Exploring clonal dynamics of B cell infiltrates in solid tumors

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Abstract

Characterizing the antigen receptor repertoire of adaptive immune cells in solid tumors is crucial for unraveling the dynamics of immune responses and tertiary lymphoid structures across diverse cancer types. In this research project, we are refining and establishing a bioinformatic workflow for the annotation of full-length immunoglobulin transcripts that were sequenced using Nanopore long-read sequencing. We utilize Unique Molecular Identifiers (UMIs) and high-accuracy base-calling algorithms to enhance the accuracy of our sequencing data analysis, enabling robust identification and quantification of individual immunoglobulin transcripts and facilitating the characterization of clonal dynamics and affinity maturation of tumor-associated B cells. The adaptive immune receptor features are integrated with other clinical and molecular data layers to determine whether and how tumor-associated adaptive immune cells and tertiary lymphoid structures are linked to anti-tumor immunity and patient prognosis.

Tertiary Lymphoid Structures

- **IDH wildtype glioblastoma:** most common malignant diffuse glioma
  - Immunosuppressive tumor microenvironment in the brain
  - Currently incurable (immune checkpoint response is limited)[1]
- Highly organized immune aggregates (TLS)
  - Hubs for antitumor immune responses in several cancer types[2,3]
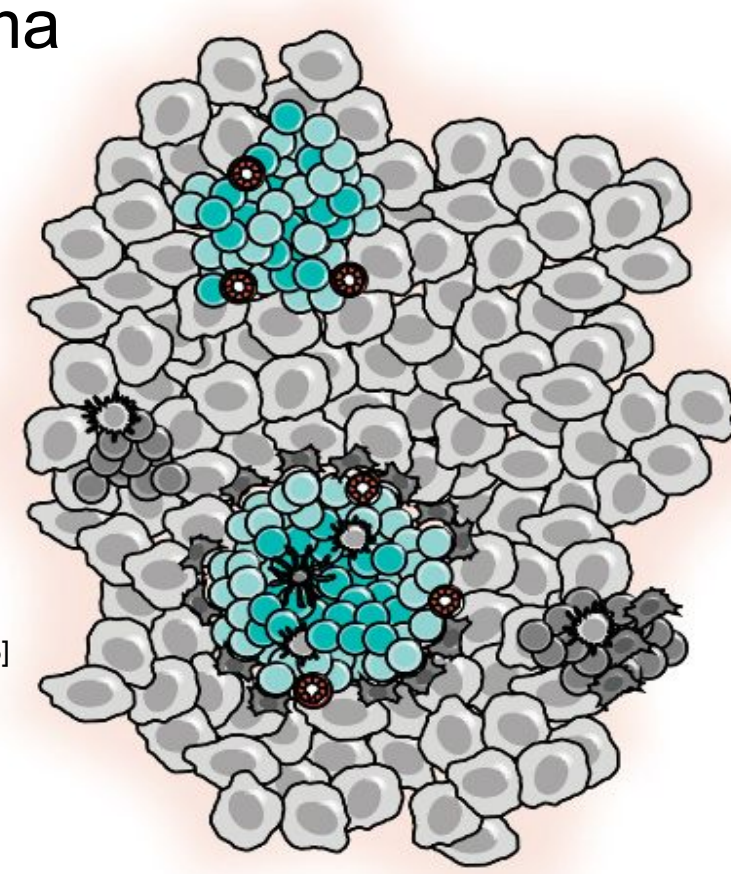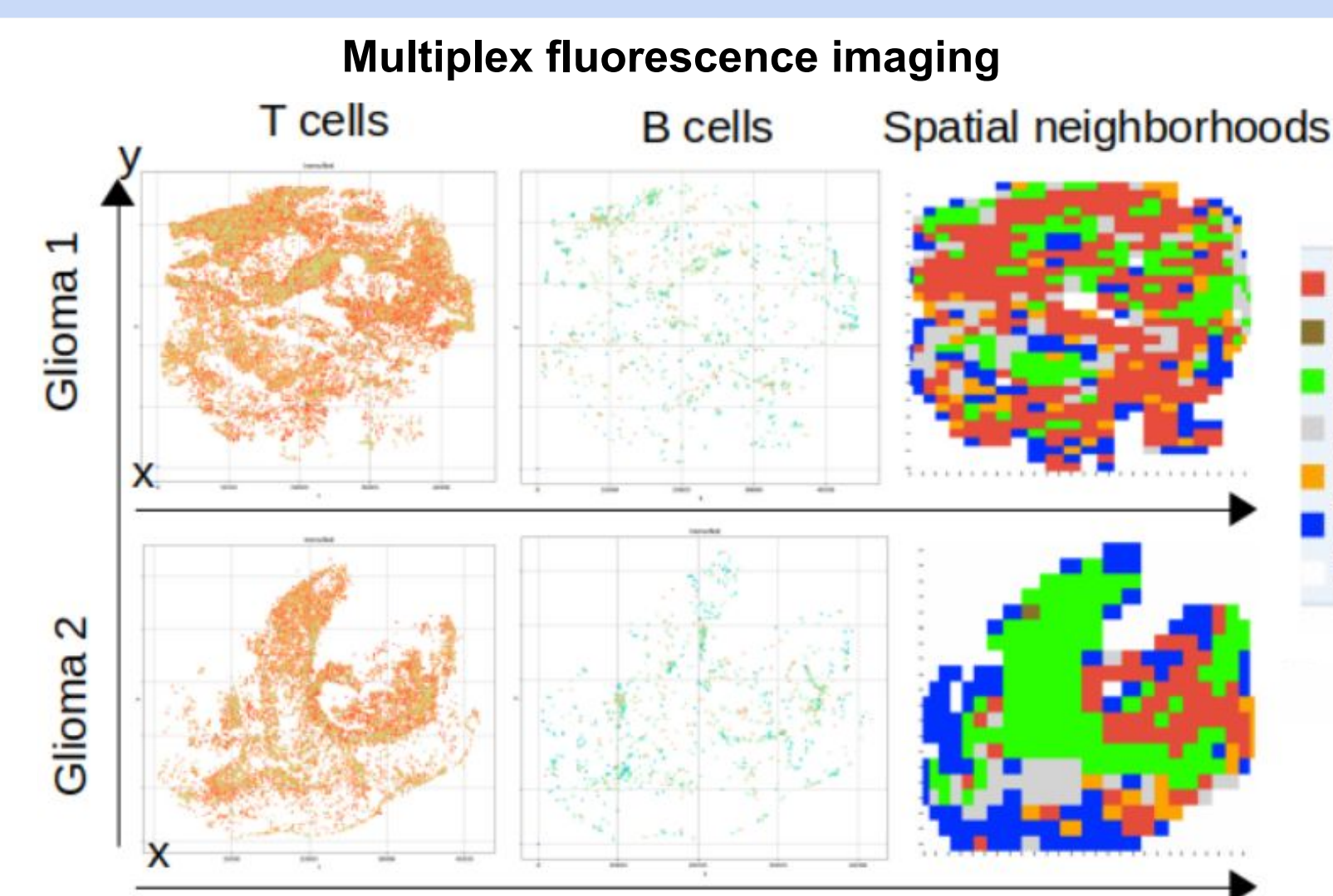- **B cells and TLS:** Unknown functionality in anti-glioma immunity

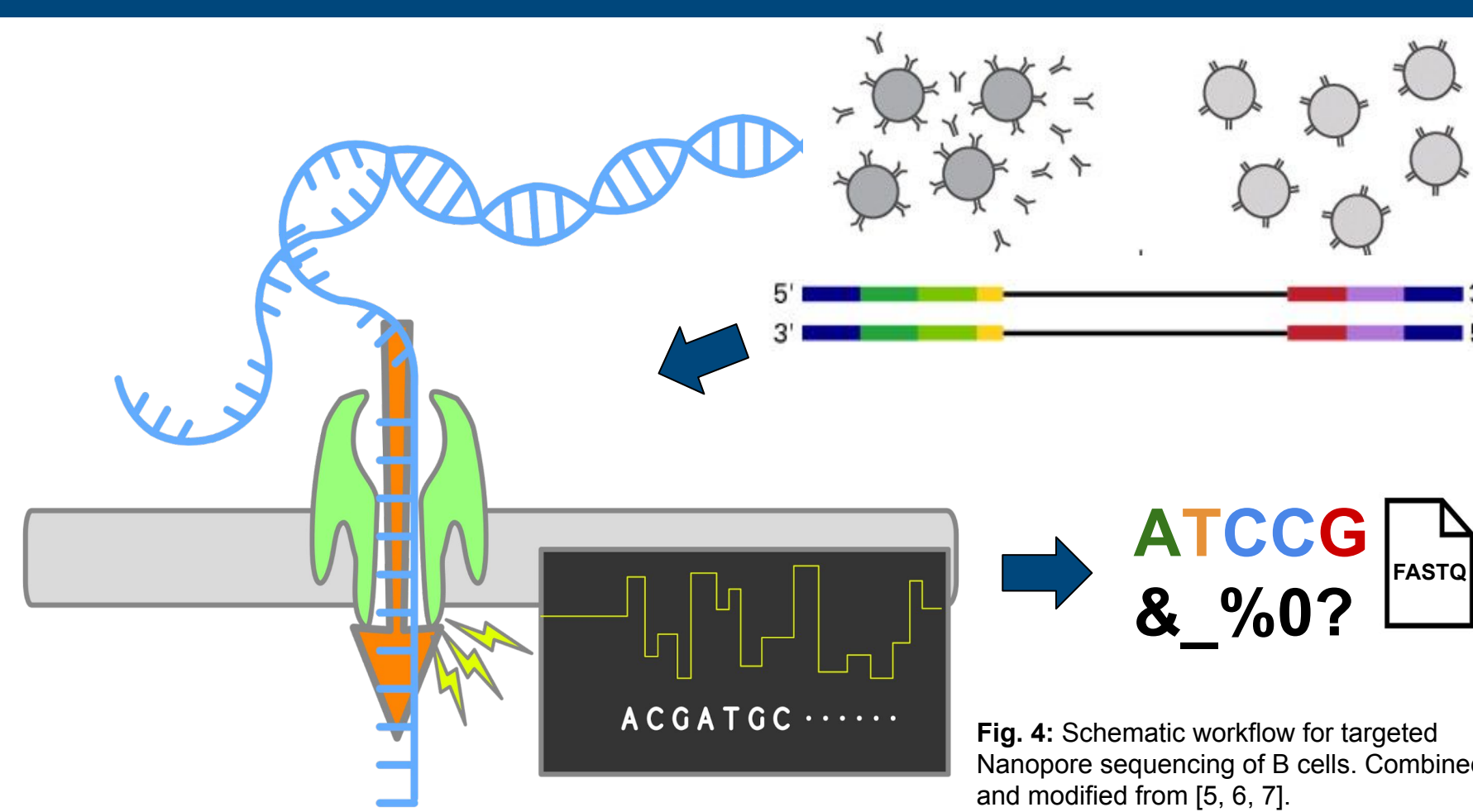**Fig. 1:** Schematic of TLS forming in tumor cells. Modified from [4].
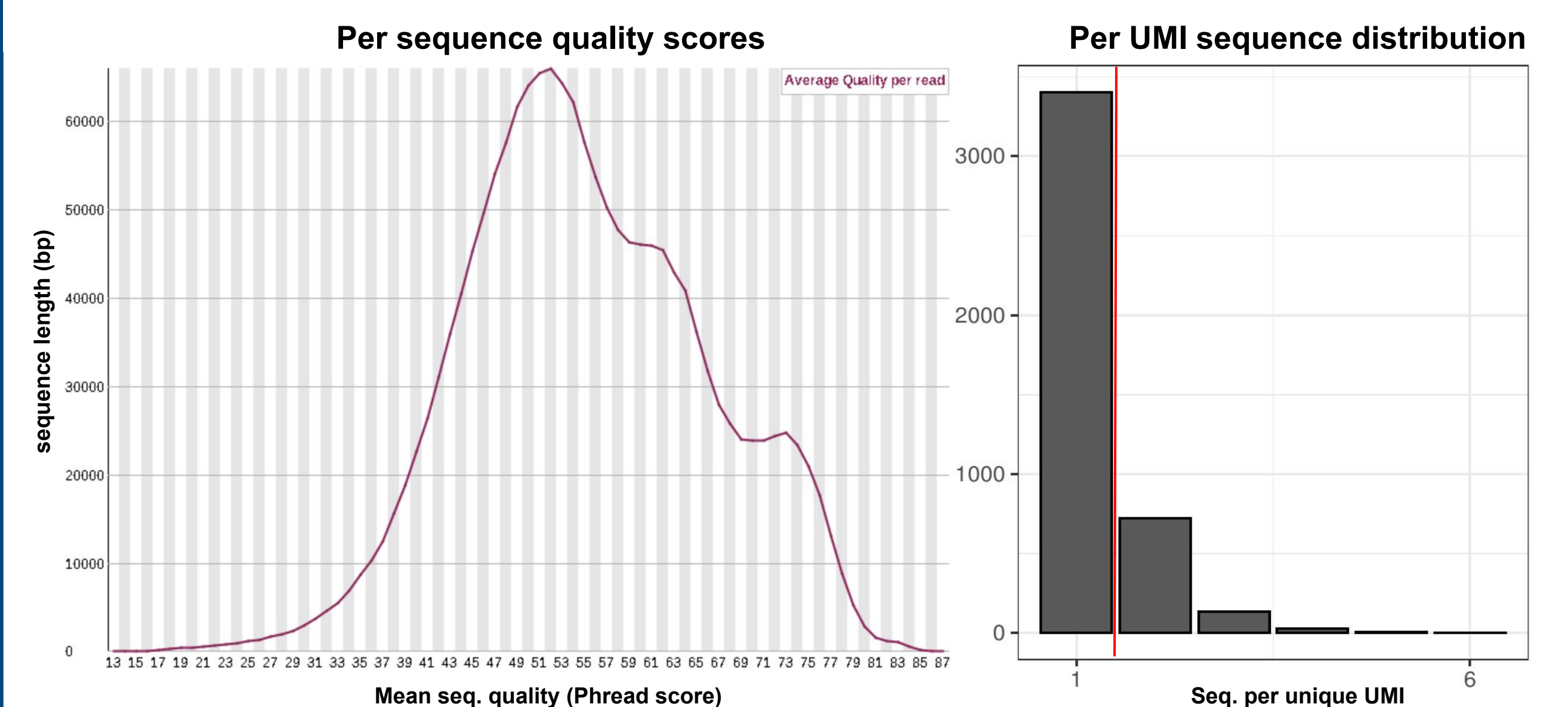
Identify Immune cell infiltration

- Fresh frozen tissue (2015-2022)
- **Glioblastoma**

Preliminary work:
- Multiplex fluorescence imaging
- Cell-type & -region annotation
- **Selection of TLS+ tumors**
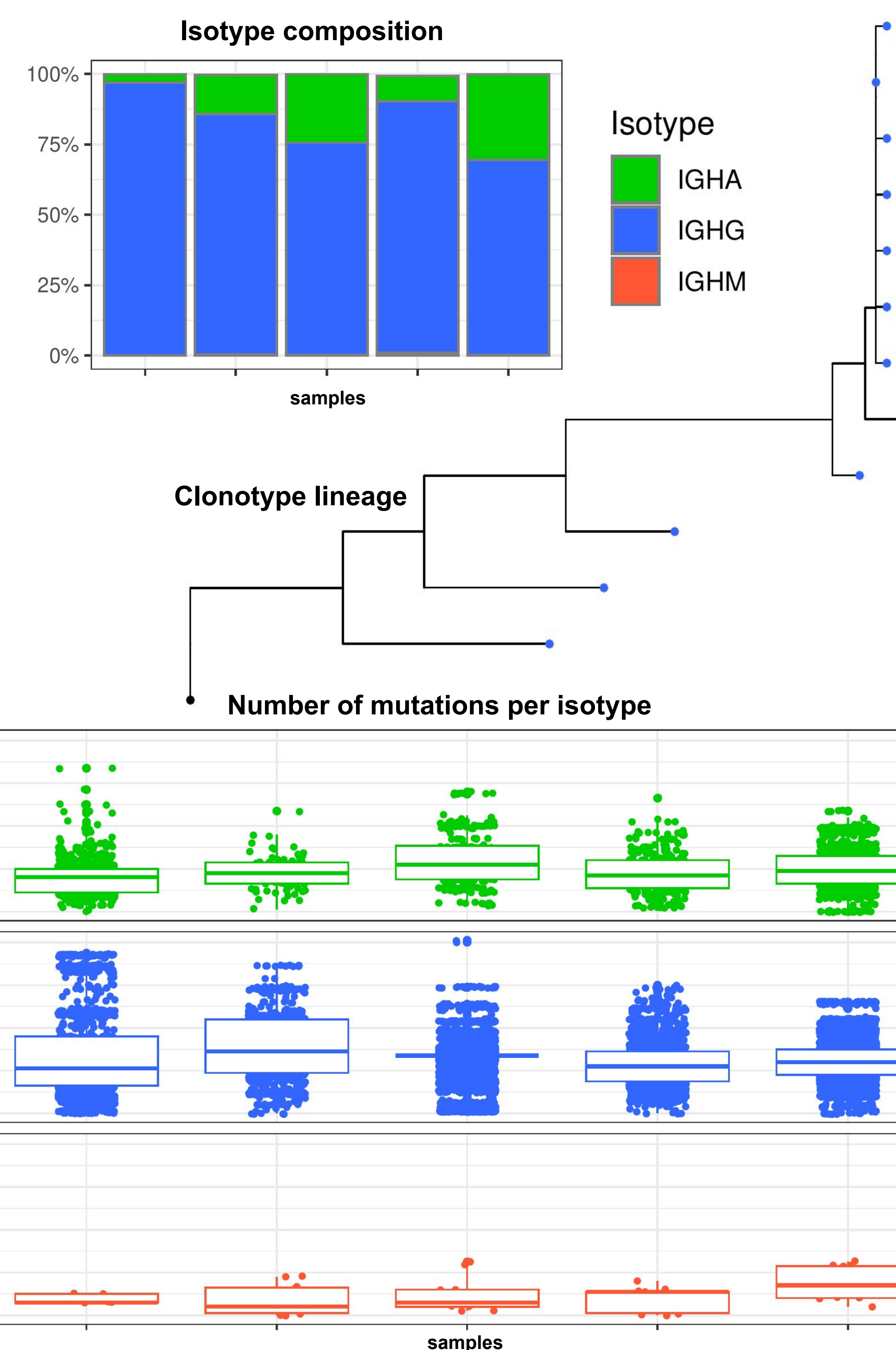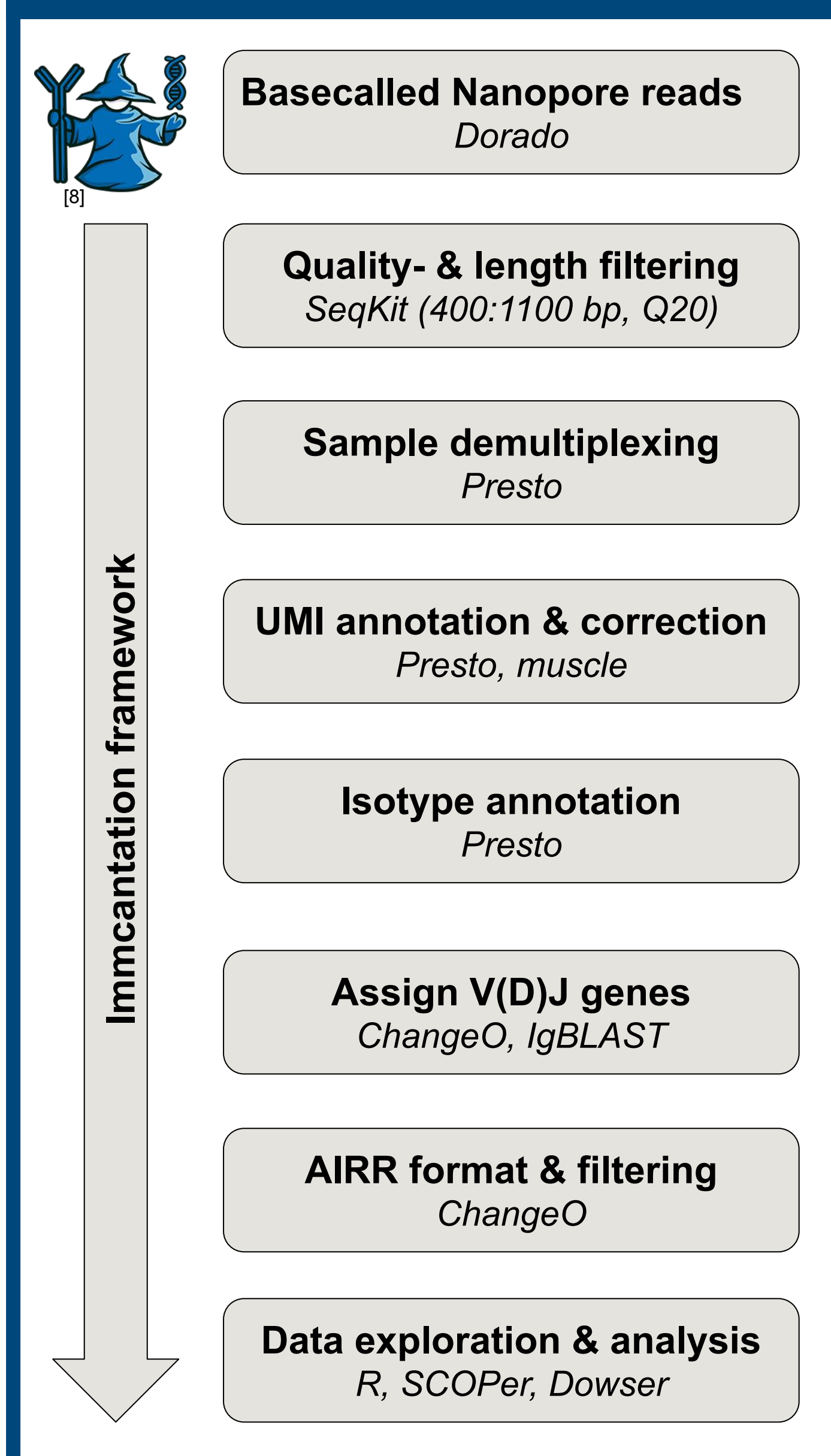
B Cell Repertoire Analysis Workflow
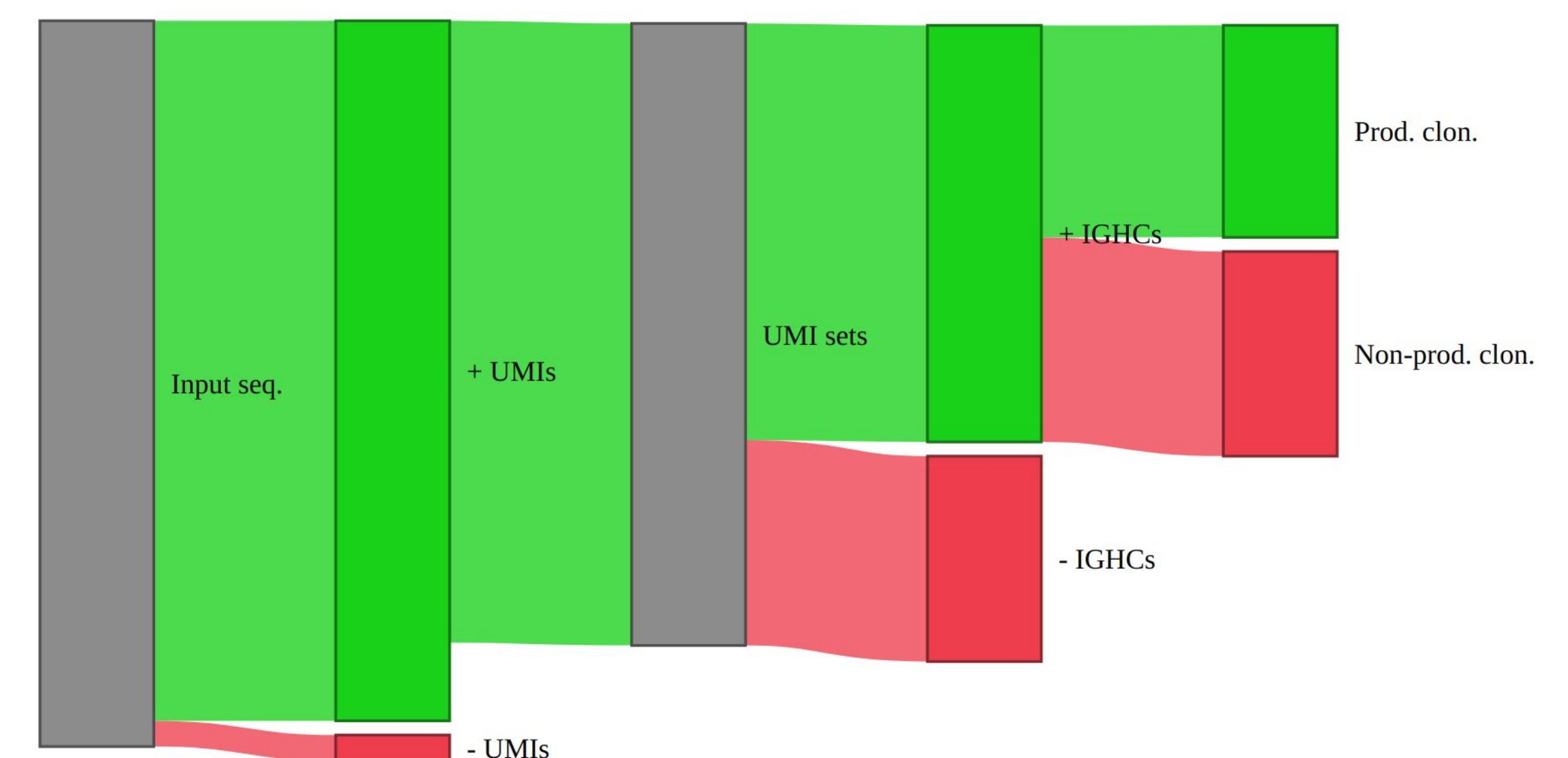
Nanopore Sequencing Workflow

- BCR targeted bulk approach
- Full length immunoglobulin transcripts
- Utilizing unique molecular identifiers (UMIs)
- Duplex pair reads
- SUP Duplex ONT Basecalling
- Sequence length and quality filtering (Q20)

Fig. 4: Schematic workflow for targeted Nanopore sequencing of B cells. Combined and modified from [5, 6, 7].

Bioinformatic Workflow

Immcantation framework

- **Basecalled Nanopore reads** *Dorado*
- **Quality- & length filtering** *SeqKit (400:1100 bp, Q20)*
- **Sample demultiplexing** *Presto*
- **UMI annotation & correction** *Presto, muscle*
- **Isotype annotation** *Presto*
- **Assign V(D)J genes** *ChangeO, IgBLAST*
- **AIRR format & filtering** *ChangeO*
- **Data exploration & analysis** *R, SCOPer, Dowser*

Conclusion

- **Sequencing workflow** using Nanopore sequencing for full length immunoglobulin transcripts from **TLS+ glioma samples**
- **Bioinformatic workflow** allows identification of **B cell repertoires** in GBM
- High-quality reads (>Q30) and UMIs enable accurate error correction, ensuring reliable downstream analysis
- Despite the highly immunosuppressive TME in glioma, this workflow accurately annotates BCR reads, revealing a **high abundance of IgA and IgG across multiple samples**