

## Immune Profiling with a New NGS-based Repertoire Analysis

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NGS-based TCR and BCR repertoire analyses are powerful tools to analyze specificities and diversities of T and B cells. Here, we developed a new TCR/BCR repertoire analysis methods using unbiased amplification (adaptor-ligation PCR) and an in-house developed analysis software following a paired-end MiSeq sequencing. This method enables a highly accurate and quantitative analysis. With regard to TCR, we examined gene usage, diversity and similarity of TRA/TRB repertoires with PBMCs of 20 healthy individuals. TRA repertoires were more similar between individuals than TRB repertoires were. The inter-individual similarity of TRA depended largely on the frequent presence of shared TCRs among two or more individuals. The shared TRA sequences often contained invariant TCR $\alpha$  derived from invariant natural killer T (iNKT) and mucosa-associated invariant T (MAIT) cells. Regarding BCR, we sequenced five isotypes of IgH (IgM, IgD, IgG, IgA and IgE) in 12 individuals and analyzed frequencies of somatic hypermutation (SHM) among Ig subclasses. Shared sequences were frequently observed within multiple Ig subclasses and the frequency of SHM varied among the Ig subclasses. Respite the sequential class-switch recombination (CSR) from upstream to downstream subclasses, the shared clones had the almost same SHM levels while subclass-specific clones had a different levels of SHM dependent of genomic location. These results gave us an interesting insight into the development and the maturation of B cells. In conclusion, NGS-based TCR/BCR repertoire analysis will provide us critical information on T and B cells playing a pivotal role in immune system.