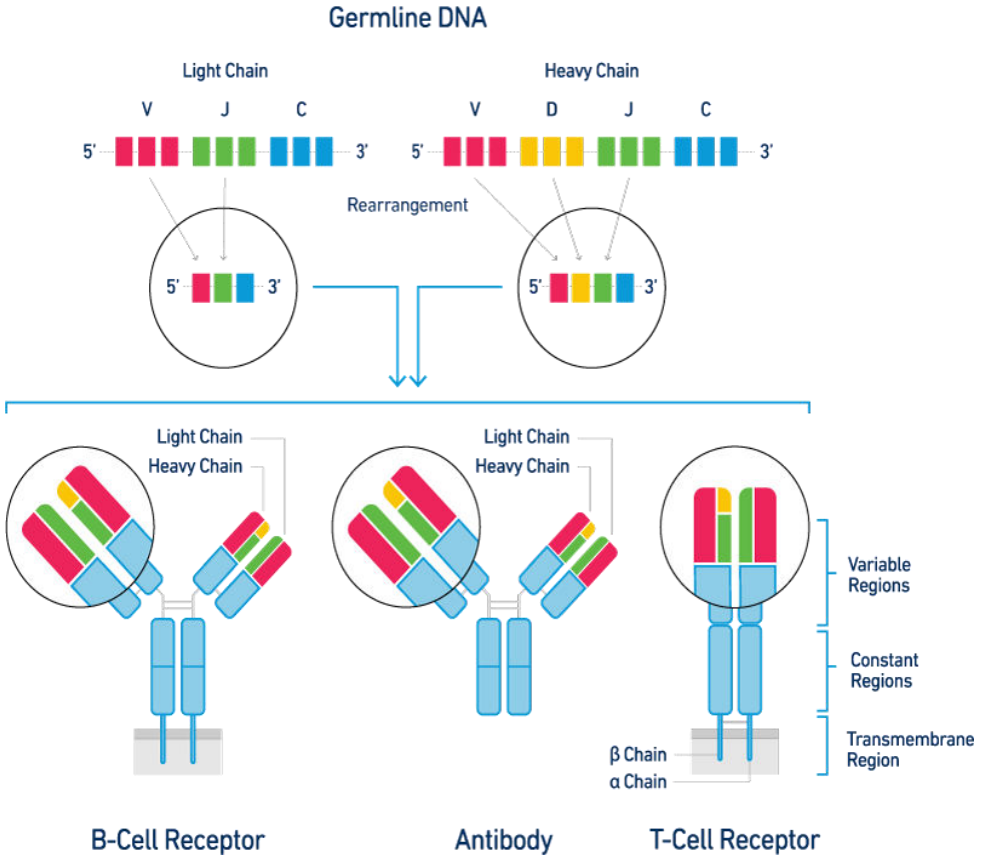
10x Genomics Chromium 5’ sequencing (including VDJ region)

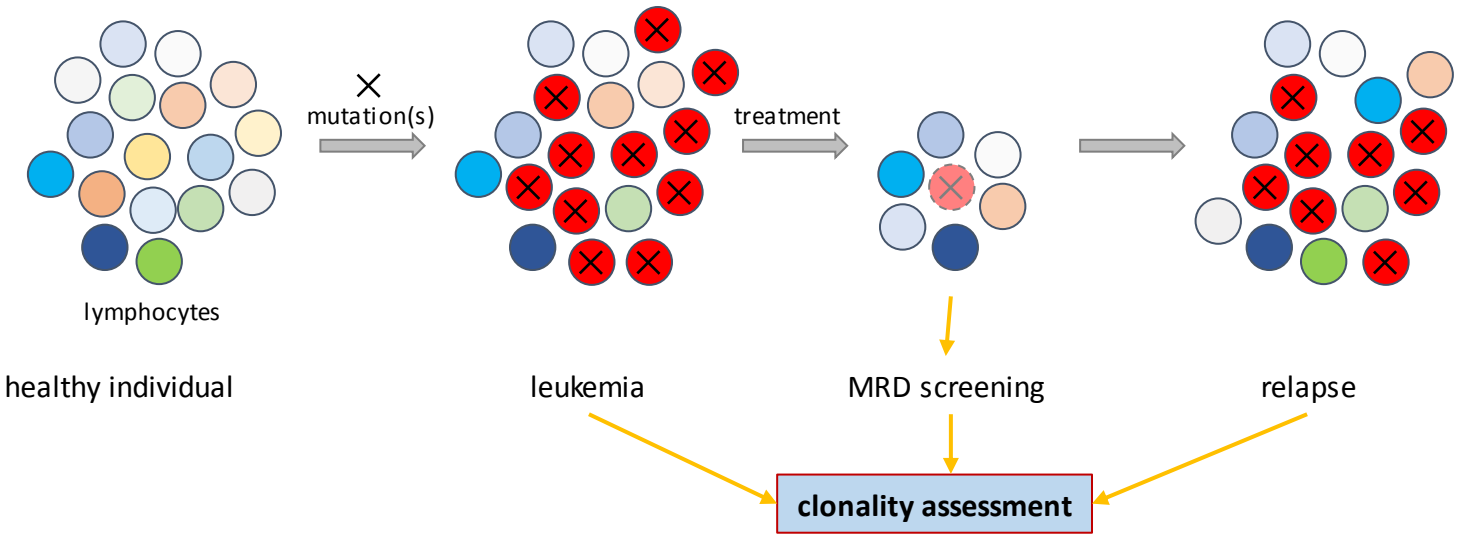
Group 3

Application of 5’ end sequencing method:

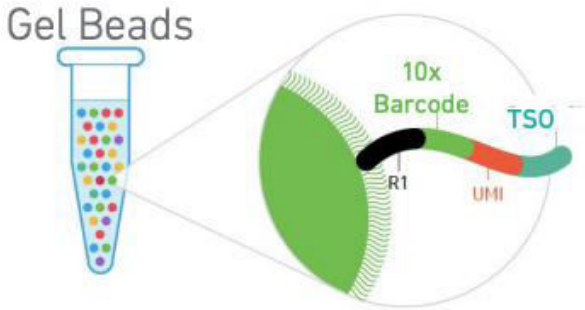
- VDJ – Mechanism of genetic recombination in T and B cells: generates diverse repertoires
- Variable part of immunoglobulin receptor or TCR is in 5’ end.
- Allows characterization of Immune repertoire:
  - Targets VDJ genes (B cells or T cells) or
  - 5’ enriched transcriptome (same cells)
- 100-10’000 cells / sample

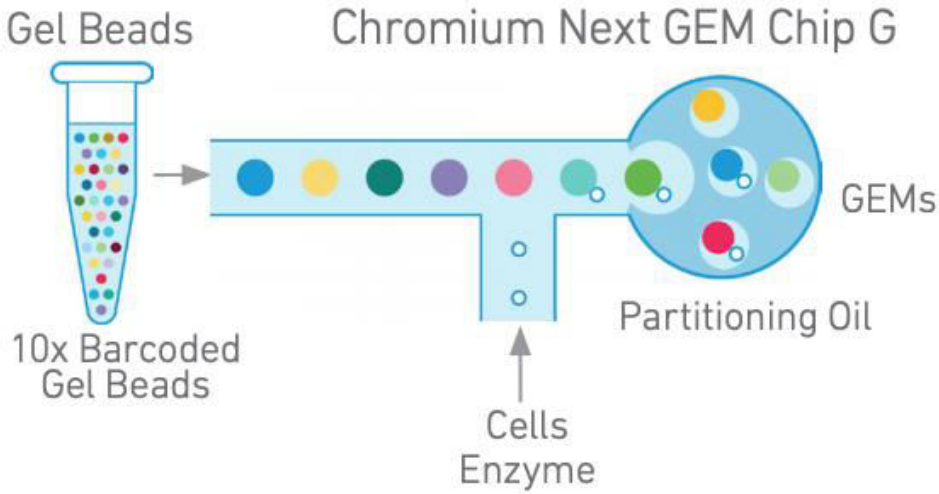


Why check immune repertoire?



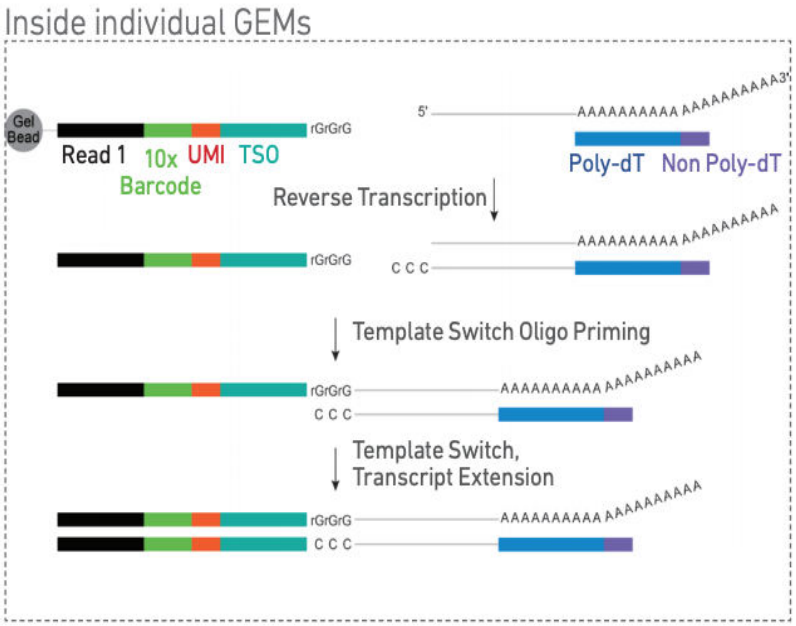
Library preparation: Gel Bead in Emulsion generation and Barcoding



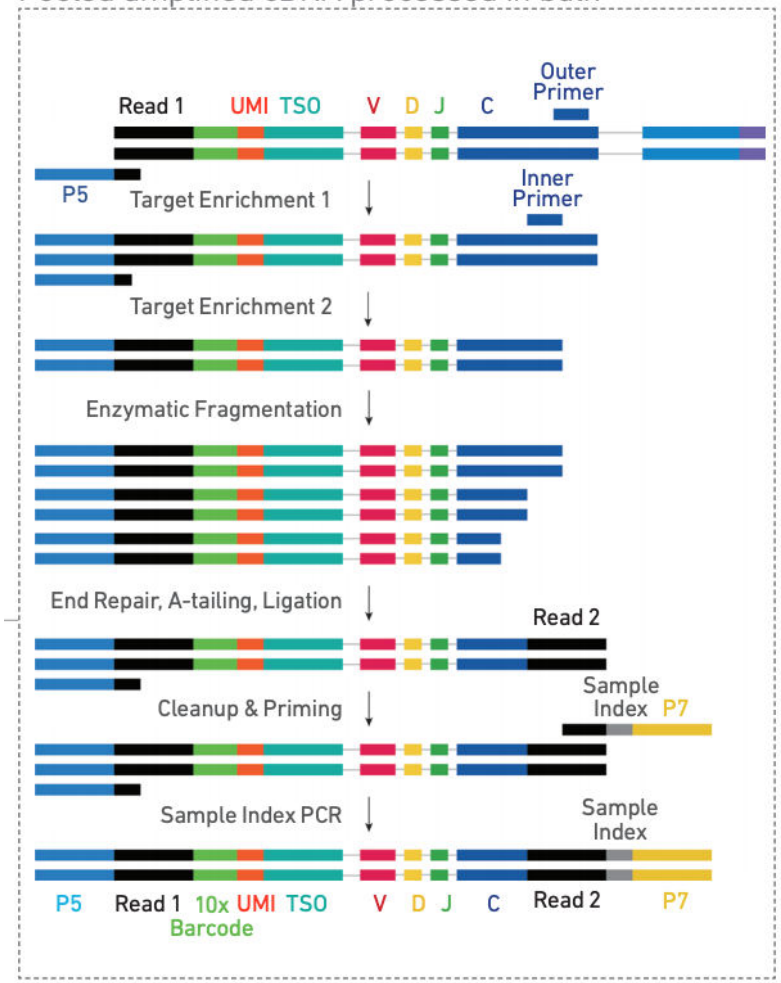


Library preparation: (a) Targeted enrichment

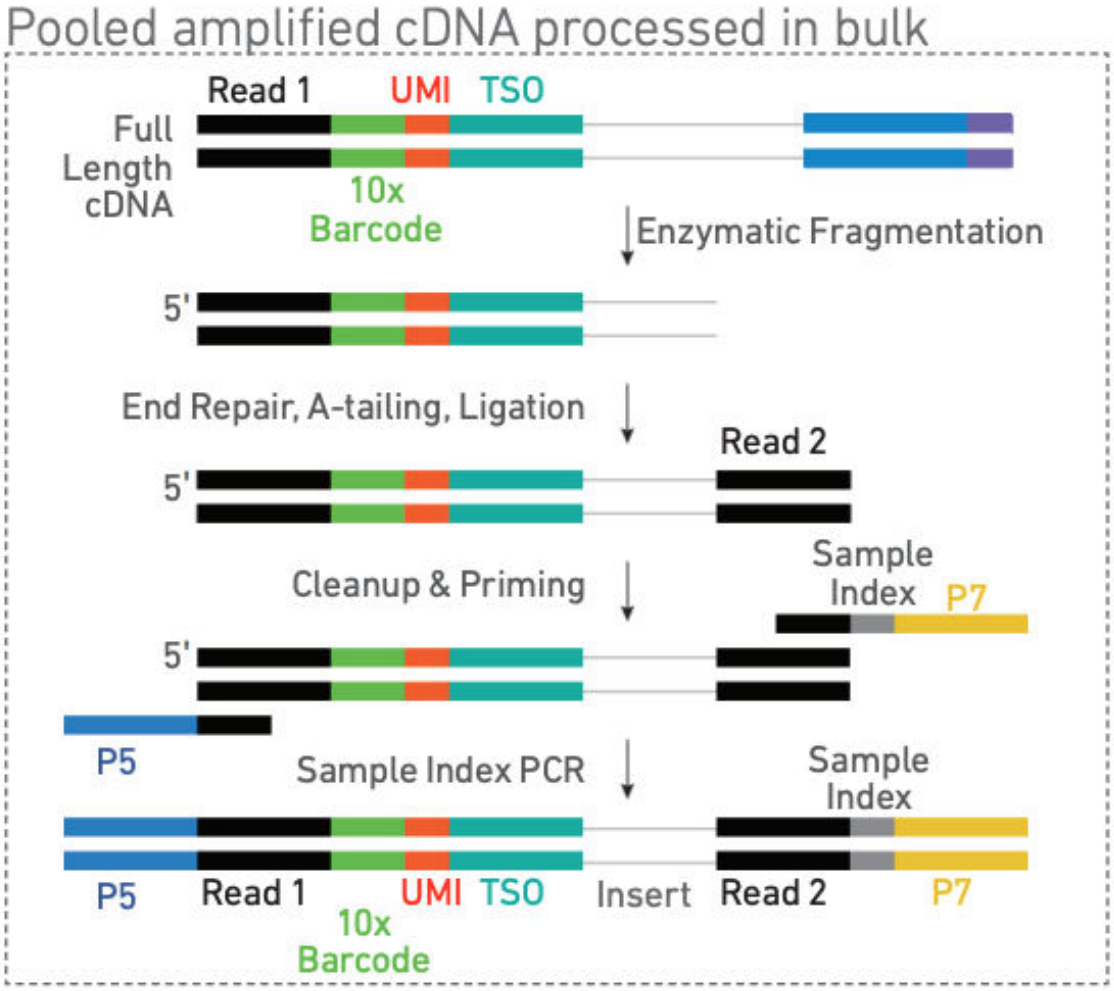
- Captures 5’ end
- UMI and barcodes
- No spike-ins / controls



cDNA amplification



Library preparation: (b) 5’ gene expression



Library preparation: (C) sequencing of libraries at recommended depth and run parameters

Chromium Single Cell V(D)J Enriched Library

Chromium Single Cell 5' Gene Expression Library

Biases, advantages, disadvantages

Main advantages / disadvantages

- + Paired information from a single cell + pair with whole transcriptome or other methods (i.e. cell surface protein expression)
- + 1 UMI per transcript
- - We don't sequence the full transcript
- - Requires specific equipment (expensive)

Biases:

- Enriches for 5'end (less validated)
- Depends on polyA capture efficiency
- Size of VDJ varies between cells
- For full transcriptome you are more likely to capture the most abundant transcripts
- Very short and very long transcripts will be less likely to be captured
- GC content is always an issue