

Supplementary Table 1

|  |                   | BROAD SPECTRUM PIPELINES        |   |  |  |                               |
|--|-------------------|---------------------------------|---|--|--|-------------------------------|
|  |                   | IgReC                           | ImmunediveRsity   | Immcantation Framework   | IGGalaxy   | VDJServer                     |
| BASIC PROCESSING                         | PREPROCESSING     | Amplification methods supported | ▸ RACE  | <b>pRESTO</b><br>▸ Multiplex PCR<br>▸ RACE<br>▸ Can perform demultiplexing   |  | ▸ Access to pRESTO or VDJPipe |
|  |                   | Sequencing supported            | ▸ Single-end<br>▸ Paired-ends   | ▸ Single-end<br>▸ Paired-ends (overlapping or not)   |  |                               |
|  |                   | Quality filtering               | ▸ Read quality  | ▸ Read quality   |  |                               |
|  |                   | Amplification error correction  | ▸ UMI based correction<br>▸ Amplification correction without the need for UMI   | ▸ Amplification correction without the need for UMI<br>▸ UMI based correction  |  |                               |
|  | VDJ ALIGNMENT     | V(D)J Alignment                 | VJ  | <b>TigER</b><br>▸ Takes already aligned reads and corrects it detecting new alleles  | ▸ Wraps IgBlast<br>▸ Wraps IMGT HighV-QUEST  | ▸ Wraps IgBlast               |
|  | CLONAL GROUPING   | Clonotype definition(s)         | ▸ Grouping by Hamming graphs  | ▸ Unique antibodies  | <b>Change-O</b><br>▸ VJ + CDR3 (common ancestor)<br>▸ VJ + CDR3-AA   | ▸ Wraps Change-O and Alakazam |
|  |                   | Removes nonfunctional reads     |   | ✓  | ✓  |                               |
|  |                   | Lineage Tree                    | ▸ Lineage tree and Hamming graphs   | ▸ Network graphical representation   | <b>Alakazam</b>  |                               |
|  |                   |                                 |   |  |  |                               |
| REPERTOIRE CHARACTERIZATION AND ANALYSIS | DIVERSITY         | Basic Characterization          | <b>IgDiversityAnalyzer</b><br>▸ Relative/absolute frequency of clonotypes   |  | <b>Alakazam</b><br>▸ Physicochemical properties of AA sequences<br>▸ V(D)J allele/ gene usage  | ▸ Wraps Alakazam              |
|  |                   | Diversity Index                 | <b>IgDiversityAnalyzer</b><br>▸ Simpson Index (SI)<br>▸ Clonal Simpson Index (CSI)<br>▸ Ratio SI/CSI  | ▸ Gini index<br>▸ Shannon Weiner index<br>▸ Shannon normalized   | ▸ Species richness<br>▸ Shannon Weiner<br>▸ Inverse simpson<br>▸ Berger Parker<br>▸ Total diversity estimation: Chao1  |                               |
|  |                   | PLOTS                           | <b>IgDiversityAnalyzer</b><br>▸ CDR3 length distribution<br>▸ CDR3 nucleotide content per position<br>▸ CDR3 aminoacid content<br>▸ VJ usage heatmap  | ▸ Histogram of CDR3 length distribution<br>▸ Proportion of AA in CDR3<br>▸ AA distribution of CDR3<br>▸ VJ use heatmap<br>▸ Rarefaction curve  | ▸ Clonal abundance distribution<br>▸ V(D)J allele/gene usage graphs<br>▸ Rarefaction curve   |                               |
|  | MUTATION ANALYSIS | Mutation Analysis               | <b>IgDiversityAnalyzer</b><br>▸ Identifies mutation type (substitution, insertion, deletion)<br>▸ V region mutation and AA change table<br>▸ Identifies synonym or stop codon mutations   | ▸ Identifies synonymous and non synonymous mutations   | <b>SHazaM</b><br>▸ Quantification of mutational load<br>▸ Statistical models of SHM targeting patterns<br>▸ Tools to build SHM targeting models from data<br>▸ Analysis of selection pressure using BASELINE | ▸ Wraps SHazaM                |
|  |                   | PLOTS                           | <b>IgDiversityAnalyzer</b><br>▸ SHM vs relative position<br>▸ Number of reads vs SHM<br>▸ Distribution of indel SHMs positions<br>▸ Distribution of indel SHMs lengths<br>▸ Distribution of synonymous SHMs positions<br>▸ Heatmaps of amino acids, or nucleotide substitutions | ▸ Mutability frequencies<br>▸ Visualization of SHM targeting model<br>▸ Probability density functions resulting from selection analysis<br>▸ Plot summary statistics resulting from selection analysis |  |                               |
|  | EVOLUTION         | Clonal dynamics                 |   |  |  |                               |
|  | CONVERGENCE       | Convergence of Repertoire       |   | ✓  | <b>RDI</b>   |                               |
|  | OTHER             |                                 |   |  |  |                               |

Supplementary Table 1. (continued)

|  |                   | MODULAR PIPELINES               |   |   |   |  |   |   |
|--|-------------------|---------------------------------|---|---|---|--|---|---|
|  |                   | MIGEC                           | IMSEQ   | MIXCR   | LymAnalyzer   | Partis   | ImmuneDB  | Vidjil  |
| BASIC PROCESSING                         | PREPROCESSING     | Amplification methods supported | <ul style="list-style-type: none"> <li>▸ Multiplex PCR, RACE</li> <li>▸ Can perform demultiplexing</li> </ul> |   | <ul style="list-style-type: none"> <li>▸ RACE</li> <li>▸ RNA seq</li> </ul>   |  |   |   |
|  |                   | Sequencing supported            | <ul style="list-style-type: none"> <li>▸ Paired-ends</li> </ul>   | <ul style="list-style-type: none"> <li>▸ Single-end</li> <li>▸ Paired-ends</li> </ul>                         | <ul style="list-style-type: none"> <li>▸ Single-end</li> <li>▸ Paired-ends</li> </ul>   |  |   |   |
|  |                   | Quality filtering               | <ul style="list-style-type: none"> <li>▸ Cluster average quality</li> </ul>                                   | <ul style="list-style-type: none"> <li>▸ Read quality filtering</li> <li>▸ Group quality filtering</li> </ul> | ✓   |  |   |   |
|  |                   | Amplification error correction  | <ul style="list-style-type: none"> <li>▸ UMI based correction</li> </ul>                                      | <ul style="list-style-type: none"> <li>▸ Amplification correction without the need for UMI</li> </ul>         | <ul style="list-style-type: none"> <li>▸ Amplification correction with and without UMI</li> </ul>                             |  |   |   |
|  | VDJ ALIGNMENT     | V(D)J Alignment                 | ✓   | VJ  | VDJ + C   | VDJ  | VDJ   | VJ  |
|  | CLONAL GROUPING   | Clonotype definition(s)         | <ul style="list-style-type: none"> <li>▸ Unique antibodies</li> </ul>   | <ul style="list-style-type: none"> <li>▸ Unique antibodies</li> <li>▸ Can group by C</li> </ul>               |   | <ul style="list-style-type: none"> <li>▸ Common ancestor</li> </ul>  | <ul style="list-style-type: none"> <li>▸ VJ+ CDR3-AA</li> </ul>   | <ul style="list-style-type: none"> <li>▸ Unique antibodies</li> </ul>                         |
|  |                   | Removes nonfunctional reads     |   | ✓   |   |  |   |   |
|  |                   | Lineage Tree                    |   |   |   |  | ✓   |   |
|  | DIVERSITY         | Basic Characterization          |   |   | <ul style="list-style-type: none"> <li>▸ CDR3 extraction and characterization</li> </ul>                                      |  | <ul style="list-style-type: none"> <li>▸ V length distribution</li> <li>▸ CDR3 length distribution</li> <li>▸ V, J usage</li> </ul> |   |
|  |                   | Diversity Index                 |   |   |   |  |   | <ul style="list-style-type: none"> <li>▸ Shannon-Wiener</li> <li>▸ Inverse Simpson</li> </ul> |
|  |                   | PLOTS                           |   |   |   |  |   |   |
| REPERTOIRE CHARACTERIZATION AND ANALYSIS | MUTATION ANALYSIS | Mutation Analysis               |   |   |   | <ul style="list-style-type: none"> <li>▸ Mutation frequencies</li> <li>▸ Identification of non-templated insertions</li> </ul> |   |   |
|  |                   | PLOTS                           |   |   | <ul style="list-style-type: none"> <li>▸ Mutation tree (for each CDR3 one can visualize the hypermutation process)</li> </ul> |  |   |   |
|  | EVOLUTION         | Clonal dynamics                 |   |   |   |  |   | ✓   |
|  | CONVERGENCE       | Convergence of Repertoire       |   |   |   |  |   |   |
|  | OTHER             |                                 |   |   |   |  |   |   |
|  |                   |                                 |   |   |   |  |   |   |
|  |                   |                                 |   |   |   |  |   |   |

Supplementary Table 1. (continued)

|  |                   | SPECIALIZED PIPELINES           |  |   |   |  |   |  |  |
|--|-------------------|---------------------------------|--|---|---|--|---|--|--|
|  |                   | SONAR                           | TRIGS  | IMEX  | IRProfiler  | VDJtools   | DivE  | BASELINE   | AbSim  |
| BASIC PROCESSING                         | PREPROCESSING     | Amplification methods supported | ▸ Single-end   |   |   |  |   |  |  |
|  |                   | Sequencing supported            | ▸ Paired-ends  |   |   |  |   |  |  |
|  |                   | Quality filtering               | ✓  |   |   | ▸ Frequency based correction<br>▸ Frequency based filtering  |   |  |  |
|  |                   | Amplification error correction  |  |   |   |  |   |  |  |
|  | VDJ ALIGNMENT     | VDJ Alignment                   |  |   | ▸ Wraps IMGT HighV-QUEST<br>▸ 11 filtering criteria                       |  |   |  |  |
|  | CLONAL GROUPING   | Clonotype definition(s)         | ▸ VJ + CDR3 (Common ancestor)<br>▸ VJ + CDR3-AA              | ▸ VJ + CDR3 (Common ancestor)<br>▸ VJ + CDR3-AA   | ▸ VDJ + CDR3-AA<br>▸ VJ+ CDR3-AA<br>▸ V + CDR3-AA<br>▸ J + CDR3<br>▸ CDR3 |  |   |  |  |
|  |                   | Removes nonfunctional reads     |  | ✓   |   | ✓  |   |  |  |
|  |                   | Lineage Tree                    | ✓  | ✓   |   |  | ✓   |  |  |
| REPERTOIRE CHARACTERIZATION AND ANALYSIS | DIVERSITY         | Basic Characterization          |  | ▸ Number of productive / unproductive reads<br>▸ VDJ gene total and relative frequencies            | ▸ Clonotype quantification<br>▸ V, J gene usage                           | ▸ Read counts<br>▸ Mean clonotype sizes<br>▸ Non-functional reads<br>▸ Histogram of CDR3 nucleotide length   |   |  |  |
|  |                   | Diversity Index                 | ▸ Gini index   | ▸ IMEX calculates sequence diversity using a more elaborated data mining approach.                  |   | ▸ Shannon-Wiener<br>▸ Inverse Simpson<br>▸ D50 index, a recently developed immune diversity estimate<br>▸ Lower bound total diversity: Chao1 or Efron-Thisted estimate   | ▸ DIVE species richness estimate (fits different models and selects the top 5 model to estimate species richness) |  |  |
|  |                   | PLOTS                           | ▸ CDR3 length distribution<br>▸ Histograms for VJ gene usage | ▸ VDJ usage (heatmaps or histogram)<br>▸ Productive/ non productive pie charts<br>▸ Diversity curve |   | ▸ V, J, VJ usage (heatmap and hierarchical clustering)<br>▸ Clonality plot (Plots a three-layer donut chart to visualize the repertoire clonality)<br>▸ Rarefaction plot | ▸ Rarefaction plot  |  |  |
|  | MUTATION ANALYSIS | Mutation Analysis               | ▸ V, J usage   |   |   |  |   | ▸ Identifies point and silent mutations<br>▸ Identifies and quantifies positive or negative selection<br>▸ Germline normalization for direct comparison of different repertoires |  |
|  |                   | PLOTS                           |  |   |   |  |   |  |  |
|  | EVOLUTION         | Clonal dynamics                 |  |   |   | ✓  |   |  |  |
|  | CONVERGENCE       | Convergence of Repertoire       |  | ✓   | ✓   | ✓  |   |  |  |
|  | OTHER             |                                 | ▸ longitudinal phylogenetic "birthday" trees                 |   |   |  |   |  | Generates simulations of Ig repertoires with the desired characteristics |