Immune Repertoire Profiling Service

DESCRIPTION

Immune repertoire often represents an individual's current immunological status; whether the person is healthy, vaccinated, diseased, or infected. Only high-throughput NGS analysis can comprehensively profile an individual's immune repertoire. The Immune Repertoire Profiling Service provides effective data acquisition, integration, and interpretation for the customers.

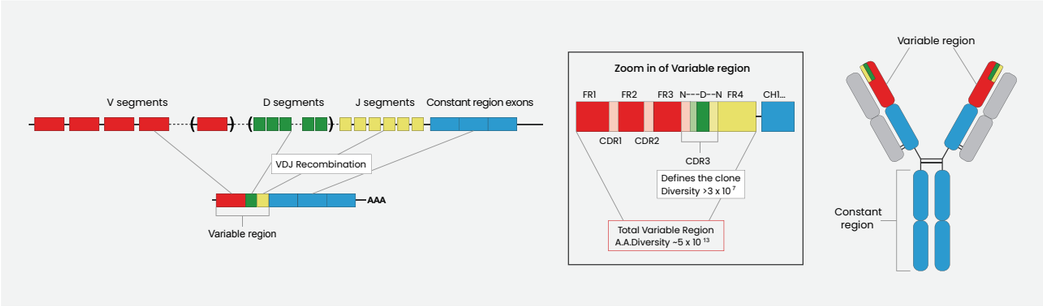
KEY FEATURES

1. Quantitative analysis of library diversity	- NGS-based analysis of complex antibody library consisting of millions (10^6~10^{12}) of sequences in a single experiment - Analysis of immunoglobulin and T-cell receptor repertoire; analysis of BCR/TCR for each clone - Frequency analysis of individual antibody clones within the library, identifying major and minor clones
2. Tracking of clonal frequencies for each sample	- For antibody discovery, analysis of library diversity according to its panning degree enabling monitoring changes in clonal frequency - Minimized omission of potentially significant antibody clones - Analysis of immune repertoire characteristics from blood sample and monitoring of each clone
3. Various analysis options for immune system studies	Perform the experiment with drastically reduced time and cost enabled by the advanced technology of MSSIC developed by Celemics

REQUIREMENTS

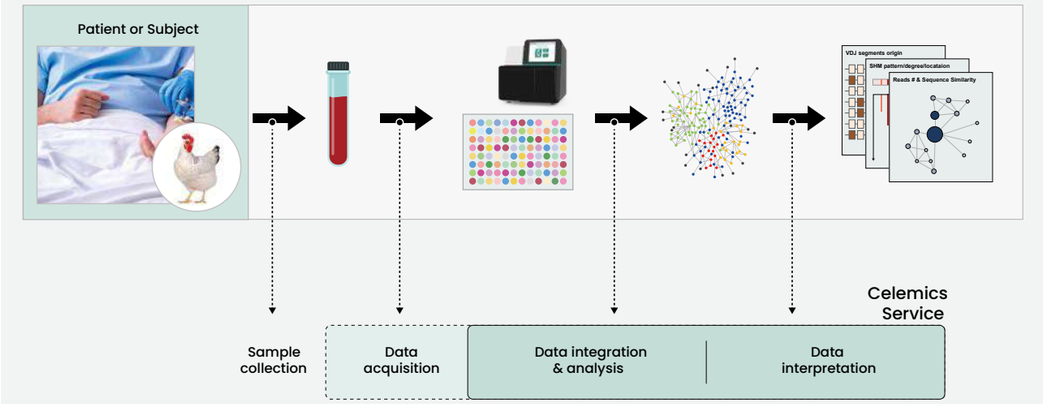
Sample type	Total RNA from B-Cell or/and T-Cell, DNA from B-Cell or/and T-Cell, DNA/RNA Amplicons
Concentration	100 ng/μl
Amount	1 μg
Turnaround time	Within 4-6 business weeks from sample collection
Temperature	RT for storage and shipment

DIVERSITY OF ANTIBODY



The antibody genes are composed of many different segments. The antibodies are presented in B cells with great diversity of 10^{13} repertoires.

GENERAL WORKFLOW



Celemics provides service for data acquisition, integration, and analysis, and interpretation.

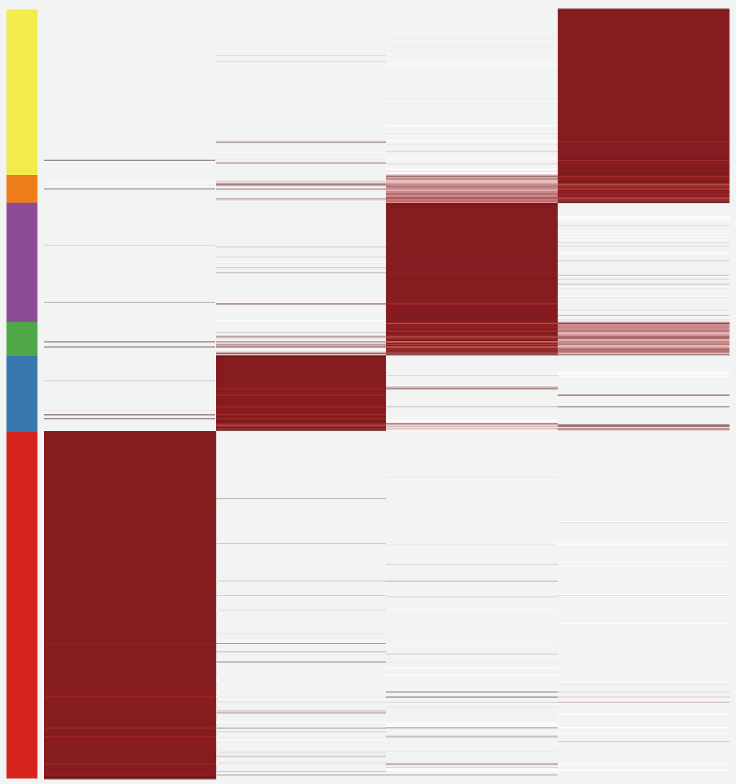
IMMUNE PROFILING EXAMPLES

Example 1 - Discovery of candidate antibodies from actively immunized chickens

CDR3 sorting results

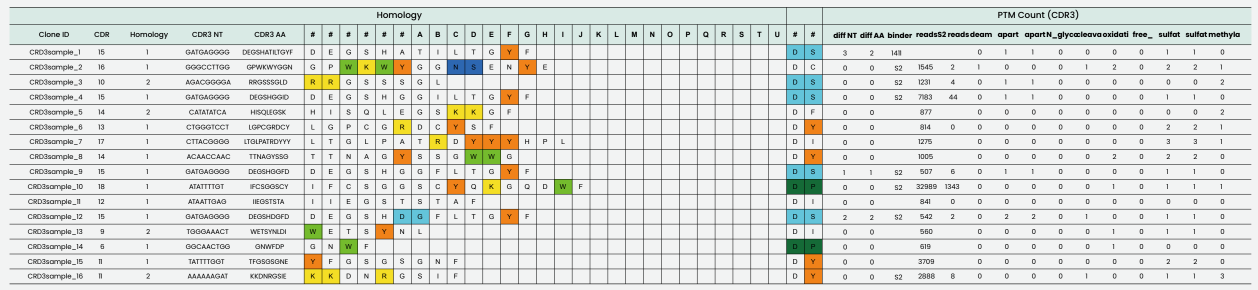
CDR3 ID	CDR3 AA	p_pre-immune	p_immune	p_1st_boost	p_2nd_boost	p_tissue	p_BM_2nd
CDR3sample_1	GSRDSSASTI	2433	773	31	0	2	461
CDR3sample_2	GSYDSSYVGI	1756	1444	2269	895	1058	789
CDR3sample_3	GSIDSSYVGI	1019	402	876	938	541	346
CDR3sample_4	ANFDSSSGAGI	46	25	338	483	1707	345
CDR3sample_5	GGYDSSAGI	231	268	934	207	966	7770
CDR3sample_6	GSFDSSTYAGI	3678	1034	425	290	547	431
CDR3sample_7	GSRDSSASTI	2433	773	31	0	2	461
CDR3sample_8	GSRDSSYVGI	6427	6370	10151	5756	10089	2680
CDR3sample_9	GGYDGSTYVGI	279	211	2047	178	271	88
CDR3sample_10	GSRDSNYVGI	407	567	974	749	868	224
CDR3sample_11	GSSSGTGI	1563	2580	899	1999	114	24702
CDR3sample_12	GSYDSSAGI	1195	875	1342	743	746	288
CDR3sample_13	GSRDSTYVGI	461	795	1355	998	983	136
CDR3sample_14	GGYDSSTDAGI	1167	1129	1353	1617	892	405

Sequence abundance clustering result

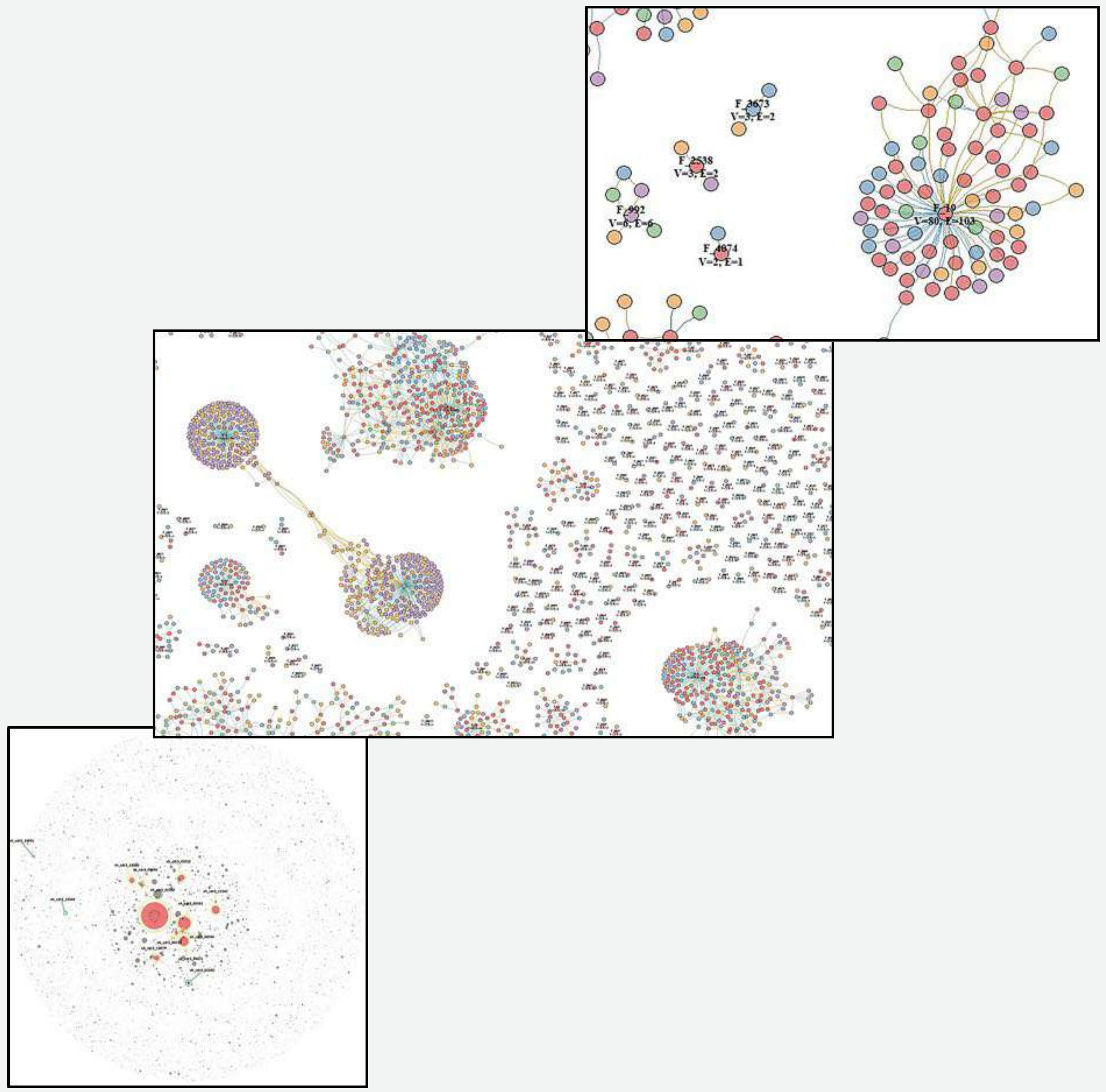


Example 2 - Discovery of novel drug candidates with antibody analysis from respiratory infection patient samples

CDR3 similarity analysis



Network Analysis Between Antibody Sequences



TrueRepertoire™ Service

DESCRIPTION

The TrueRepertoire™ is a NGS-based antibody library sequencing platform developed to overcome the key issues of existing methods such as sequencing error, short-read length, and high-cost gene synthesis for further characterization. Celemics has developed a cloning microchip, barcode assay technology, and laser-based non-contact clone retrieval system and integrated into the newly developed platform, TrueRepertoire™ assay. This service allows for full sequence analysis of over 10,000 clones in a single experiment and thereby discovering rare clones. The TrueRepertoire™ service contains the client's antibody clone of interest within the library itself, eliminating the need to perform new gene synthesis and significantly reducing time and cost.

KEY FEATURES

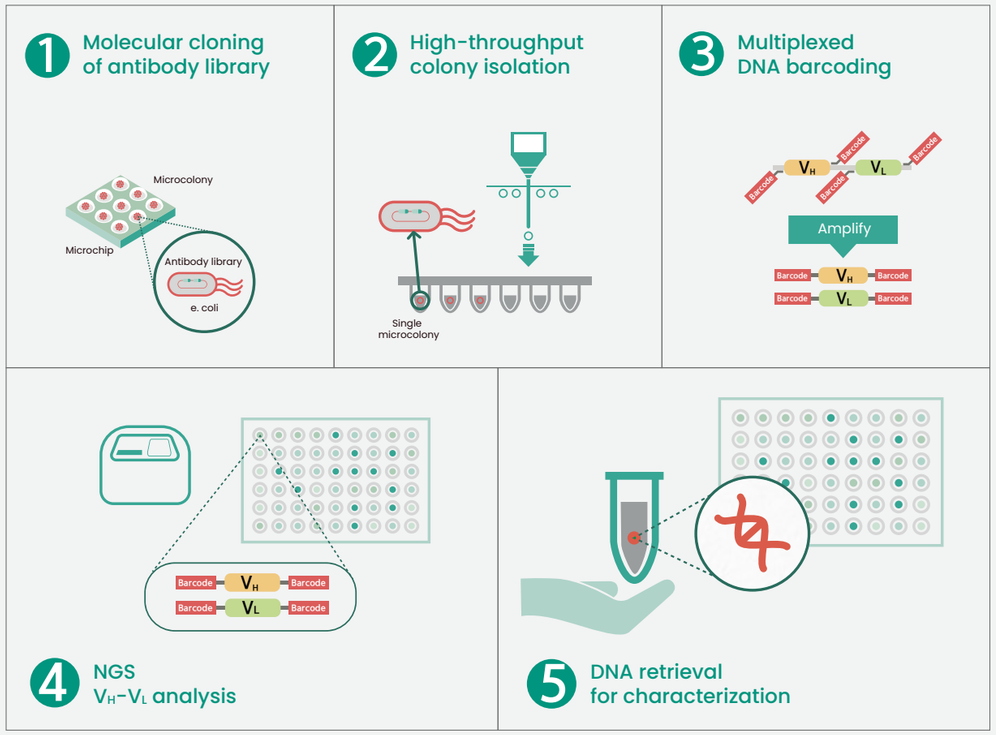
1. Provision of antibody DNA sequence library containing over 10,000 errorless strains	NGS-based sequence analysis and high-capacity clone separation and molecular barcode assays using Celemics proprietary MSSIC technology
2. V_H-V_L linkage analysis of each antibody	Receive V_H-V_L linkage information, an area difficult to analyze through NGS due to its short read length
3. Provision of physical property analysis of each antibody through bioinformatics analysis	Clone frequency distribution within the library V_H-V_L sequence length distribution, post-translation modification information, CDR and frame amino acid information, etc.
4. Retrieval of selected physical antibody allowing for convenient workflow	Eliminates the need to perform new gene synthesis and reduces time and cost due to the antibody clones within the library itself, enabling isolation of physical DNA for further characterization

REQUIREMENTS

Sample type*	Total RNA from B-Cell or/and T-Cell, DNA from B-Cell or/and T-Cell, DNA/RNA Amplicons
Concentration	100 ng/µl
Amount	1 µg
Turnaround time	Within 4-6 business weeks from sample collection**
Temperature	RT for storage and shipment

* ~30 bp of Consensus upstream & downstream sequence over V_H and V_L region required
** TAT depends on colony size

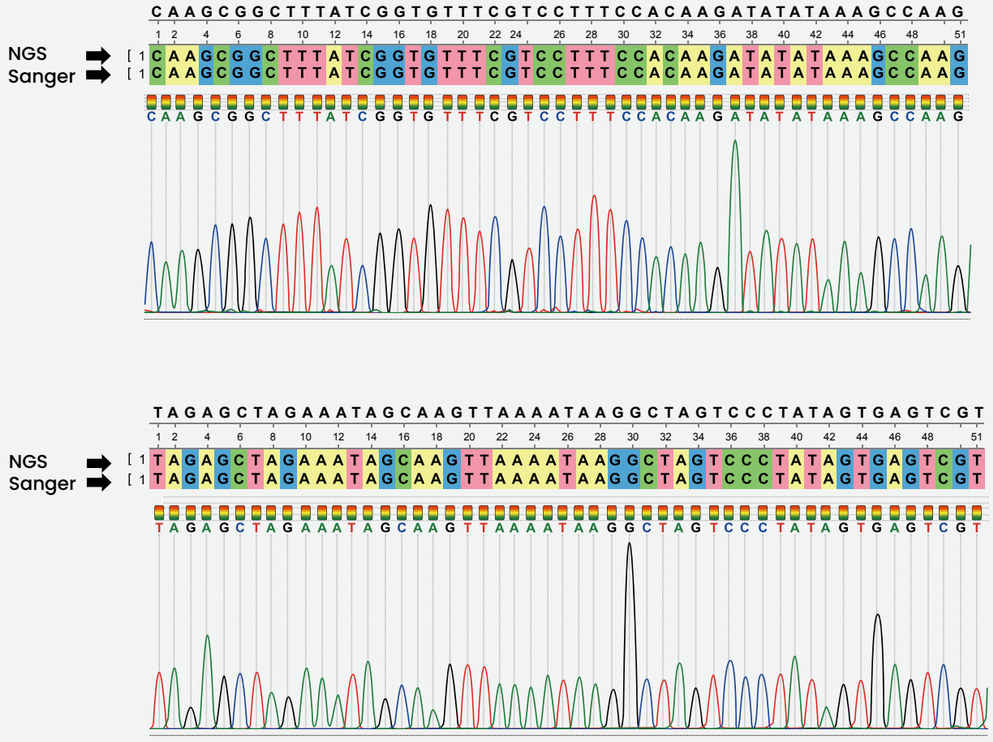
HOW TrueRepertoire™ WORKS



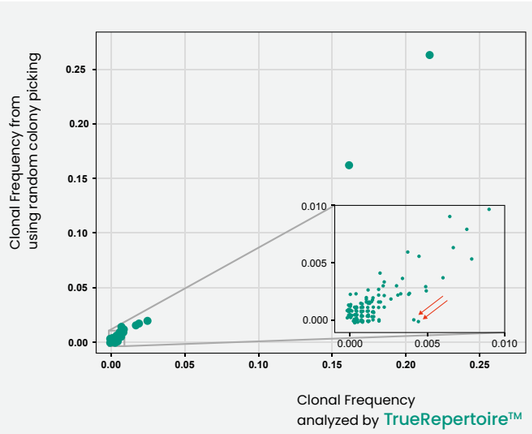
1. Celemics proprietary microcolony chip formation with high density, each colony starts from a single E. coli.
2. Extraction of the colonies from the microchip into microwell by Celemics' proprietary laser system
3. Multiplex PCR with barcoded primers from the isolated colonies
4. NGS and computation of the consensus sequences with cognate pairing of V_H and V_L
5. Clonal DNA retrieval based on the consensus sequence for further characterization

VALIDATION TESTS

Validation I. Result of 480 randomly selected antibody clones from TrueRepertoire™ perfectly matched (480/480) Sanger sequencing results of their physical DNA

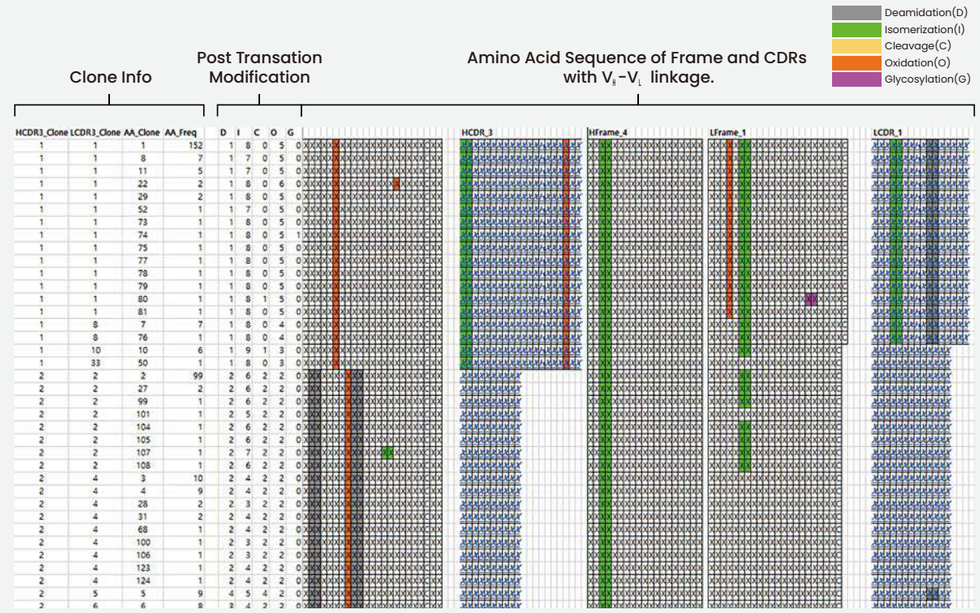


Validation II. Similar clonotype frequencies of major clones between TrueRepertoire™ and random colony picking

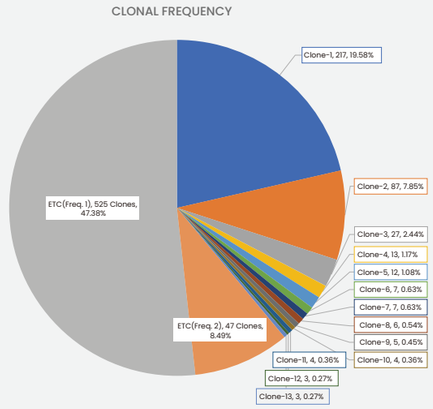


1. Major clones showed similar clonotype frequency in both platforms - random colony picking followed by Sanger sequencing and TrueRepertoire™
2. The result showed that there were newly identified clones found only in the TrueRepertoire™ results (red arrows)

USER FRIENDLY TrueRepertoire™ REPORT



Clonal Frequency Distribution



V_H, V_L Sequence Length Distribution