Immune Repertoire Profiling

a window into TILs diversity

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Your precision medicine partner

American Society of Gene & Cell Therapy

Conference 2023, May 16th – May 20th

Advancing Research, Advancing Health

Dr. Dirk Van Alewijk | Senior Scientist | Cerba Research

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Chimeric Antigen Receptor (CAR) - Cell therapy

Our portfolio for your cell therapy development

- ✓ **Scientist to scientist** communications.
- ✓ **Standardized processes** and global centralized database.
- ✓ **Integrated specialty testing** to your existing central laboratory process.
- ✓ **Large global PBMC network** with state-of-the-art processing.
- ✓ A **Global network** of Labs **and Pathologists.**
- ✓ Specialized in **Tech Transfers** and **New Assay Development**

1. **NGS** (Immune repertoire profiling, TILs characterization)
2. **NanoString** (OMICS platform for tumor profiling, immune landscape characterization)
3. **Flow Cytometry** (immunophenotyping, CAR-T characterization and monitoring)
4. **Genomics** (NGS, 4-digit HLA Typing, Immune repertoire sequencing, ddPCR, qPCR, FISH, ctDNA)
5. **Histopathology** (simplex and multiplex, 250+ biomarkers available, digital imaging, pathologist review)
6. **Biobanking** (access to >3000 blocks and growing tumor microarrays)
7. **Circulatory biomarkers/CRS** (cytokines/soluble proteins by MSD and ELISA)
8. **Safety Testing** (RCL/RCR, VCN, hematology, biochemistry, urinalysis, coagulation, serology, CRP, thyroid function, sPEP, uPEP, D-dimer)
9. **PBMC** Isolations (harmonized isolation procedures with 40+ locations globally)

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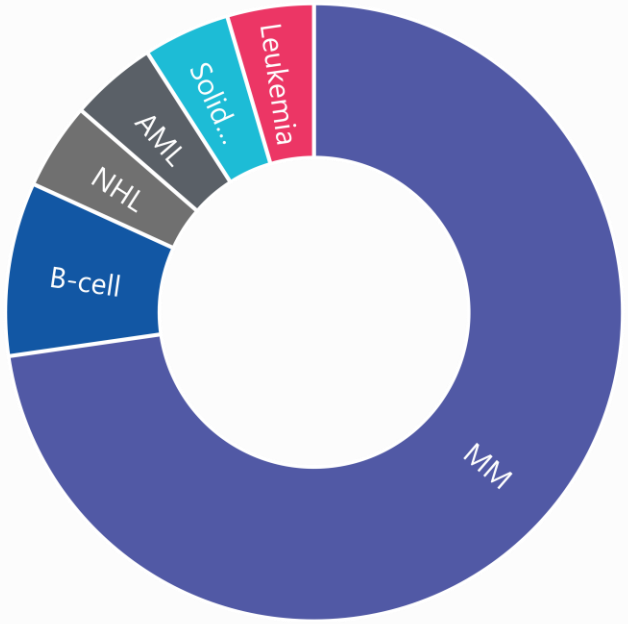
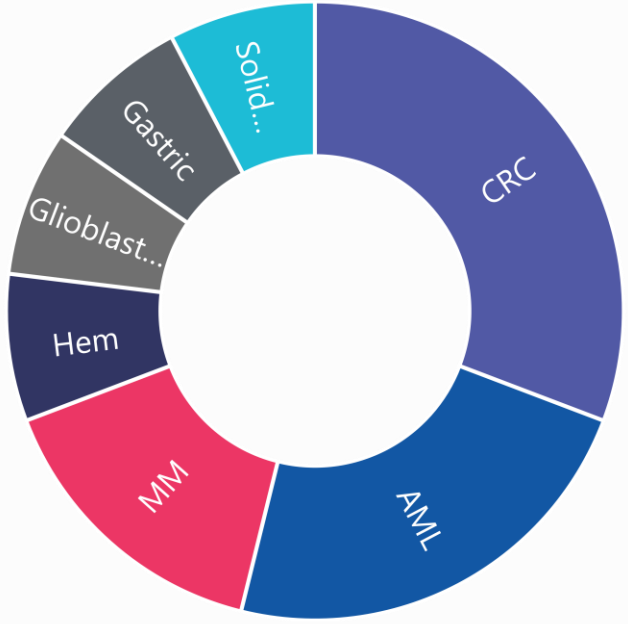
Our Cell Therapy Trial Experience By Indication

>40 CGT trials in the last 5 years

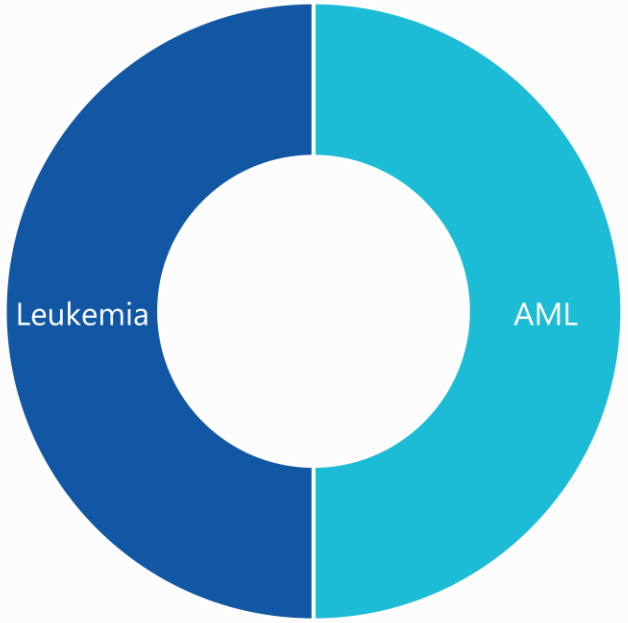
CAR T trials



NK cell trials



Engineered donor graft trials



AML = Acute Myeloid Leukemia; B-cell = B-cell lymphoma; CRC = Colorectal Cancer; Gastric = Gastric cancer; Glioblastoma; Hematological malignancies; NHL = Non-Hodgkin Lymphoma; MM = Multiple Myeloma; Solid tumors

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Immune Repertoire Profiling And TILs

The solution for the cell therapy of solid tumors

Liquid vs Solid

Liquid tumors:

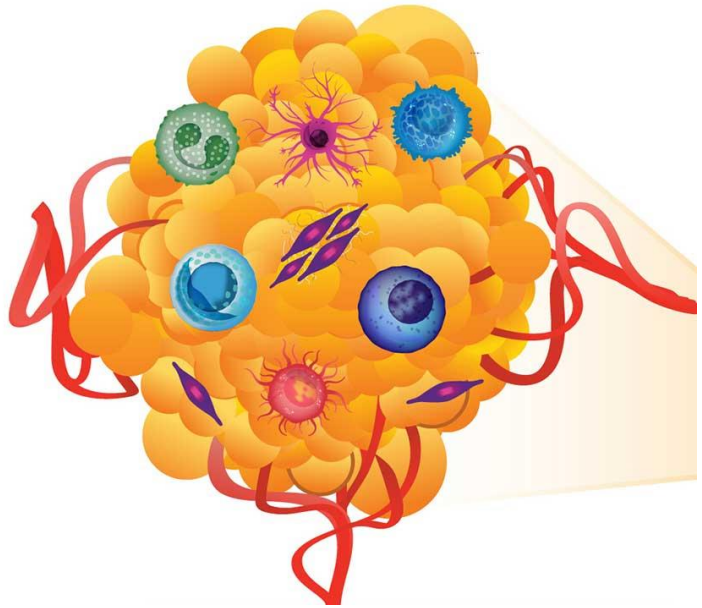
Leukemias
Lymphomas
Myelomas

- Easily accessible for treatment
- CAR approaches
- FDA approved therapies
- Several successful trials

- Difficult to treat
- Diverse immunogenicity
- More complex biology
- On target off tumor toxicity
- CAR & TCR-T approaches
- No FDA approved therapies
- No complete remission achieved

Solid tumors:

Tissue cancers



Immune Repertoire Sequencing

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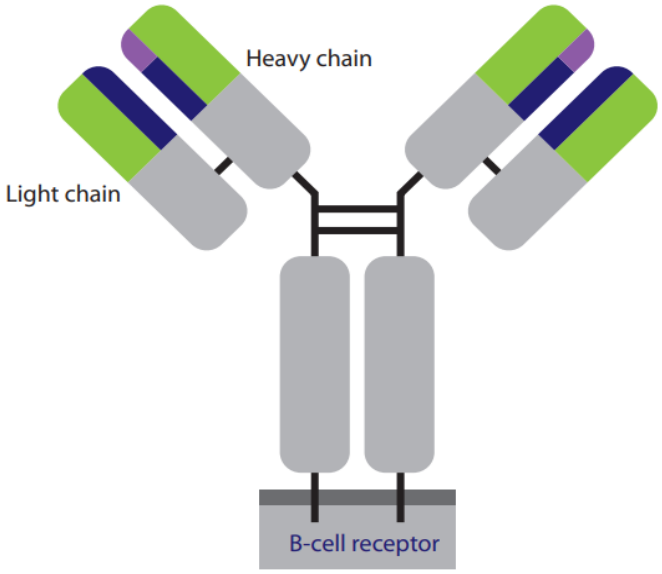
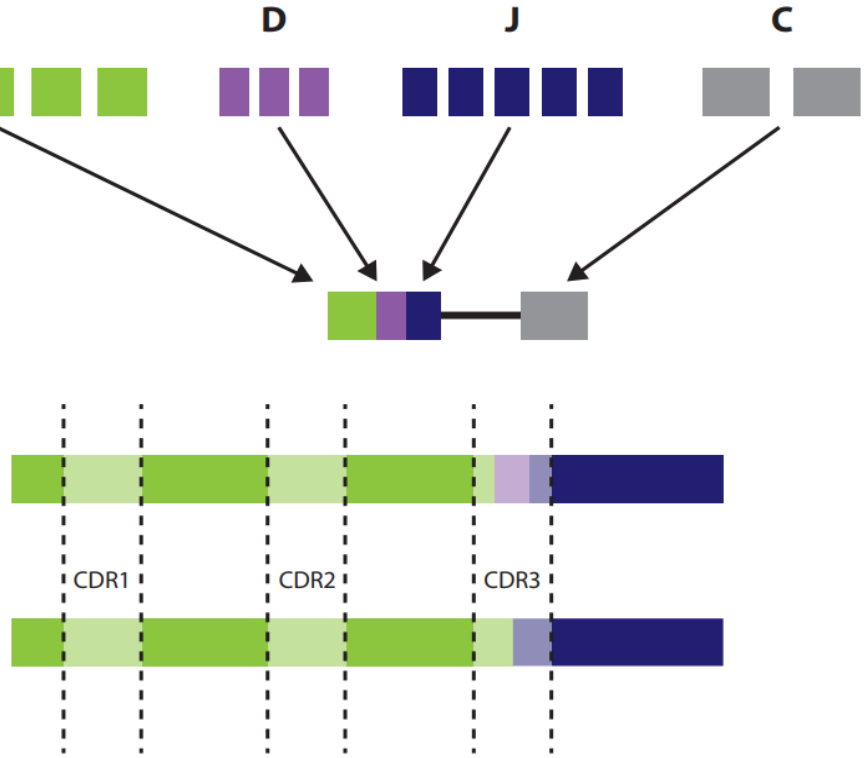
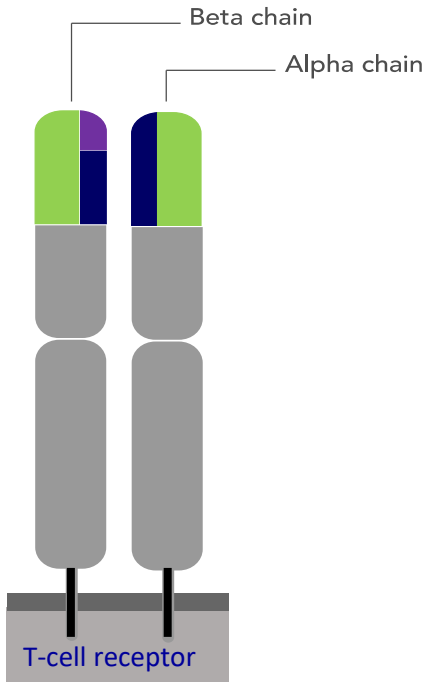
Immune Repertoire Focus: B&T Cell Profiling & Analysis



~10^{11} potential receptors (before SHM)



~10^{12} lymphocytes in the human body



~10^{16} potential receptors

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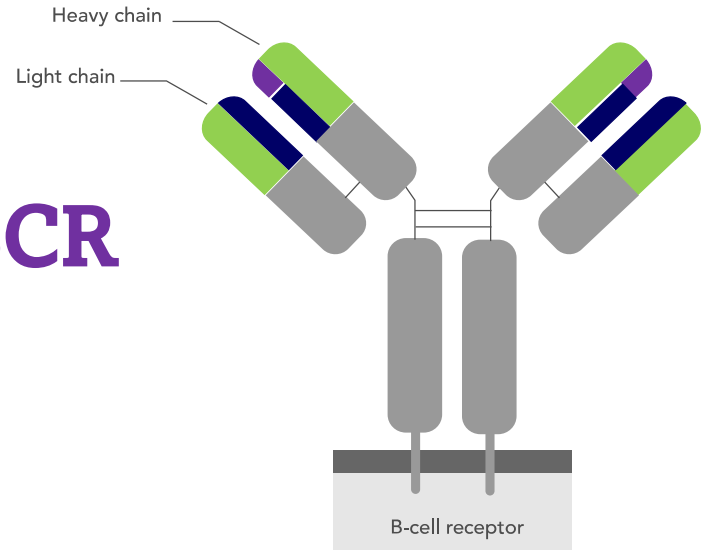
Profiling Immune Cell Receptors… What Can We Learn?

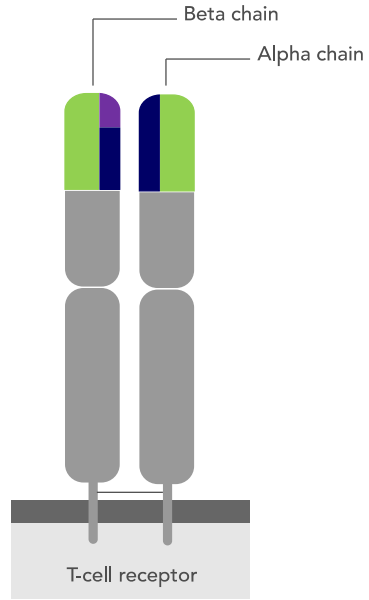


BCR

CLINICAL TRIALS

DIAGNOSTICS

RESEARCH



TCR

USAGE BY FIELD

- Infectious diseases
- Vaccine development
- Autoimmunity
- Oncology
- Aging

- Oncology: Biomarker of response to therapy
  Biomarker of therapy toxicities
  Tumour infiltrating leukocytes (TILs)
  CAR-T cell therapy
  TCR-T cell therapy
- Vaccine development
- Autoimmunity

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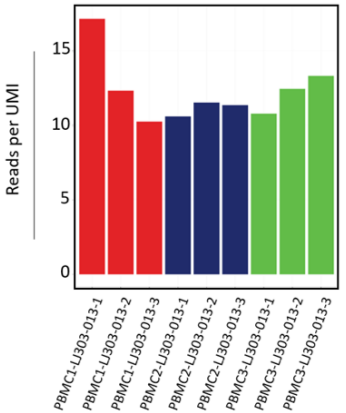
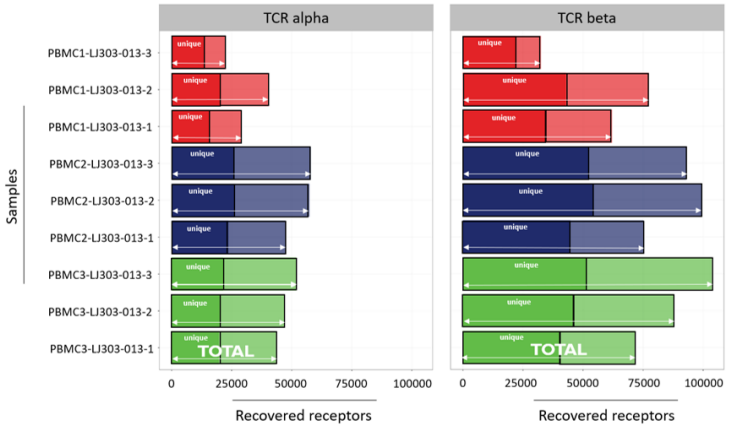
TCR Repertoire Analysis, Some Standard Parameters…

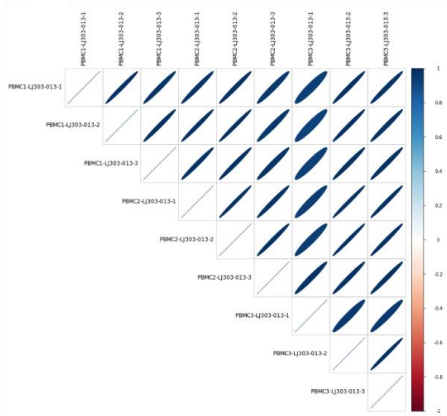
1 Reads per UMI



2 Unique/ Total clones

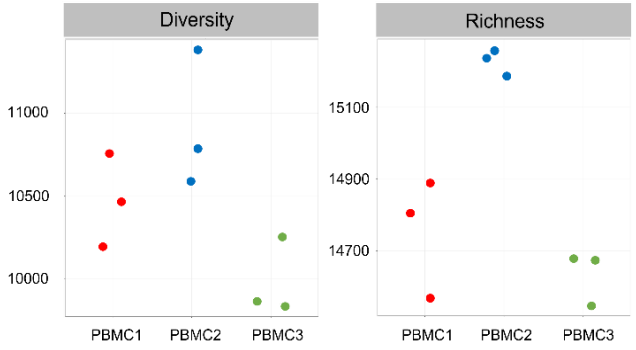


3 TCR beta Similarity

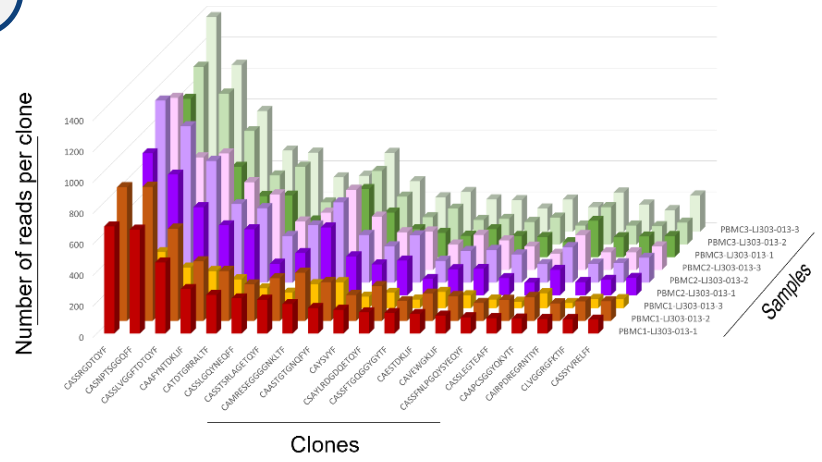


4 Diversity & Richness

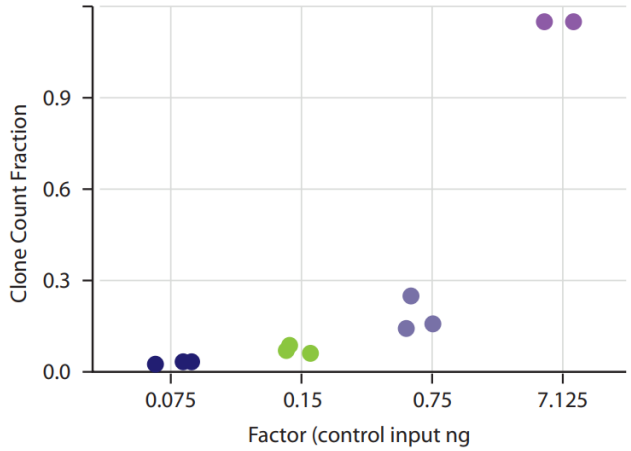
TCR beta



5 Top clones



Use of spike-ins as a correction for amplification bias



TCR Repertoire Sequencing In Immuno-oncology

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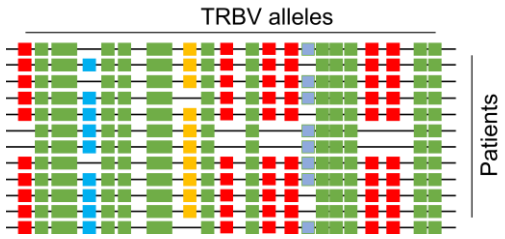
Select A Suitable Patient Group Or Monitor Disease

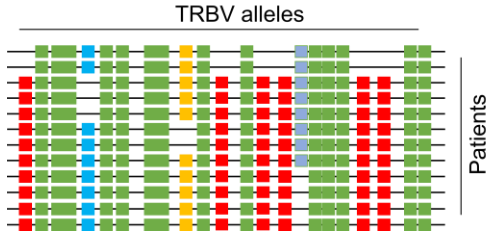
Adverse effect of the therapy related to the TCR haplotype

Clustering of haplotypes matched with observed severity of toxicities reveals haplotype most responsive to the therapy

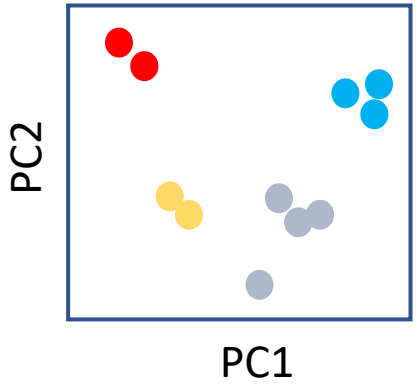


Patient stratification

Missing red alleles
Missing blue alleles
Missing yellow alleles
Missing gray alleles

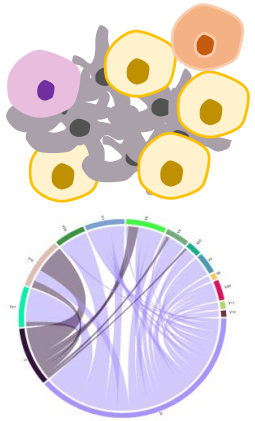


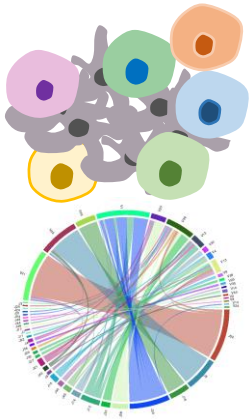
Adverse effect monitoring



Therapy effectiveness - Clonality & diversity

Therapy is efficient, favorizes one clone; Thus, clonality is increased, diversity decreased





Therapy is not efficient, no dominant clone; Thus, clonality is decreased, diversity increased

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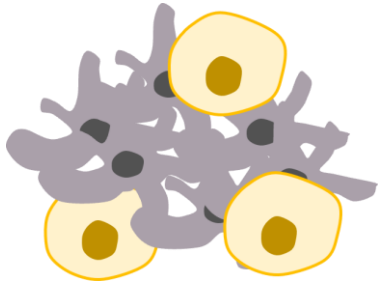
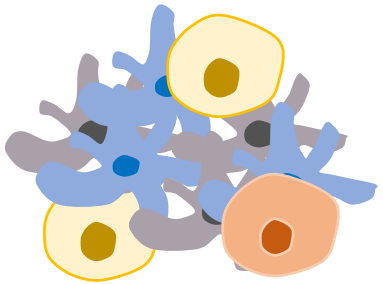
Monitor The Effectiveness Of The Therapy

Tumor evolution - CDR3 NT Sequence



ATTGGCGGTAGT

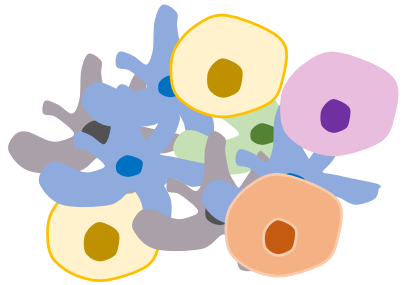


ATTGGCGGTAGT

ATTGGCTGTAGT

Tumor evolution

Increased number of T cell clonotypes



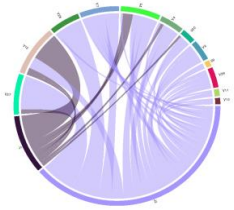
ATTGGCGGTAGT

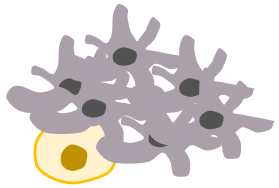
ATTGGCGGTAGT

ATTGGCTATAGT

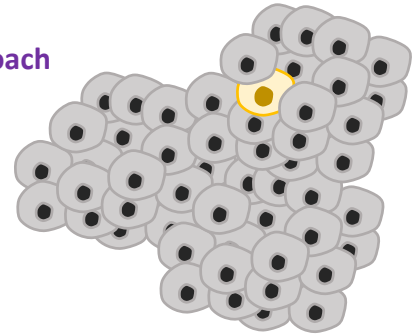
Minimal residual disease – Detection of rare clones

Tumor responsive clone

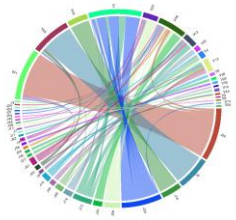




Therapeutic approach



Detection of the rare tumor responsive clone



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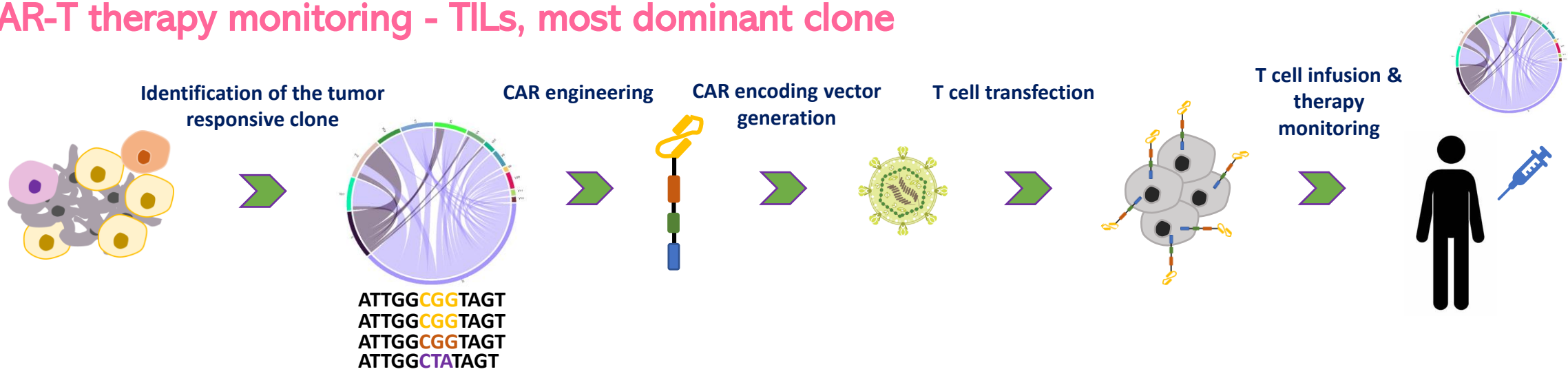
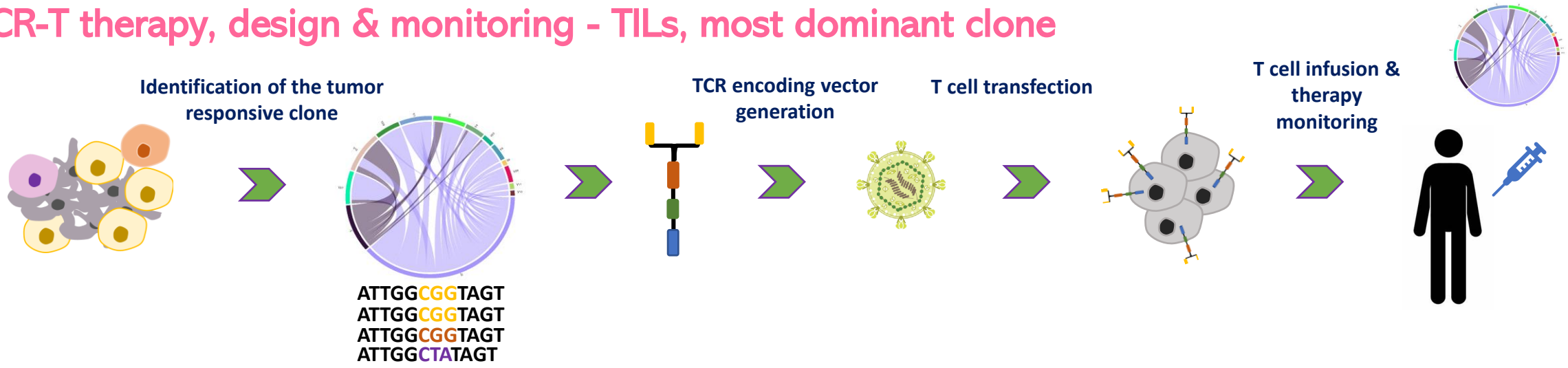
Analyze TILs, Find Rare Clone And Monitor Therapeutic Cell Population

CAR-T therapy monitoring - TILs, most dominant clone



TCR-T therapy, design & monitoring - TILs, most dominant clone



Our QC Workflow & Standard Report Elements

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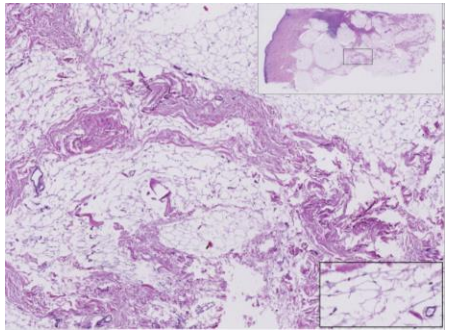
Features Of The Analyzed Tumors

Breast cancer



20 % pathological tissue

Type: Breast cancer

No necrosis

No fibrosis

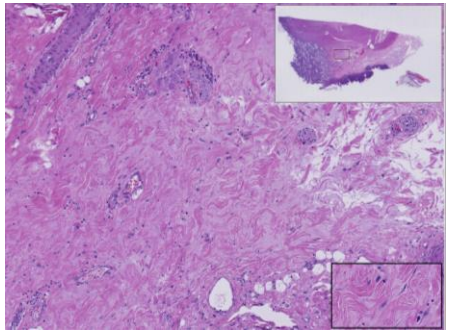
All cell type present

Immunogenicity: Low

FFPE block: 2 years old

Storage condition: RT

Colon cancer



30 % pathological tissue

Type: adenocarcinoma

No necrosis

No fibrosis

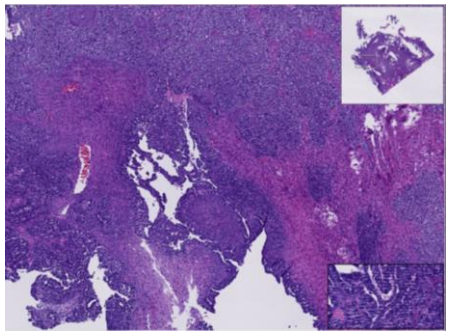
All cell type present

Immunogenicity: Medium

FFPE block: 7 years old

Storage condition: RT

Ovary cancer



100 % pathological tissue

Type: Serous adenocarcinoma

No necrosis

No fibrosis

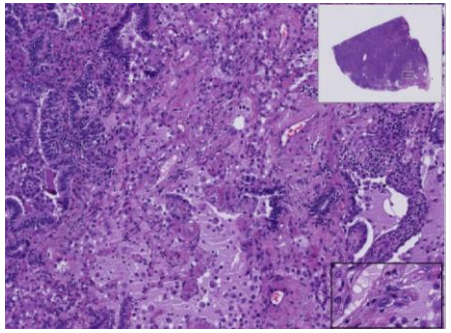
All cell type present

Immunogenicity: Hi

FFPE block: 1,2 years old

Storage condition: RT

Lung cancer



61 % pathological tissue

Type: NSCLC

No necrosis

No fibrosis

All cell type present

Immunogenicity: Medium

FFPE block: 7,5 years old

Storage condition: RT

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CD247, For Assessing T Cell Content In Your FFPE Samples

1 Bioanalyzer

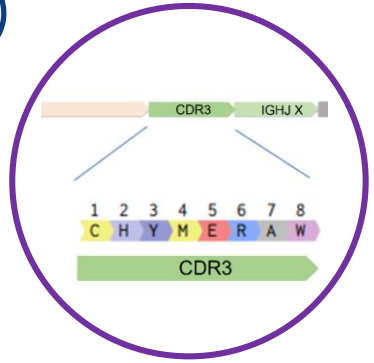
To assess RNA quality

2 RiboGreen ®

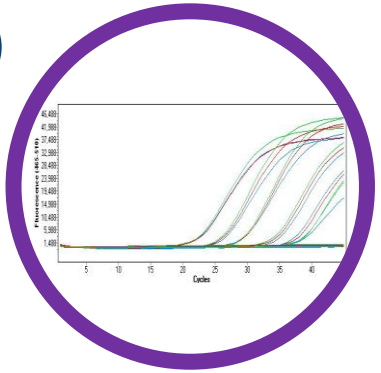
To assess RNA quantity

3 Spike-ins

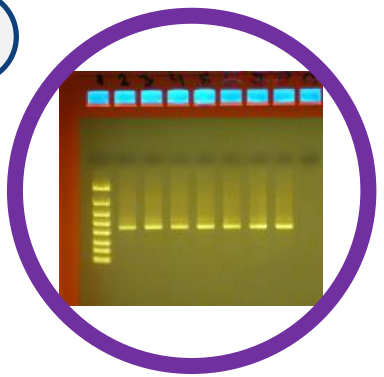
To correct for amplification bias



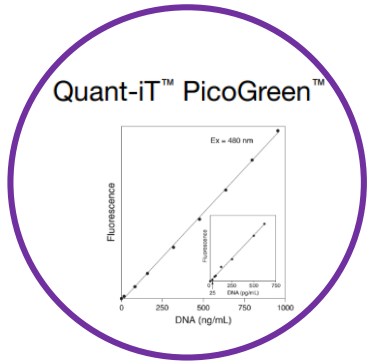
4 Lymphocyte specific transcript (FFPE)



5 Lonza FlashGel ™

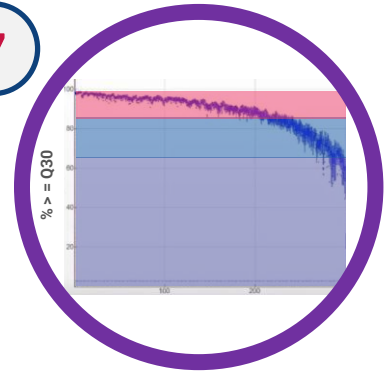


6 PicoGreen ®

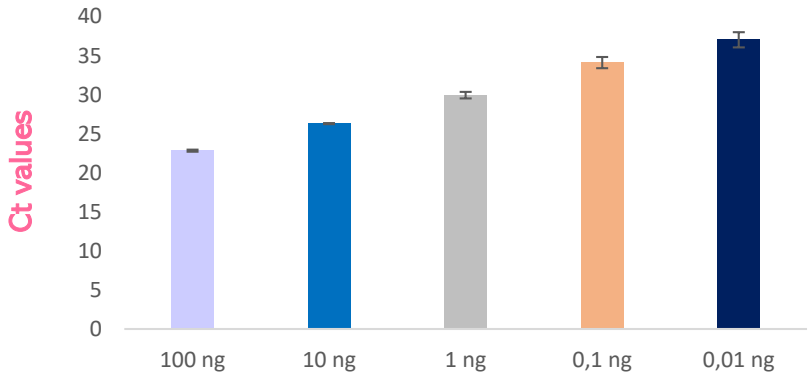


7 Q30

To assess quality of the run



CD247 dynamic range



T cell total RNA ≈ 15 pg

Assay LOD is approx. 10 T cell

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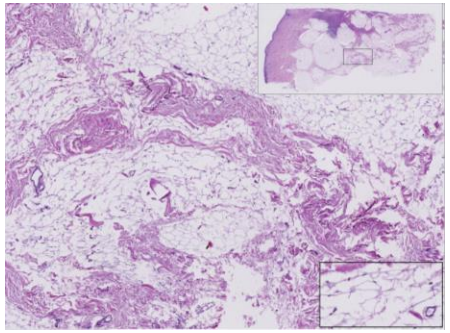
QC Parameters Of The Analyzed Tumors

Breast cancer



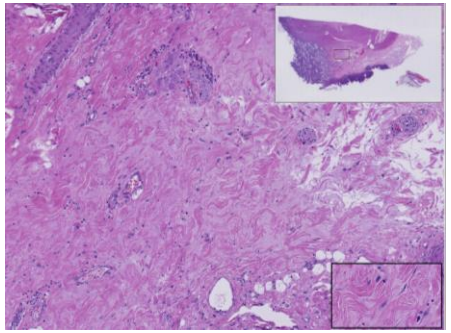
DV200 pre DNAse: >70%

DV200 after DNAse: 50-70%

Ct value for CD247: 40

Outside of the dynamic range

Colon cancer



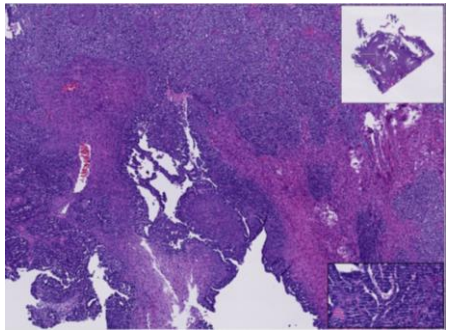
DV200 pre DNAse: >70%

DV200 after DNAse: 30-50%

Ct value for CD247: 38,02

Outside of the dynamic range

Ovary cancer



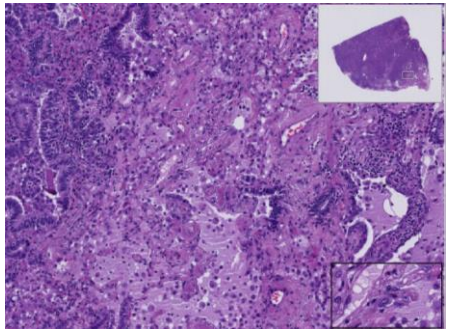
DV200 pre DNAse: >70%

DV200 after DNAse: 30-50%

Ct value for CD247: 34,78

Within the dynamic range

Lung cancer



DV200 pre DNAse: 50-70%

DV200 after DNAse: <30%

Ct value for CD247: 36,88

At the limit of the dynamic range

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Limitations And Guidelines For TILs Profiling In FFPE Samples

- Storing conditions: Preferred 4 °C instead of room temperature
- Age of the FFPE block: Preferred age is under 2 years
- Isolation method: Avoid solvent-based approaches
- Isolate enough quantity of the starting material
- Avoid any steps not essential for the process
- DNAse treatment reduces both quantity and quality of the isolated material
- Assess the amount of T cell material

Only samples that fulfill all the above stated requirements are suitable for further analysis

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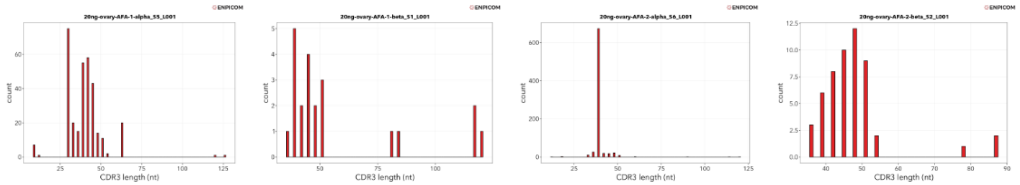
Standard Report When Profiling FFPE Samples

Our portfolio for your cell therapy development

CDR3 Length

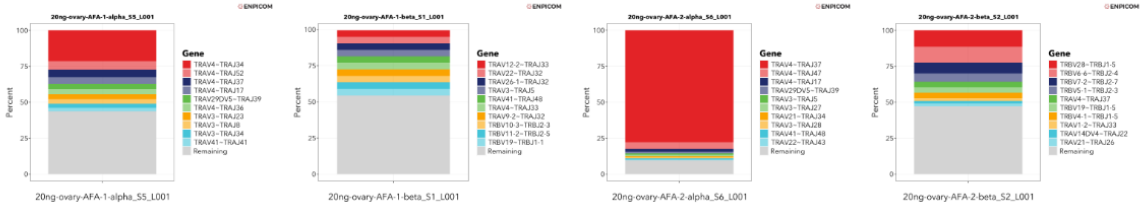
In this section, we examine the length distribution of the CDR3 region of the immune receptor. We generate histograms of the CDR3 length distribution. The histograms how the number of clones with different lengths of CDR3 nucleotide sequences. These histograms can help understand the diversity and complexity of the TCR or BCR repertoire and highlight potential quality control issues.

The output of repertoire processing is often restricted to functional immune receptors (i.e., in the correct reading frame, no stop codons or indels, no missing regions); in this case, the plot below will have bars only at lengths that are divisible by 3.



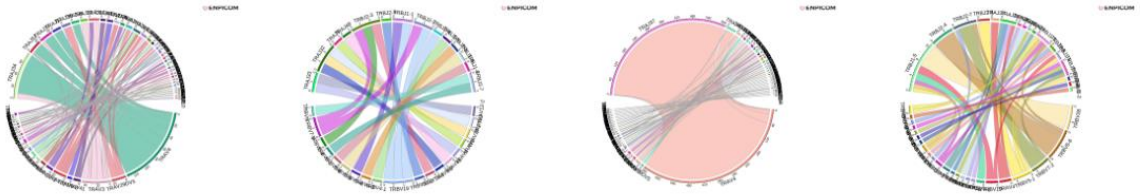
Combined V- and J-Gene Usage

Gene usage is shown per sample. Only the top 10 most frequently used genes per sample are named.



V and J-Gene Co-occurrence

Plots showing the co-occurrence of V and J-genes in the same samples. Ribbons connect V and J-genes found in the same VDJ with sizes scaled to the total number of co-occurrences.



Combinations of V- and J-genes per sample, depicted as circosplots.

Combinations of V- and J-genes per sample, depicted as circosplots.

Combinations of V- and J-genes per sample, depicted as circosplots.

Combinations of V- and J-genes per sample, depicted as circosplots.

Top 10 clones per sample

Top 10 clones with a clone count of at least 3. Samples with no clones are omitted.

Top 10 clones (with ties) for sample '20ng-ovary-AFA-2-alpha_S6_L001'

Vgene	Jgene	CDR3.aa	clone_count
TRAV4*00	TRAJ37*00	CLVETSNTGKLIF	529
TRAV4*00	TRAJ47*00	CLVGTLGGNKLVF	13
TRAV4*00	TRAJ17*00	CLVGDLVYAAGNKLTF	11
TRAV21*00	TRAJ34*00	CAVPGGDTDKLIF	6
TRAV3*00	TRAJ28*00	CAVRDLGSGAGSYQLTF	6
TRAV3*00	TRAJ5*00	CAVRDTGRRALTF	6
TRAV4*00	TRAJ37*00	SLVETSNTGKLIF	6
TRAV4*00	TRAJ47*00	CLVGDKAYGNKLVF	6
TRAV3*00	TRAJ27*00	CAVSLNAGKSTF	5
TRAV4*00	TRAJ37*00	CLVGTSNTGKLIF	5

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Analysis Summary Report

Report of results of TCR/BCR repertoire analysis

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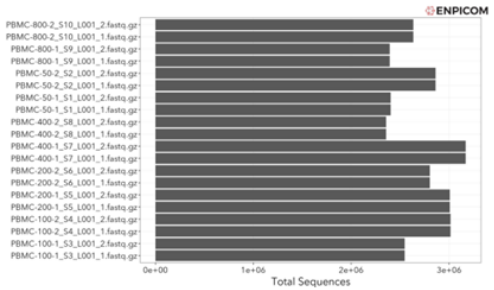
Contents

Fastq Quality Metrics

To assess the quality of the data that serves as an input to the TCR/BCR repertoire processing pipeline, the basic characteristics of the input data are assessed.

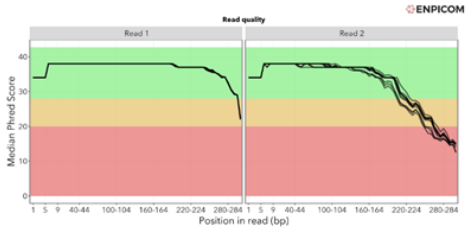
Number of Reads per Sample



Phred Score Plots

The figures below display the per-base quality metric plot generated by FastQC, which provides a graphical overview of the quality scores for each nucleotide position across all reads in a sequencing dataset. These plots can be used to evaluate the quality of the sequencing data, identifying potential issues such as variations in quality scores across the read length or the presence of low-quality bases at certain positions or in certain samples.

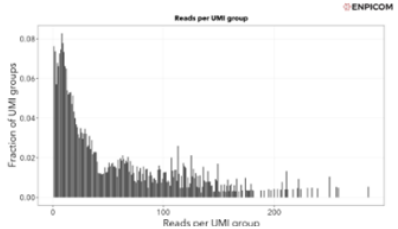
Both reads



Reads per UMI

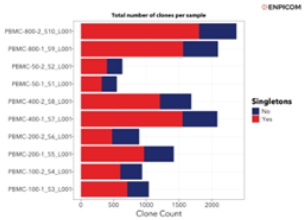
To assess if the addition and processing of unique molecular identifiers (UMI) is successful, the number of reads per UMI group is examined. With more than one read per UMI group, error correction can be performed; conversely, if there is only a single read per UMI group, no error correction can be performed.

If the number of reads per UMI group is close to 1, it might be better to omit UMI processing altogether.



Total Clone Count

We report the total number of clonotypes detected in the dataset and highlight singletons. This depends partially on the clonotype definition used in the analysis and thus depends partially on the processing parameters. The number and distribution of the clonotypes gives us a sense of the overall repertoire characteristics of the sample, and can help identify potential issues with sequencing or sample preparation.



CDR3 Length

In this section, we examine the length distribution of the CDR3 region of the immune receptor. We generate histograms of the CDR3 length distribution. The histograms how the number of clones with different lengths of CDR3 nucleotide sequences. These histograms can help understand the diversity and complexity of the TCR or BCR repertoire and highlight potential quality control issues.

The output of repertoire processing is often restricted to functional immune receptors (i.e., in the correct reading frame, no stop codons or indels, no missing regions); in this case, the plot below will have bars only at lengths that are divisible by 3.

Distribution of CDR3 length in nucleotides. Divide length by 3 to obtain the length in amino acids.

V and J-Gene Co-occurrence

Plots showing the co-occurrence of V and J-genes in the same samples. Ribbons connect V and J-genes found in the same VDJ with sizes scaled to the total number of co-occurrences.

Combinations of V- and J-genes per sample, depicted as circosplots.

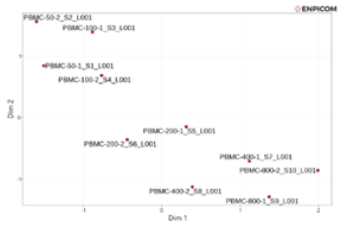
Sample similarity

Sample similarity is shown using different metrics. To increase comparability rare clones and short CDR3 sequences are removed. Thus low quality samples could be missing from the comparisons.

Umap

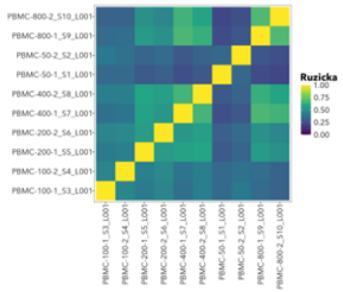
UMAP plot of BCR or TCR clonotypes. UMAP was performed on the OTU table representing the clonotypes identified from a BCR or TCR assay.

The x and y axes represent the first and second principal components of the UMAP embedding, respectively, and each point corresponds to a different sample.



Ruzicka Heatmap

Ruzicka similarity heatmap of BCR or TCR clonotype samples. The Ruzicka similarity between all pairs of samples was calculated using the OTU table representing BCR or TCR clonotypes and visualized as a heatmap. Each cell in the heatmap represents the similarity between two clonotype samples, with higher values indicating greater similarity.



Top 10 clones per sample

Top 10 clones with a clone count of at least 3. Samples with no clones are omitted.

Top 10 clones (with ties) for sample 'PBMC-100-1_S3_L001'

Vgene	Jgene	CDR3.aa	clone_count
IGHV4-28*00	IGHJ5*00	CSKHGDCRGGDCYSDSW	197
IGHV3-73*00	IGHJ4*00	CARGGYCDGGKCPLIEDW	75
IGHV4-34*00	IGHJ1*00	CARSGIVKYLQVW	73
IGHV3-7*00	IGHJ4*00	CAQNPKYGELQW	69
IGHV3-30*00	IGHJ3*00	CARGRDYAFDIW	68
IGHV3-73*00	IGHJ4*00	CARGGFCADAKCPLIEGW	61
IGHV4-61*00	IGHJ4*00	CAANPVYDDDGFVVGAIRFW	58
IGHV3-74*00	IGHJ4*00	CANLLRGV	55
IGHV3-74*00	IGHJ4*00	CVREINWRNNYW	55
IGHV3-7*00	IGHJ4*00	CSSREKTTARAAW	52

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