Service Profile

Immune Repertoire Profiling Services

Helping you unveil adaptive immune responses with an all-in-one solution

Accurate characterization of the T cell receptor (TCR) repertoire is key to understanding adaptive immune responses and has applications across vaccine development, autoimmunity, monitoring treatment response in lymphoid malignancies and immunotherapy. Compared to traditional methods, next-generation sequencing (NGS) provides an unprecedented, high-resolution picture of the immune repertoire. However, the complex workflows and high time investment required can make developing automated NGS workflows challenging. In addition, this approach involves multiplex PCR with primers targeting different V or J regions, which can introduce substantial amplification bias.

QIAGEN Genomic Services has risen to meet these challenges with our convenient, all-in-one Immune Repertoire Profiling Services. Extend your in-house resources with the expertise, powerful bioinformatics and custom services that you expect from QIAGEN®. Using our proven sample preparation and targeted NGS technologies, we deliver end-to-end expertise to support your adaptive immune response studies. Our profiling solution offers the following benefits:

- **End-to-end service**: we take care of every step, from sample preparation to data analysis
- **Guidance and flexibility**: we aid in designing your project and help you make the right decisions
- **Optimized TCR variable region enrichment**: we use unique molecular indices to reduce amplification bias, providing accurate and sensitive clonotype and repertoire diversity assessment
- **Ready-to-publish data**: we deliver comprehensive reports and data packages, and provide guidance on the next steps

Partner with us for expert guidance and dedicated service – from Sample to Insight – for immune repertoire profiling.

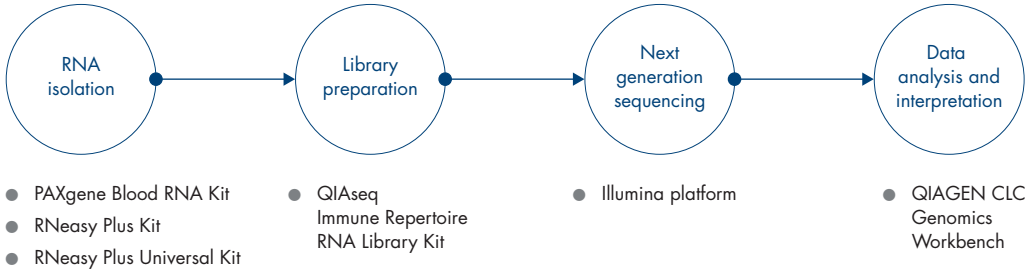


Figure 1. Sample to Insight immune repertoire profiling workflow.

An innovative workflow that supports uniform amplification

The QIAseq Immune Repertoire RNA Library Kit detects all four chains of the expressed human or mouse TCR repertoire (α, β, γ and σ subunits), including the CDR1, CDR2 and CDR3 regions, using 10–1000 ng RNA from cells, tissues or biofluids. The kit relies on a highly efficient, TCR-specific cDNA synthesis reaction, followed by ligation of sample index adapters containing unique molecular indices (UMIs) and TCR gene-specific primer enrichment for sensitive TCR clonotype and diversity assessment. The complete TCR variable region is enriched by PCR amplification with gene-specific primers targeting TCR constant regions and a universal primer complementary to the ligated adapter. This approach greatly improves amplification uniformity compared to multiplexed V and J primer pools. The incorporation of UMIs before the amplification step further reduces amplification bias and allows accurate and sensitive TCR clonotype and repertoire diversity assessment.

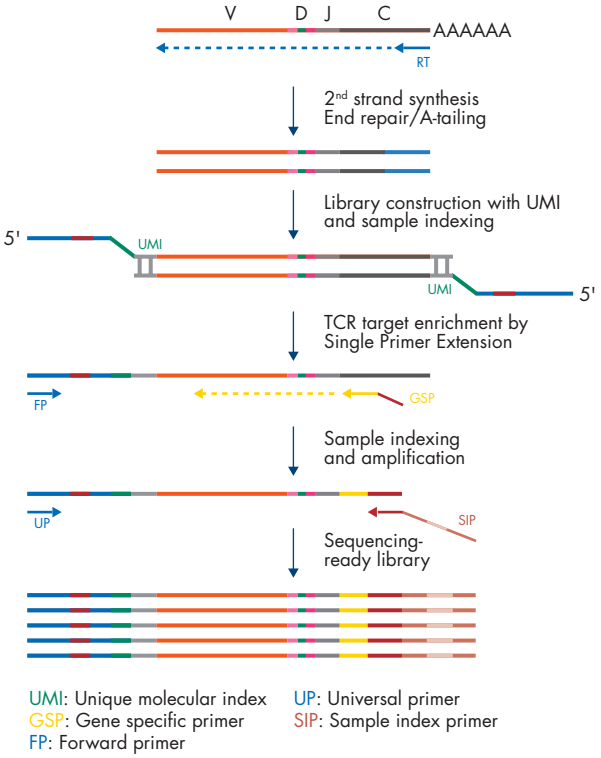


Figure 2. QIAseq Immune Repertoire RNA Library Kit workflow.

A comprehensive view of the T cell immune repertoire – delivered

Following sequencing, your data is analysed in a ready-to-use, optimized workflow in our CLC Genomics Workbench. This online, UMI-aware pipeline delivers key sequencing QC metrics, alignment of V, D and J regions against IMGT reference sequences and reports of frequency and identity for each unique clonotype. Additional downstream analyses, including V and J segment usage, CDR3 length distribution and diversity metrics, are provided in your comprehensive report.

Table 1. An example clonotype table that will be included in your report

Chain	V	J	CDR3 nucleotide sequence	CDR3 amino acid sequence	CDR3 length	Count	Productive
TRA	V-13-1	J-40	TGTGCAGCAAGA…	CAARTGTYKYIF	36	1	Productive
TRB	V-19	J-2-2	TGTGCCAGTAGC…		50	1	Out of frame
TRA	V-20	J-32	TGTGCTGTGCAT…	CAVHSFYGGATNKLIF	48	1	Productive

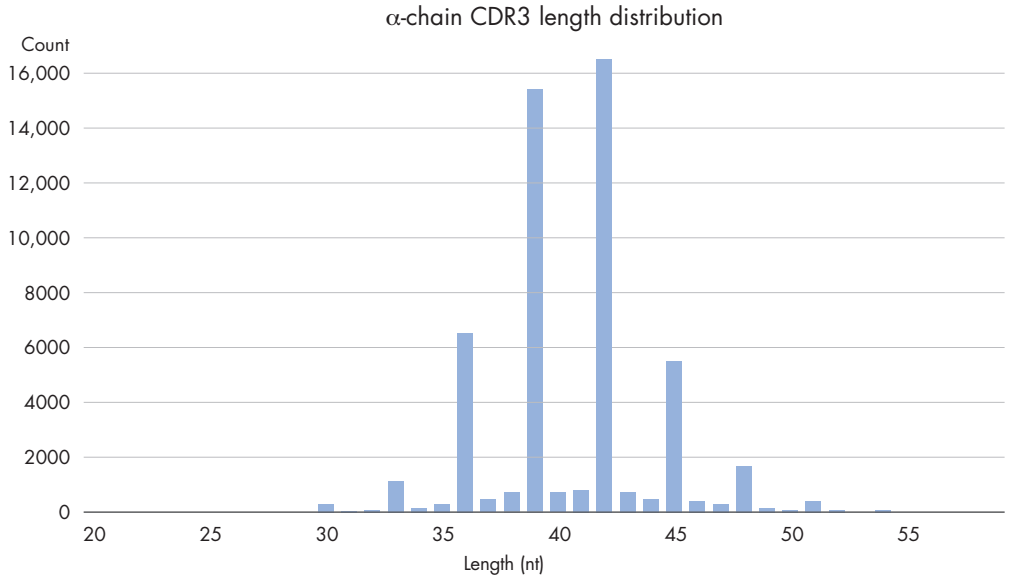


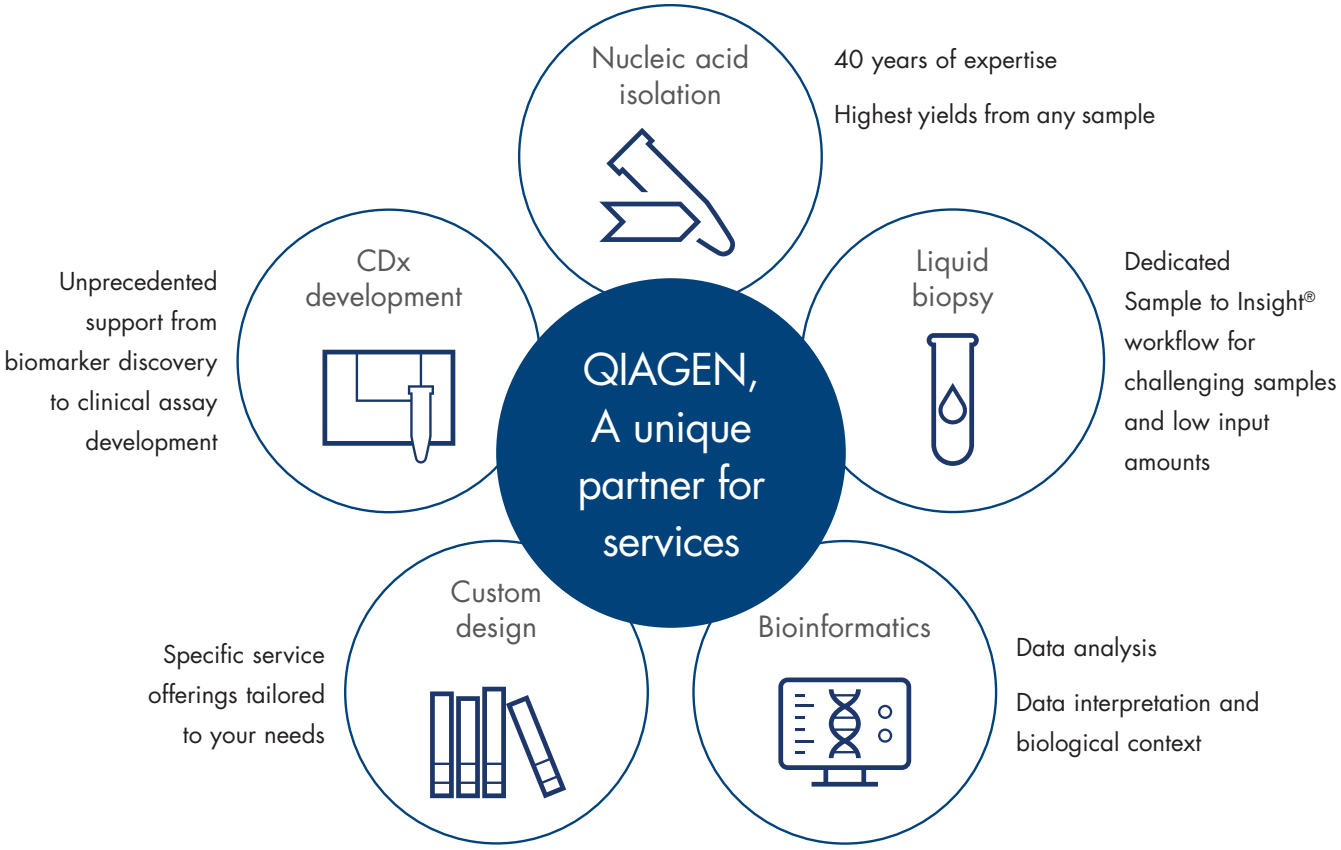
Figure 3. An example plot of CDR3 length distribution for the α-chain with peaks every 3 nt.

Service specifications

Consultation	Free consultation with an expert to design an experimental setup that best meets your needs and budget.		
RNA extraction	**Input sample**	**Isolation kit**	**Input requirements**
	Customer-isolated DNA	Not applicable	Minimum: 450 ng (>50 ng/µl)
	Whole blood	PAXgene® Blood RNA Kit	1 tube
	Fresh-frozen tissue	RNeasy® Plus Universal Kit	Minimum: 4–5 mg Maximum: 50 mg
	Cells	RNeasy Plus Kit	Minimum: 2 x 10^6 cells pelleted and frozen Maximum: 1 x 10^7 cells pelleted and frozen
	Other	Please inquire	
RNA quality control	RNA quality control (QC) is assessed by fluorometric measurement to measure the sample concentration and (capillary) gel electrophoresis to determine the RIN value. We will notify you about the QC results. This is a STOP/GO point where it is possible to omit samples, replace samples before proceeding or terminate the project.		
Library preparation and quality control	Library preparation is performed using the QIAseq Immune Repertoire RNA Library Kit.		
	Library QC is assessed by gel electrophoresis to check for the right fragment size and concentration. Library quantification is performed using qPCR. We will notify you about the QC results. This is a STOP/GO point where it is possible to terminate or adjust before proceeding to the sequencing.		
DNA sequencing parameters	Sequencing with Illumina® NextSeq®500, NextSeq550, MiSeq® or NovaSeq™ systems Paired-end reads, 2 x 250 bp for complete variable region sequencing Shallow: 2 x 1–2 M reads Deep: 2 x 5 M reads		
Complete data analysis	After analysis is complete, you will receive a comprehensive report with all the relevant data from your project. The table below lists some of the data that may be included in your report, depending on the services requested.		
	Raw data	De-multiplexed FASTQ files	
	Raw data QC	CLC graphical QC report (per sample)	
		CLC supplementary QC report (per sample)	
	Data trimming	CLC trim report (per sample); removal of adapters, low-quality, short sequences and ambiguous nucleotides)	
	Read deduplication	UMI groups report (per sample; reads collapsed by UMIs)	
	TCR analysis report	Summary Diversity indices Rarefaction CDR3 length	V and J usage Cumulative frequencies of clonotypes Productive summary
	Clonotypes	Table presenting the clonotypes identified in the sample with the following information:	
		Chain (gene family of clonotype) Identified V reference segments Identified J reference segments CDR3 nucleotide sequence	CDR3 amino acid CDR3 length Count Categorization (productive, out of frame, in-frame stop)
	Data delivery	Encrypted USB/hard disk drive or cloud delivery	
Final report and consultation	The final data analysis package contains an overview of data analysis and algorithms used, the files and tables listed above and publication-ready figures (PDFs provided as standard, please inquire for SVG or other formats). A teleconference is scheduled with QIAGEN scientists to discuss analysis and validation of results. Consultation and support will be provided for 90 days following delivery of data (for data delivery only projects), or delivery of data analysis (for data analysis inclusive projects). For extended support beyond 90 days, please inquire.		

How can we accelerate your research?

Our expert team is looking forward to learning about your research project and designing your customized service with QIAGEN.



Tell us about your project

The QIAGEN Genomics Profiling Service is intended exclusively for research use only (RUO). This service is not intended for the diagnosis, prevention or treatment of a disease.

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