Sequence profiling mucosal T-cell repertoires

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International Human Microbiome Congress
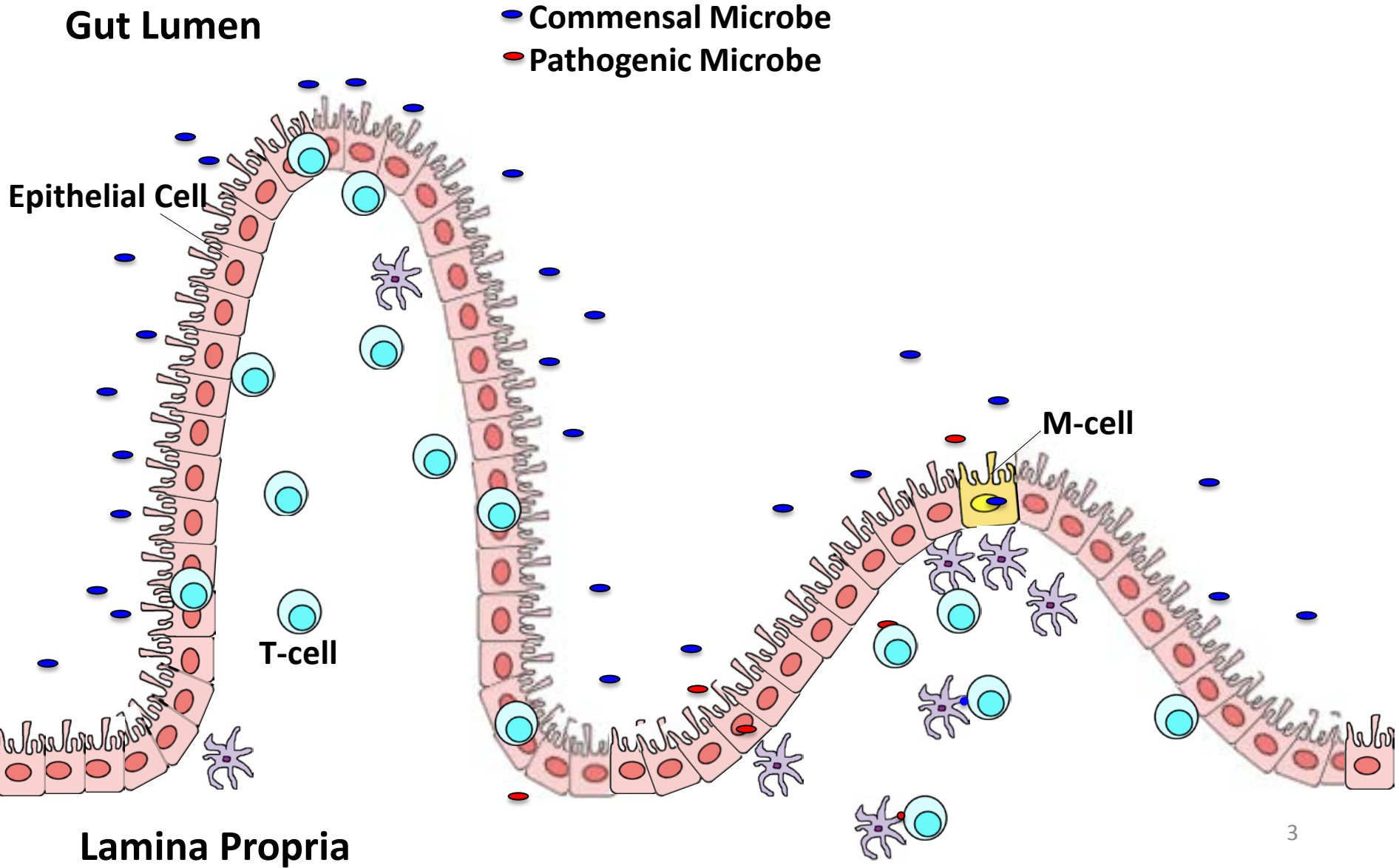Vancouver, B.C., Canada
March 9 –11 2011

BC Cancer Agency
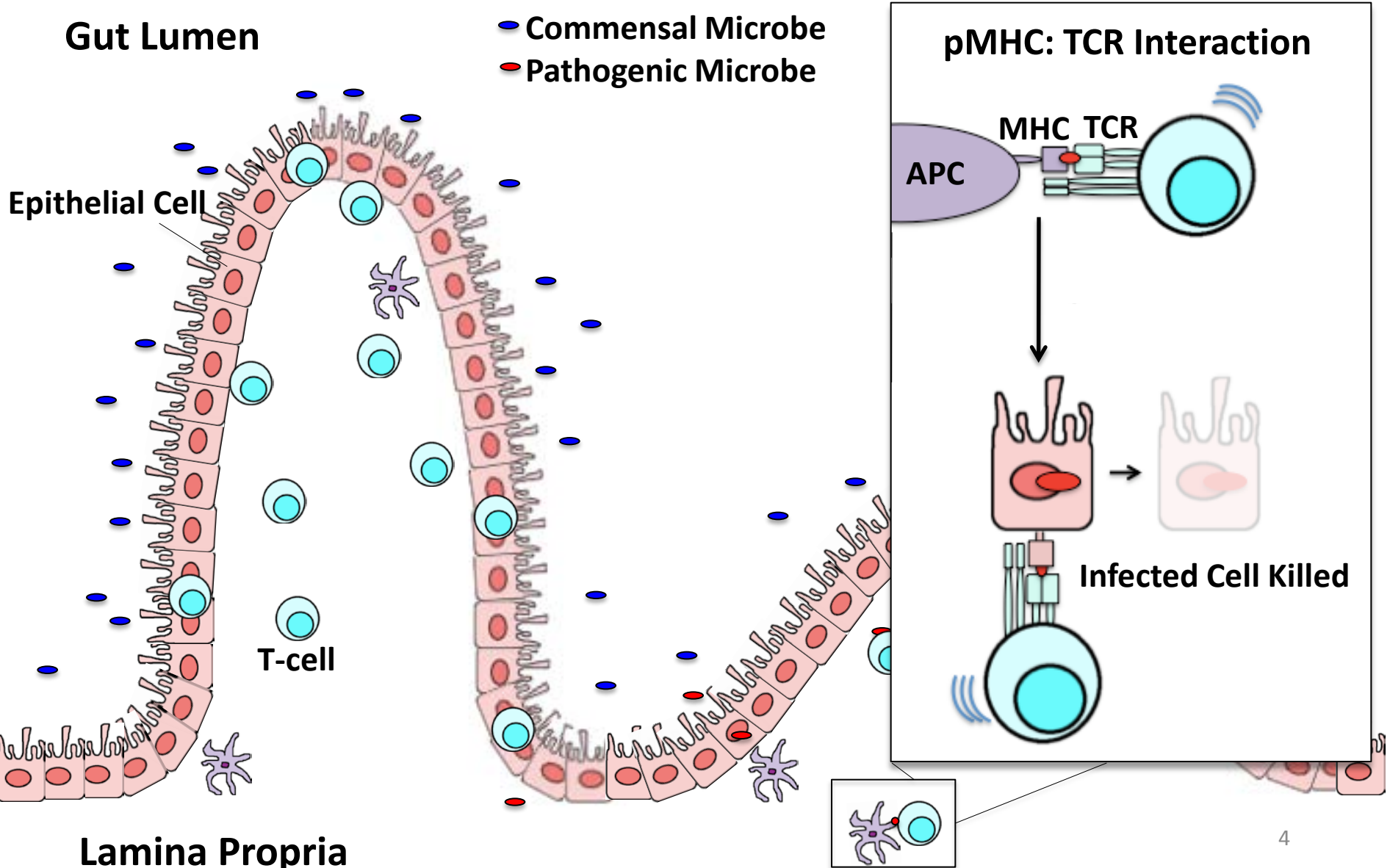CARE & RESEARCH

GEN◈M E
Sciences ◈ *Centre*

Mucosal Immune Repertoire

- Major undertaking in exploration of gut microbiome diversity

- Interaction between the host adaptive immune system to the microbiome less understood

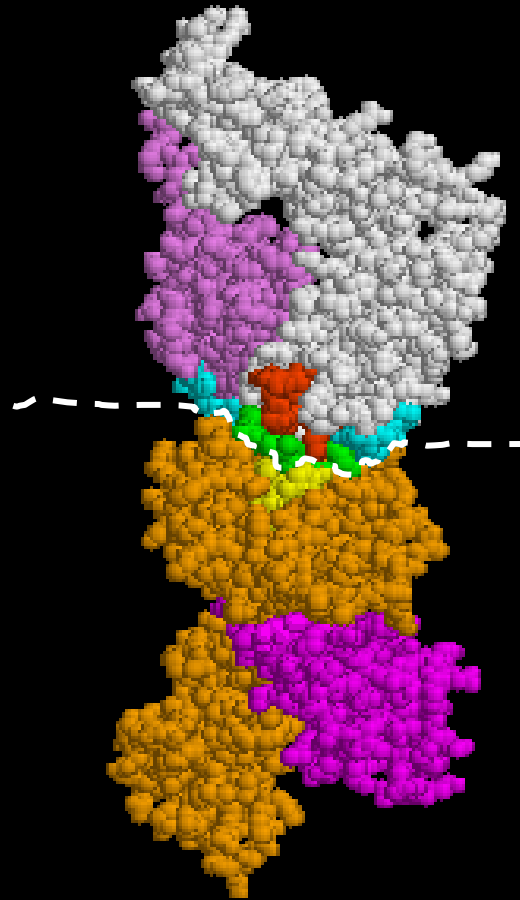- Profiling the T-cell immune repertoire

Activation of a T-cell in Gut

Gut Lumen

Commensal Microbe
Pathogenic Microbe

Epithelial Cell

M-cell

T-cell

Lamina Propria

3

Activation of a T-cell in Gut

Gut Lumen

Commensal Microbe
Pathogenic Microbe

pMHC: TCR Interaction

MHC TCR

APC

Epithelial Cell

T-cell

Infected Cell Killed

Lamina Propria

pMHC : TCR interaction

TCR alpha subunit
TCR beta subunit
CDR1
CDR2
CDR3
Peptide antigen
MHC
B2 Microglobulin

Generation TCRβ CDR3 diversity

TCRβ Locus

Somatic Recombination

TCRβ chain mRNA

Generation TCRβ CDR3 diversity

TCRβ Locus

Somatic Recombination

TCRβ chain mRNA

CDR3

CDR3

+ / -   + / -

T-cell Repertoire Profiling Approach

Profiling the T-cell receptor beta-chain repertoire by massively parallel sequencing

J. Douglas Freeman, René L. Warren, John R. Webb, Brad H. Nelson, and Robert A. Holt. 2009. *Genome Res.* 19: 1817-1824.
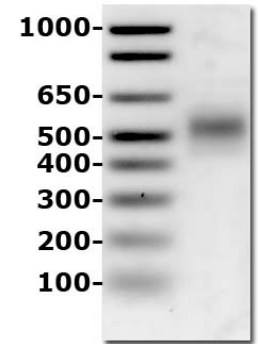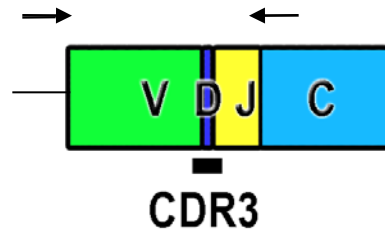
Exhaustive T-cell repertoire sequencing of human peripheral blood samples reveals signatures of antigen selection and a directly measured repertoire size of at least 1 million clonotypes

René L. Warren, J. Douglas Freeman, Thomas Zeng, Gina Choe, Sarah Munro, Richard Moore, John R. Webb and Robert A. Holt. 2011. *Genome Res.* Published online in advance. Feb 24. doi: 10.1101/gr.115428.110
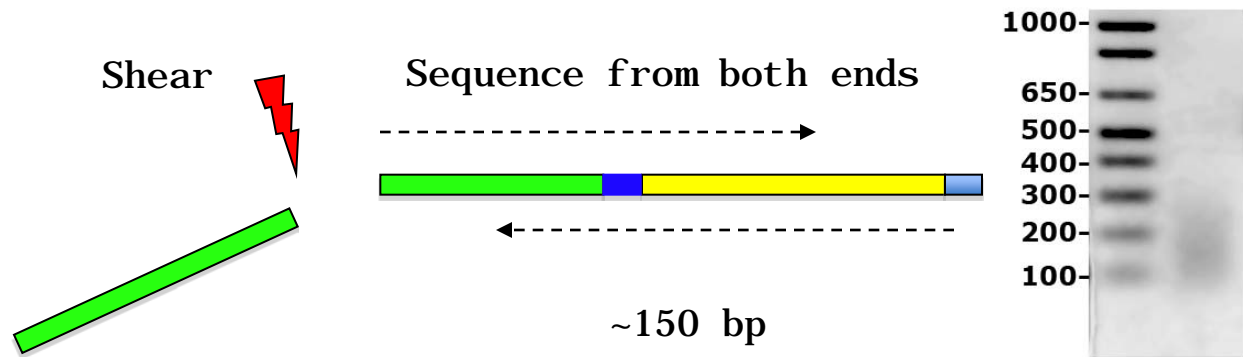
T-cell Repertoire Library Construction

1. Obtain mRNA from PBMC prep (Peripheral Blood Mononuclear Cells, which include T-cells)

2. PCR amplify the TCR using 5' RACE

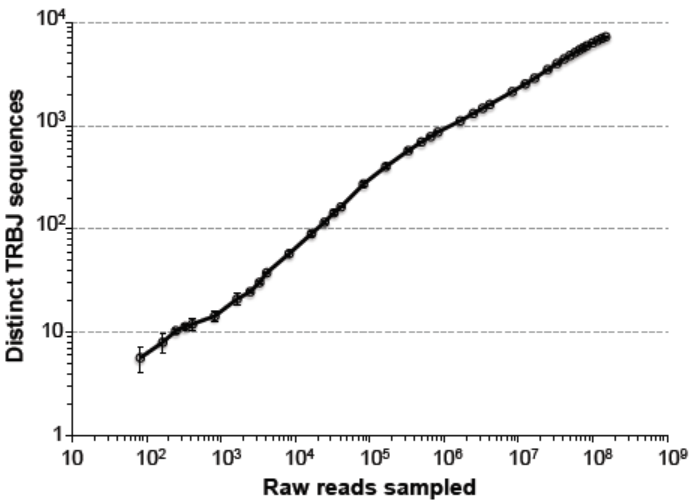3. Shear off uninformative region of V region (by sonication) and sequence

Handling Sequencing Error

- Real clonotypes can differ **by a single nucleotide**

- **Aggressive filtering** of raw data
    - **-** Double stranded coverage
    - - Q30 assignment to each base
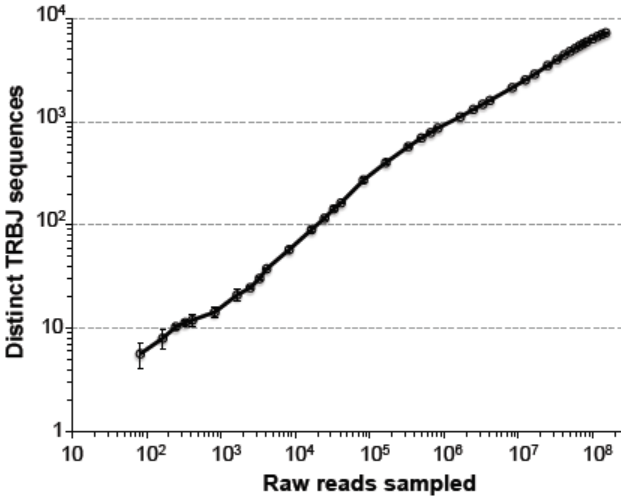   - - No high quality discrepancies

J-gene as Benchmark for Error Handling
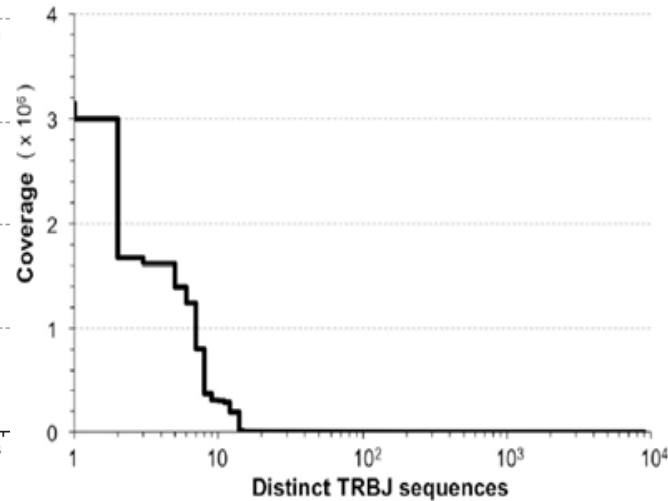
Filtered Data

J-gene as Benchmark for Error Handling
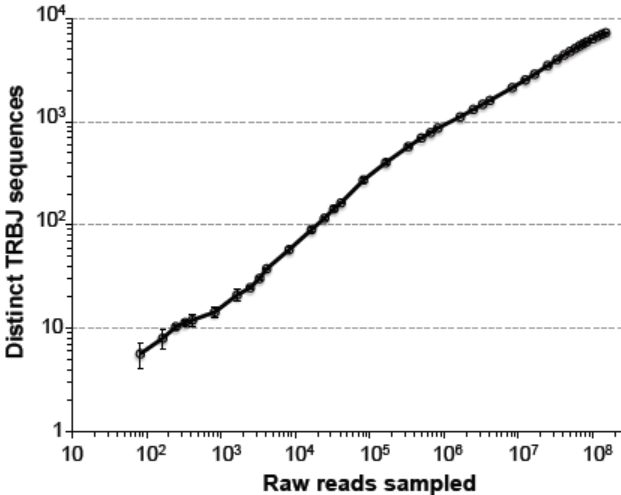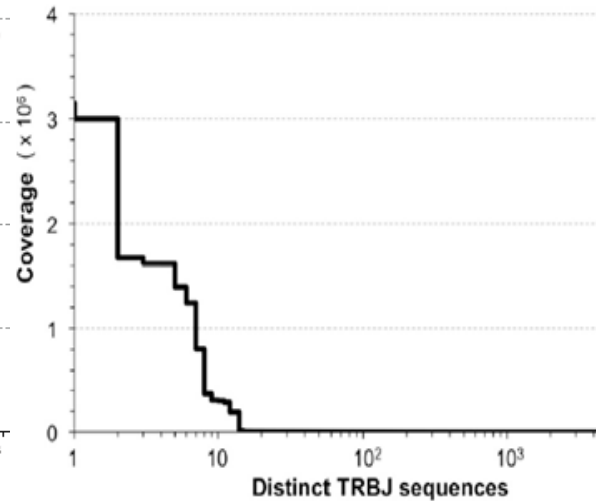
Filtered Data

J gene sequences
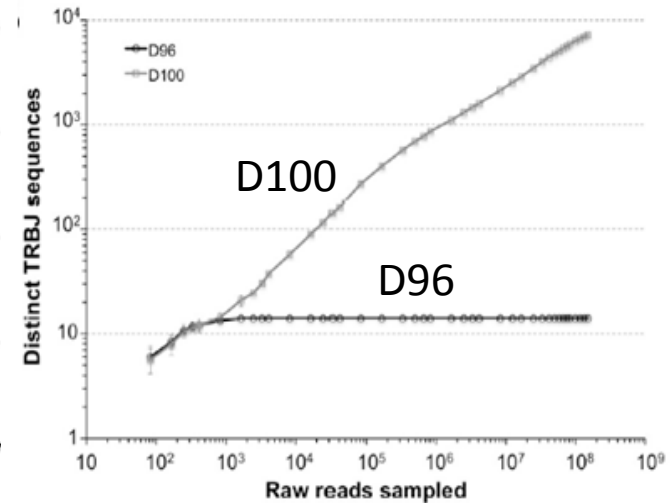
J-gene as Benchmark for Error Handling

Filtered Data

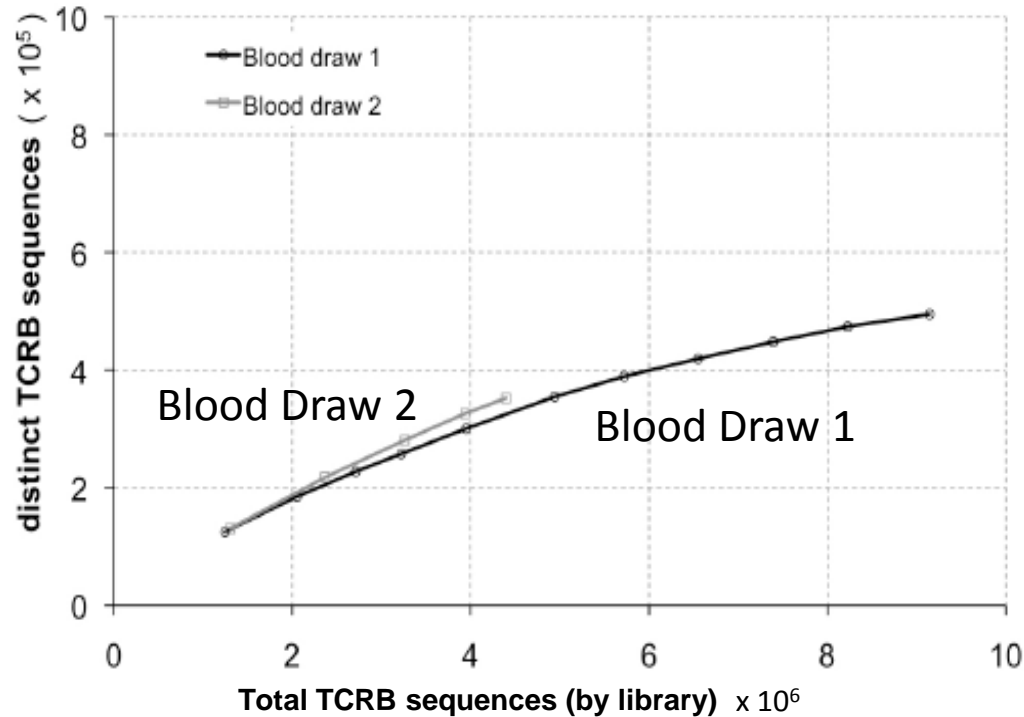J gene sequences

Apply D96 cutoff

Very rare clonotypes cannot be distinguished from sequence errors

TCRβ CDR3 Diversity

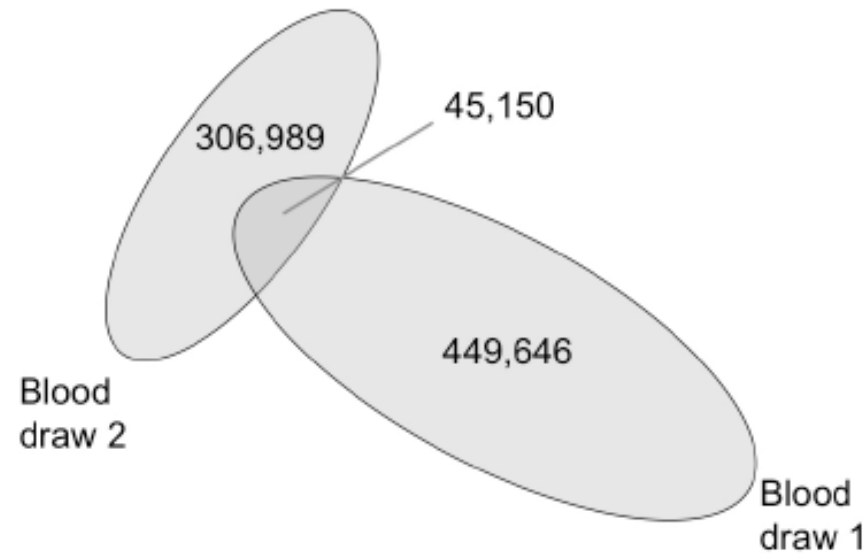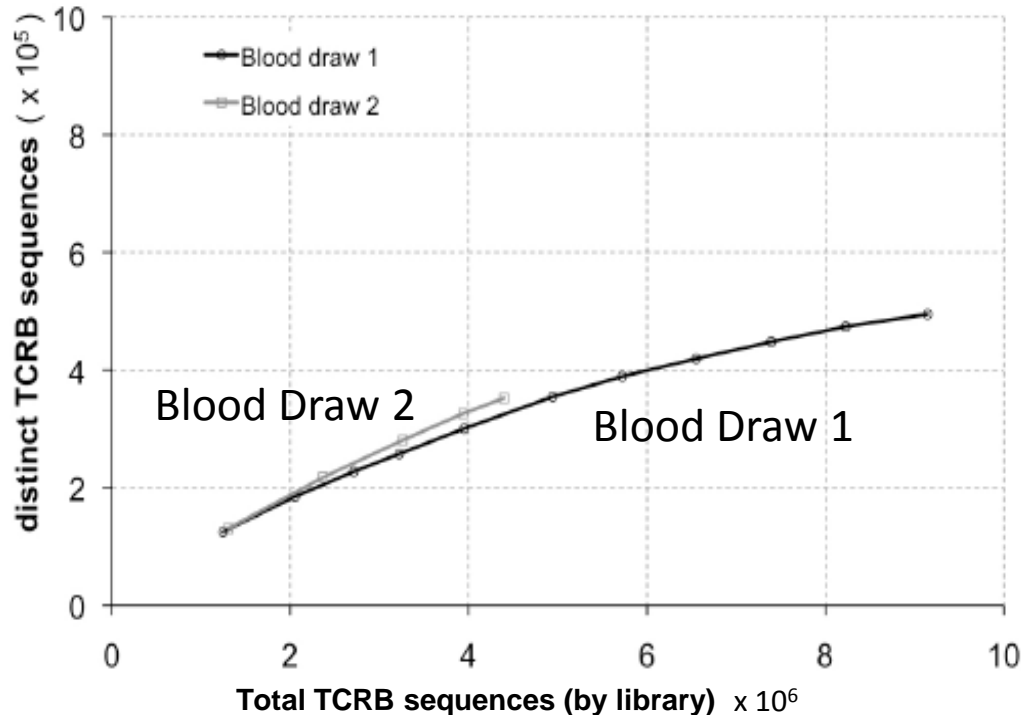| Subject	Blood Draw	Raw reads Sequenced	Total TCRβ sequences	Distinct TCRβ sequences
Male 1	1,2	1,564,567,374	209,671,125	846,935
Male 2	1,2	307,058,456	6,219,383	193,551
Female	1,2	91,110,650	1,069,612	93,990

TCRβ diversity in peripheral blood

Donor 1:

Multiple libraries are required to
capture full diversity of a blood sample

TCRβ diversity in peripheral blood

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Multiple libraries are required to capture full diversity of a blood sample

Repertoire diversity is substantially greater than the diversity captured in a single blood sample

Sharing of TCRβ repertoires

Female shared 1.1% nucleotide CDR3β sequences with male1.
Female shared 14.2% amino acid CDR3β sequences with male 1.

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	CDR3 (aa)	CDR3 (nt)[a]	CDR3(aa) / CDR3(nt)
♀ total	86,255	89,663	0.96
♀ unique	73,006	73,947	0.99
♀ shared with ♂①	13,249	15,716	0.84
♂② total	165,931	177,763	0.93
♂② unique	144,781	150,992	0.96
♂② shared with ♂①	21,150	26,771	0.79

Profiling the mucosal T-cell repertoire

- Normal tissue samples from 43 people

- Use same approach for profiling
  - Add barcoding step

TCRβ CDR3 Diversity in colon/rectum

Sequencing and Assembly Statistics

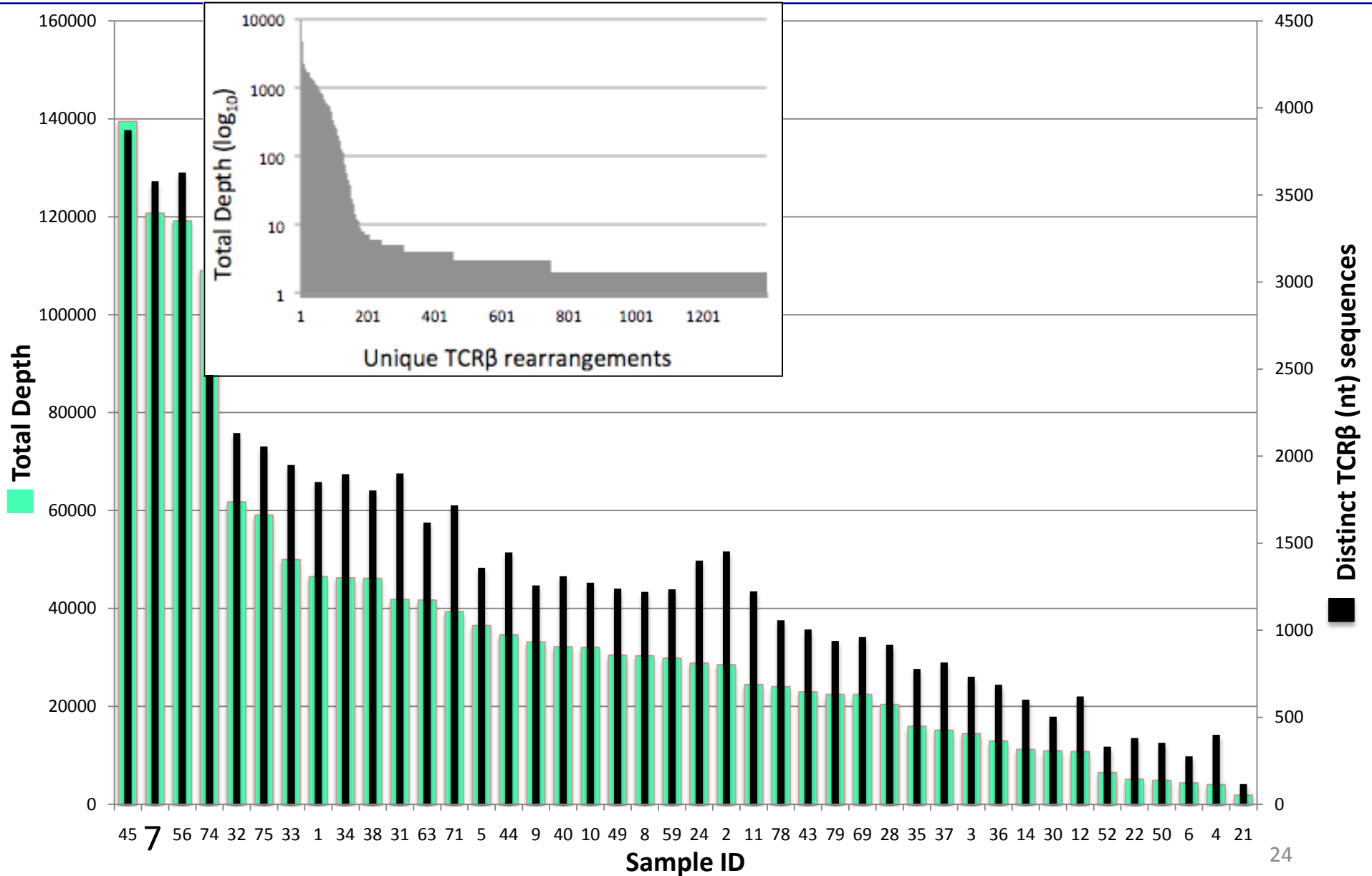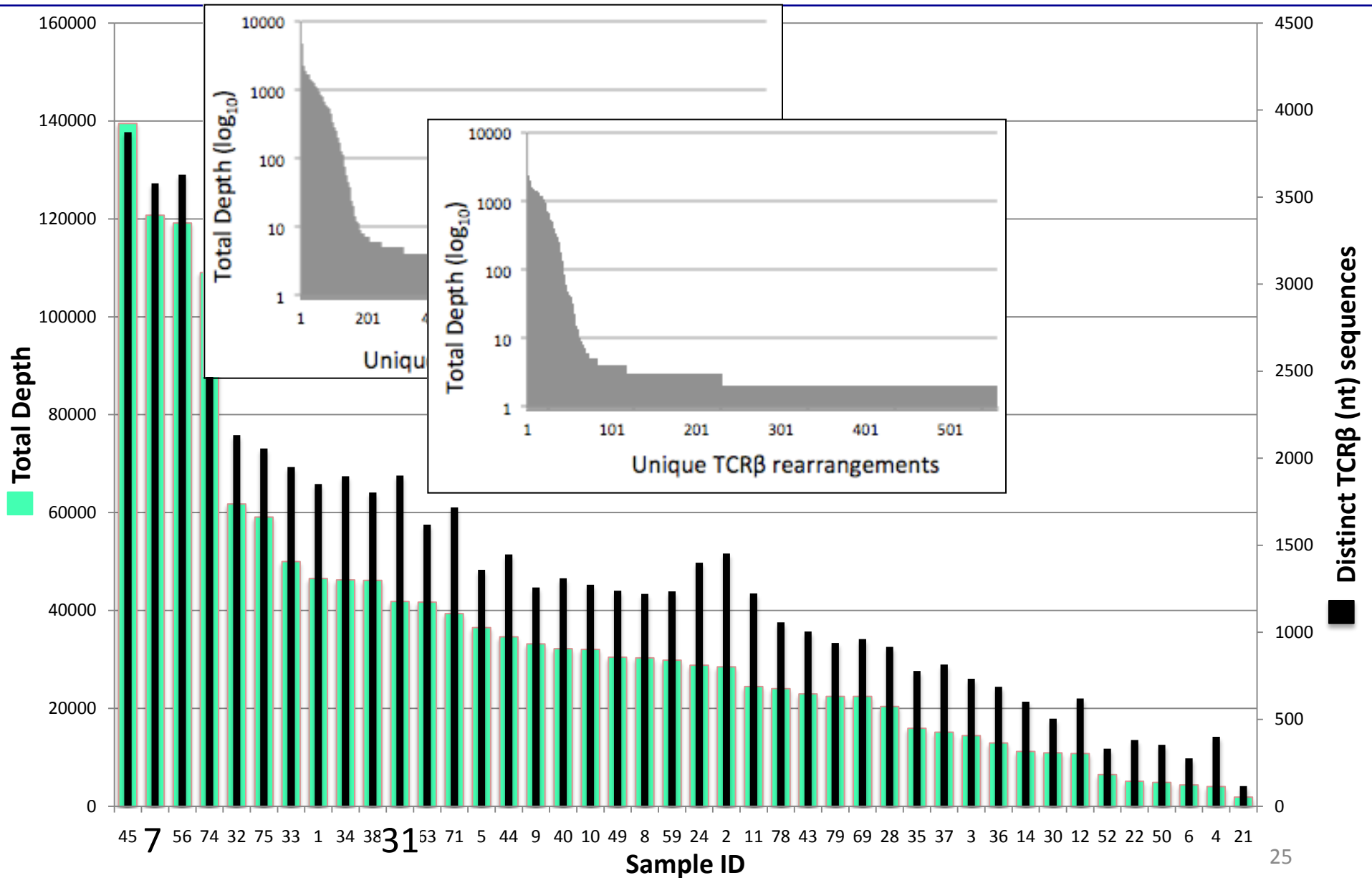|	
Number of samples	43
Total read pairs	25,954,853
Total CDR3β sequences assembled*	60,234
Distinct ntCDR3β sequences assembled*	16,179
Distinct aaCDR3β sequences assembled*	12,439

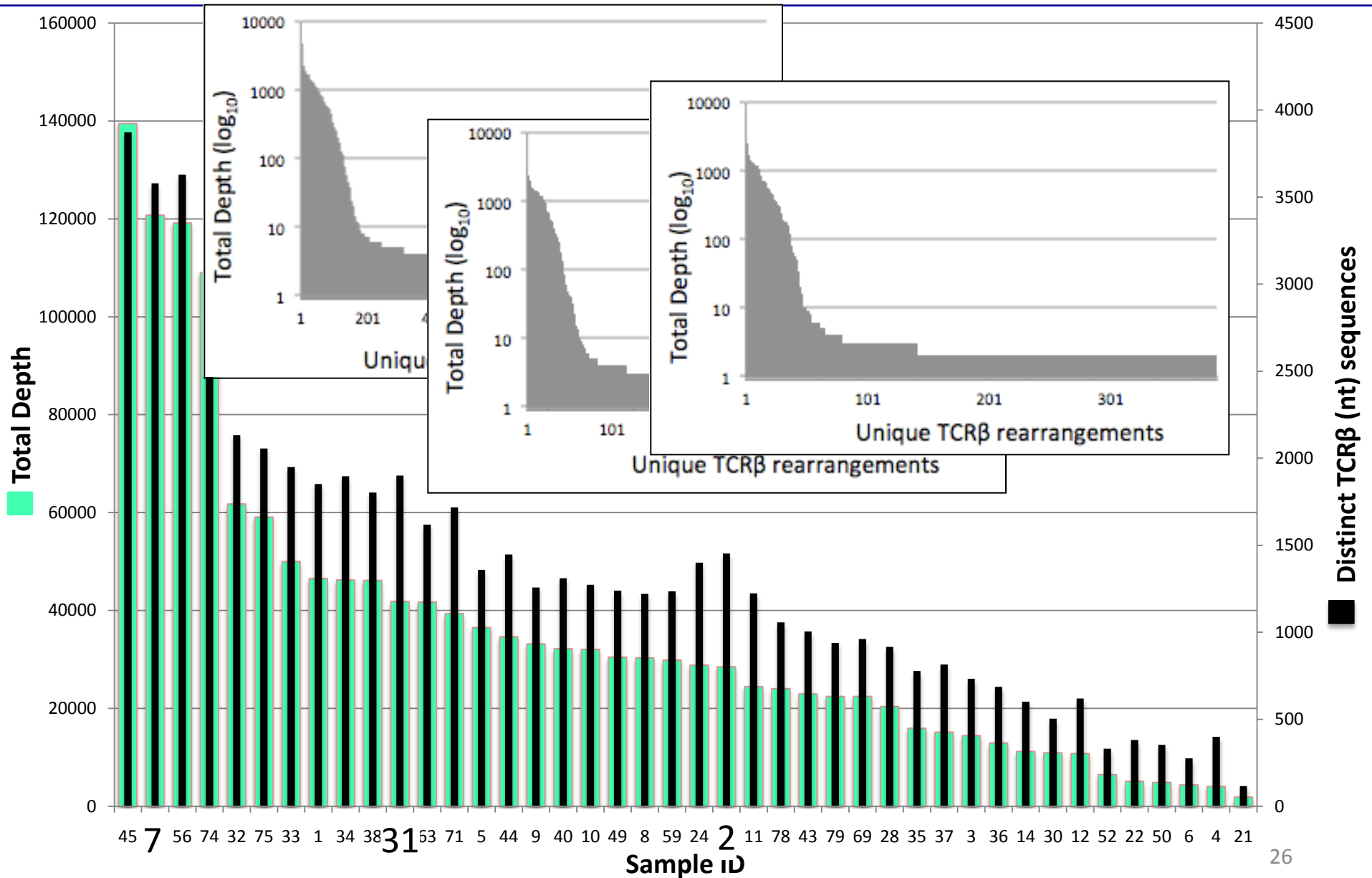*Values reported are in correct reading frame

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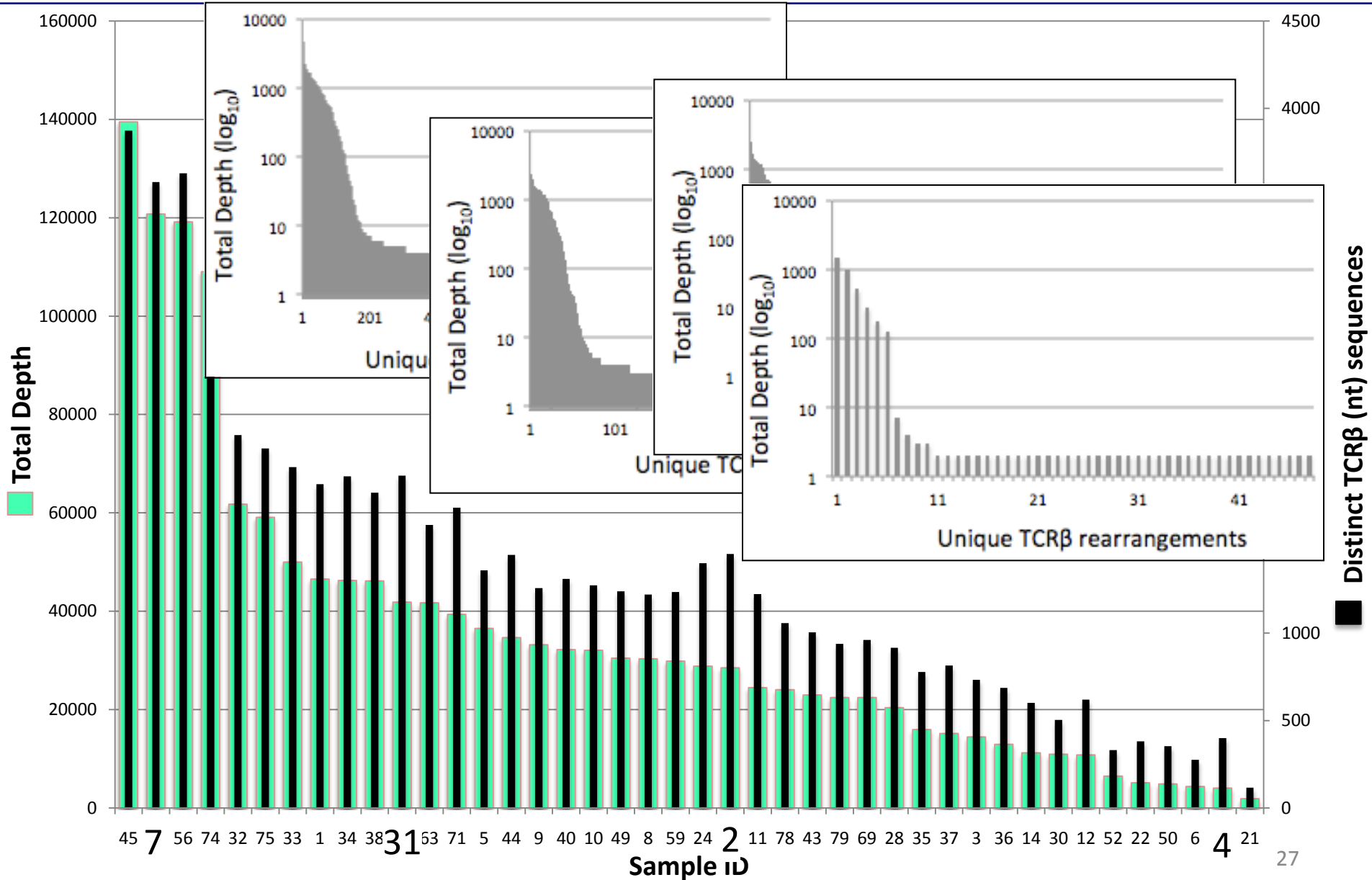|	
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Convergence

*Values reported are in correct reading frame

TCRβ Diversity Across Samples

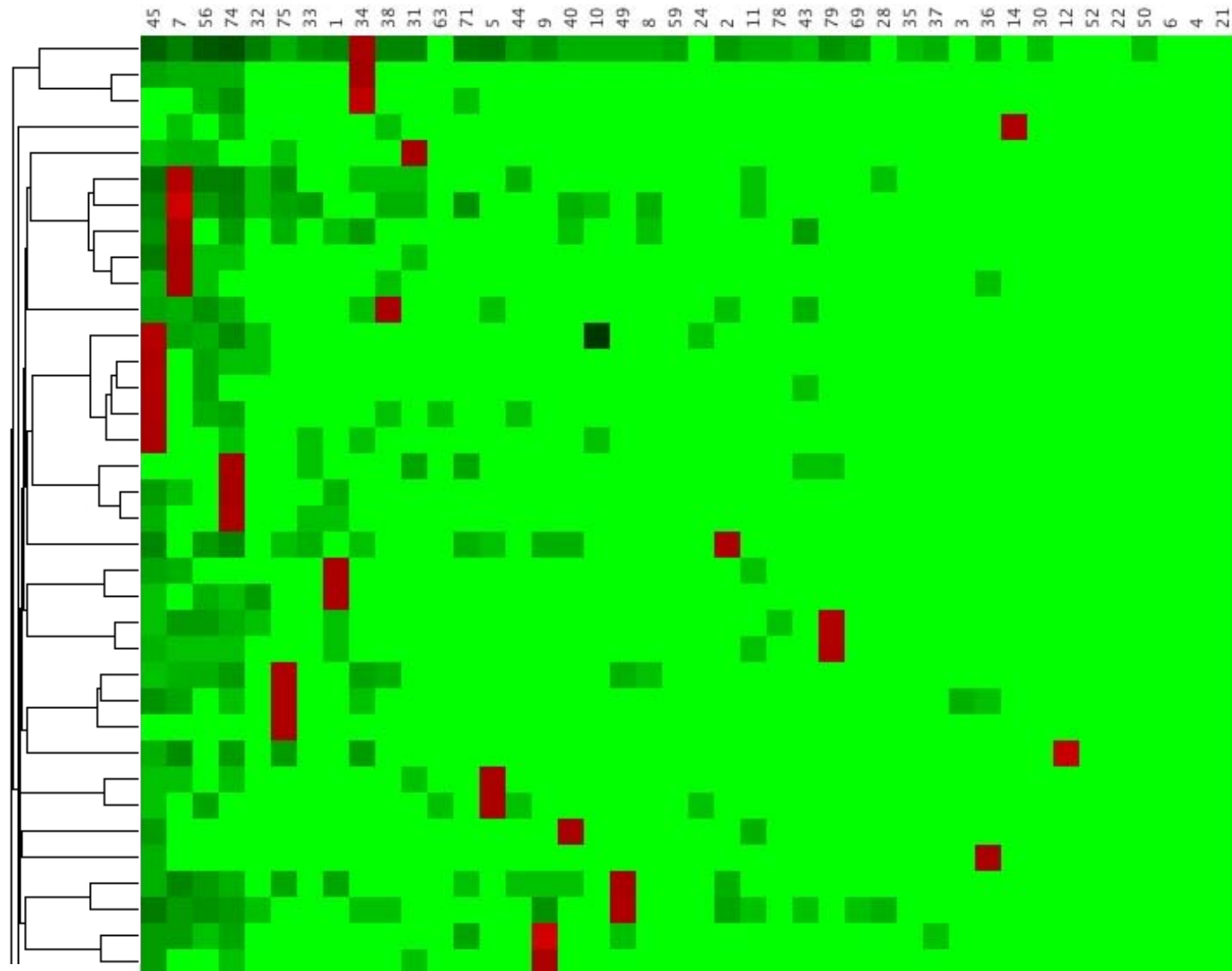# TCRβ Diversity Across Samples

TCRβ Diversity Across Samples

TCRβ Diversity Across Samples

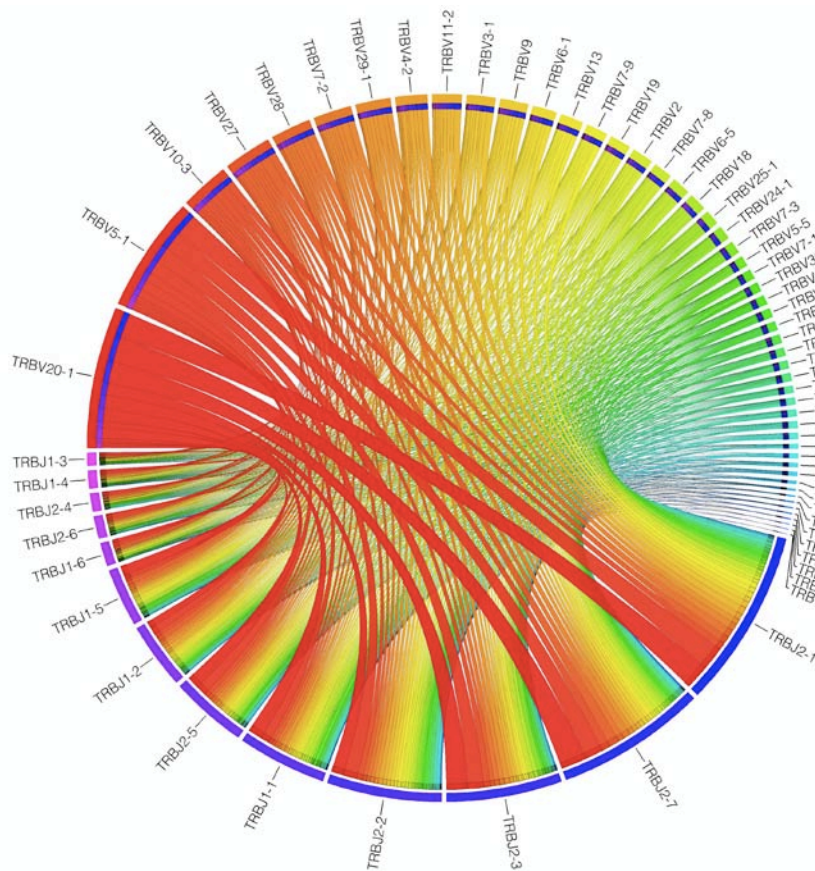# TCRβ Diversity Across Samples
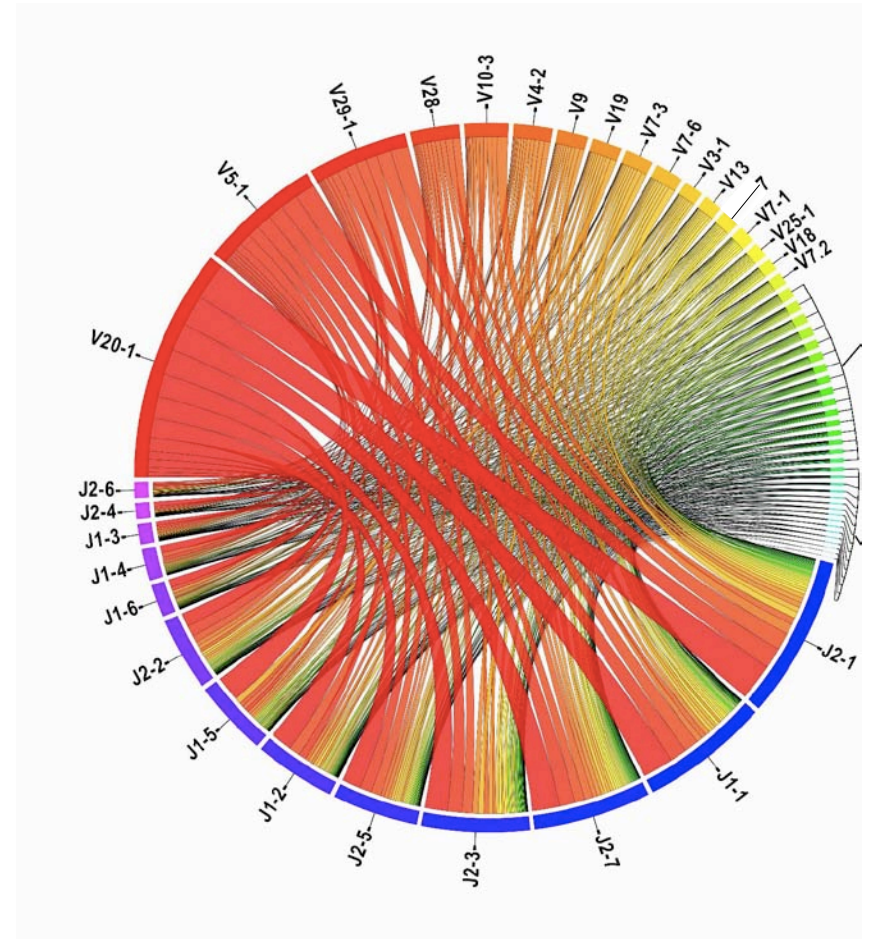
TCRβ aaCDR3 sequence sharing

TCRβ V-J Gene Usage

Mucosal samples

Public Samples
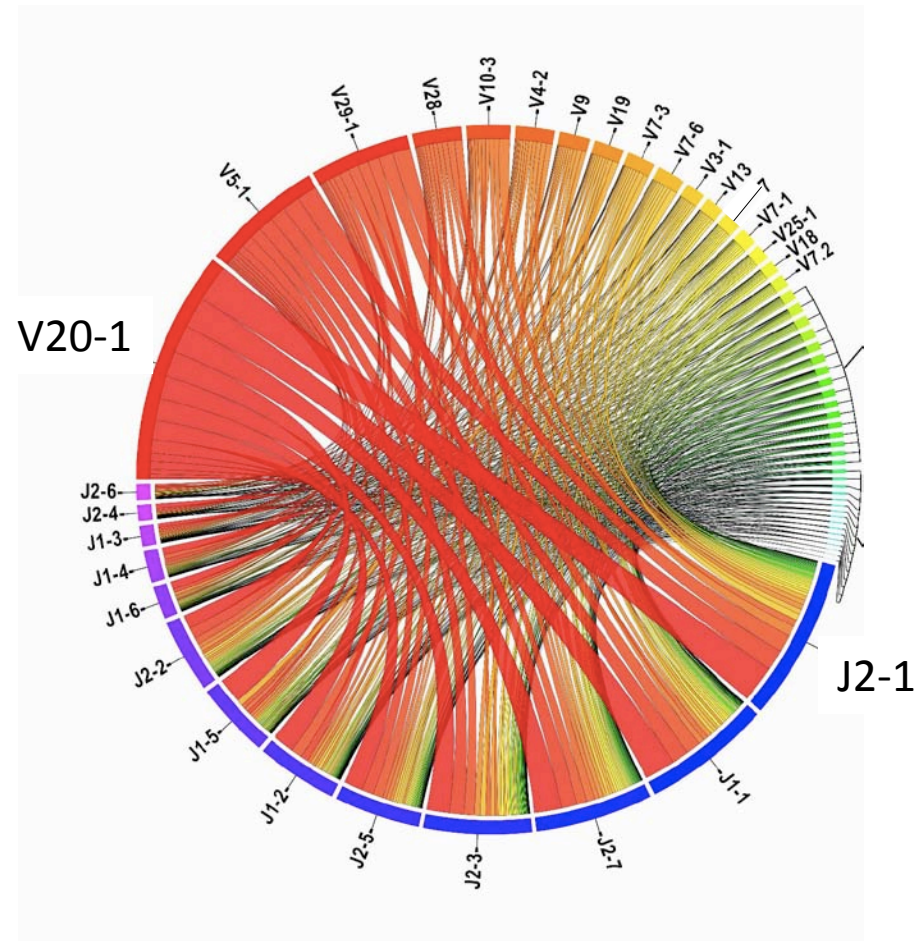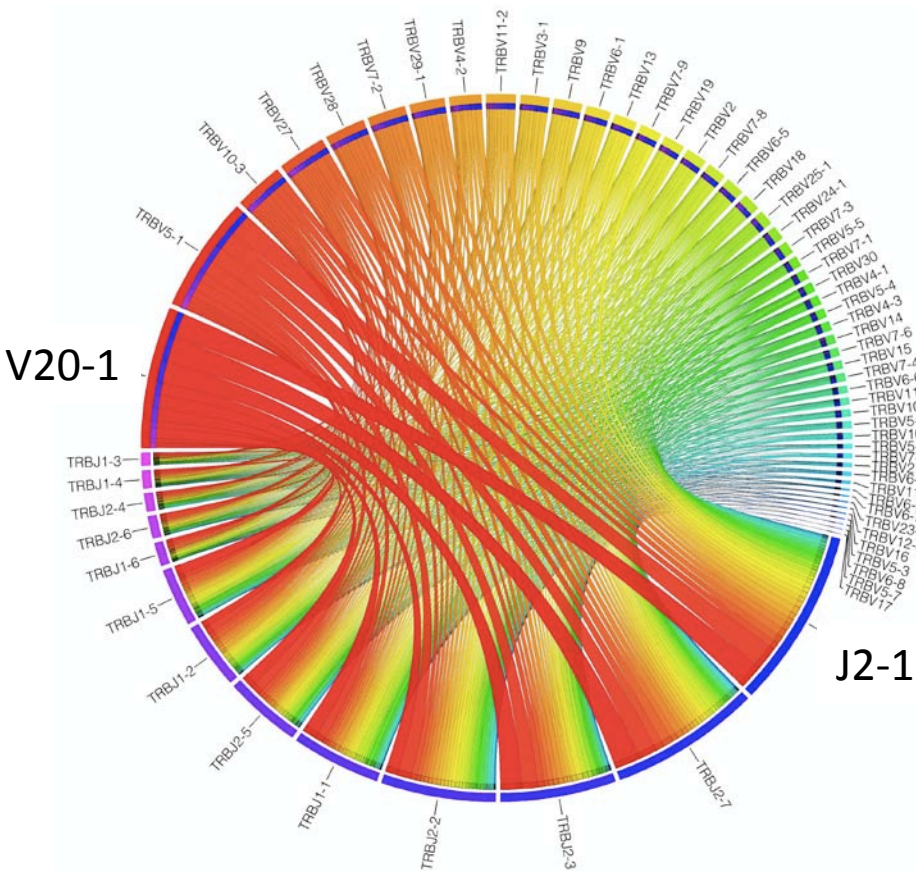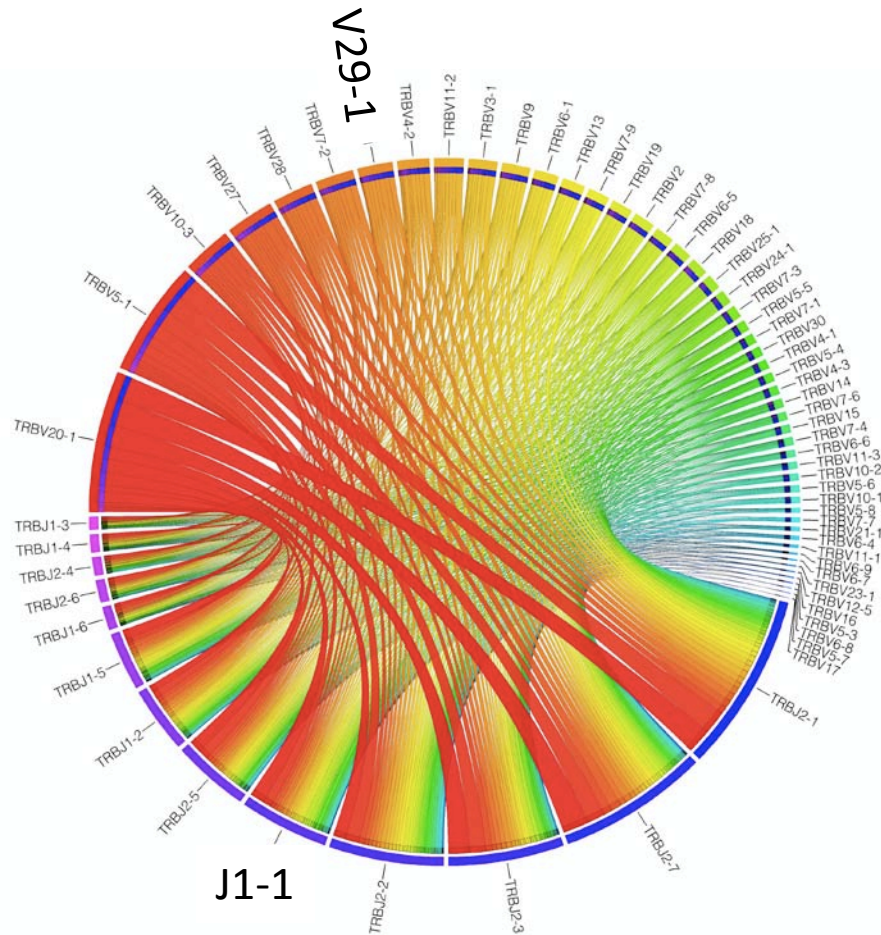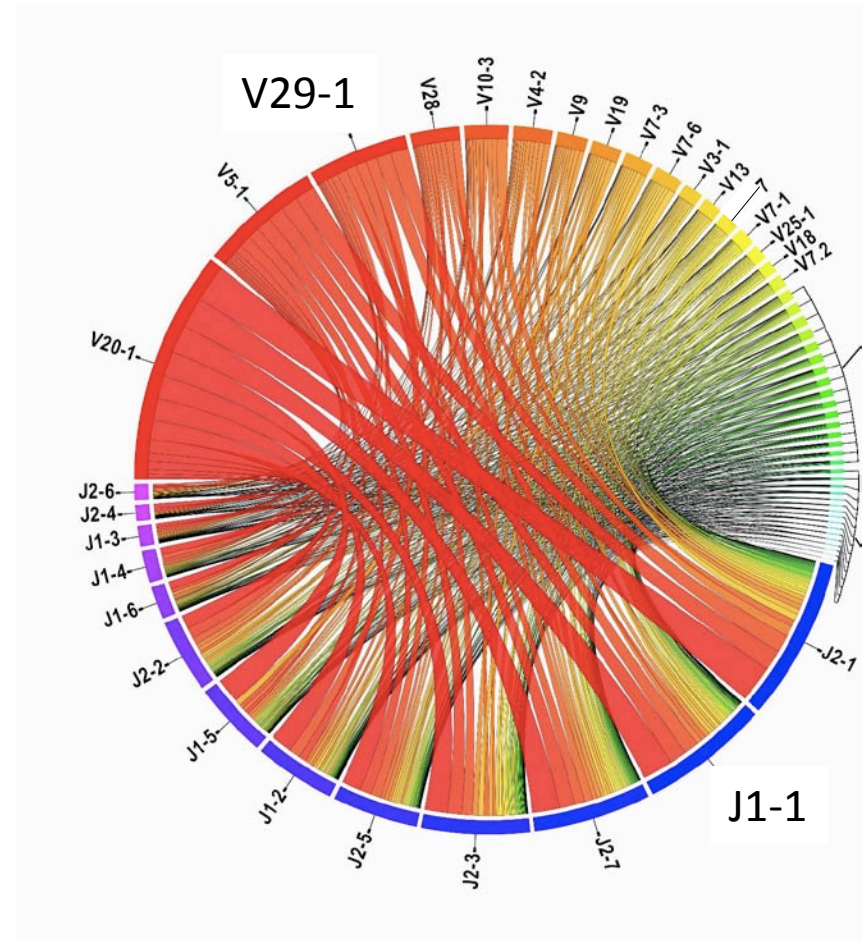(Freeman *et al*. 2009)

Plots generated using Circos software (Krzywinski *et al*. 2009)

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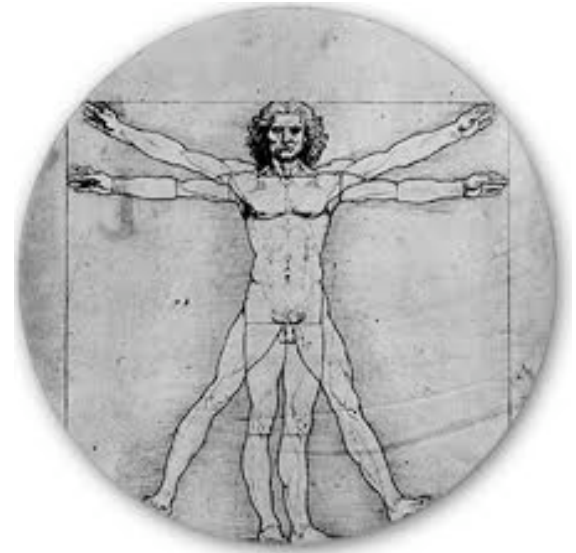
Mucosal samples

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(Freeman *et al*. 2009)

Plots generated using Circos software (Krzywinski *et al*. 2009)

Conclusion

•Approach can be used to **characterize mucosal immune response** to microbes at sequence level resolution

•Approach can be applied in the investigation both **normal and abnormal** mucosal immune repertoires

Acknowledgements