Using NGS to accelerate Immuno-Oncology Research

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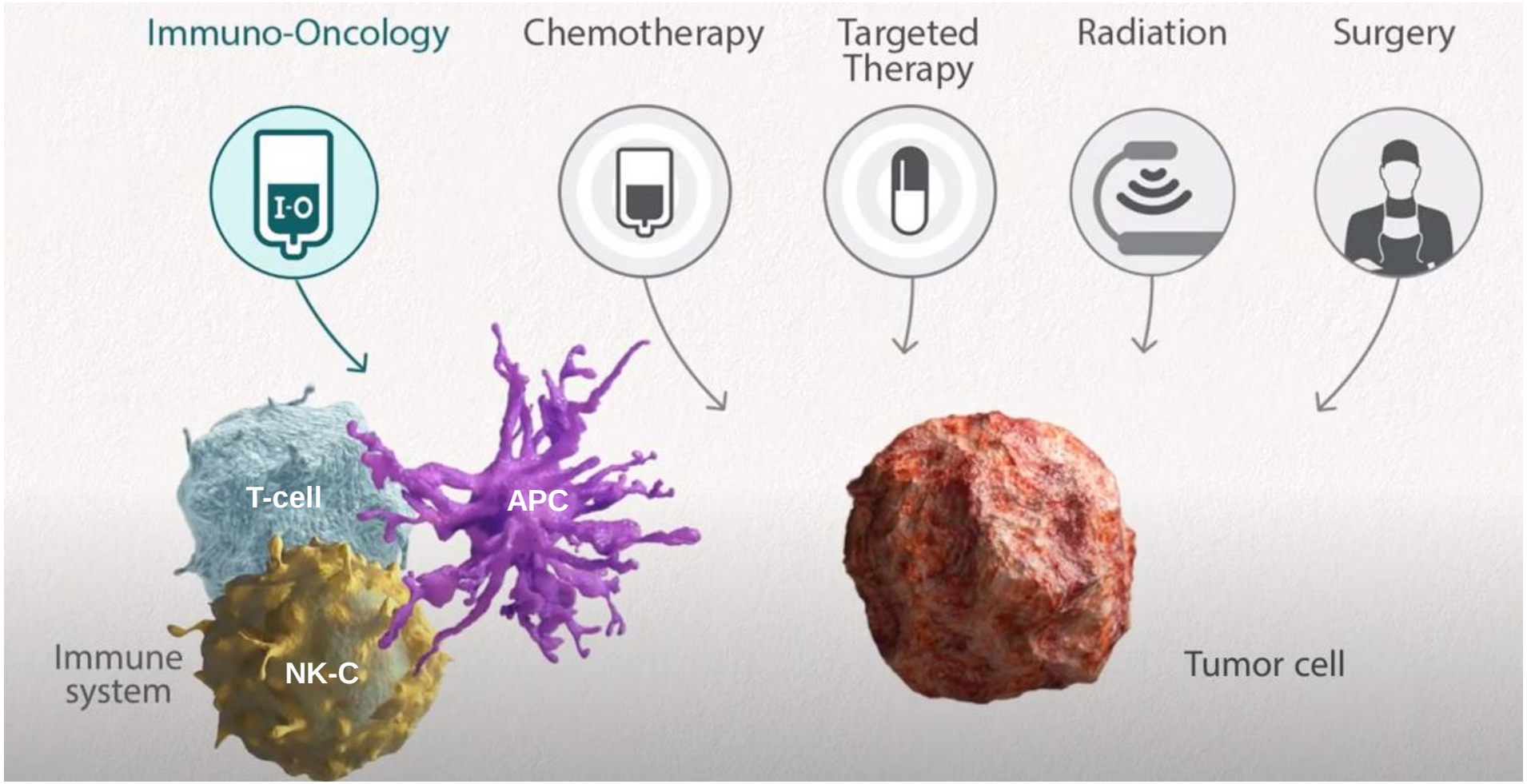
Agenda

Elements & Challenges of Immuno-Oncology

NGS solutions for Immuno-Oncolgy

Summary

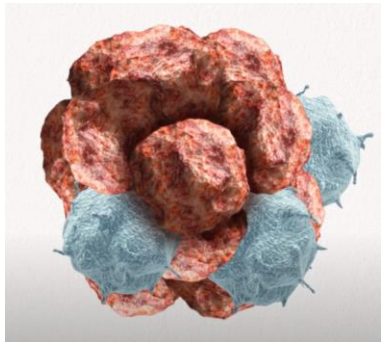
Immuno-oncology therapies target body’s immune system to fight cancer



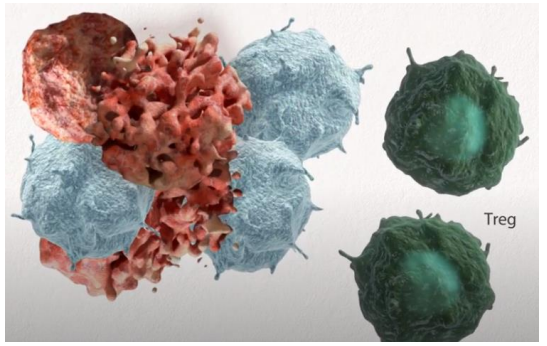
Immune response to cancer and challenges

Presentation

Infiltration



Elimination



Typical immune response timeline

Immune response to cancer and challenges

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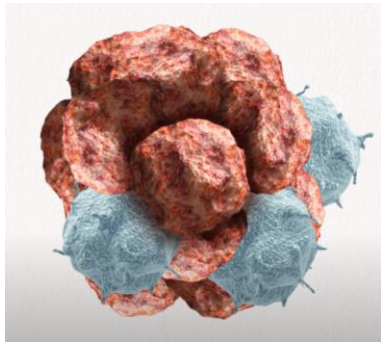

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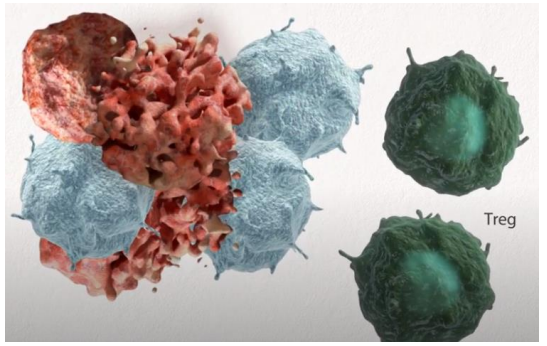
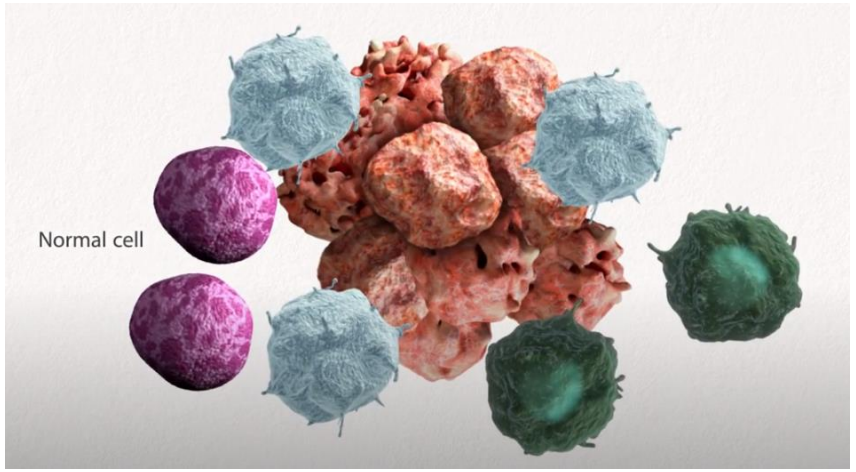


Typical immune response timeline



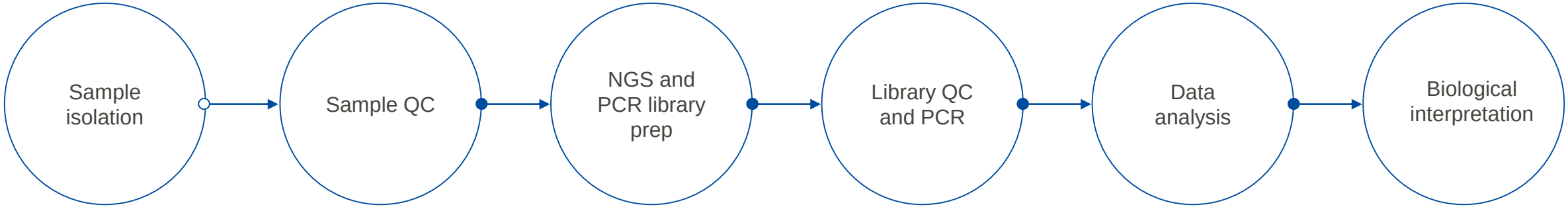
Challenges

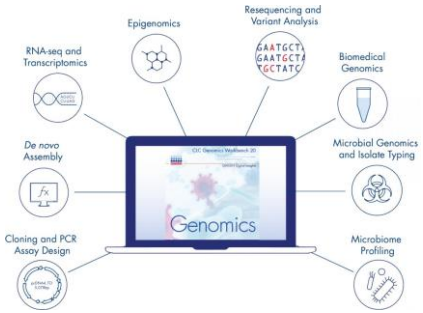
Are normal cells affected ?

Are the tumor cells escaping ?

Approaches for precise monitoring…

QIAGEN Sample to Insight workflows





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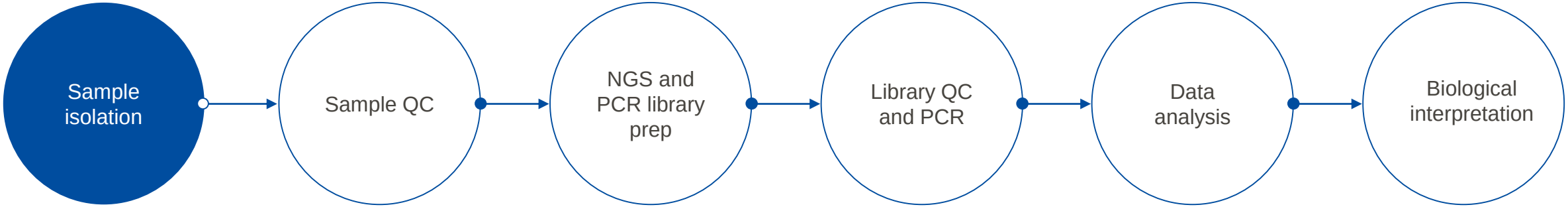
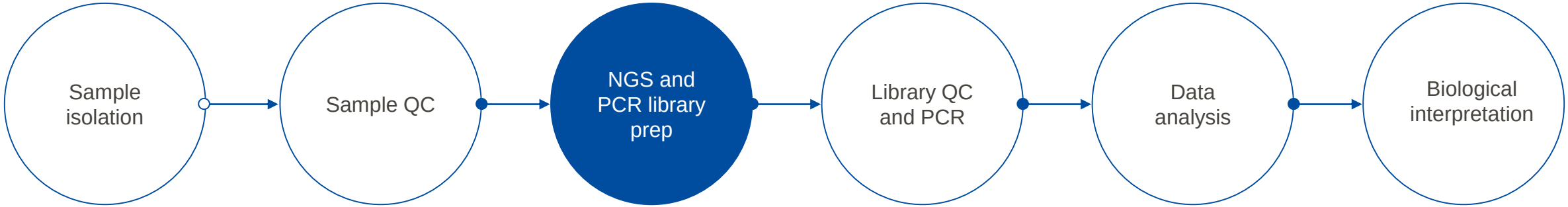


ARTICLE

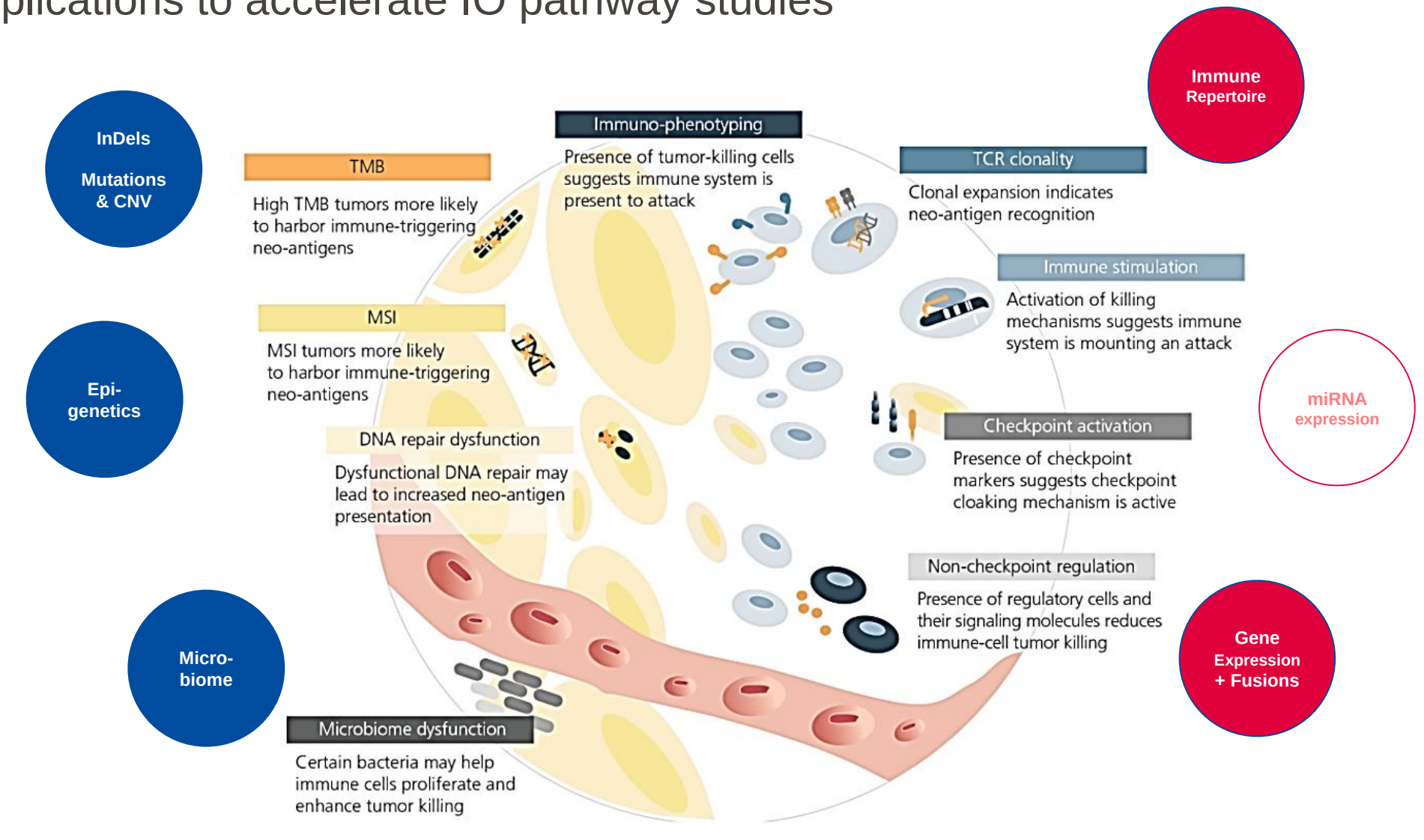
https://doi.org/10.1038/s41467-019-12159-9 OPEN

DNA methylation loss promotes immune evasion of tumours with high mutation and copy number load

QIAGEN Sample to Insight workflows

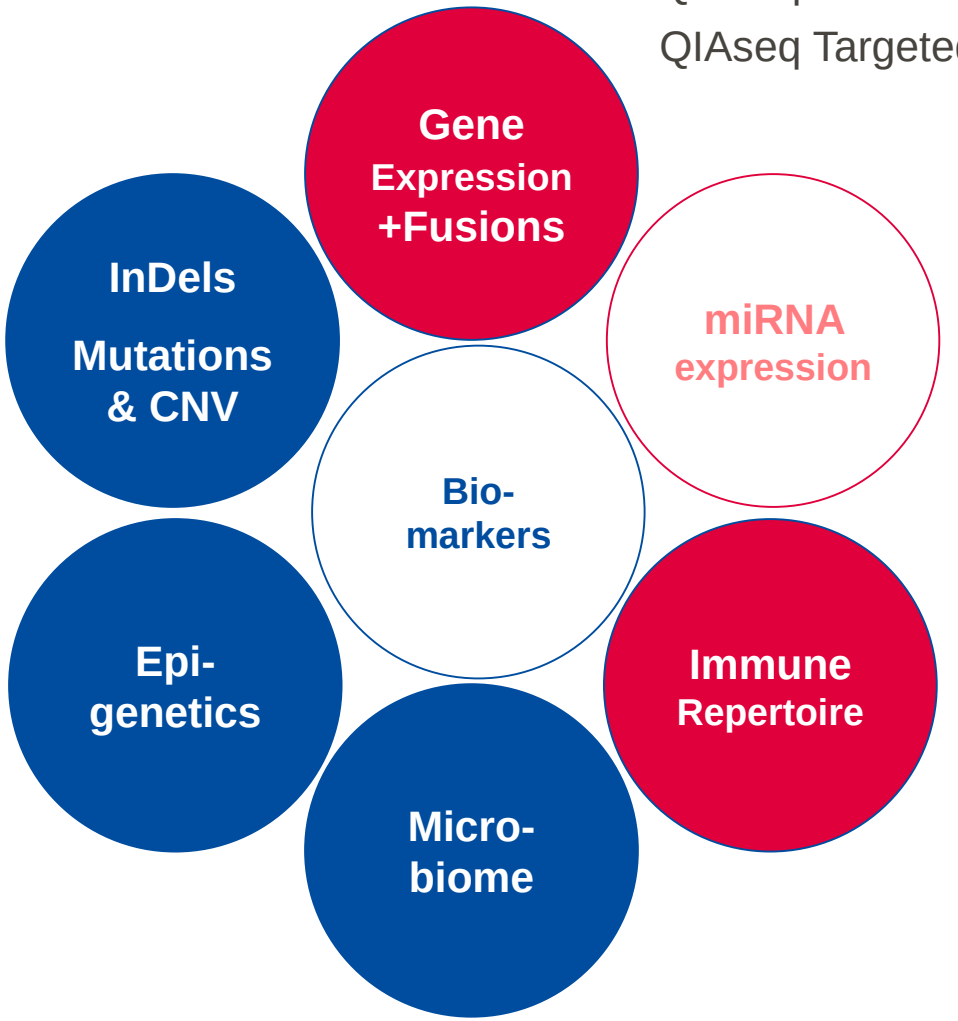


NGS applications to accelerate IO pathway studies



QIAGEN's NGS portfolio: QIAseq for Immuno-Oncology

QIAseq Stranded RNA + FastSelect rRNA/Globin removal
QIAseq UPX 3' Whole / Targeted Transcriptome
QIAseq Targeted / RNAscan RNA Panels



QIAseq miRNA Sequencing System

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QIAseq Whole Exome Panel
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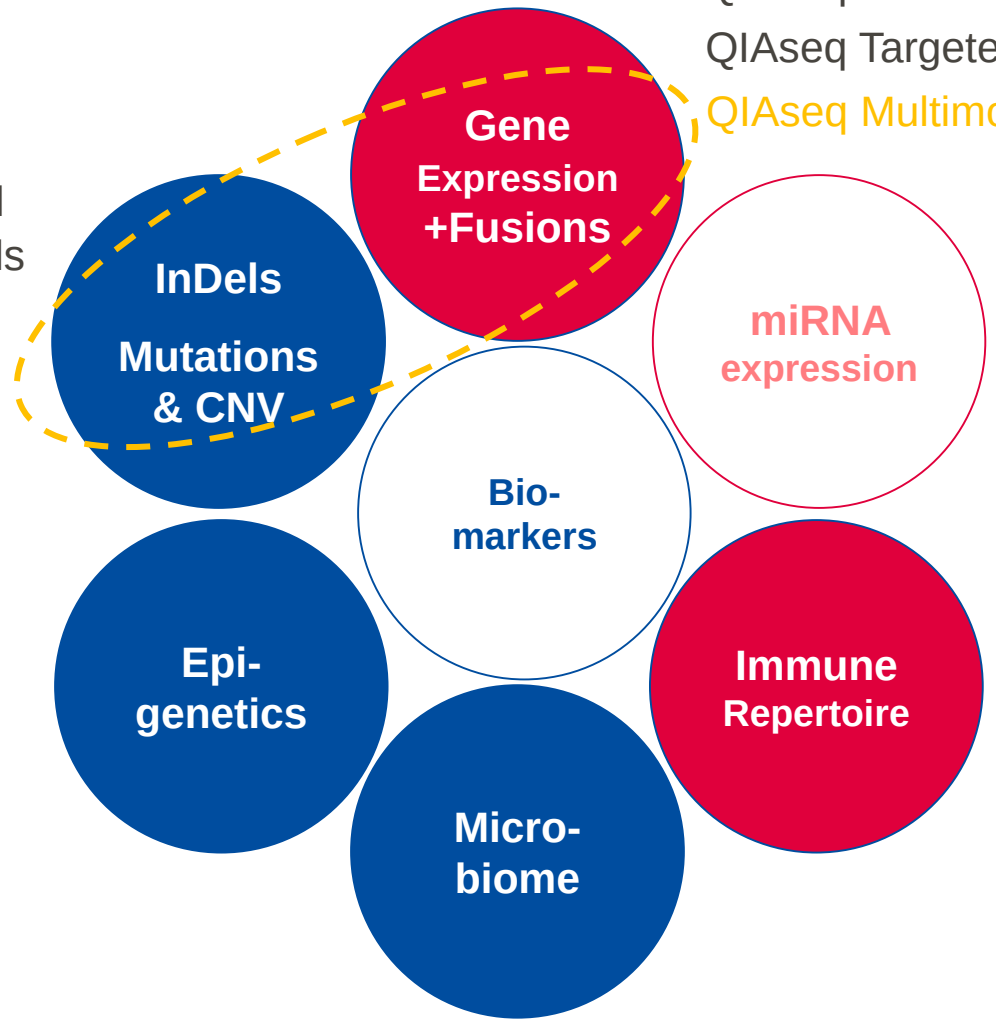

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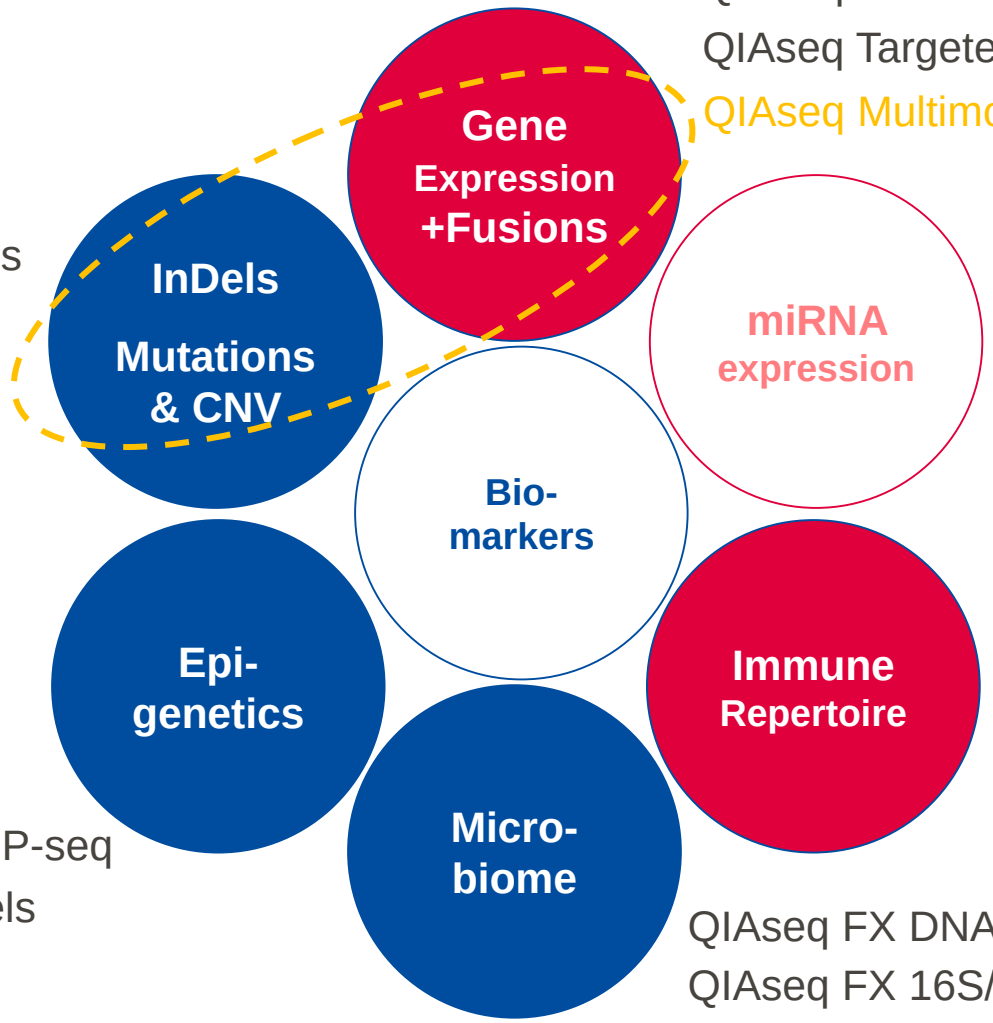
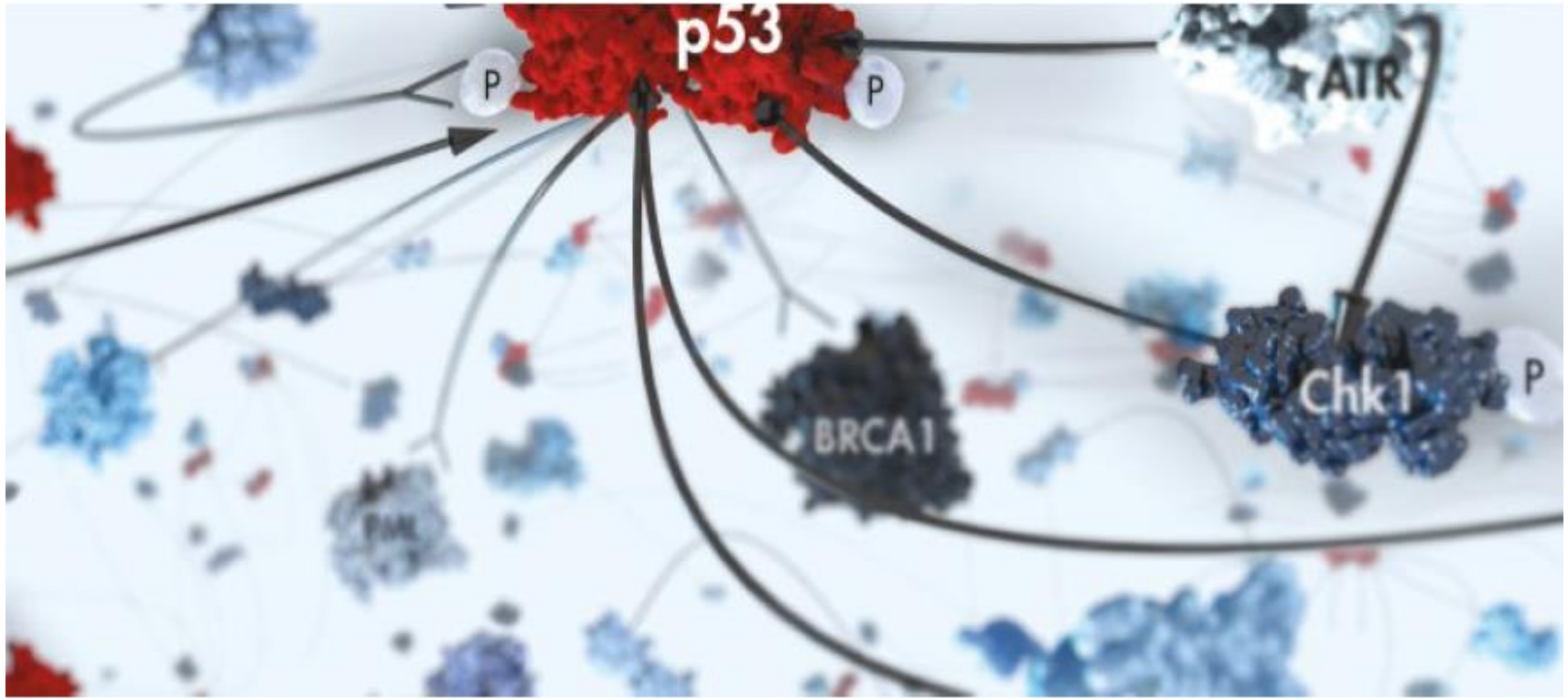


QIAseq miRNA Sequencing System

QIAseq TCR Panel (CDR3 + Full Length)

QIAseq Bisulfite Conversion
QIAseq Ultra-low DNA for ChIP-seq
QIAseq Targeted Methyl Panels

QIAseq FX DNA for Whole Genome
QIAseq FX 16S/ITS Screening + Region Panels



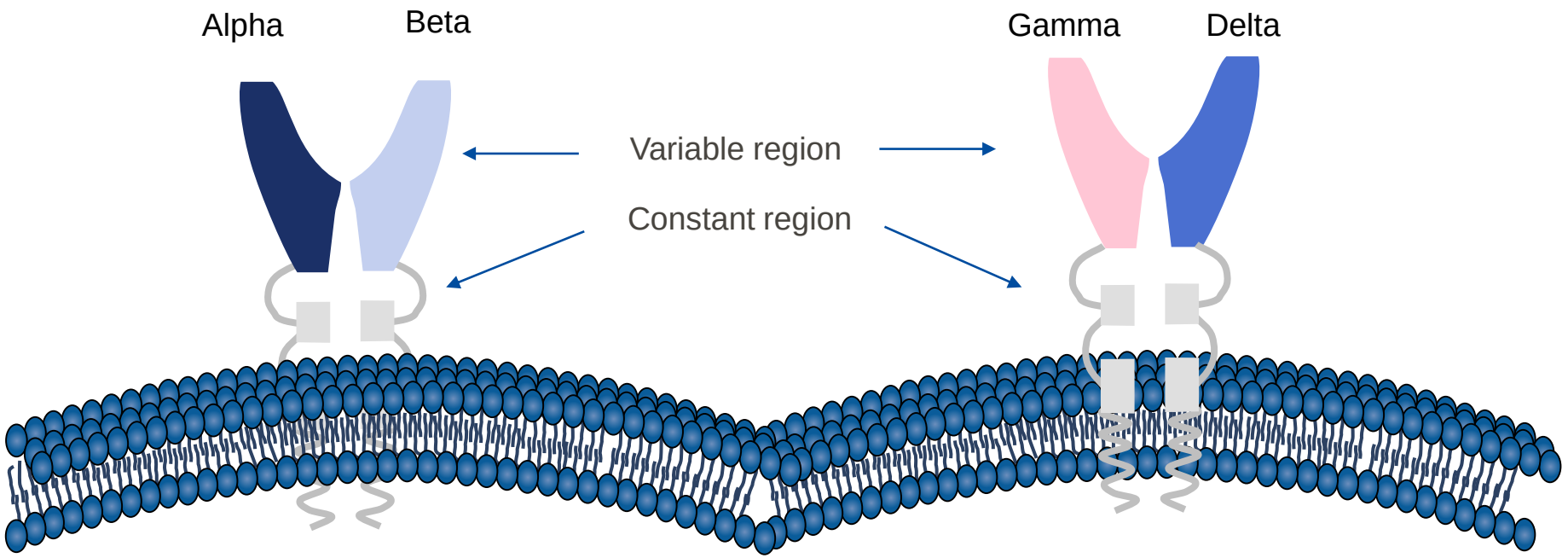
QIAseq Immune Repertoire RNA Library Kits

Precise detection of T-cell receptors using RNAseq with unique molecular indexing

Understanding T-cell receptors

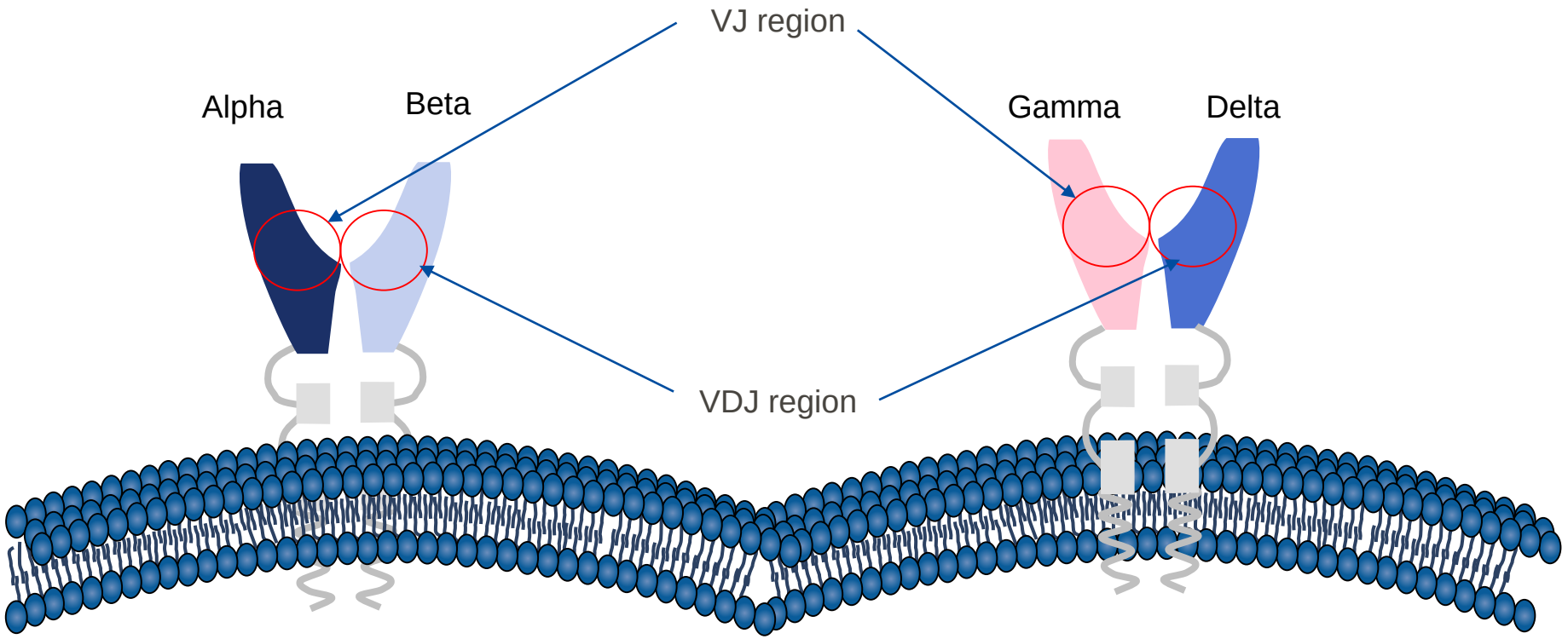
T-cells express hetero-dimeric receptors which recognize specific antigens.

- T-cell receptors are encoded by 4 different genes
  - Alpha (TRA), beta (TRB), gamma (TRG) and delta (TRD)
  - T-cells have either alpha + beta chains or gamma + delta chains
  - T-cell receptors have constant and variable regions – variable regions are more interesting to researchers as they determine the properties of a T-cell receptor



Understanding T-cell receptors

- The VDJ region determines the specificity for antigens
- QIAGEN's Immune Repertoire T-cell Receptor Kit analyzes this region by RNAseq



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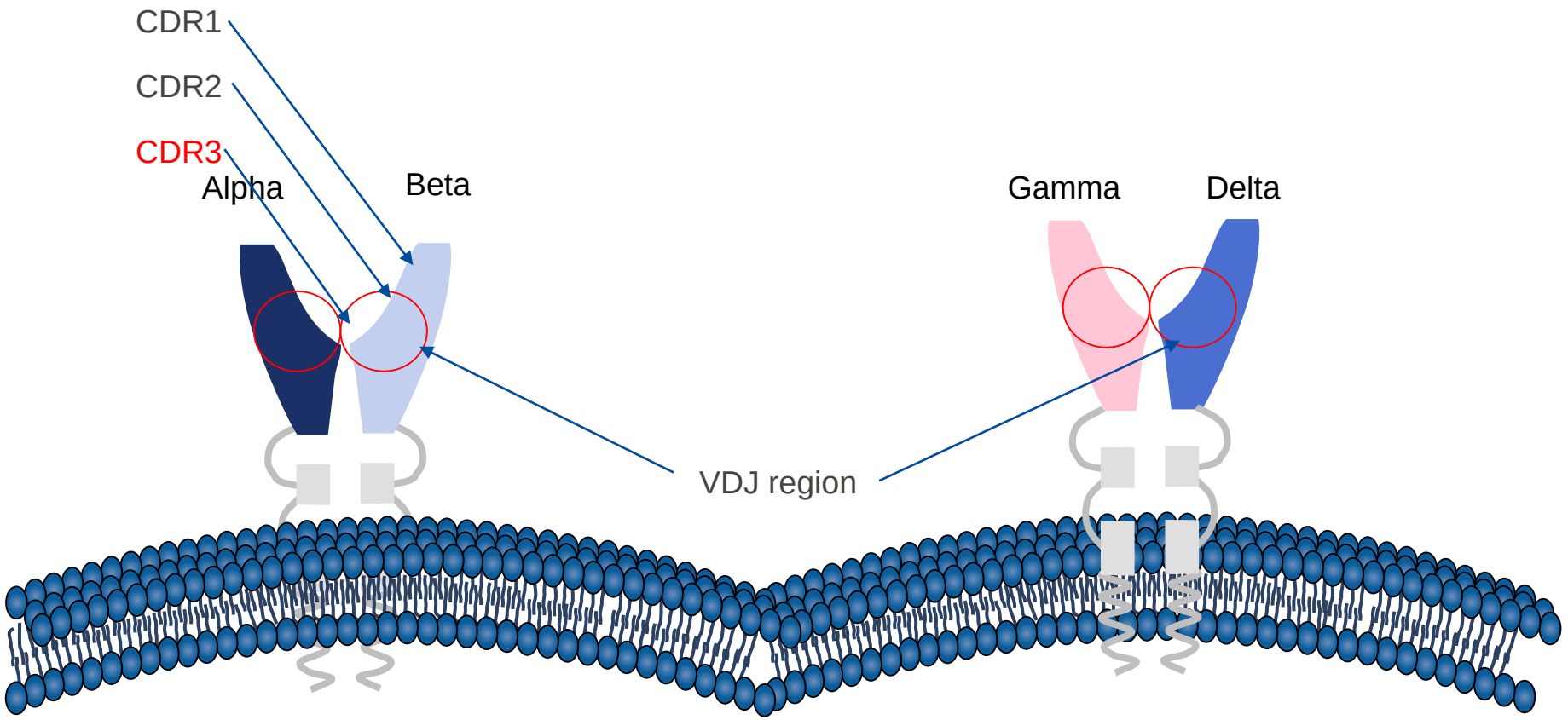
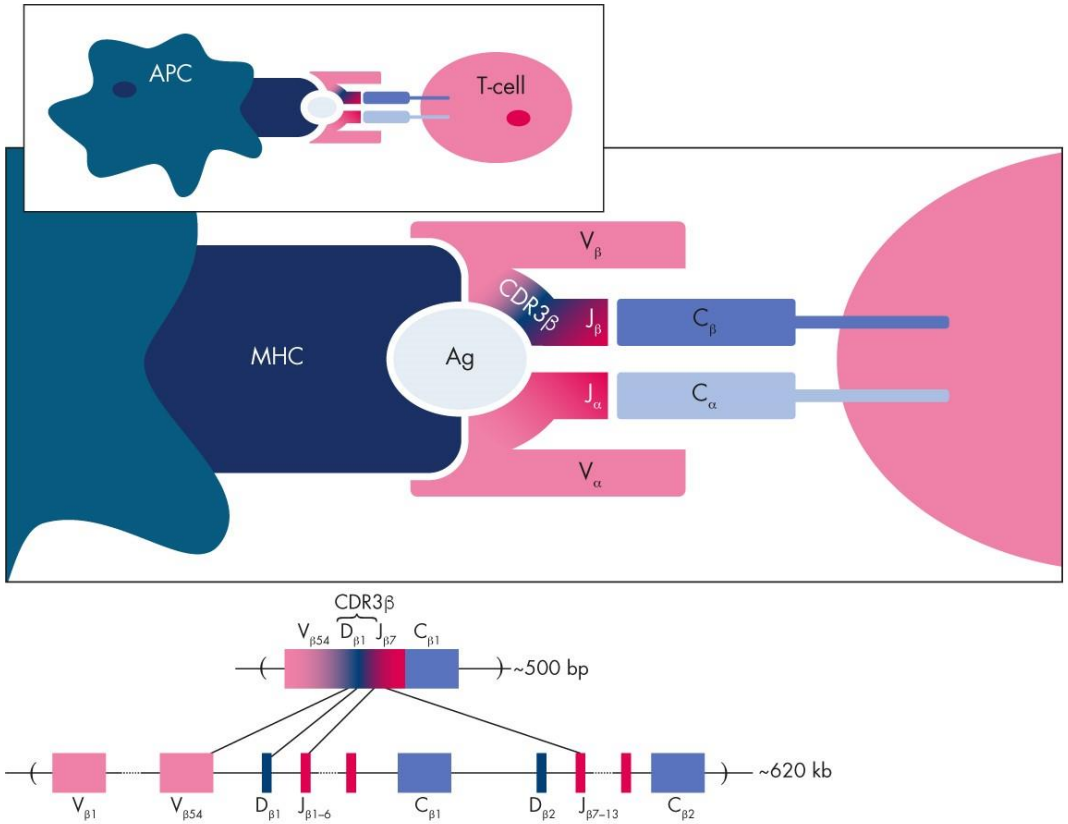


NGS approaches to study T-cell receptors

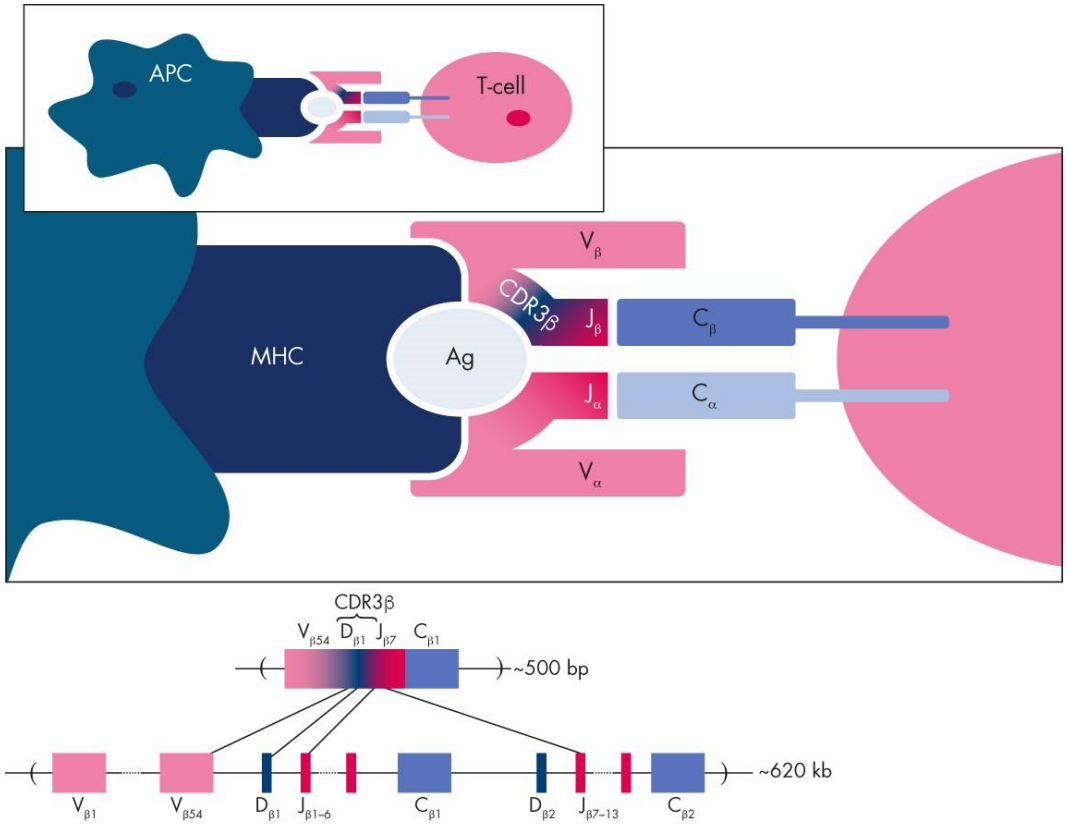


- RNA
  - TCR mRNA templates are likely to be more represented than DNA, allowing higher sensitivity
  - RNA sequencing specifically allows for the identification of expressed TCR sequences
  - The relatively shorter length of TCR mRNA templates allows for simpler library construction and for capture of complete V(D)J regions including CDR1, CDR2 and CDR3

- DNA
  - Samples are much easier to obtain; even biopsy samples from tissues or slides can be used
  - Since each cell may only have one copy of the successfully rearranged V(D)J, it may reflect the quantity of the repertoire better

Woodsworth, D. J., Castellarin, M., and Holt, R. A. (2013). Sequence analysis of T-cell repertoires in health and disease. Genome Medicine

NGS approaches to study T-cell receptors



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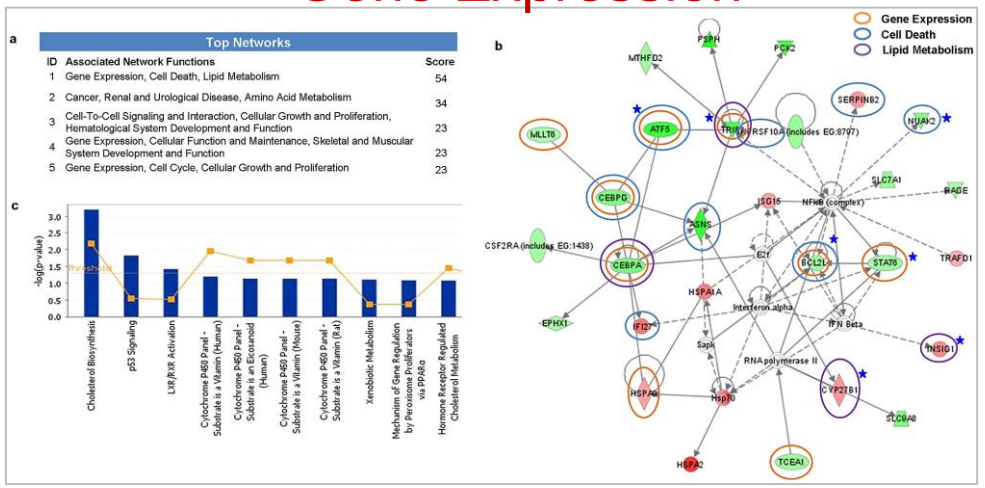
+ Gene Expression



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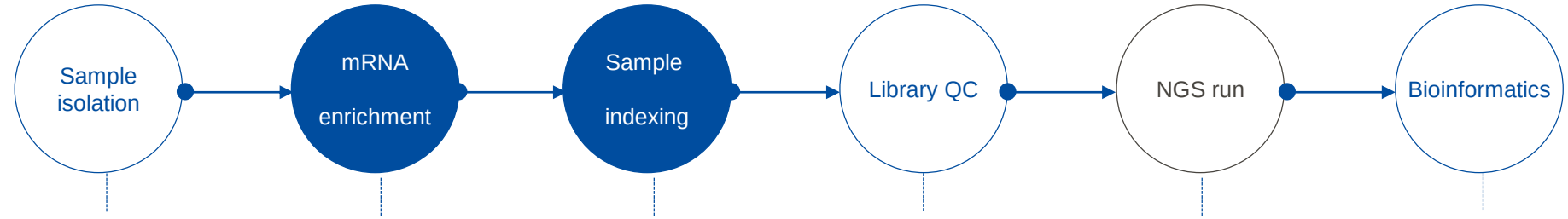
What do I need to determine the expressed V(D)J regions for TCR mRNA



- RNeasy
- RNeasy Plus

- QIAseq Immune Repertoire Library Kit
  - IMHS-001Z Human T-cell
  - IMMM-001Z Mouse T-cell

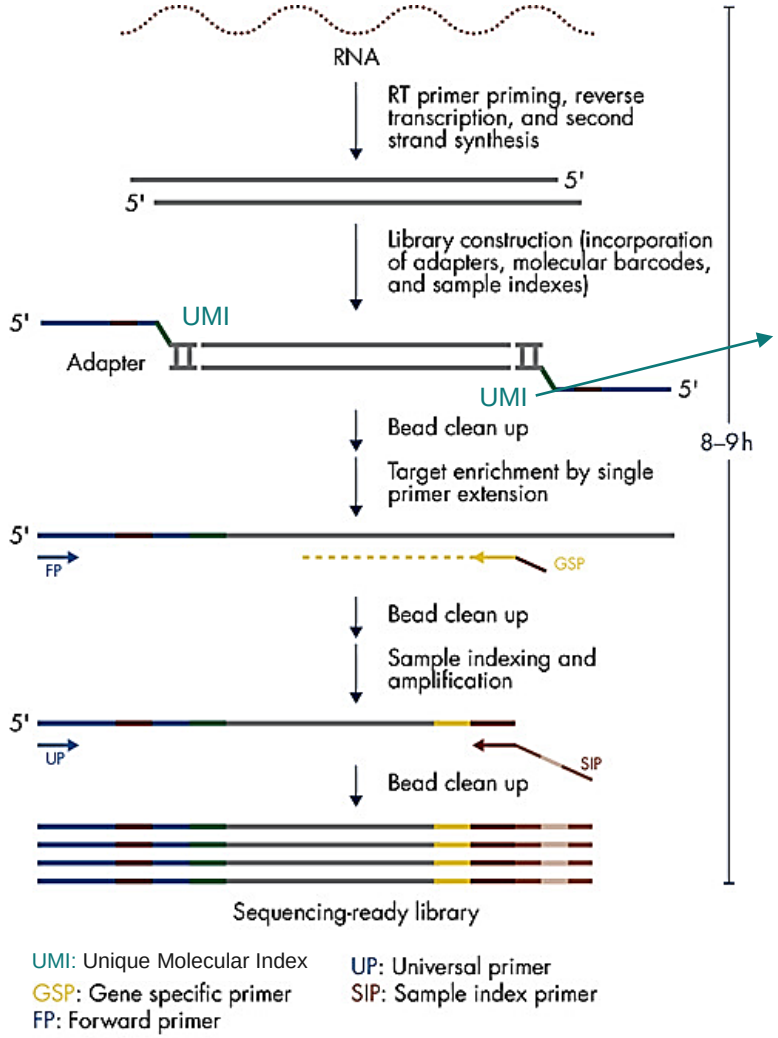
- QIAseq Index Kit
  - QIAseq 96-Index UDI Set A (384)
  - QIAseq 96-Index UDI Set B (384)
  - QIAseq 96-Index UDI Set C (384)
  - QIAseq 96-Index UDI Set D (384)

- QIAseq Library Quant Assay Kit
- QIAseq Library Quant Array Kit

- Illumina

- GeneGlobe Data Analysis Center
- CLC Workbench

QIAseq Immune Repertoire RNA Library Kit workflow



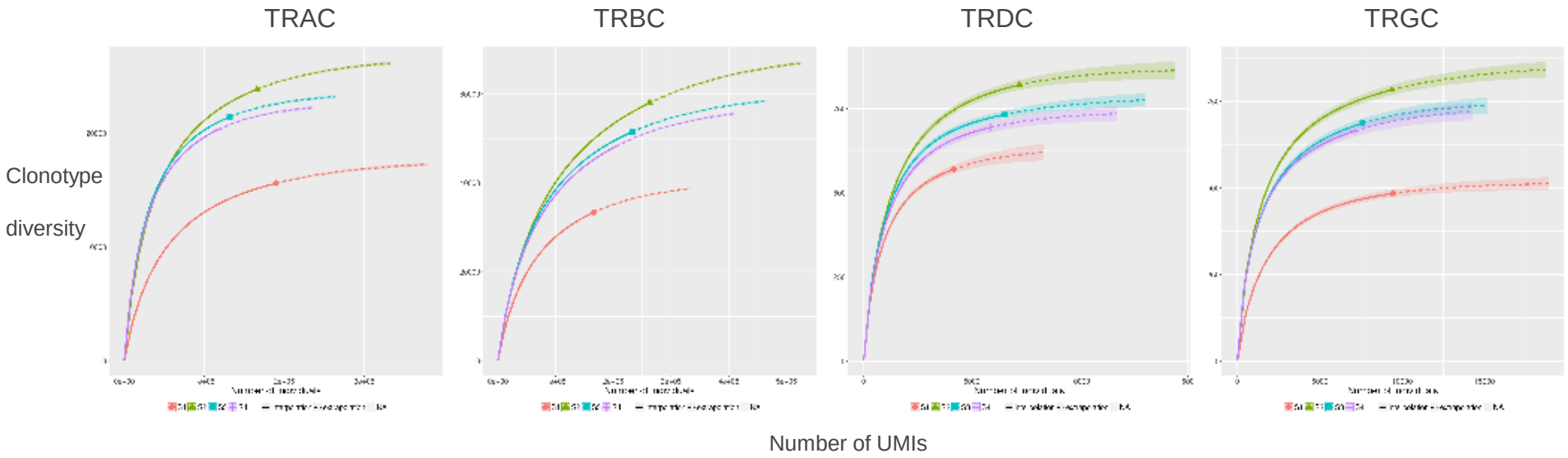
Pool of primers for gene-specific reverse transcription of alpha (TRA), beta (TRB), gamma (TRG) and delta (TRD) TCR mRNAs

UMI- provides the ability to more accurately quantify the number of individual TCR clonotypes

Gene specific pool of primers for target enrichment of alpha (TRA), beta (TRB), gamma (TRG) and delta (TRD) gene sequences

Precision sequencing with UMIs: Improved diversity modeling

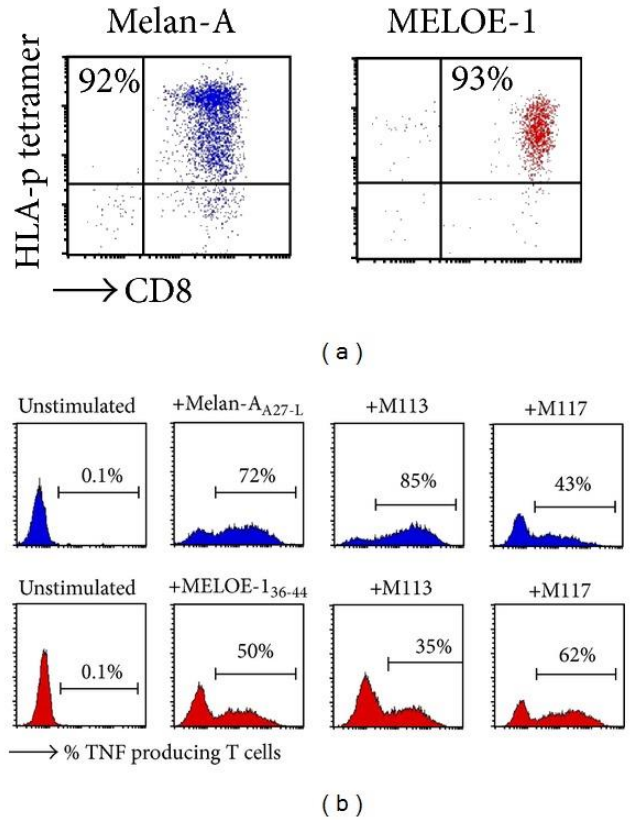
- Rarefaction plots for each sample per receptor
- Number of reads per UMI can be used to gauge if the complete repertoire was sequenced



Performance data

- T-cells sorting using flow cytometry based on available antibodies is one of the classic approaches

- Can we capture information if similar T-cells populations are subjected to RNAseq using 10 ng or 25 ng total RNA (1000 to 2500 T-cell)



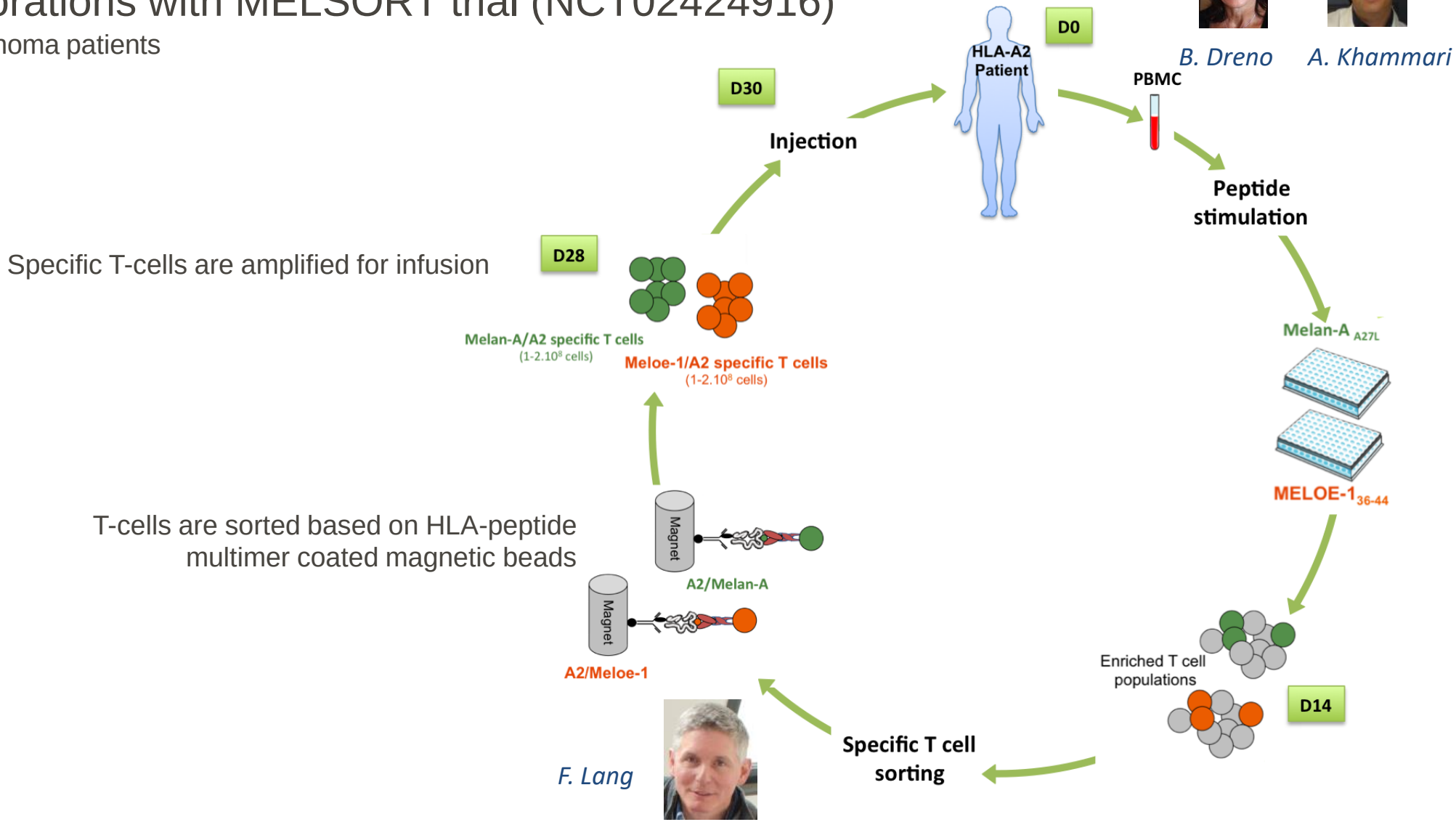
Labarriere et al. 2013

Research collaborations with MELSORT trial (NCT02424916)

To study T-cells from Melanoma patients

B. Dreno

A. Khammari



F. Lang

Labarriere et al. 2013

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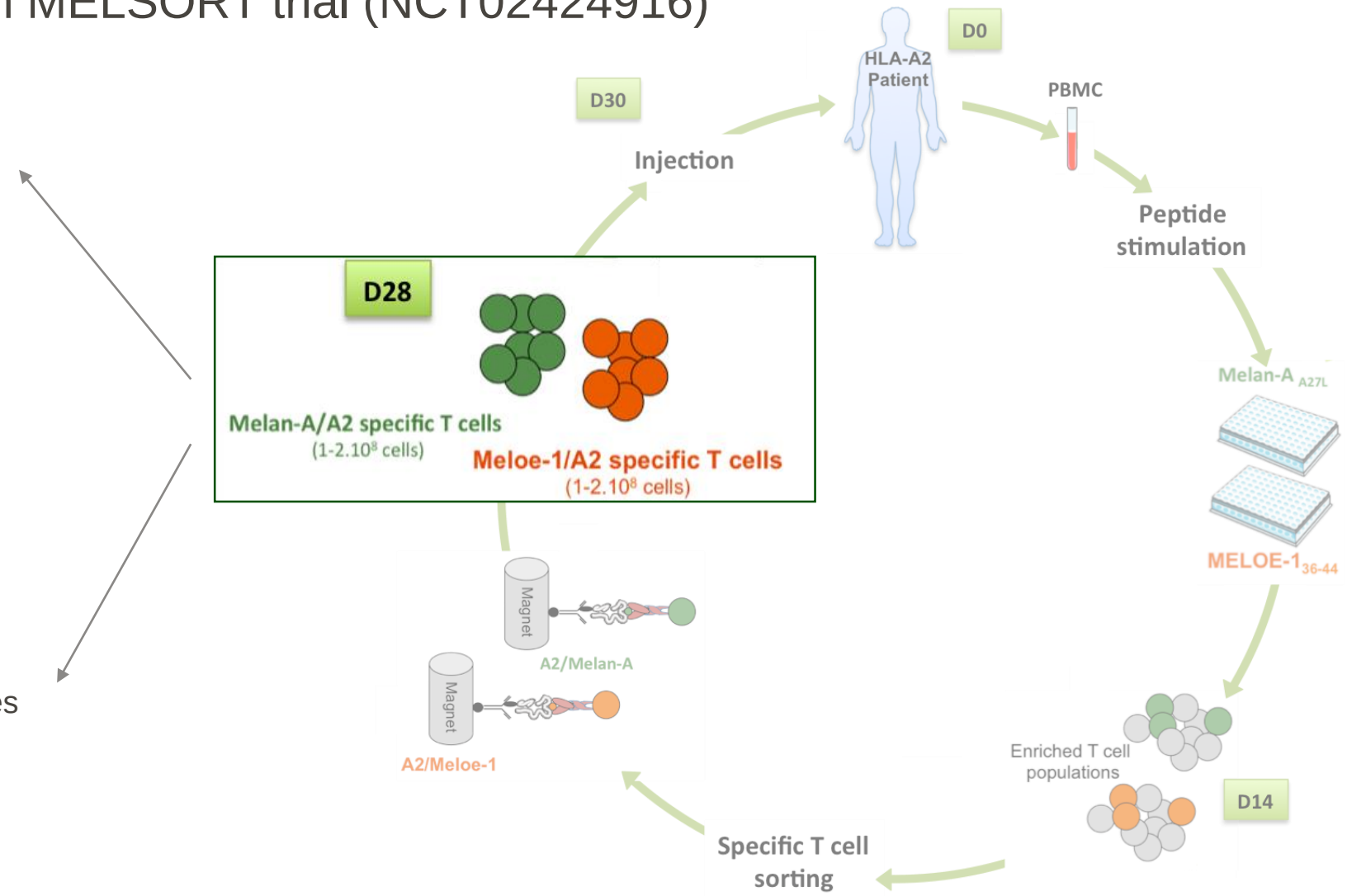
To study T-cells from Melanoma patients

Classic phenotypic analysis - FACS

Transcriptional profiling for TCR clonotypes



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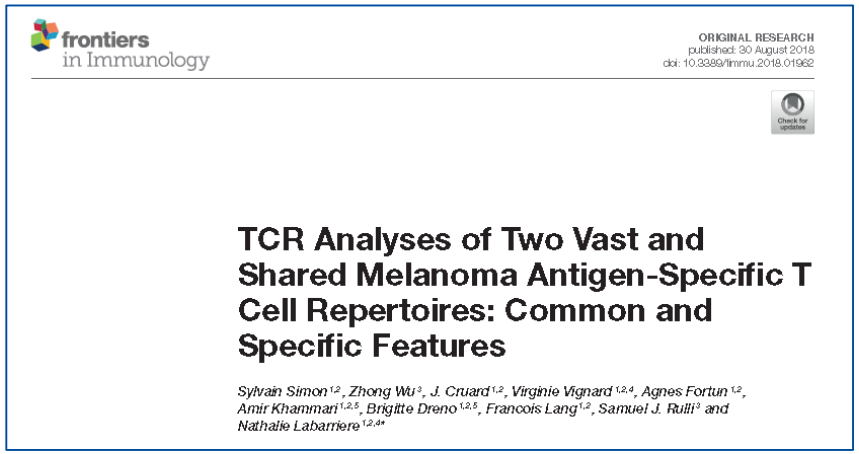
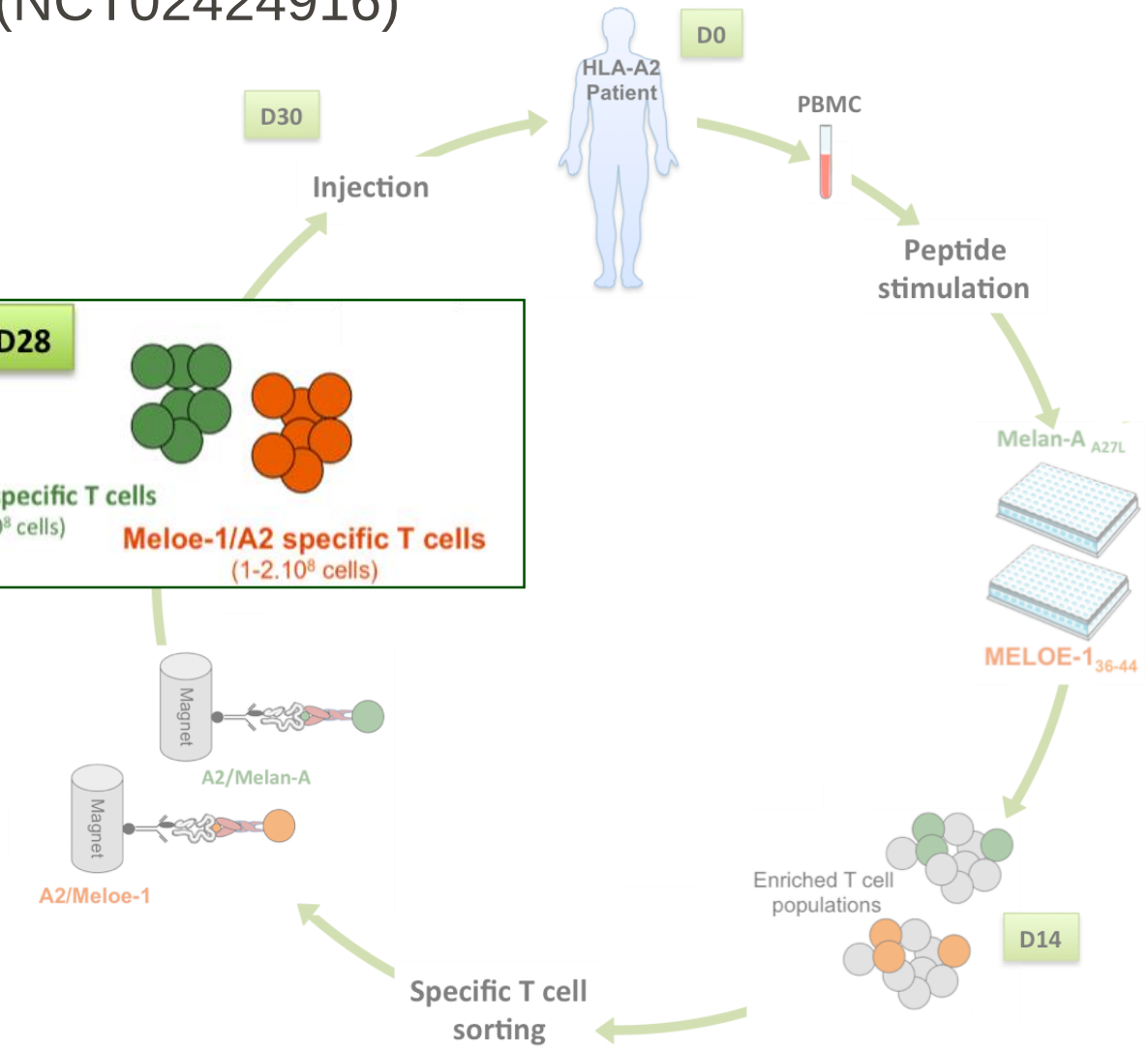


Transcriptional profiling for TCR clonotypes



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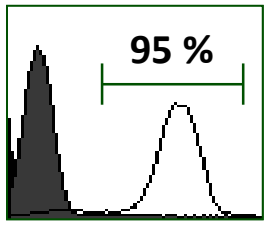
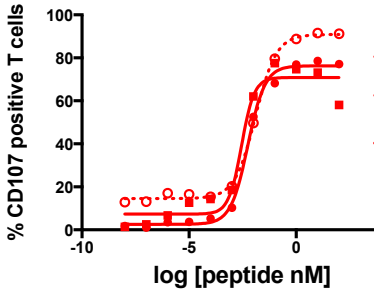
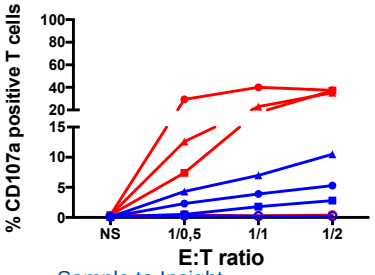


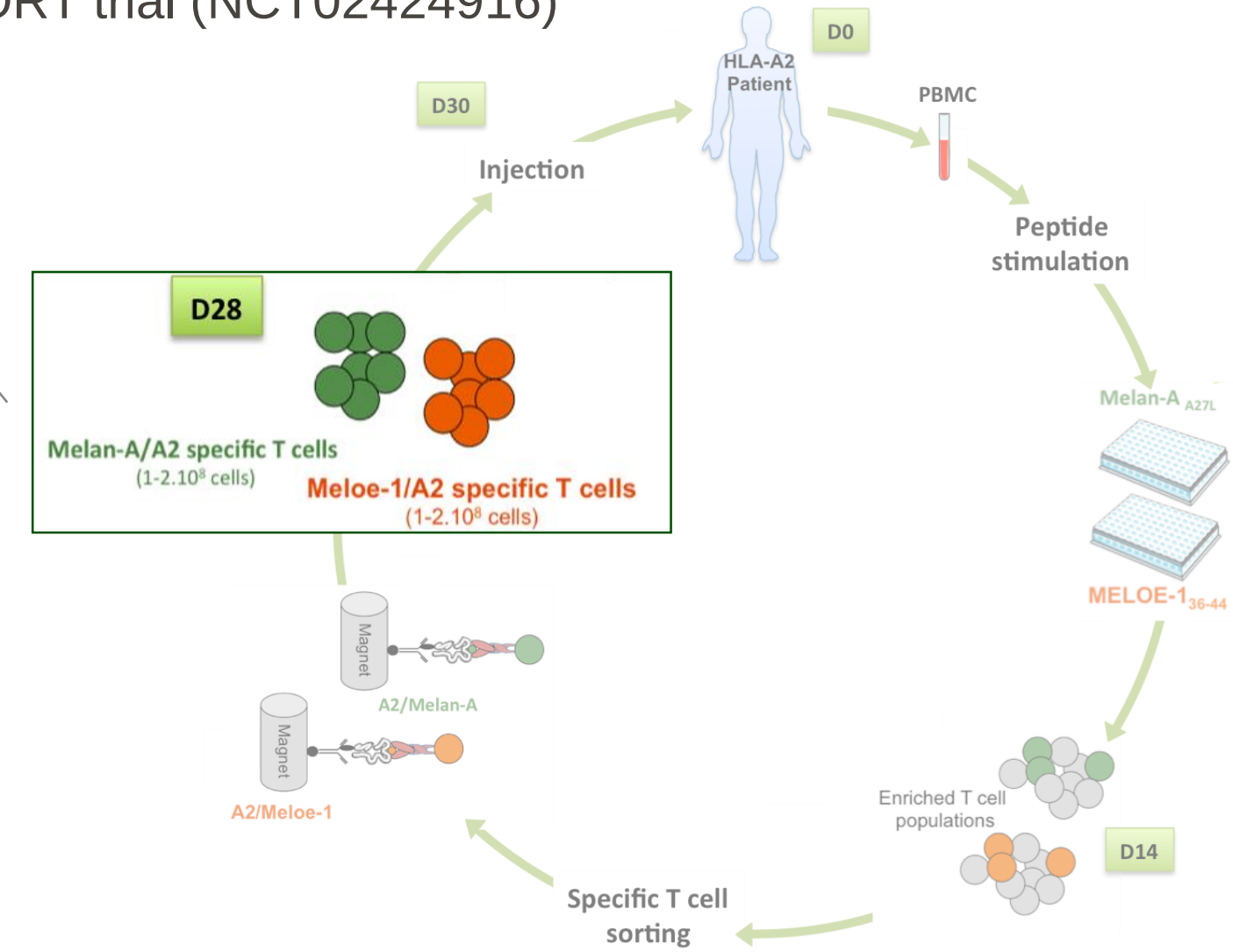
Good Purity & specificity



Reactive against the peptides



Melan-A (in red) & MELOE-1 (in blue) infused specific T-cells are reactive against melanoma specific T-cells expressing the 2 antigens



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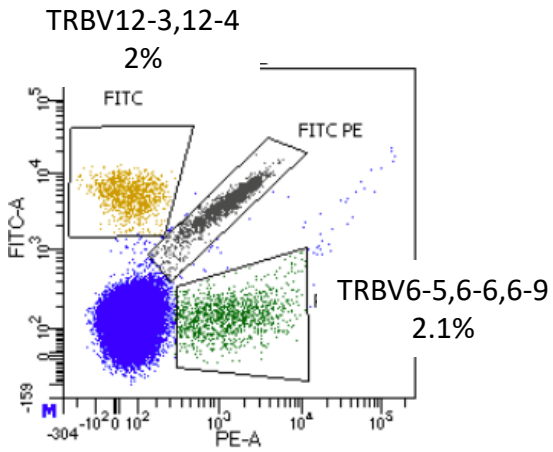
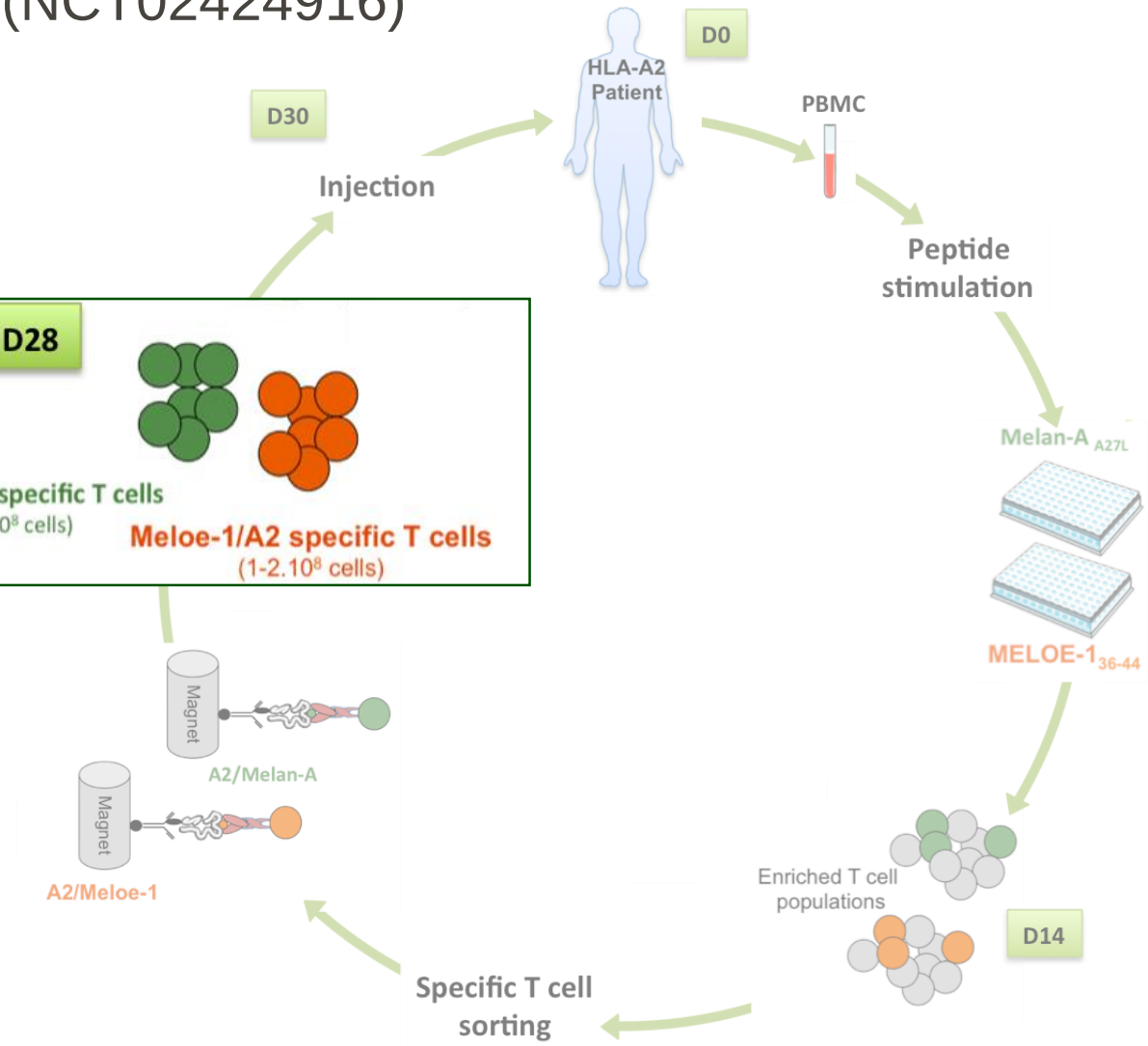
To study T-cells from Melanoma patients

Classic phenotypic analysis - FACS

Challenges



- The 24 Ab panel did not cover the entire Vbeta repertoire
- Cross-reactivity of some antibodies
- The total number of T cell clonotypes remain unknown



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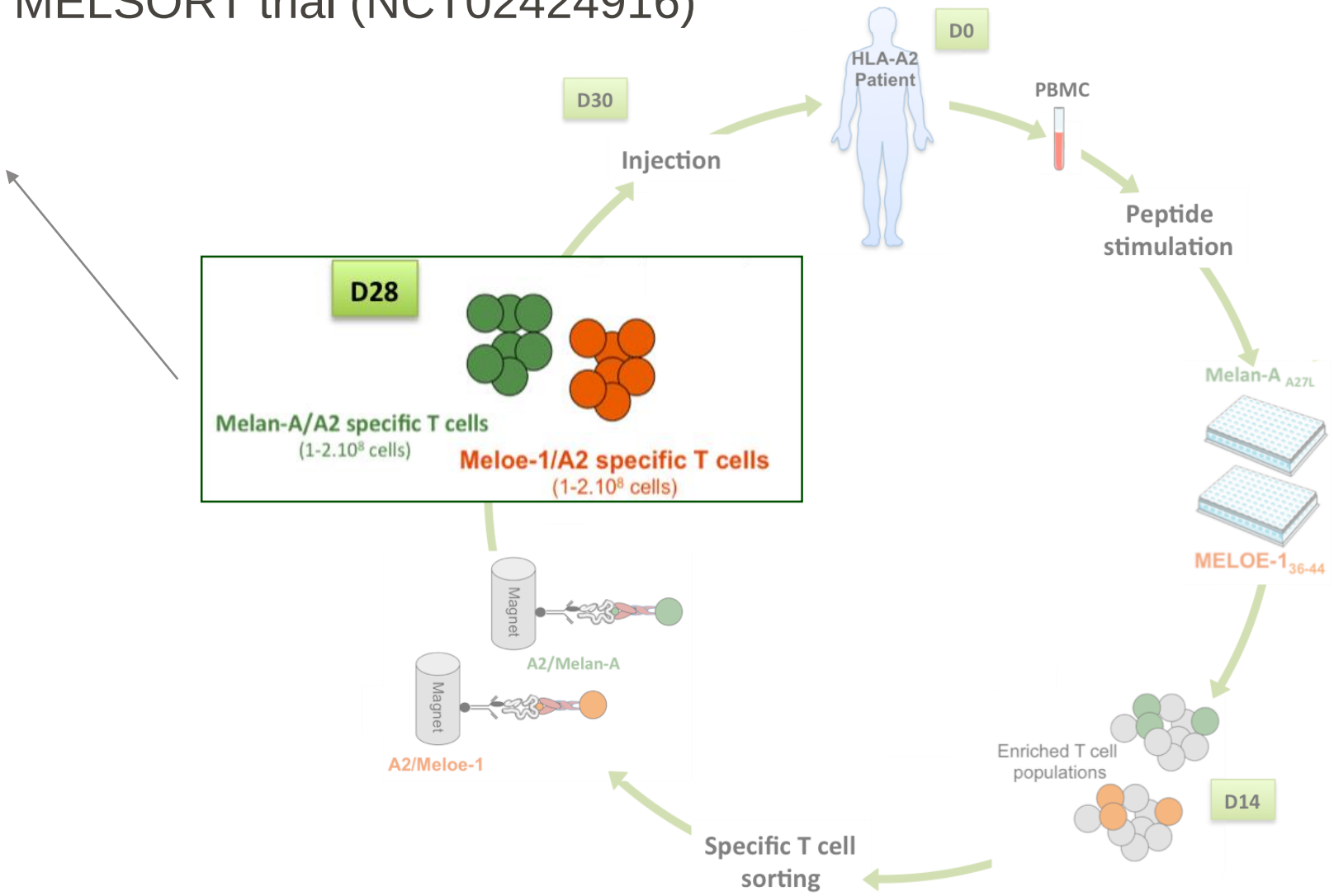
Transcriptional profiling for TCR clonotypes

QIAseq Immune Repertoire analysis

- 6 Melan-A specific T cell populations
- 4 MELOE-1-specific T cell populations

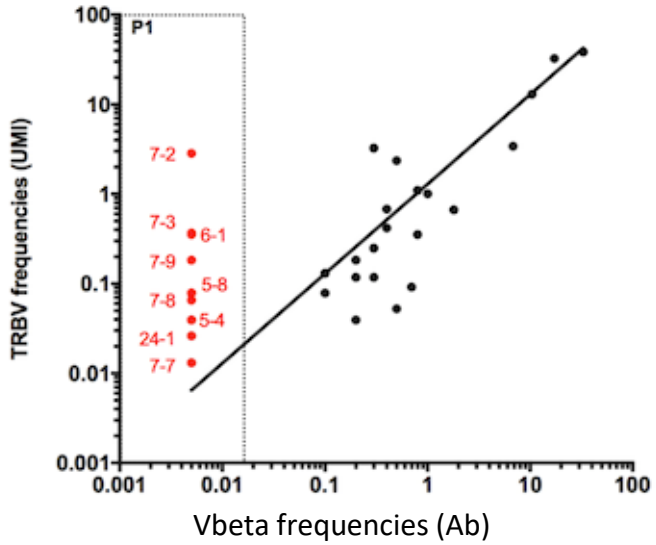


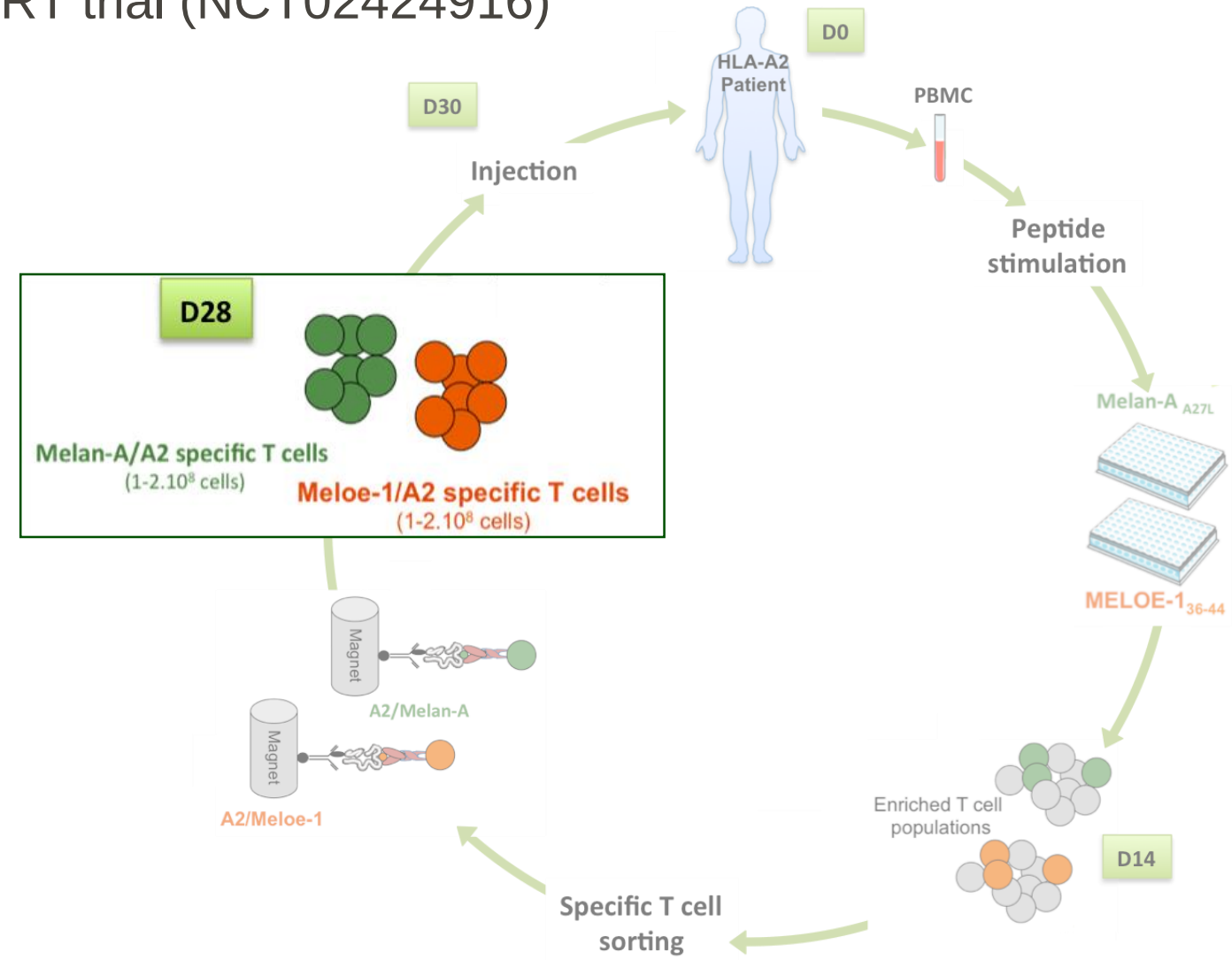
Research collaborations with MELSORT trial (NCT02424916)

To study T-cells from Melanoma patients

Transcriptional profiling for TCR clonotypes

- ✓ Major clonotypes confirmed!
- ✓ High concordance with FACS data
- ✓ Complete immune repertoire





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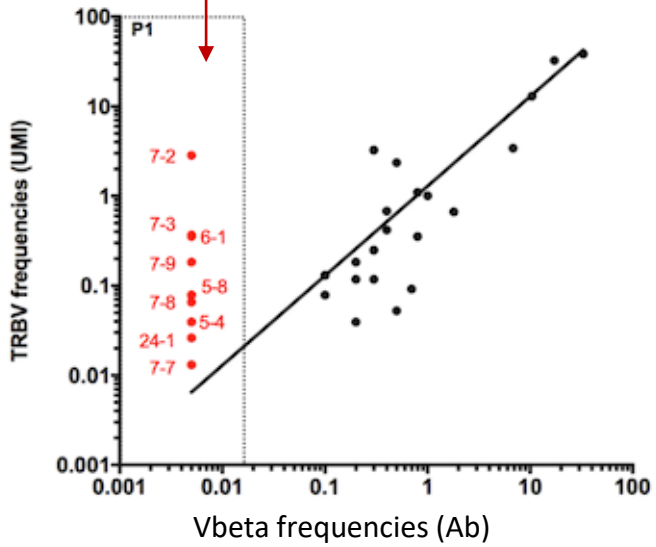
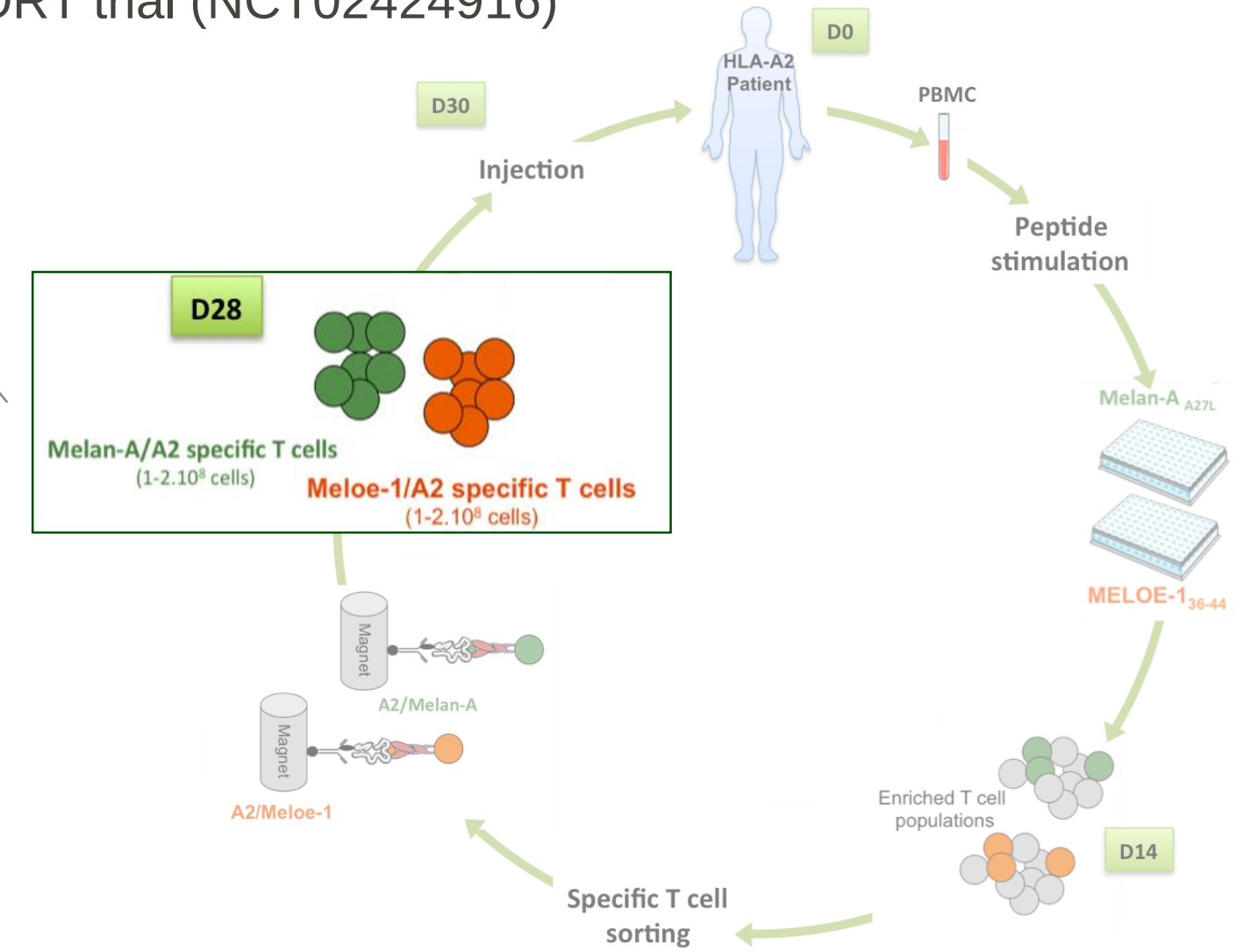
To study T-cells from Melanoma patients

Transcriptional profiling for TCR clonotypes

- ✓ Major clonotypes confirmed!
- ✓ High concordance with FACS data
- ✓ Complete immune repertoire
- ✓ TRBV repertoire NOT covered by the antibodies identified





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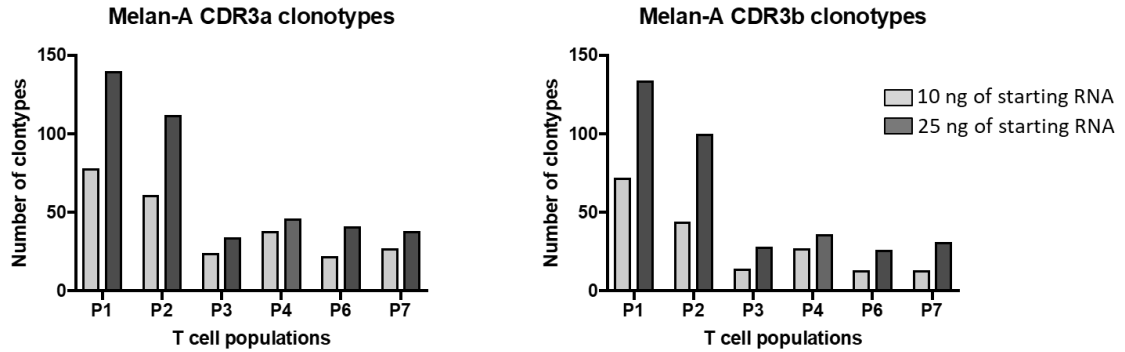
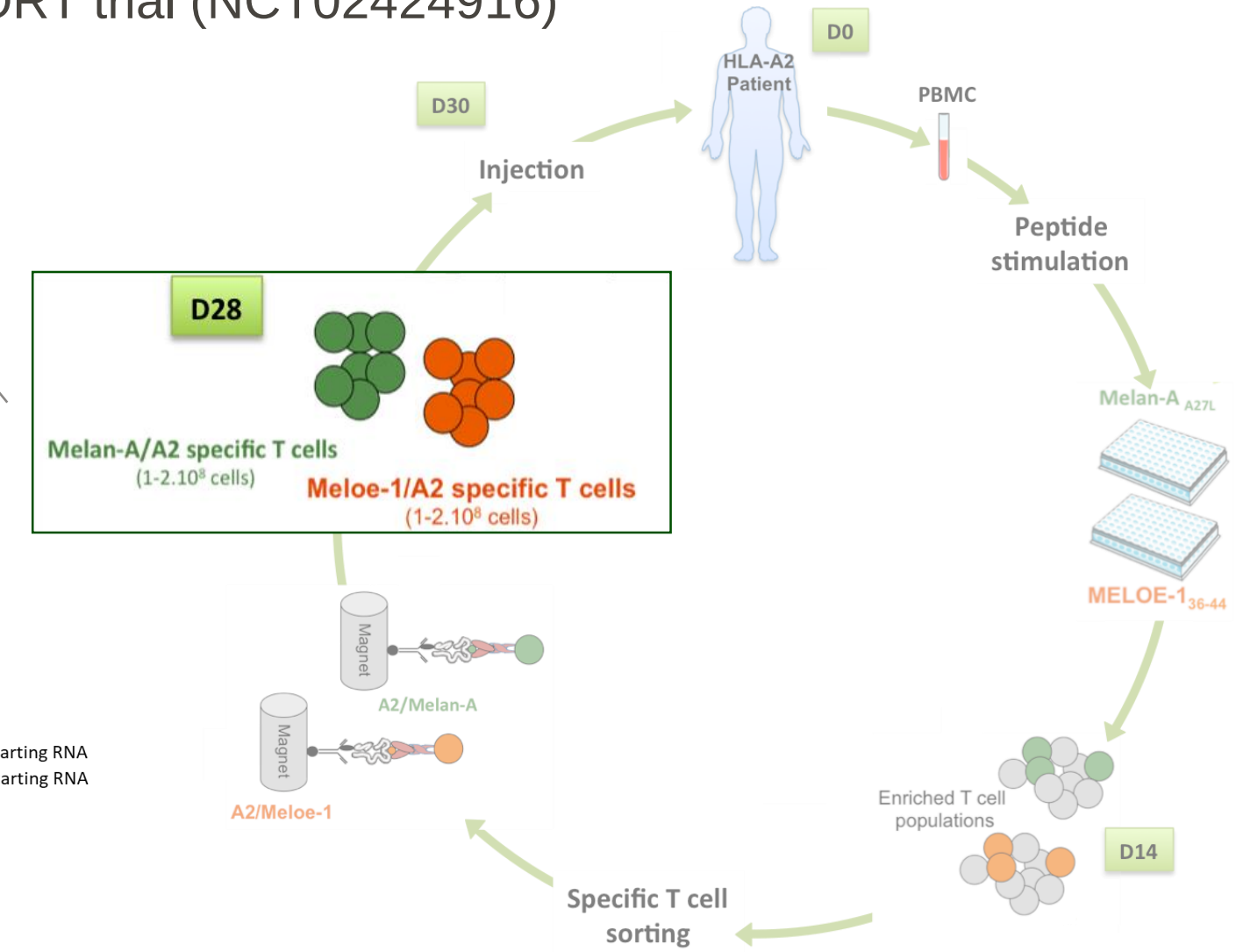
Transcriptional profiling for TCR clonotypes

- Similar numbers of CDR3a and CDR3b clonotypes
- Higher number of low frequency clonotypes (< 10^{-4}) detected when starting with a higher amount of total RNA





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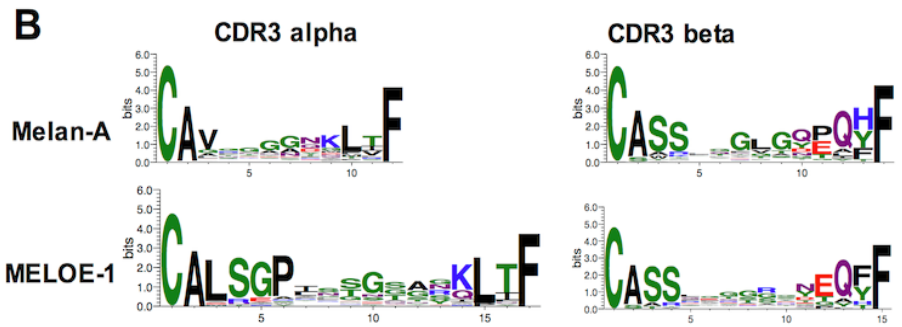
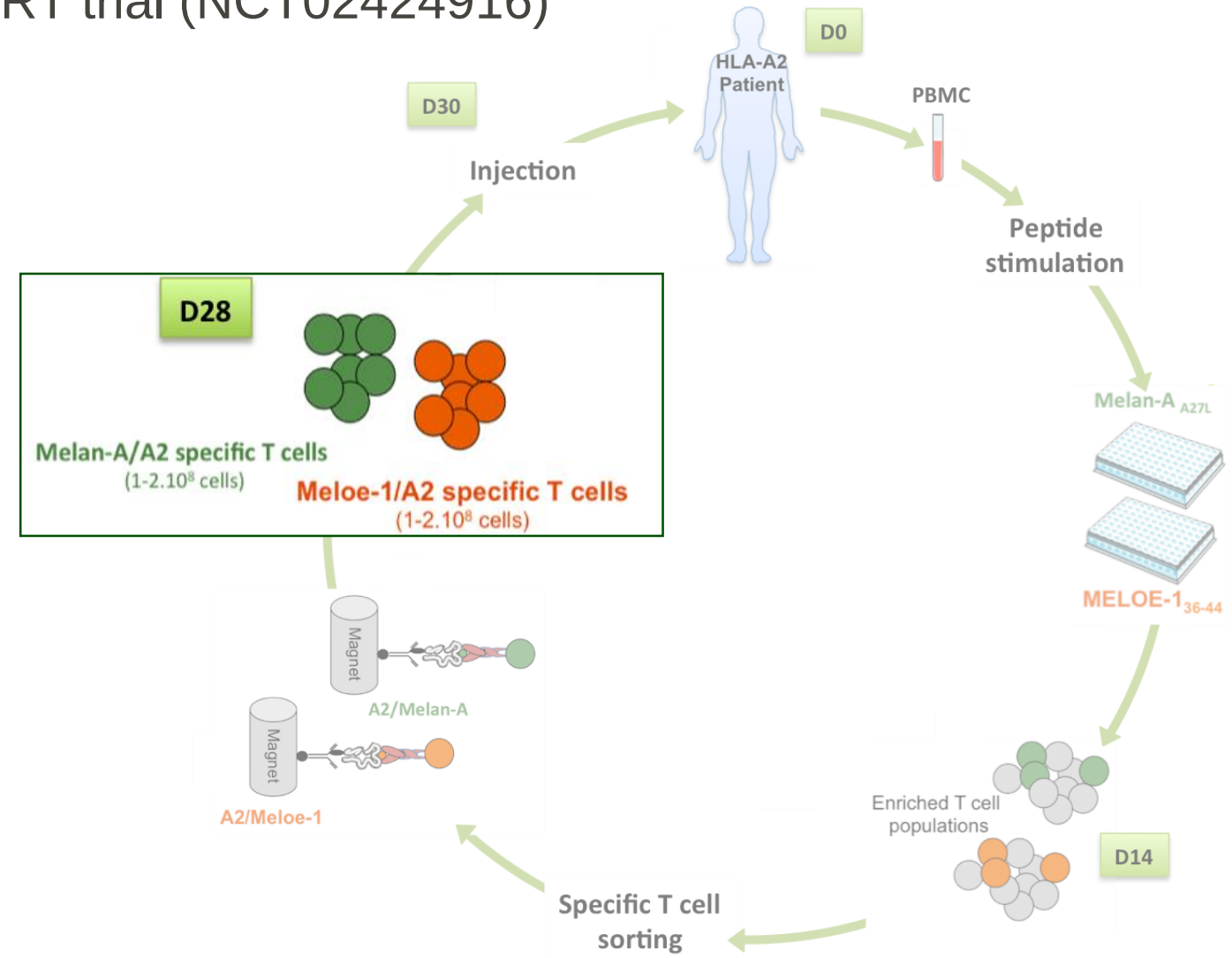
To study T-cells from Melanoma patients

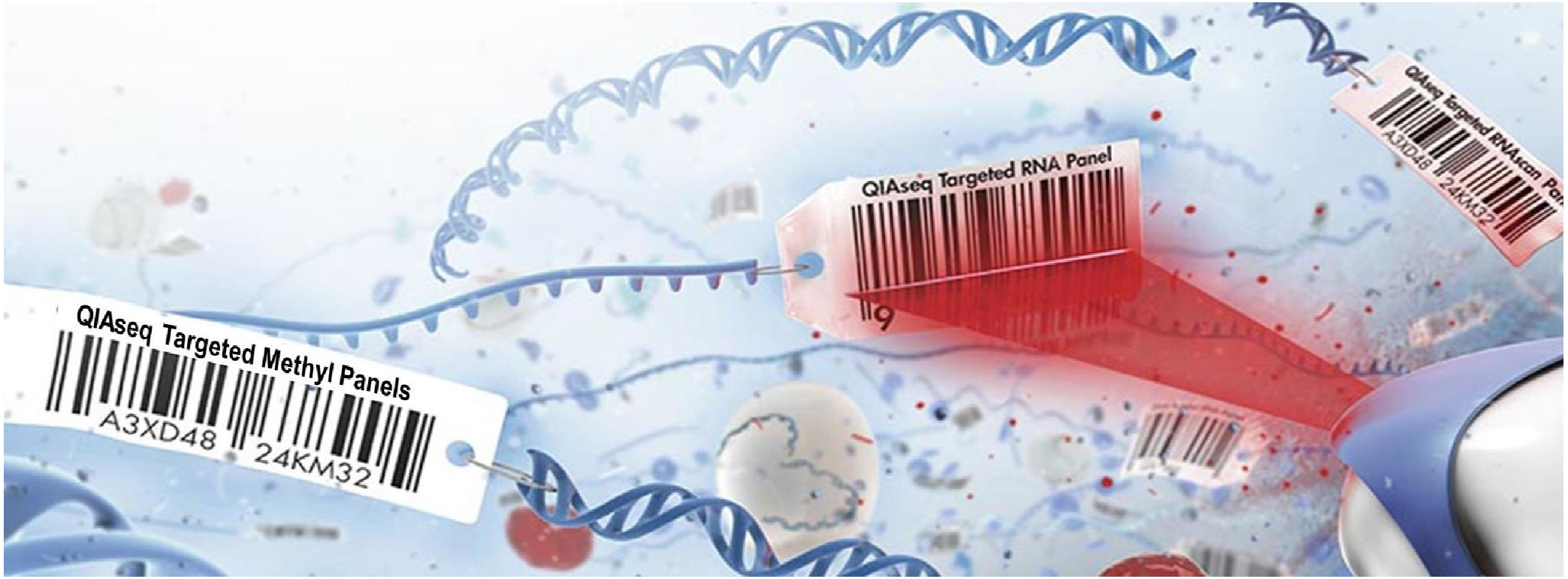
Transcriptional profiling for TCR clonotypes

QIAseq Immune Repertoire analysis

- Improves immune monitoring options by providing the details of the poly-peptide chains



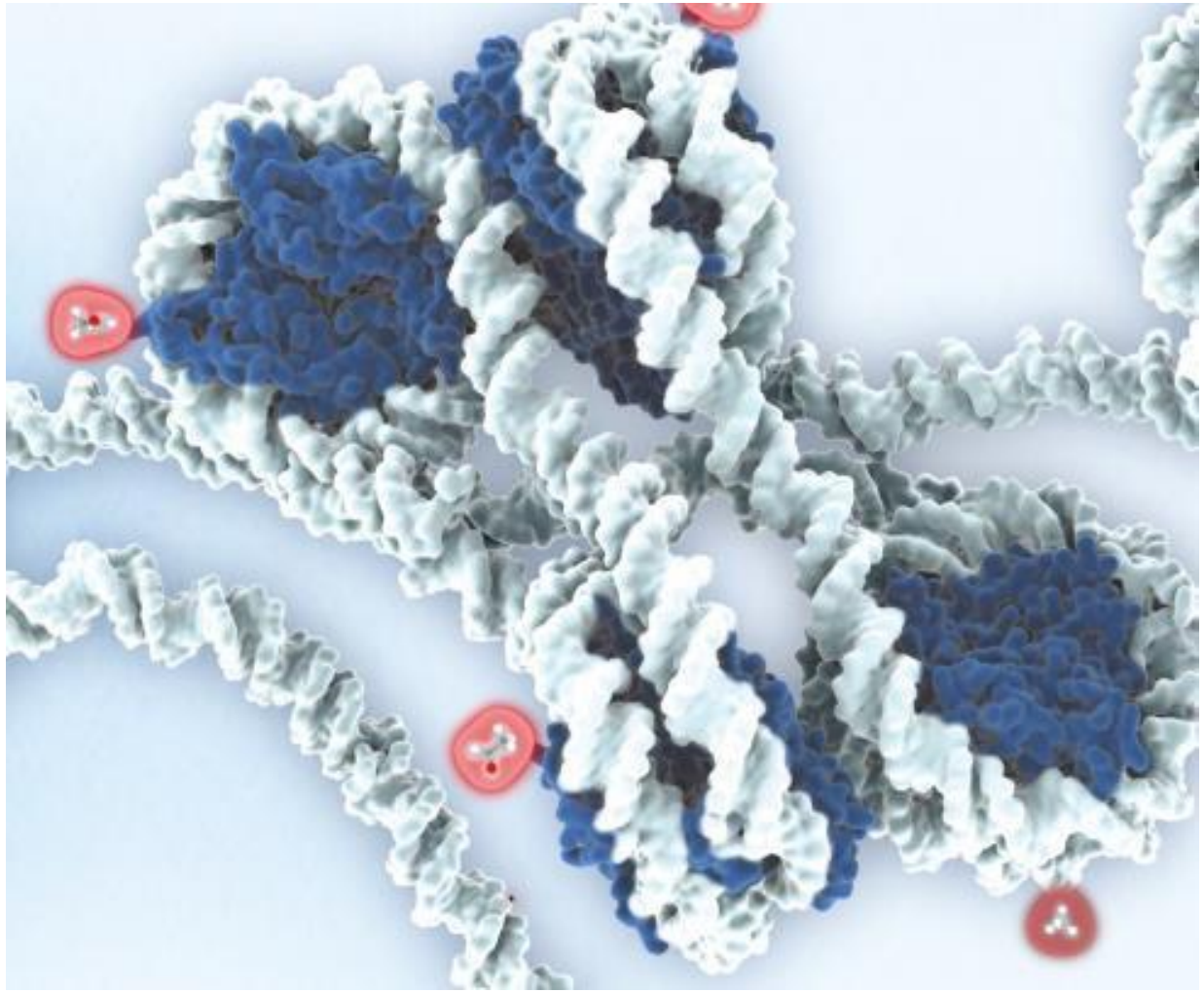




QIAseq Targeted Methyl Panels

Targeted Methylation Sequencing For Challenging Sample Types-Including FFPE and Liquid Biopsy

Epigenetic changes through DNA methylation



- Stable, heritable, covalent modifications of DNA
- Primarily at CpG dinucleotides, but are also found at non-CpG sites
- Involved in normal cell differentiation and development
- With some exceptions, CG dense promoters are unmethylated in normal tissues
- Associated with gene silencing

DNA methylation events in cancer

Hypomethylation

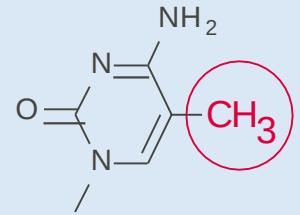
Reduction in methylated cytosines – active state of chromatin

- Activation of cancer germline genes that are normally silenced
- Increased transcriptional activity
- Less compact chromatin structure may lead to genomic instability

Hypermethylation

Increase in methylated cytosines – repressed state of chromatin

- Tumor suppressor gene silencing when regulatory sequences such as enhancer or promoter regions are affected
- Change in expression of transcript isoforms when alternative promoters are affected

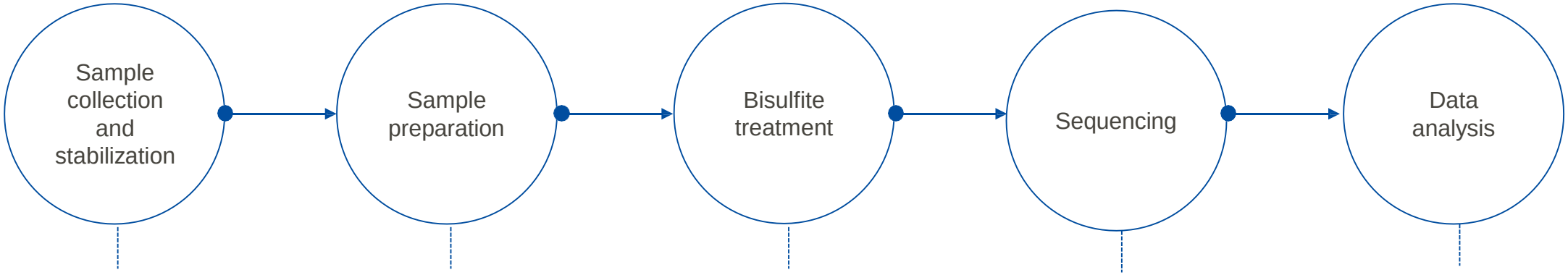


5-Methylcytosine

- Specificity of methylation patterns for different tissue types, differentiation status and disease states – promising biomarker
- Stability in circulating cell-free DNA – promising liquid biopsy biomarker
- Reversibility of methylation status – potential target for epigenetic therapies

Pfeifer, G. (2018) Defining driver DNA methylation changes in human cancer. Int. J. Mol. Sci. 19, 1166.

What do I need to determine the methylation status of DNA?



Cell-free DNA stabilization in blood samples

- PAXgene Blood ccfDNA Tubes*

- QIAamp MinElute ccfDNA Kit
- QIAamp DNA FFPE Tissue Kit
- GeneRead DNA FFPE Kit
- QIAsymphony PAXgene Blood ccfDNA Kit

- EpiTect Fast FFPE Bisulfite
- EpiTect Fast LyseAll Bisulfite

- EpiTect Fast DNA Bisulfite Conversion Kits

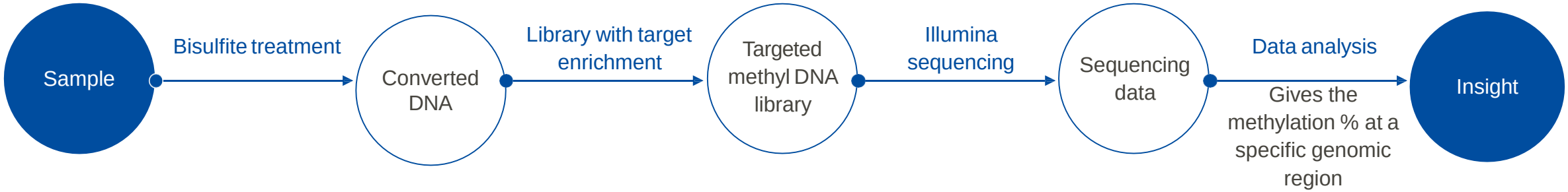
NGS

- QIAseq Methyl Library Kit
- QIAseq Targeted Methyl Panels

- QIAGEN CLC Genomics Workbench
- GeneGlobe Data Analysis Center

*CTC stabilization claim is only valid for the PAXgene Blood ccfDNA Tubes (768115; RUO) For Research Use Only. Not For Use in Diagnostic Procedures. Available in US and certain other countries outside Europe.

QIAseq Targeted Methyl Panels – an overview



Sample types : FFPE, gDNA and liquid biopsy (circulating cell-free DNA or ccfDNA)

Starting material

- 1–100 ng gDNA
- 10–200 ng FFPE DNA
- 10–200 ng ccfDNA

Total workflow time: 7.5–9 h

Total hands-on time. 2.5–4.5 h

Panels for targeted methylation sequencing

- Human Breast Cancer Panel
- Human Colorectal Cancer Panel
- Immuno-Oncology Panel
- Human T-cell Infiltration Panel

Compatible with Illumina sequencers

Panel customization: Fully design-novel panel content based on genomic coordinates or CG identifiers

Data analysis options

- GeneGlobe Data Analysis Center
- QIAGEN CLC Genomics Workbench

QIAseq Targeted Methyl Panels powered by unique technologies

UMIs reduce bias

Unique molecular indices (UMIs) are unique index sequences that are ligated on to each bisulfite-treated DNA strand

- UMIs help to overcome bias during PCR and bridge amplification
- Data is now representative of the unique number of molecules in a sample
- 12 base single UMI

SPE enhances CpG targeting

Single primer extension (SPE) enables increased targeting and multiplexing capacity

- Targeting only a single region in intronic regions reduces the need for paired primers
- A universal primer is used to create sequencing ready libraries

Liquid biopsy and FFPE compatible

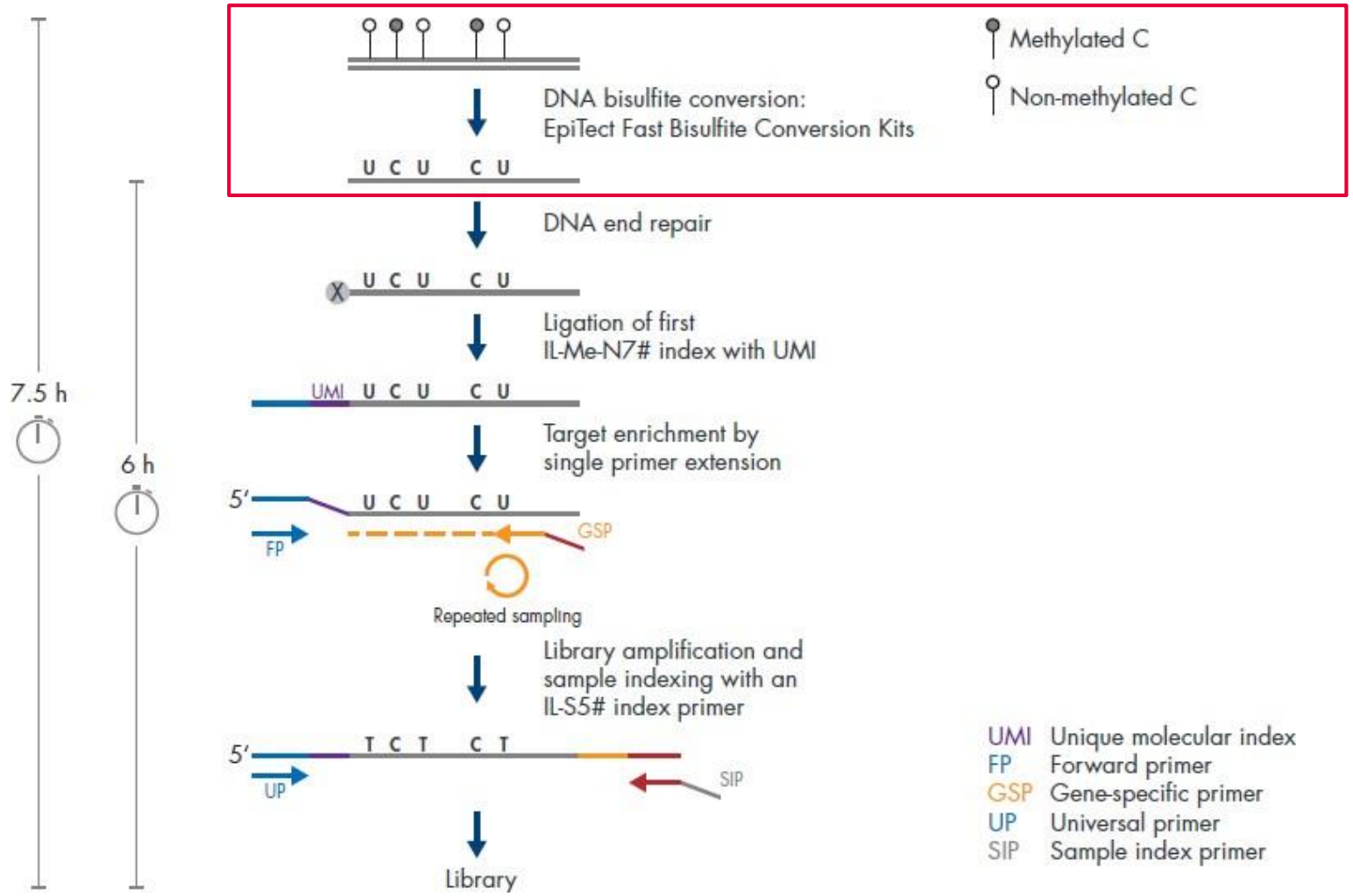
Methylation can be indicative of genomic alterations and identify certain cell and tissue types. The kit is compatible with ccfDNA and ultralow input levels, even from FFPE to help determine these patterns.

- 10–40 ng of DNA needed for liquid biopsy applications
- Minimum of 1 ng of purified gDNA for cells and tissue for the rarest samples

QIASeq Targeted Methyl Panels- efficient and high-throughput compatible workflow

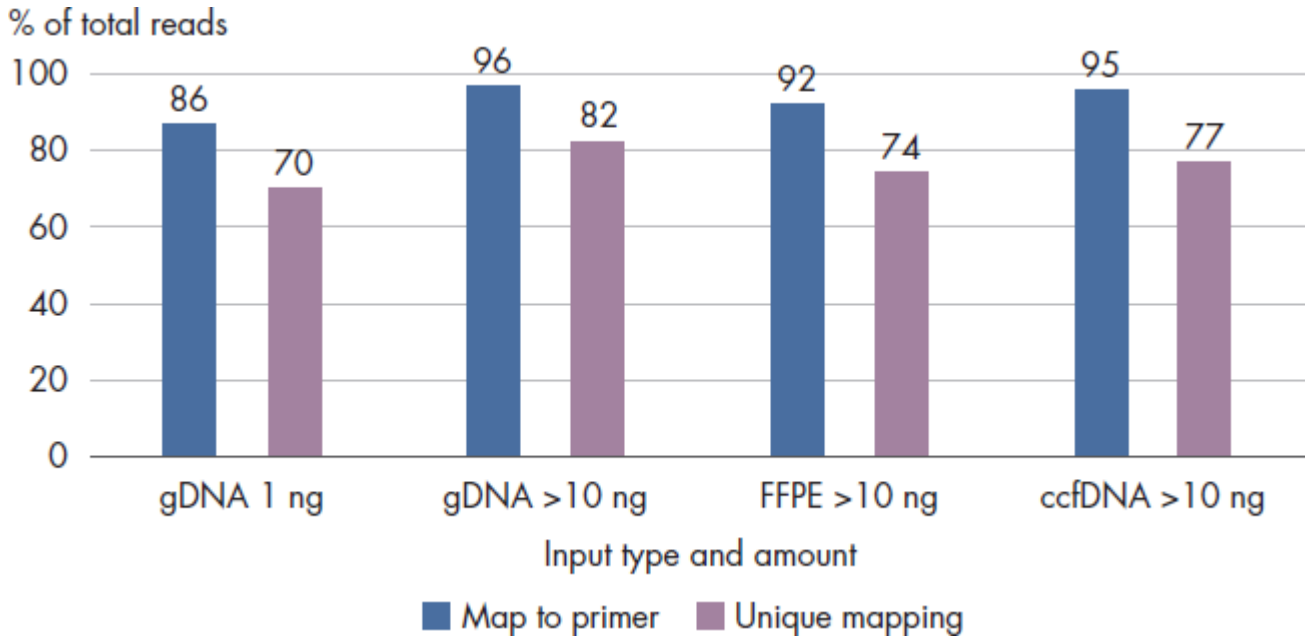
Input requirements:	250 ng – 1 µg	250 ng – 1 µg	1–40 ng
	Microarray	**Hybrid capture NGS**	**QIAseq**
Day 1	Amplify and incubate DNA	Amplify and incubate DNA	Bisulfite conversion, single primer extension; to sequencing
Day 2	Fragment DNA to hybridization	Fragment DNA to hybridization	
Day 3	Extend and stain Image chip and call		

Single day to sequencer: QIAseq Targeted Methyl Panel sequencing workflow



High performance from tissue and liquid biopsy: 1–10 ng of input range

QIAseq Targeted Methyl Panel: High mapping efficiency even at 1 ng of input

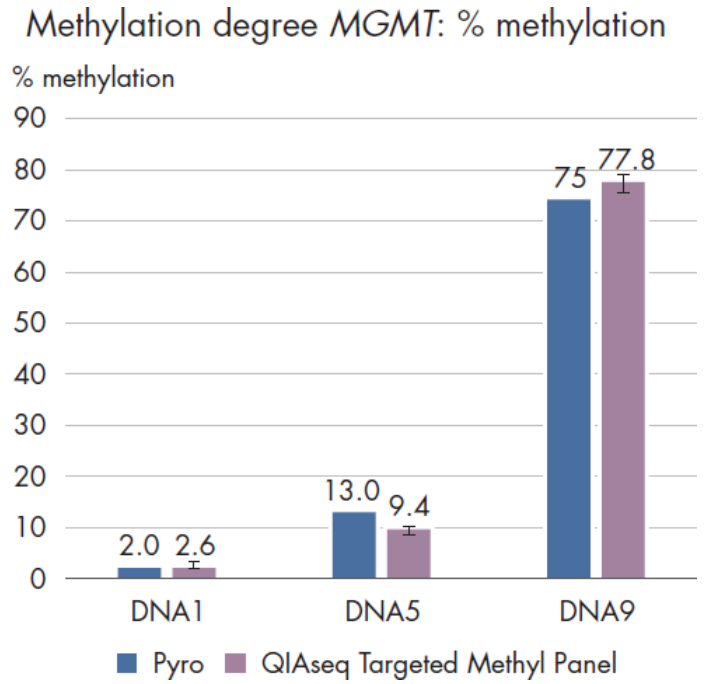


- gDNA was run at both 1 and 10 ng
- FFPE was run at 10 ng
- ccfDNA was run at 10 ng

Results show high mapping on primer and unique reads even from 10 ng inputs

Much lower input amounts with high correlation to established methods

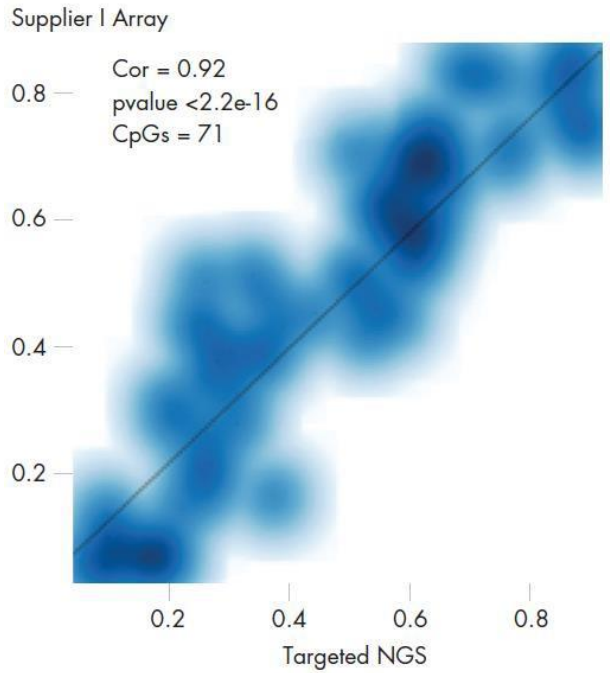
High correlation to Pyromark assays



Input: 40 ng FFPE DNA

Primers: 93 primers covering 566 CpG sites; 7 CPG sites on the *MGMT* gene, previously validated with pyrosequencing, were compared to the targeted methyl result

92% correlation to EPIC array despite 1/5th the input

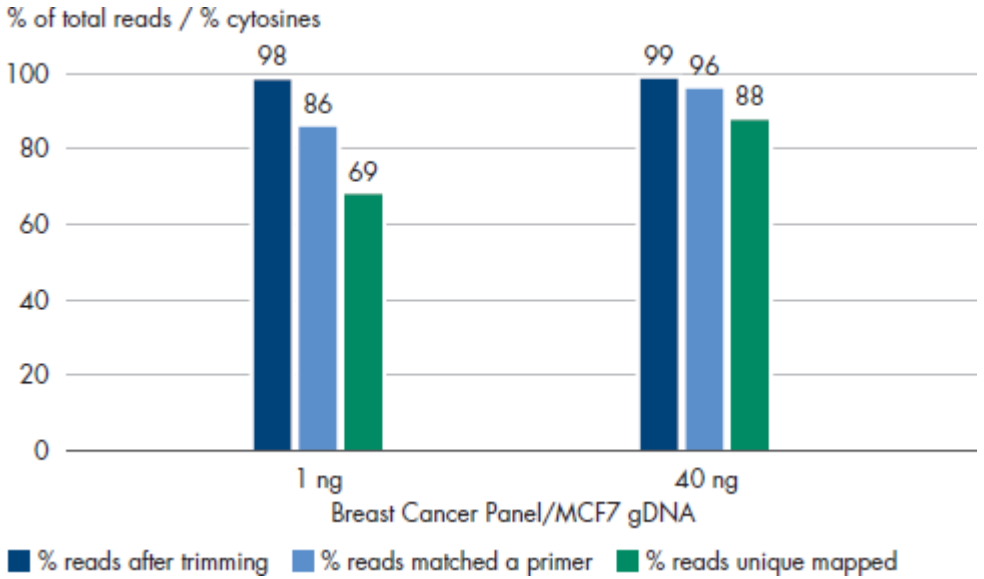


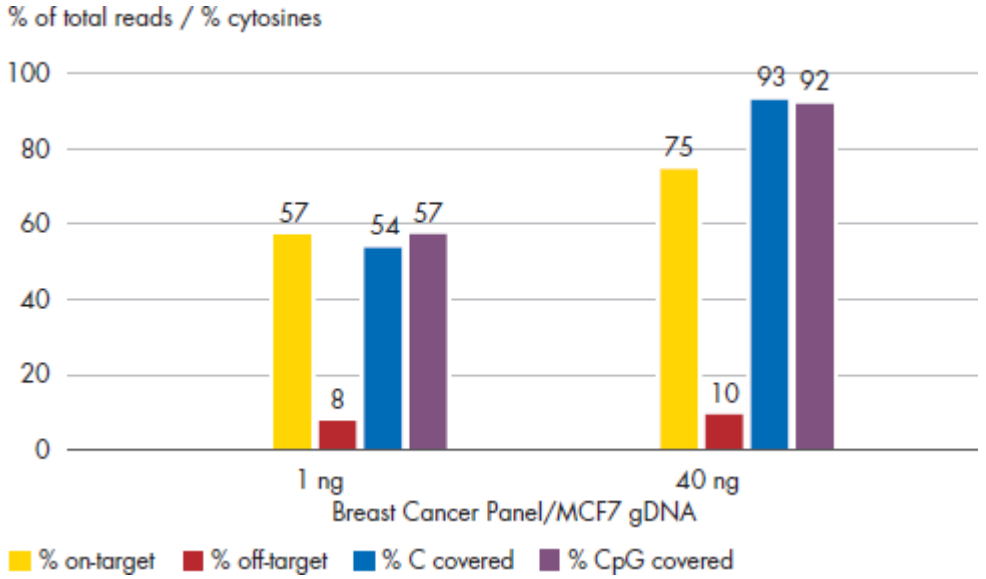
Input: 40 ng gDNA from hepatocytes

Primers: 102 primers covering 71 CpGs

QIAseq Targeted Methyl Panel: Human Breast Cancer Panel

High performance at ultra-low input levels leading to high-quality data

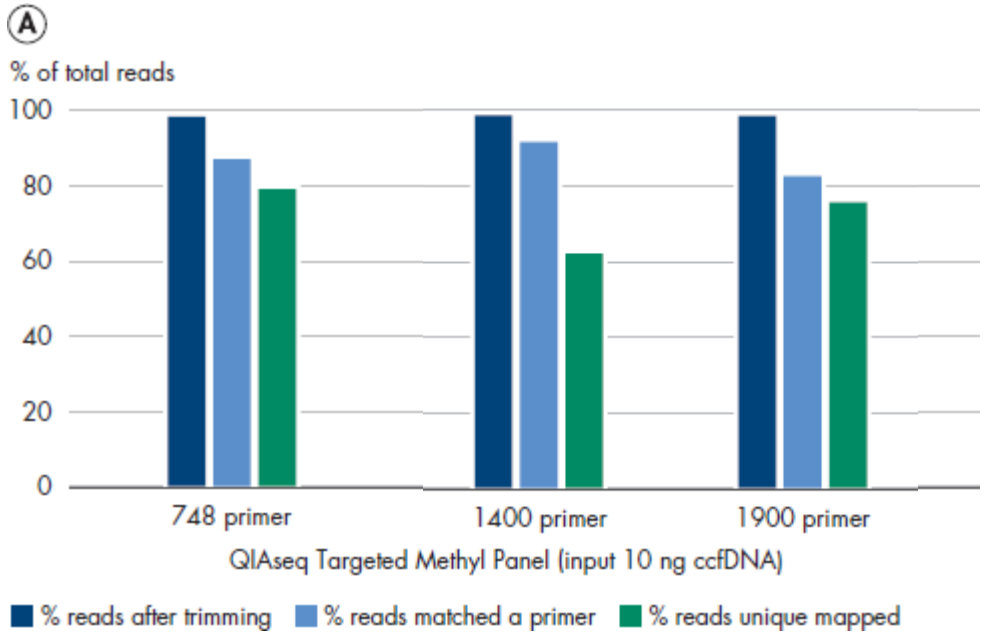




- To evaluate library characteristics of the Human Breast Cancer Panel (cat. no. MHS-001Z), 1 ng and 40 ng gDNA were used to generate libraries
- Libraries were sequenced on MiSeq, resulting in high mapping rates and high numbers of CpGs covered
- Library quality is high even with the lowest possible input used for bisulfite conversion with EpiTect Fast chemistry

Generate high-quality libraries from ccfDNA with different panel sizes

High-quality libraries that resulted in high percentages of unique reads

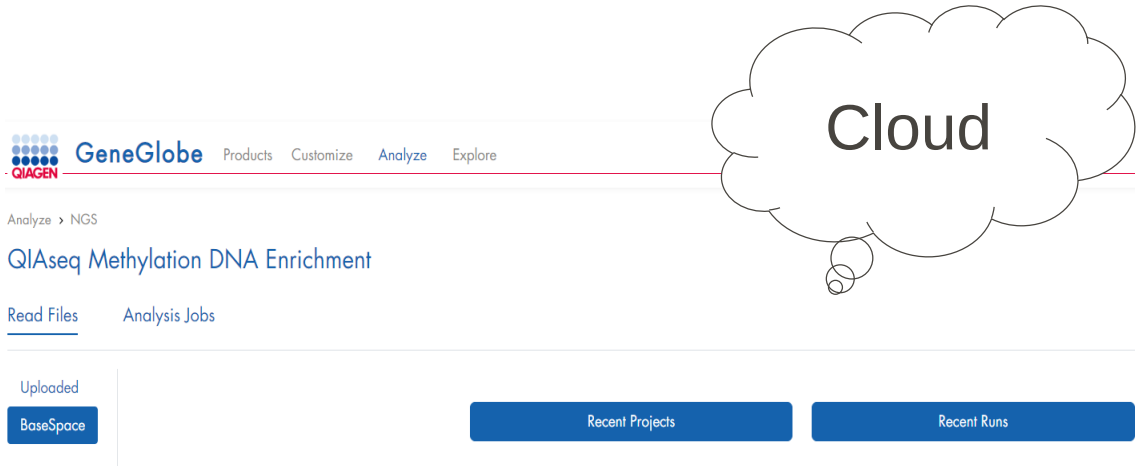


- To evaluate library characteristics, ccfDNA was purified using QIAamp chemistry and subsequently bisulfite treated using EpiTect Fast chemistry.
- 10 ng ccfDNA was processed using 3 different panels

Cloud or local data analysis solutions

Analyze your data from wherever you are

- ✓ Custom catalog numbers are provided for user-defined content
- ✓ Output files can be downloaded from your QIAGEN.com account for tertiary analysis
- ✓ Connect directly from your Illumina BaseSpace account

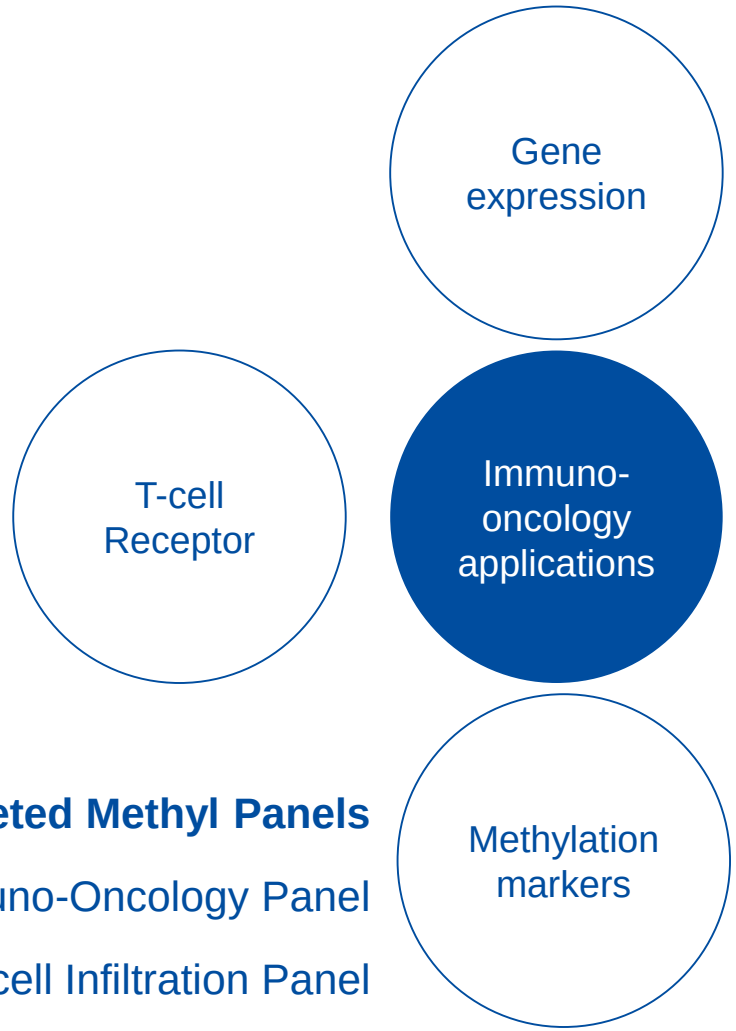


Local computer / Institution Server

- Genome-wide/ targeted methylation calling
- Bisulfite read mapping
- Detection of methylated Cs in various sequence contexts
- Statistical tests for differential methylation
- Reduced representation bisulfite sequencing support

Precision NGS for immuno-oncology applications- “off the shelf”

Targeted NGS panels with UMIs + integrated BIOX solutions = precision NGS



QIAseq Targeted RNA Panels

✓ Immunocology Panel

QIAseq Immune Repertoire

✓ RNAseq T-cell Receptor Panel

QIAseq Targeted DNA Panels

✓ Tumor Mutational Burden

QIAseq Targeted Methyl Panels

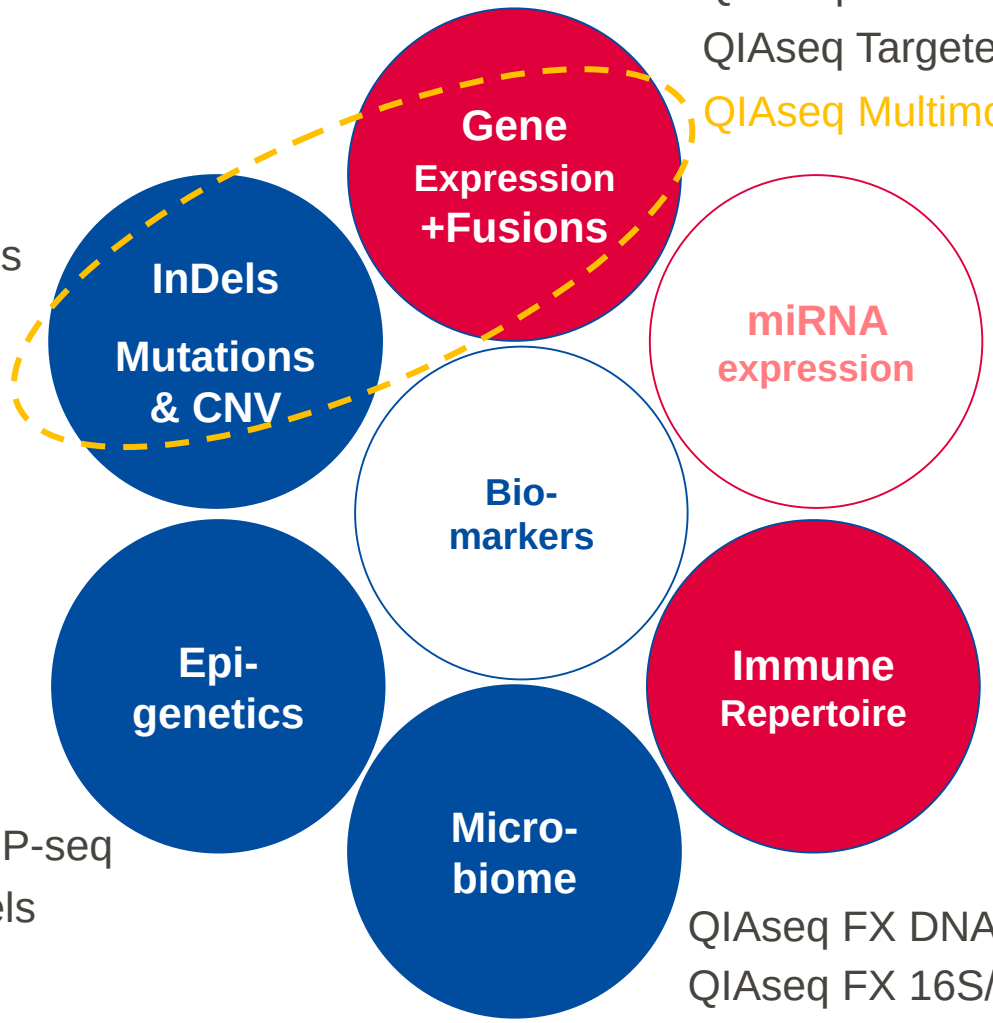
✓ Immuno-Oncology Panel

✓ Human T-cell Infiltration Panel

QIAGEN's NGS portfolio: QIAseq for Immuno-Oncology

QIAseq Stranded RNA + FastSelect rRNA/Globin removal
QIAseq UPX 3' Whole / Targeted Transcriptome
QIAseq Targeted / RNAscan RNA Panels
QIAseq Multimodal Panels

QIAseq Whole Exome Panel
QIAseq Targeted DNA Panels
QIAseq Multimodal Panels



QIAseq miRNA Sequencing System

QIAseq TCR Panel (CDR3 + Full Length)

QIAseq Bisulfite Conversion
QIAseq Ultra-low DNA for ChIP-seq
QIAseq Targeted Methyl Panels

QIAseq FX DNA for Whole Genome
QIAseq FX 16S/ITS Screening + Region Panels

QIAGEN's NGS portfolio: QIAseq for Immuno-Oncology

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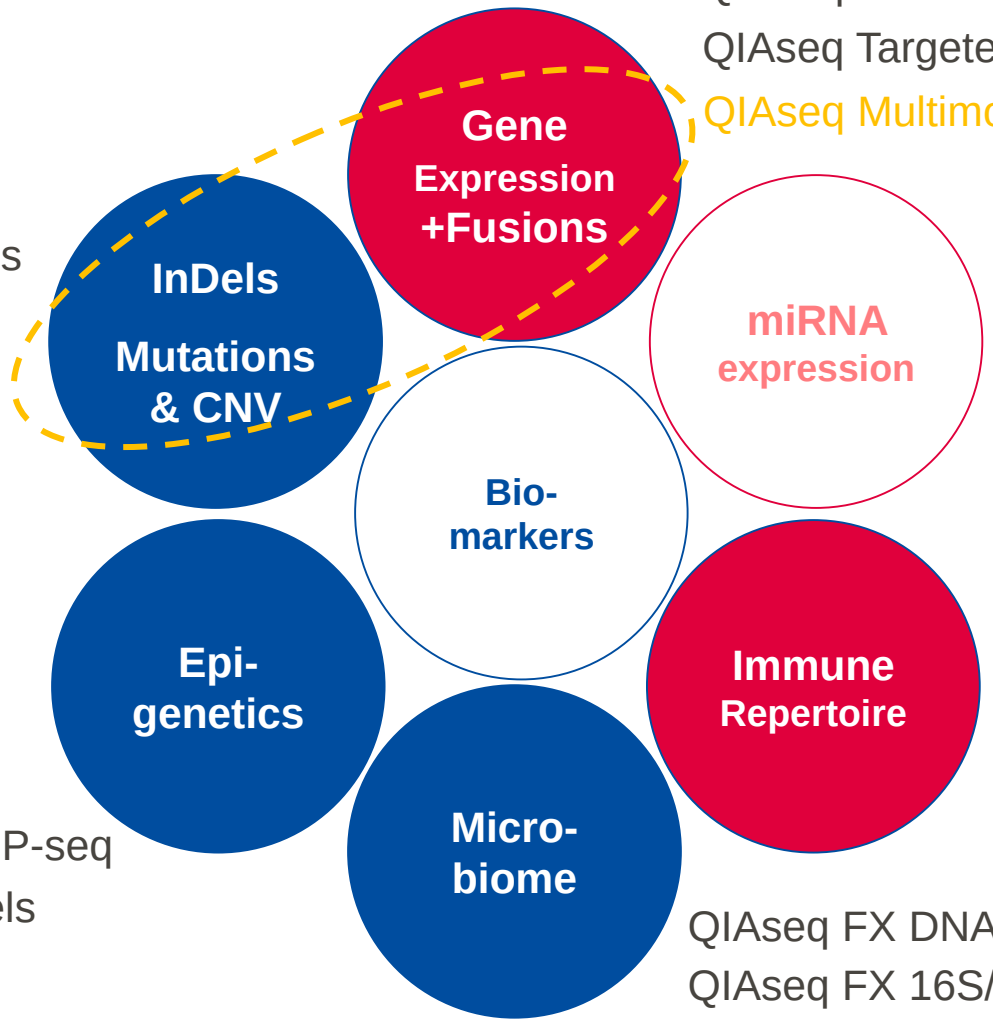

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Thank you for your attention. Questions?

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