely related TCRs is present in muscle from patients with sporadic inclusion myositis (IBM).

To summarize, the strategy of targeting the 3′ terminus of FR3 successfully allow us to minimize the number of primers and amplicon length, while preserving sequencing depth and providing a reliable capture of TCR repertoire clonal abundance, comparable to the currently available industrial sequencing platforms. We believe that Montagne et al. [1] proposed FR3AK-seq technology may pave the way to an affordable and reliable TCR sequencing, broadening our knowledge of the TCR repertoire in disease pathogenesis and immune modulating treatments.

* Corresponding author.
E-mail address: clara.ballerini@unifi.it (C. Ballerini).

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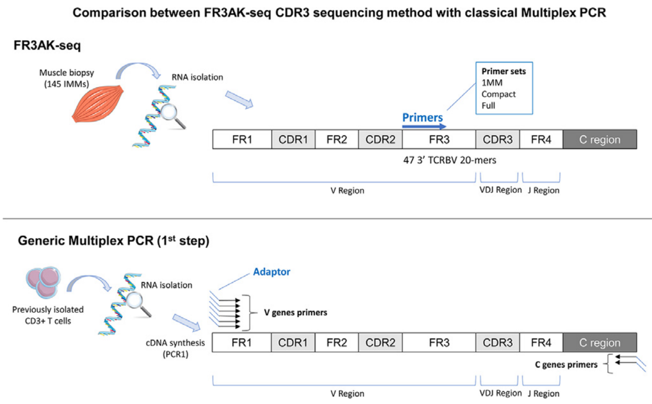

Declaration of Competing Interest

The authors declare no conflicts of interest.

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