BD Rhapsody™ VDJ CDR3 Protocols

Overview of performance data

BD Rhapsody™ VDJ CDR3 Protocol(s)

1. **Rhapsody VDJ CDR3 protocol –** Utilizes BD Targeted kit + Immune response panel and enables customers to run VDJ assay

2. **Rhapsody VDJ CDR3 + BD® AbSeq protocol -** Utilizes BD Targeted kit + Immune response panel + AbSeq and enables customers to run VDJ + AbSeq

3. **Rhapsody VDJ CDR3 + SMK protocol -** Utilizes BD Targeted kit + Immune response panel + SMK and enables customers to run VDJ on multiple samples together

4. **Rhapsody VDJ CDR3 + BD AbSeq + SMK protocol -** Utilizes BD Targeted kit + Immune response panel + AbSeq + SMK and enables customers to run VDJ + AbSeq on multiple samples together

Overall Workflow
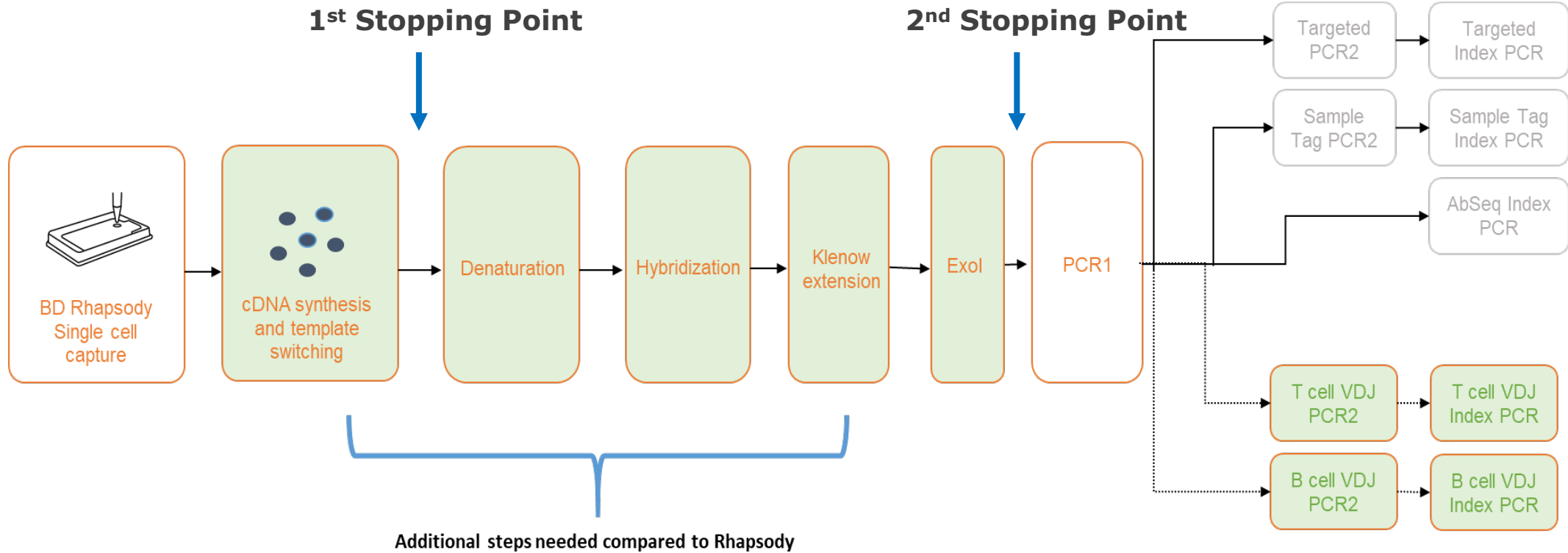
Workflow
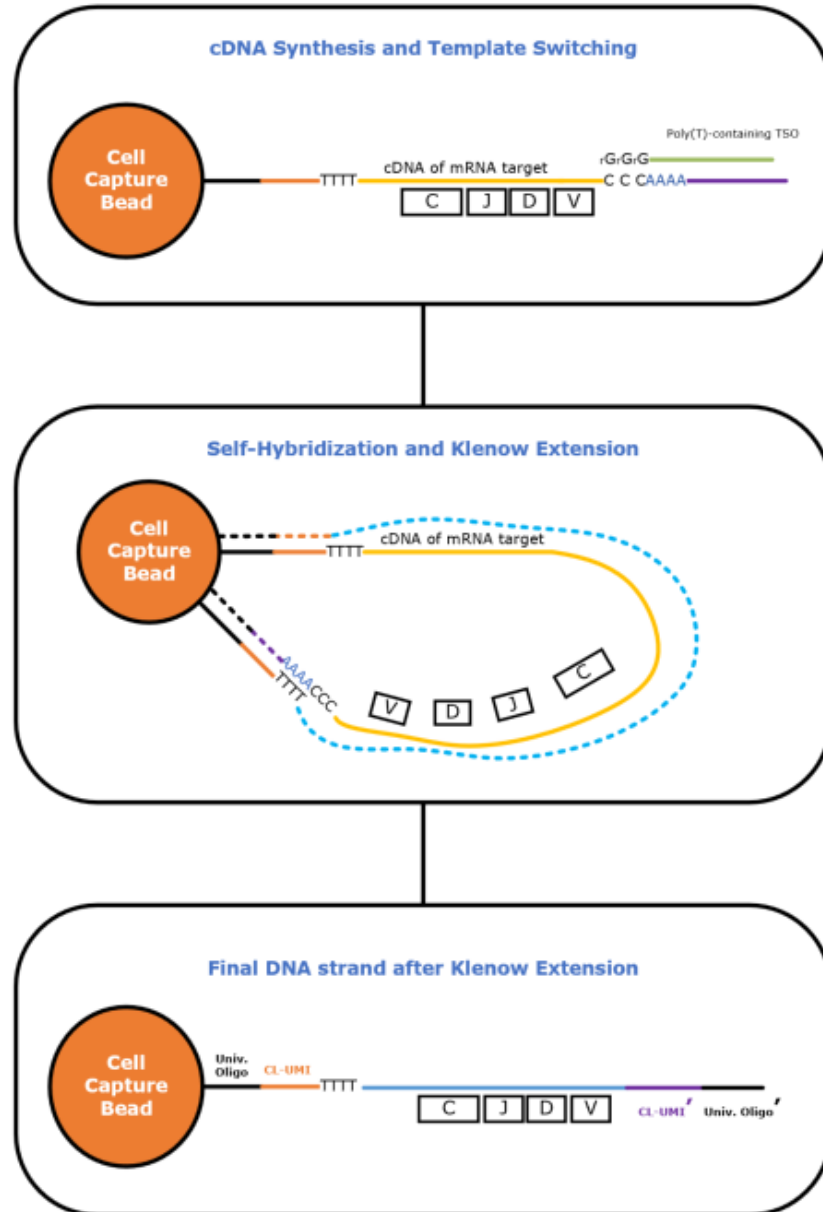
New synthetic poly-A tail is added on cDNA 3' end via template switching with a poly-T TSO (template switching oligo)

Synthetic poly-A tail on cDNA 3' end hybridizes to poly-T on its own bead.

Addition of DNA polymerase and reagents allows copying of new barcodes to cDNA 3' end (equivalent to mRNA 5' end)

Ability to amplify from the 5' end of cDNA

Workflow (cont.)
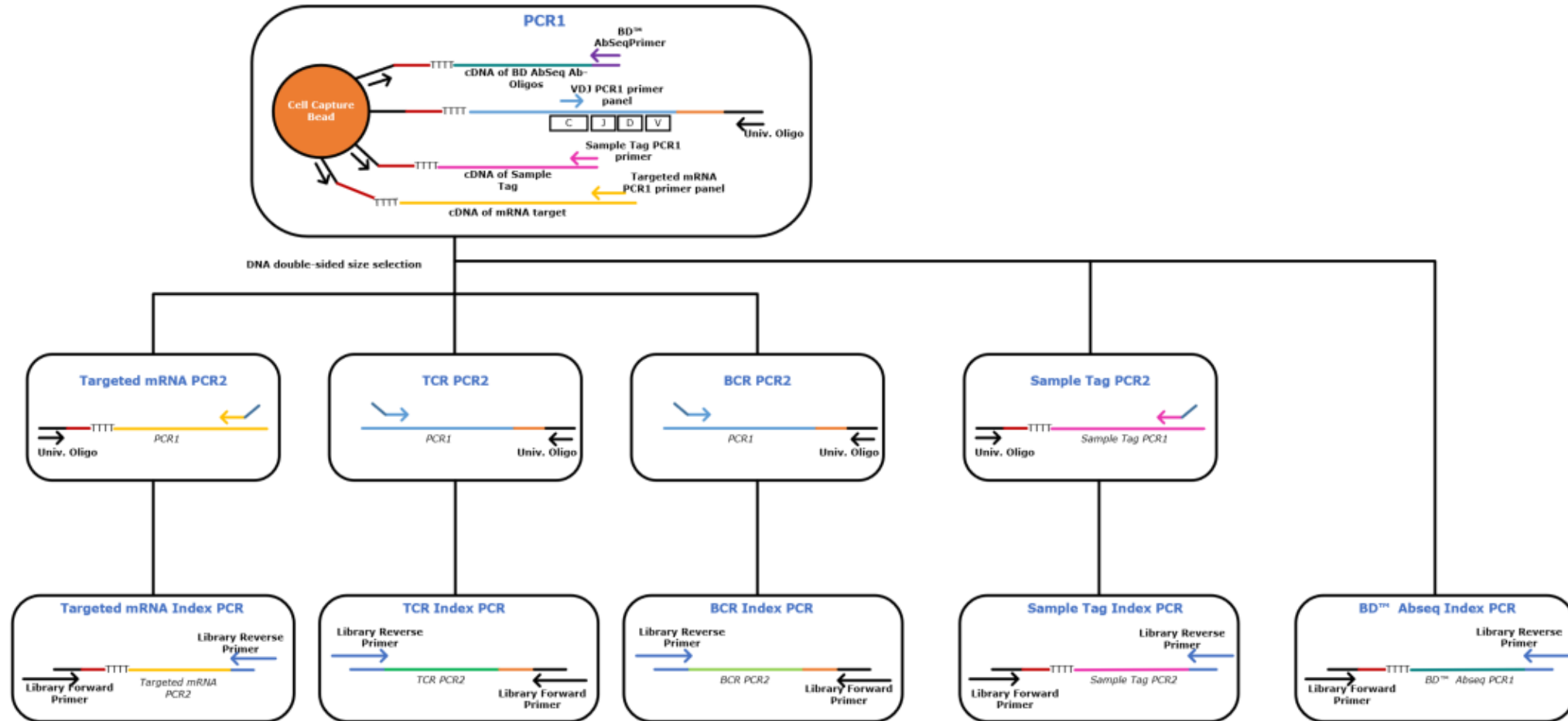
Technical Features & Performance Summary

Features	Metric	Current performance
Library quality	% reads assigned to cell labels	>80 %**
	% reads aligned to amplicons	
Sequencing depth	# of reads per cell for V(D)J assembly	<5000 **3000 (optimal)** **2000 (met specs)**
Cells processed	# of cells that can be processed	1000 - 20000
Assay time	Time of Assay (only V(D)J library)	2 working days
Workflow	1st Stopping Point	1-3 hours
Cost, usability, multiomics capability	AbSeq / SMK	Compatible
Performance	% recovery of cells loaded	>85%**
Species	Species	Human & Mouse
Compatibility	AbSeq (40-plex), SMK **Not compatible with WTA**	Compatible (optimized)
Samples tested	PBMCs, enriched samples	Optimized

** – Average performance data based on limited number of internal experiments

BD

BD Rhapsody™ VDJ CDR3 Protocol
Provided and needed components

1) Tested, optimized VDJ CDR3 protocols

2) Primer sequences for human CDR3 region of TCR and BCR

3) Bioinformatics pipeline

1) The Rhapsody Targeted mRNA and AbSeq reagent kit **(#633731, #633732, #633733, #633734)** along with immune response panel **(#633750)**

2) AbSeq or SMK (as required)

3) Need to order the **primers required for TCR and BCR** (listed explicitly in the BD protocols),

4) Order **additional reagents** required (listed explicitly in the BD protocols).

Compatibility with Targeted Assays

- While the VDJ protocol can technically work with any Targeted panel, the immune response panel with genes specific for immune cells greatly aids in immune cell calling, a feature critical for VDJ output.

- The T cell panel can work if the BD VDJ assay is being used for T cell analyses only.

- The Onco-BC panel will not be effective in cell calling for VDJ

- To work effectively with our CDR3 VDJ assay, custom panels are advised to include immune response panel markers.

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BD Rhapsody™ VDJ CDR3 Protocol
Required Materials and Primer Sequences

Required and recommended materials

Required reagents

Store the reagents at the storage temperature specified on the label.

Material	Supplier	Catalog No.
BD Rhapsody™ Targeted mRNA and AbSeq Amplification Kit	BD Biosciences	633774
Agencourt® AMPure® XP magnetic beads	Beckman Coulter	A63880
100% ethyl alcohol	Major supplier	–
10 mM Tris-HCl with 0.05% Tween-20, pH 8.0 (Tris-Tween20)	Teknova	T1485
Nuclease-free water	Major Supplier	–
Template switch oligo, TSO (5' T TTT TTT TTT TTT TTT TTT TTT TTT rG rG rG 3'). Stock concentration of TSO is 100 µM in 10 mM Tris, 0.1 mM EDTA, pH 8.0	Major Supplier	
Klenow Fragment (3' -> 5' exo-) (includes NEBuffer 2)	New England Biolabs	M0212L
10 mM dNTP	New England Biolabs	N0447L
1 M MgCl2	Major Supplier	–
1 M Tris-HCl, pH 8.0 (diluted to 50 mM Tris-HCl for the assay)	Major Supplier	–

Human T cell PCR2 primers

Primer Name	Primer Sequence (5' – 3')
TRAC_N2	CAGACGTGTGCTCTTCCGATCTATCAAAATCGGTGAATAGGCAGAC
TRBC_N2	CAGACGTGTGCTCTTCCGATCTGATCTCTGCTTCTGATGGCTCA
TRDC_N2	CAGACGTGTGCTCTTCCGATCTATATCCTTGGGGGTAGAATTCCTTC
TRGC_N2	CAGACGTGTGCTCTTCCGATCTGGGAAACATCTGCATCAAGTTG

Pooling Human T cell PCR2 primer panel

Name	Primer Stock Conc. (µM)	Volume per primer (µL)	DNA Suspension Buffer (µL)	Final volume (µL)
TRAC_N2 TRBC_N2 TRDC_N2 TRGC_N2	100	25.00	400.00	500

Human B cell PCR1 primers

Primer Name	Primer Sequence (5' – 3')
IGHA_N1	CACAGTCACATCCTGGCT
IGHD_N1	GATCTCCTTCTTACTCTTGCTGG
IGHE_N1	CGCTGAAGGTTTTGTTGTCG
IGHG_N1	TGTTGCTGGGCTTGTGAT
IGHM_N1	CGTTCTTTTCTTTGTTGCCGT
IGKC_N1	TTTGTGTTTCTCGTAGTCTGCT
IGLC_N1	TGTAGCTTCTGTGGGACTTC

Pooling Human B cell PCR1 primer panel

| Name	Primer Stock Conc. (µM)	Volume per primer (µL)	DNA Suspension Buffer (µL)	Final volume (µL)
IGHA_N1 IGHD_N1 IGHE_N1 IGHG_N1 IGHM_N1 IGKC_N1 IGLC_N1	100	15.00	420.00	525

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Sensitivity and Specificity Data

Examples of TCR and BCR sensitivity and specificity metrics

**Combined data from multiple experiments and 5 donors. Values displayed are mean +/- standard deviation.
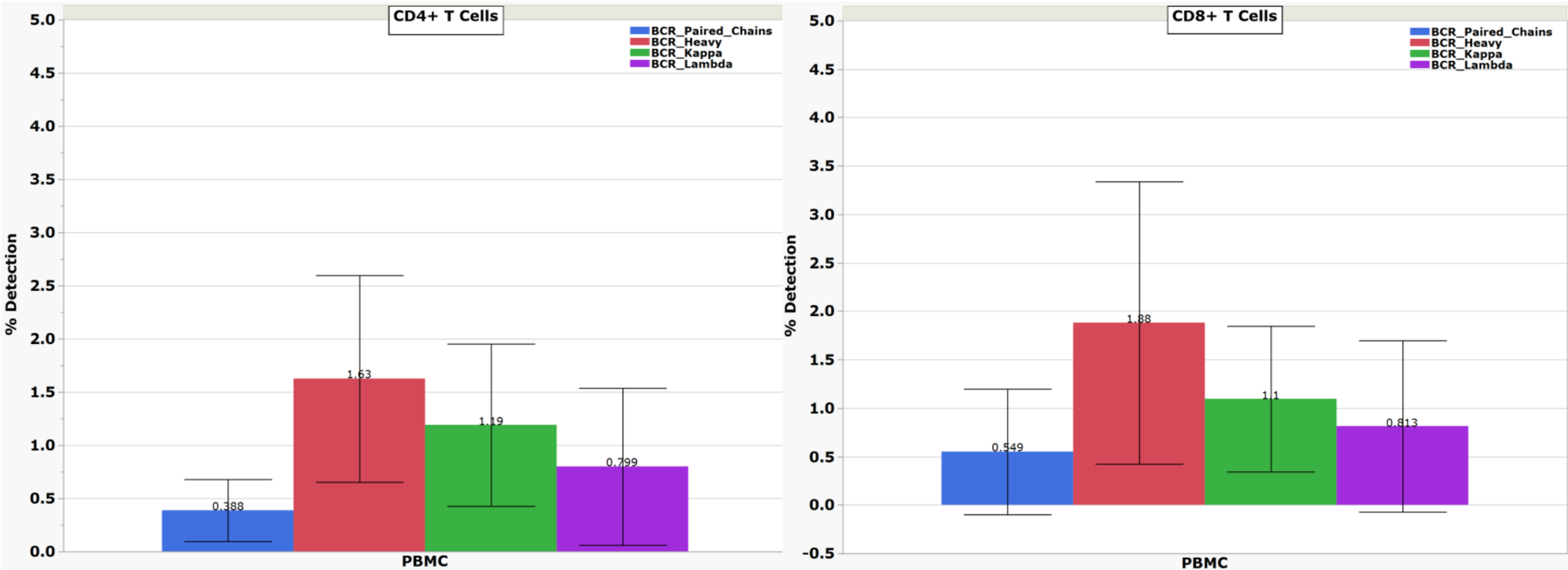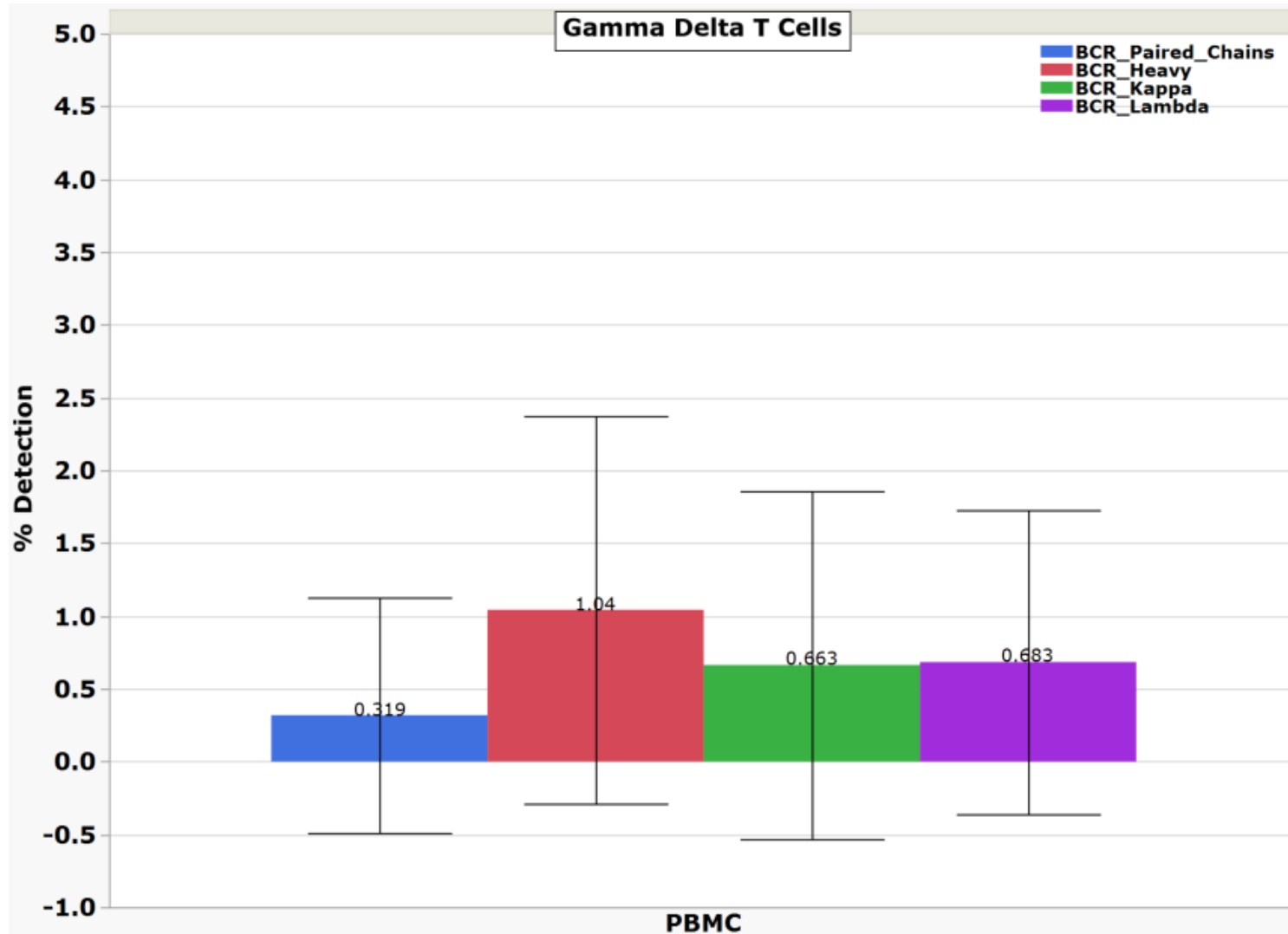
BCR Performance – Sensitivity
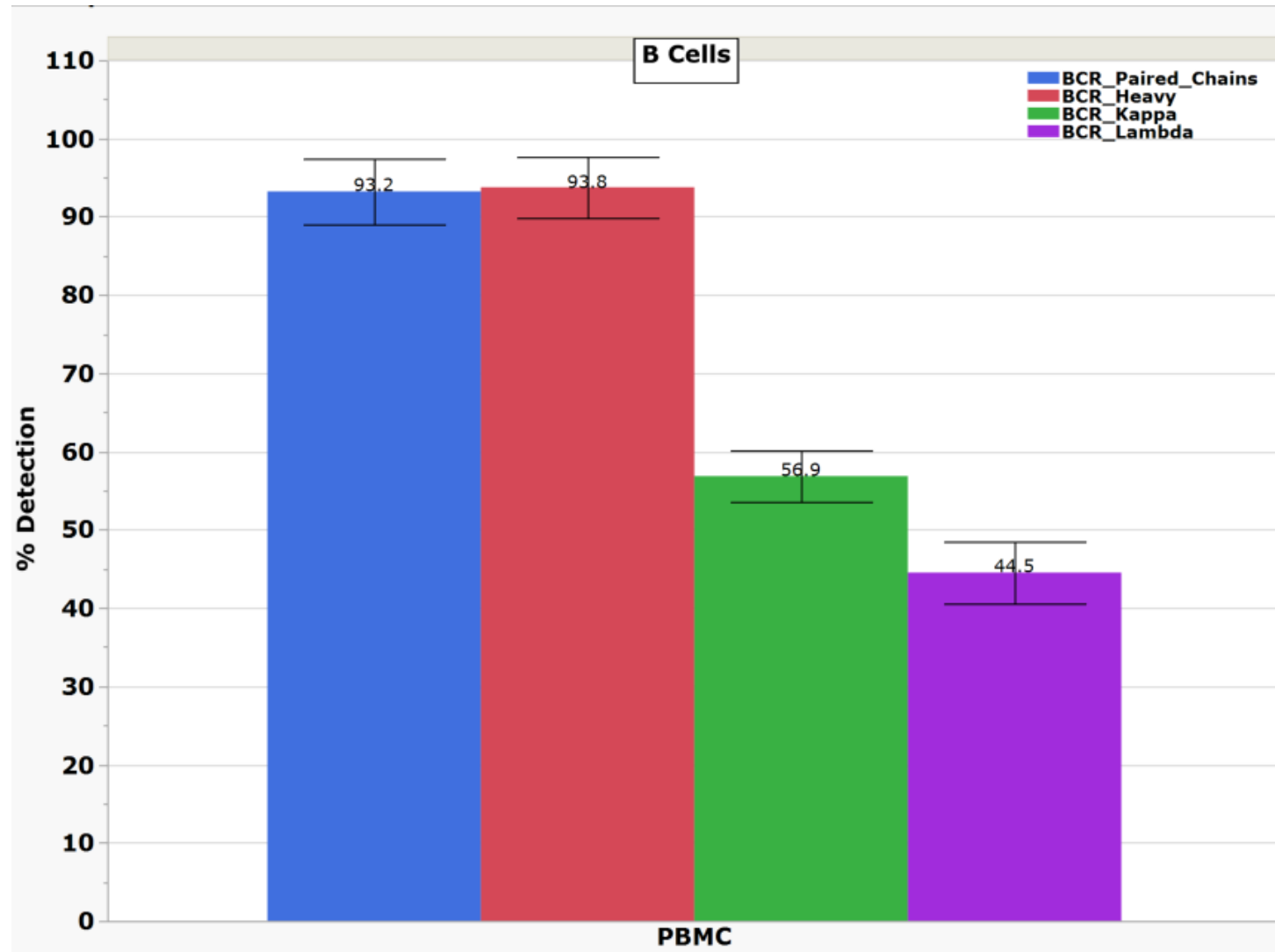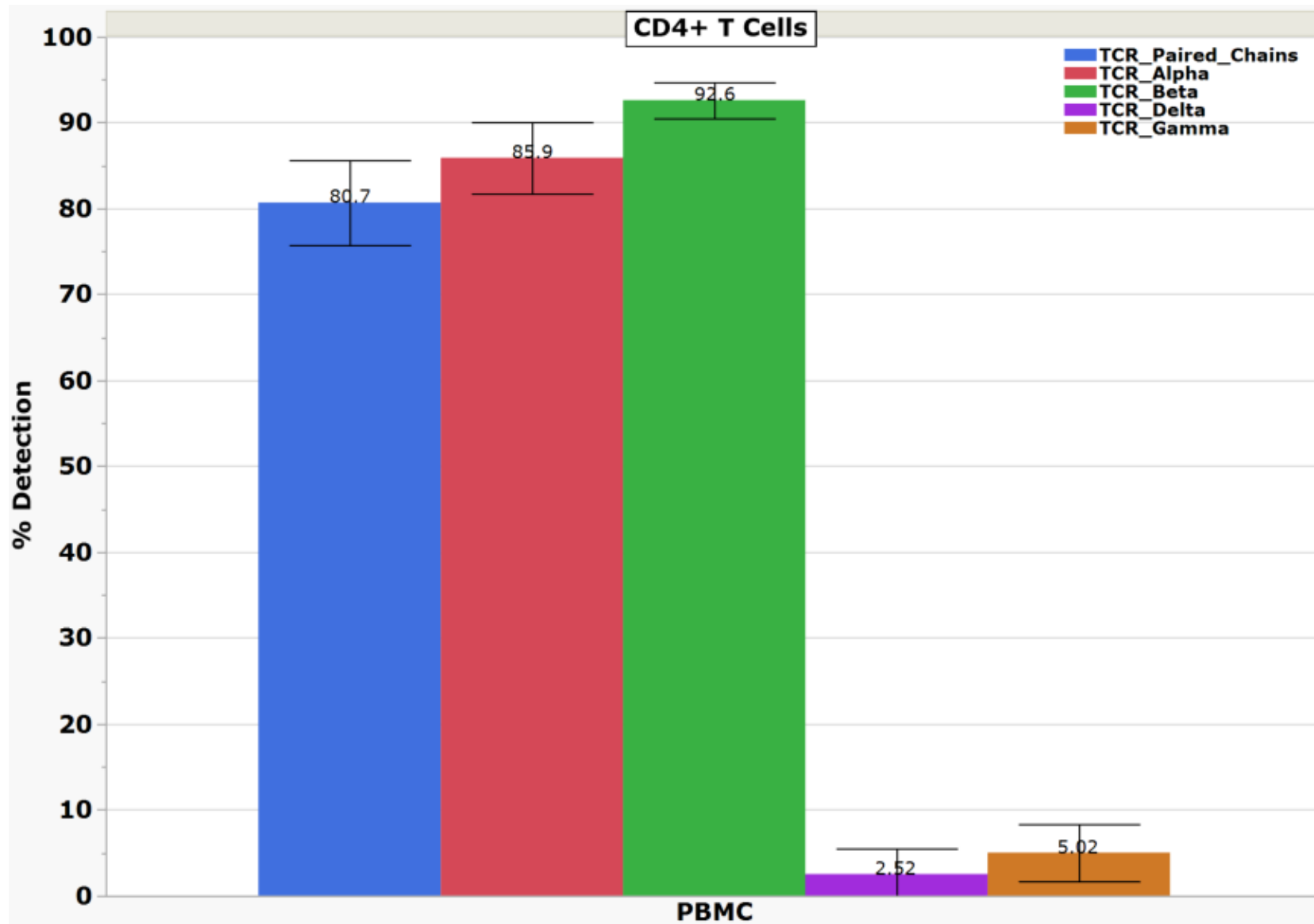
BCR Performance – Specificity
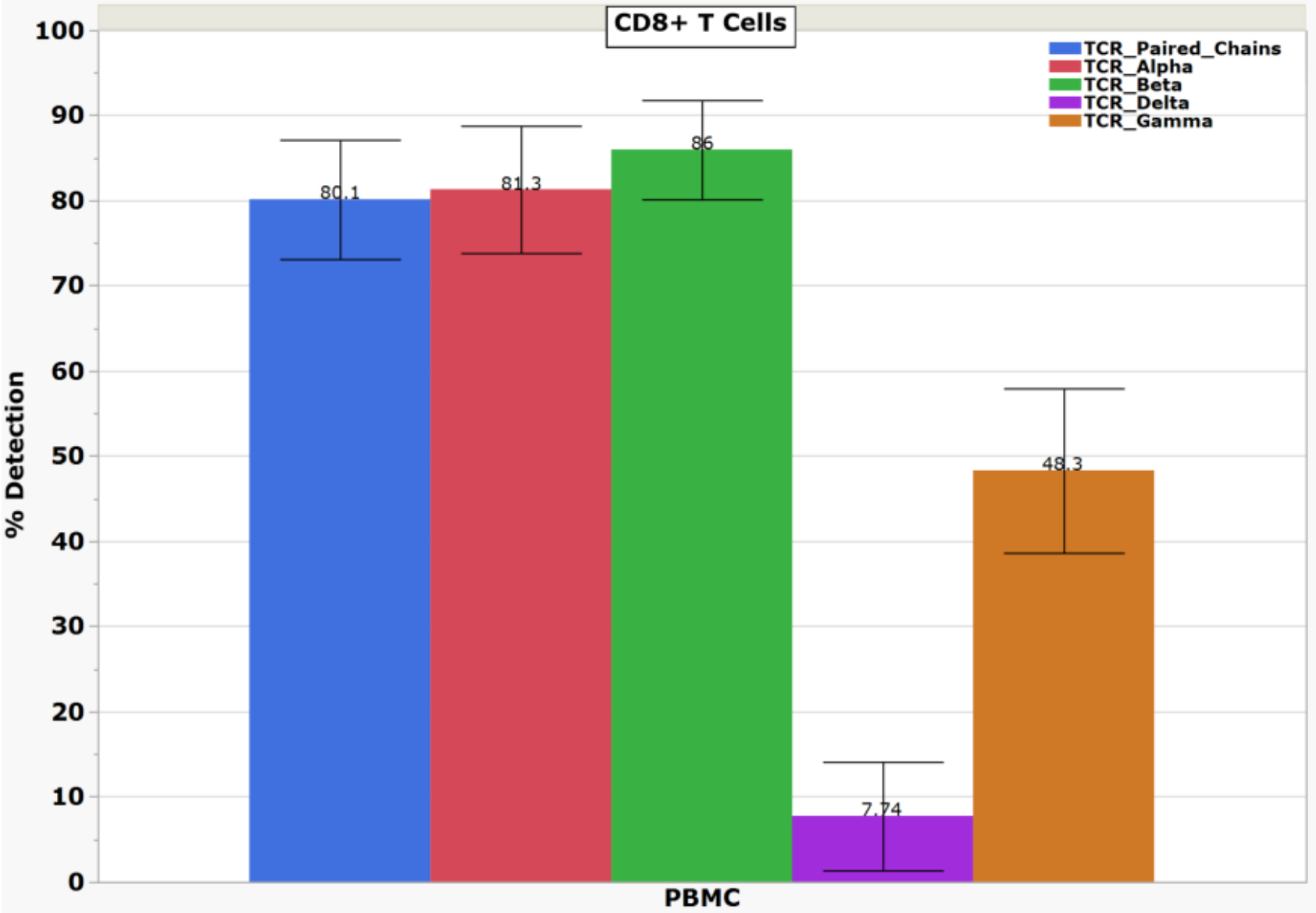## (% non-B cells with BCR CDR3 chain sequences)

BCR Performance – Specificity
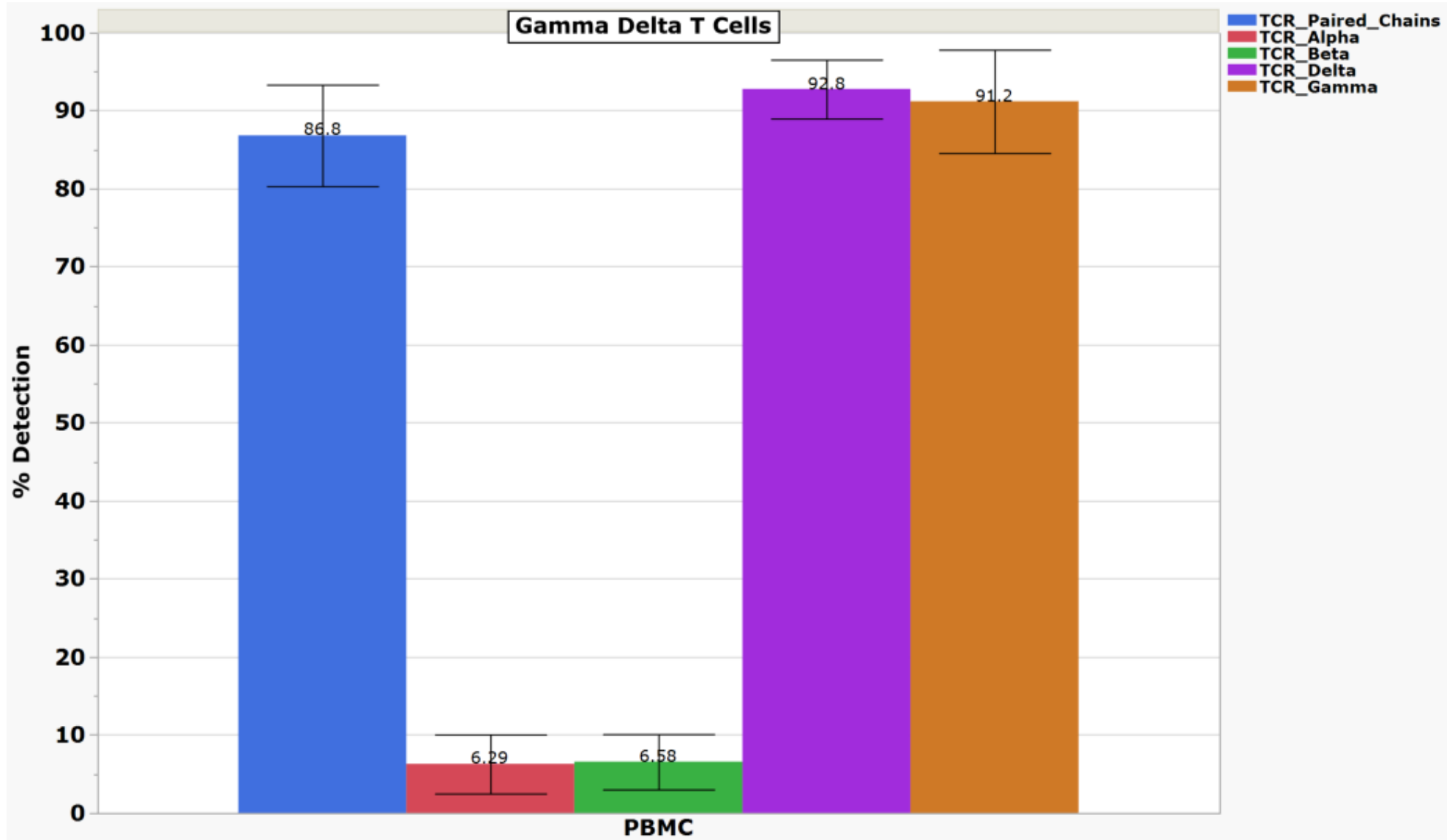## (% non-B cells with BCR CDR3 chain sequences)

TCR Performance – Sensitivity

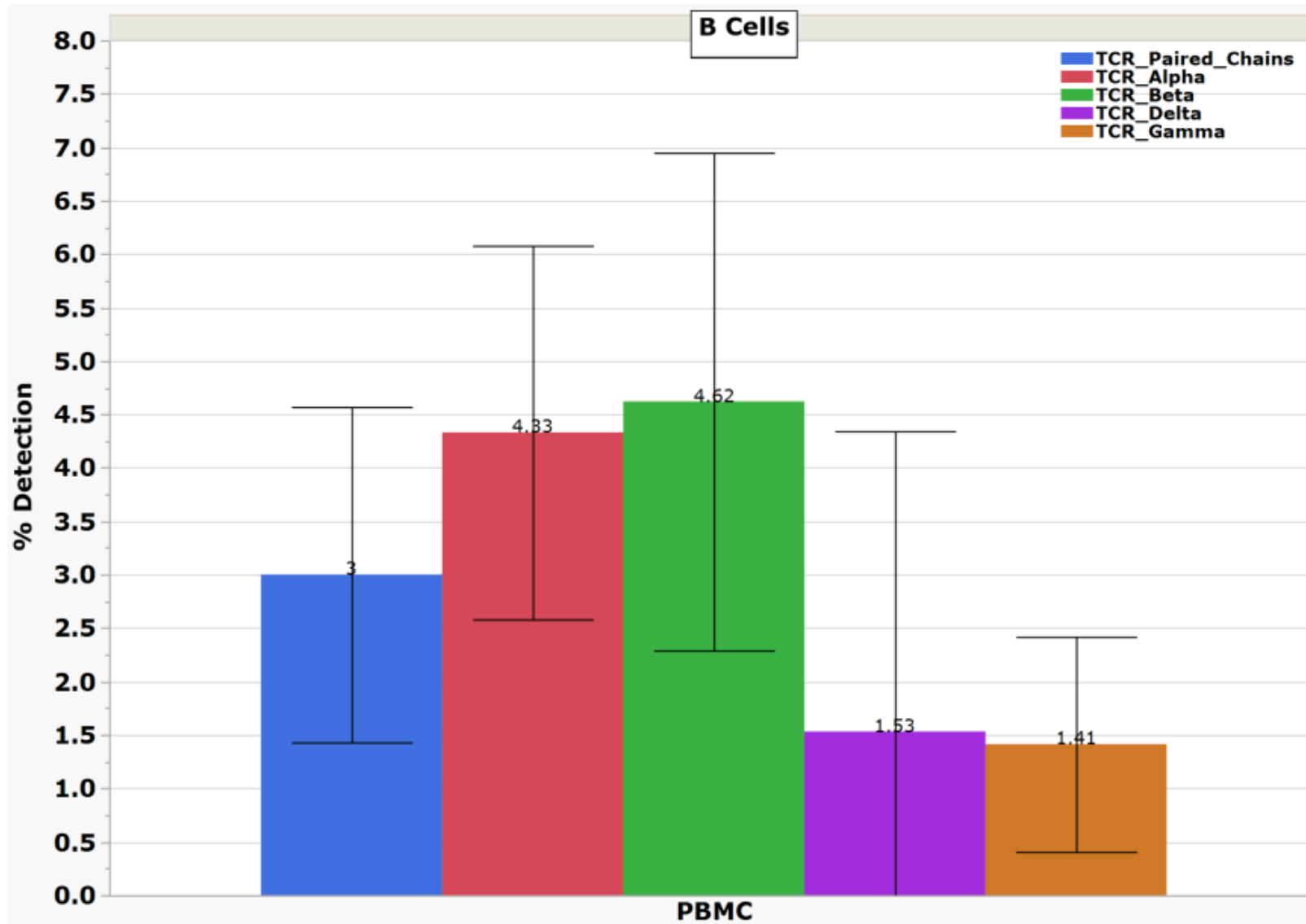# TCR Performance – Sensitivity

TCR Performance – Sensitivity
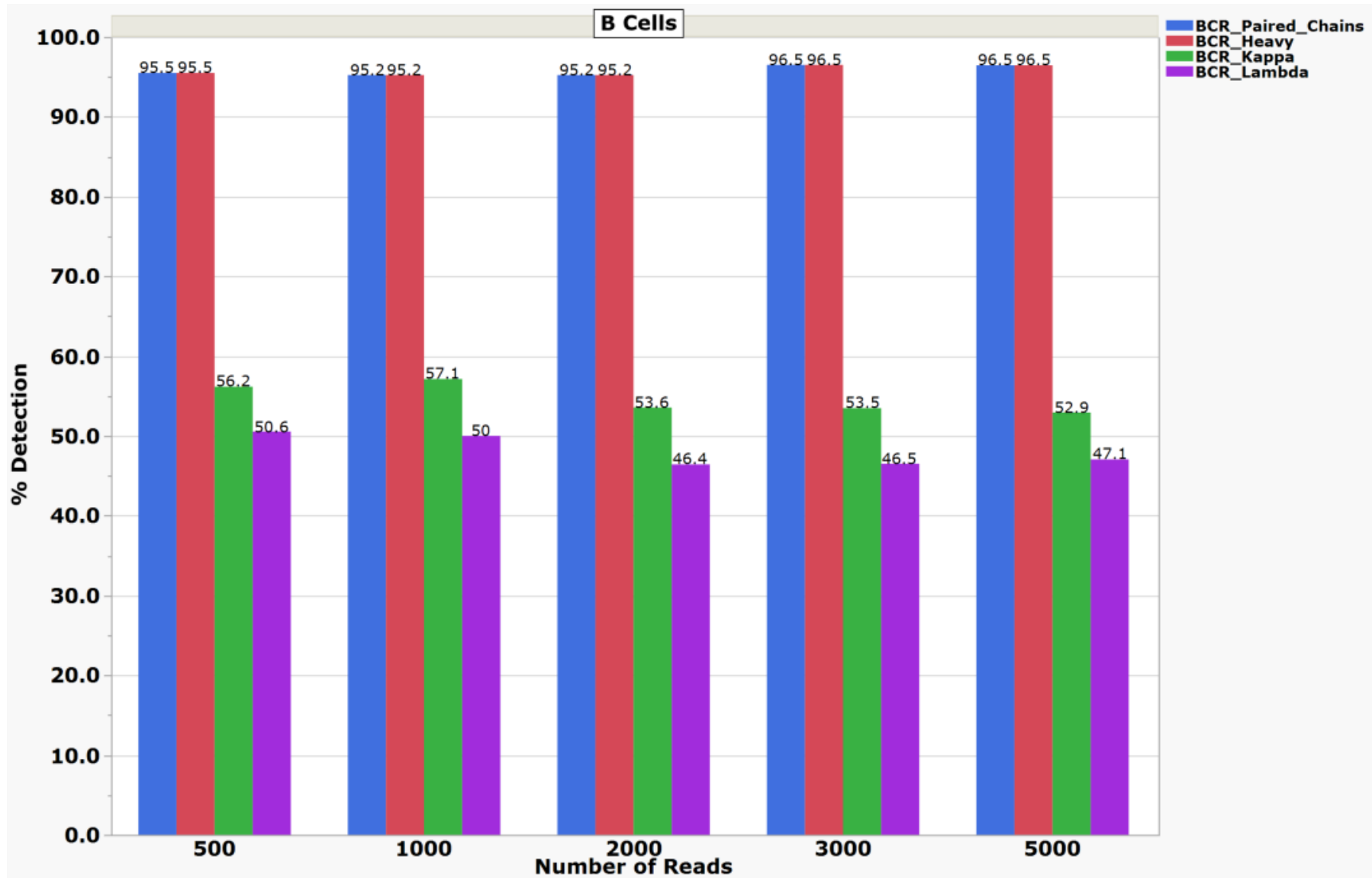
TCR Performance – Specificity
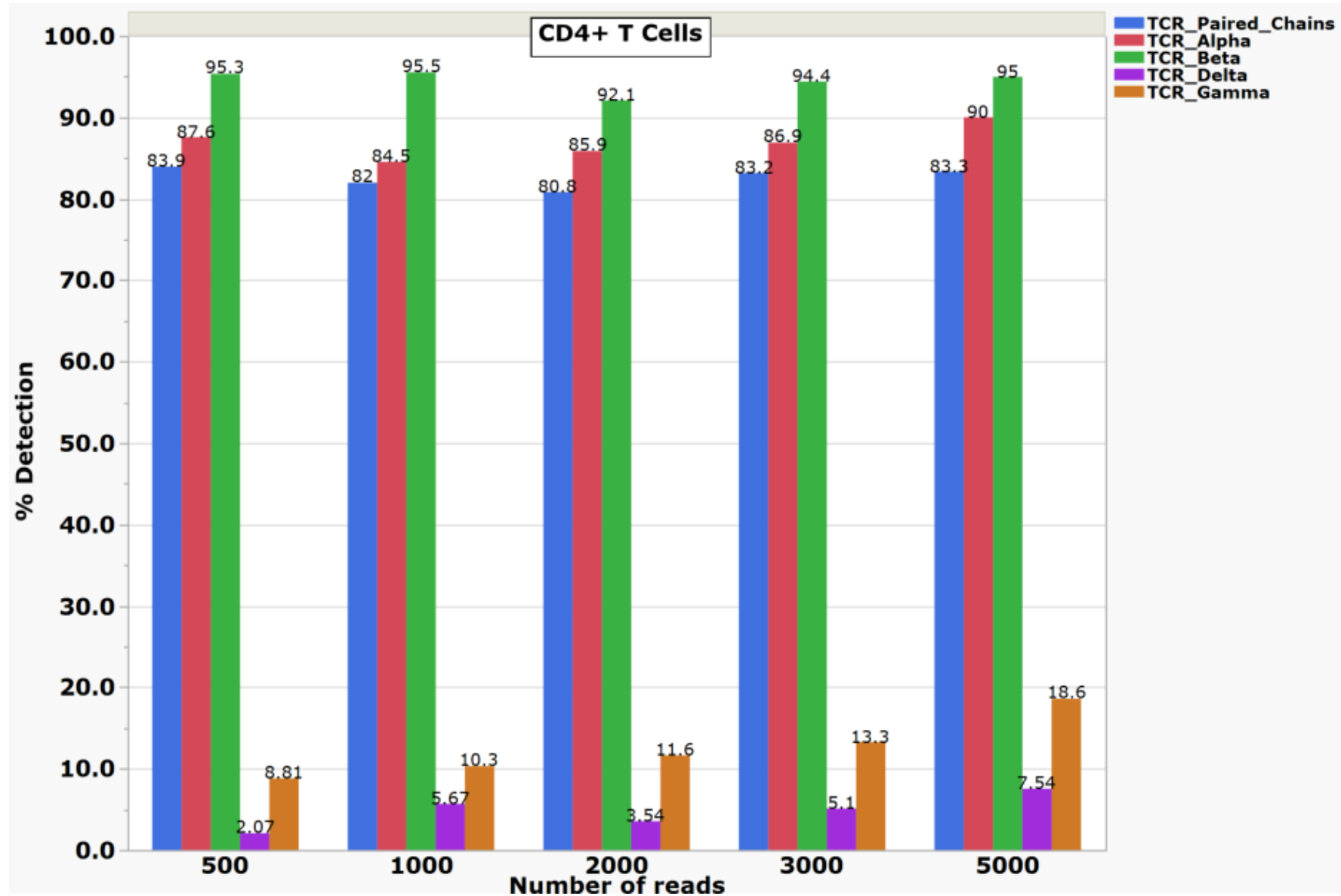## (% non-T cells with TCR CDR3 chain sequences)

Sequencing Depth Data

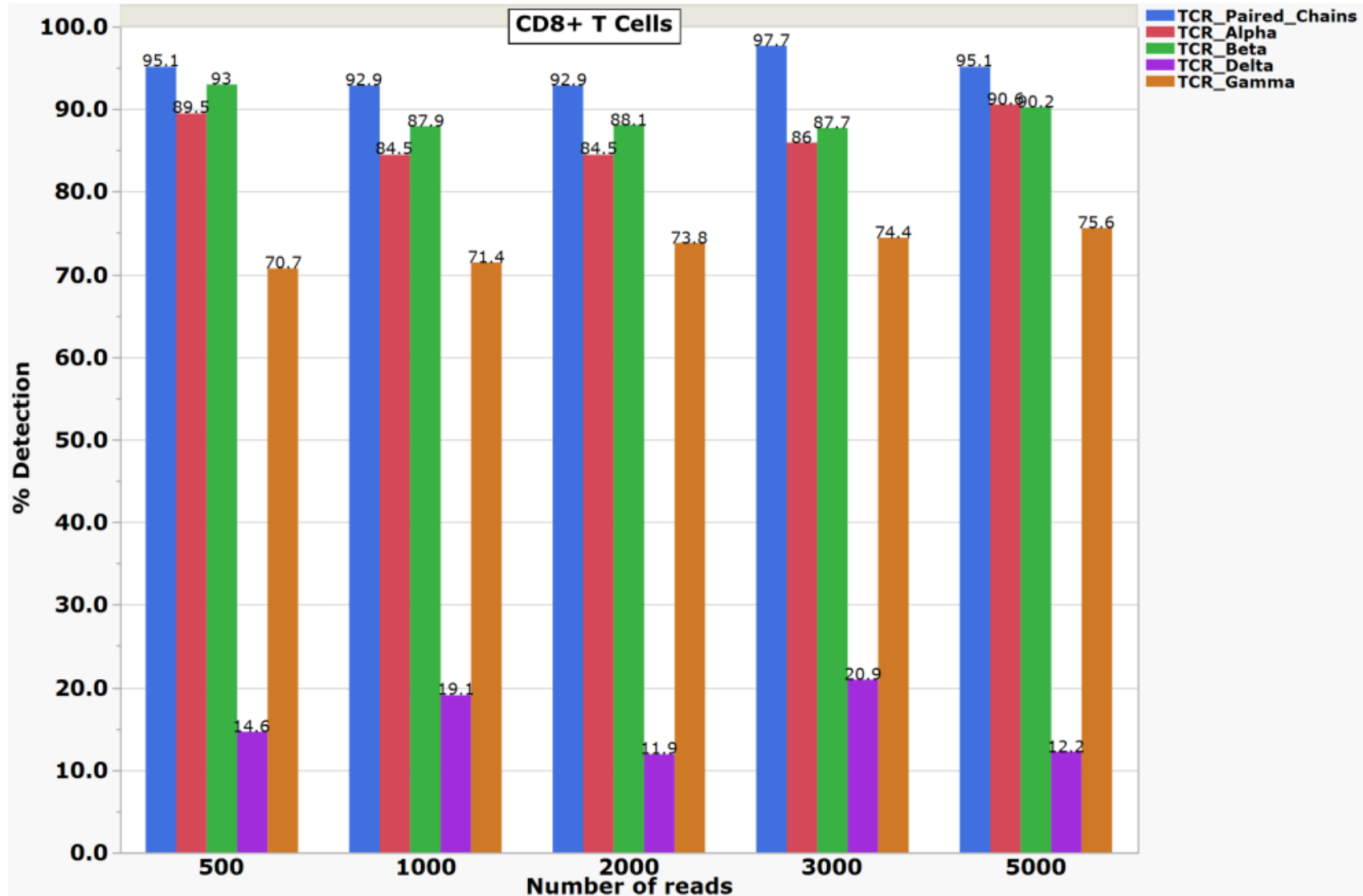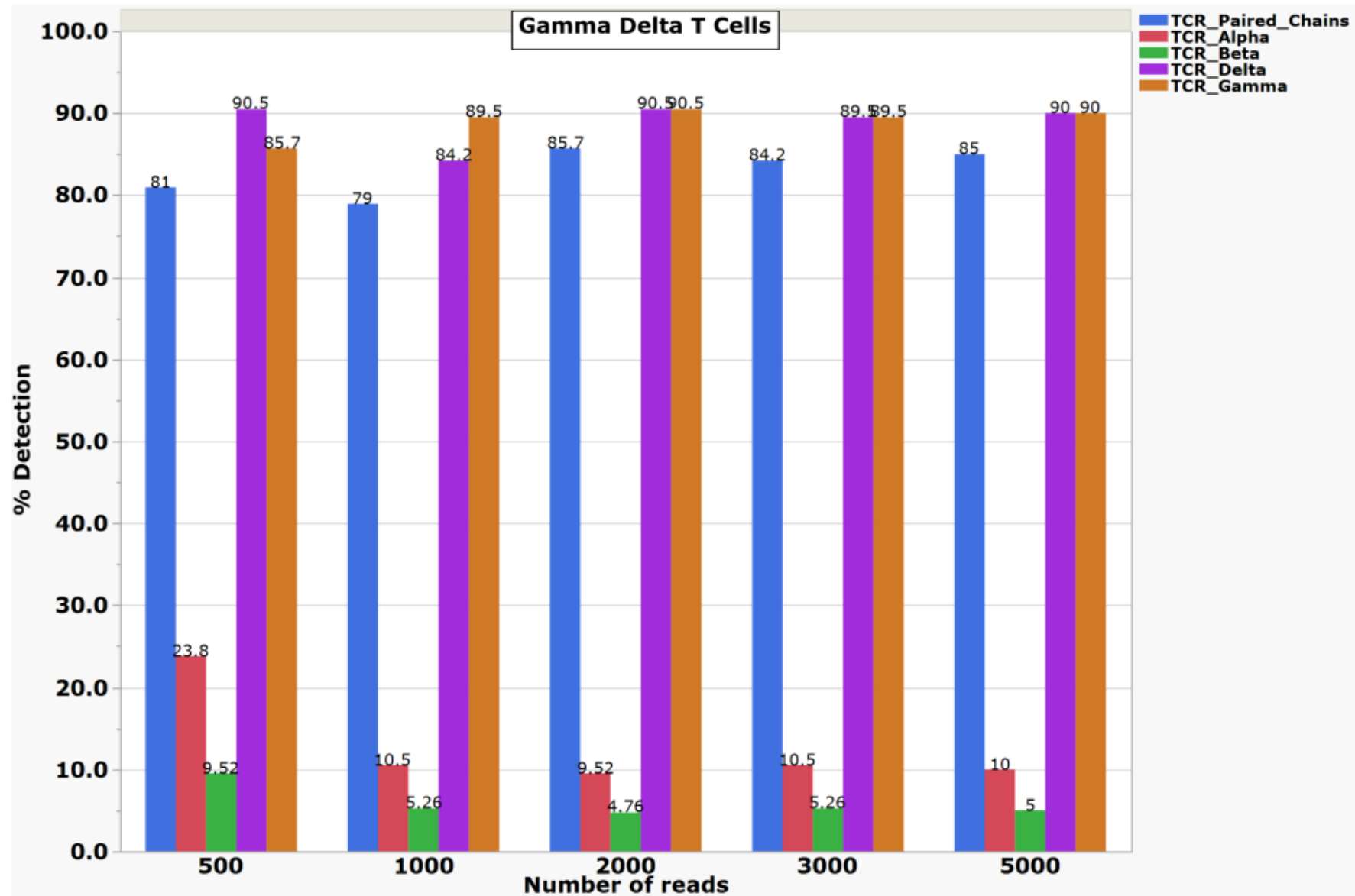*An overview of different immune cell subset detection at varying sequencing depths*

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B cells

CD4+ T cells

CD4+ T Cells

% Detection vs Number of reads

Number of reads	TCR_Paired_Chains	TCR_Alpha	TCR_Beta	TCR_Delta	TCR_Gamma
500	83.9	87.6	95.3	2.07	8.81
1000	82	84.5	95.5	5.67	10.3
2000	80.8	85.9	92.1	3.54	11.6
3000	83.2	86.9	94.4	5.1	13.3
5000	83.3	90	95	7.54	18.6

CD8+ T cells

Gamma Delta T cells

BD VDJ Bioinformatics Pipeline Output

VDJ Pipeline

All reads put into the same input

DRAFT **BD Rhapsody™ Analysis Pipeline run - 10-16-19 01:55:29** ✎

Last update by liukai1029 on Oct. 15, 2019 18:55
▾ App: BD Rhapsody™ Analysis Pipeline - Revision: 0

Task Inputs   Execution Settings

Inputs

Batching ❔   Off ⬤

▾ **AbSeq Reference** ❔ 📁 Select file(s)

No files selected

▾ **Reads** * ❔ 📁 Select file(s)

No files selected

This field is required and cannot be empty.

▾ **Reference** * ❔ 📁 Select file(s)

No files selected

This field is required and cannot be empty.

App Settings

✎ Edit parameters   Show editable ▾

▸ **Putative_Cell_Calling_Settings** (#Putative_Cell_Calling_Settings)

▸ **Subsample_Settings** (#Subsample_Settings)

▾ **VDJ_Settings** (#VDJ_Settings)

VDJ Species Version ❔

| ✓ No value |
| Human VDJ - BCR and TCR |
| Human VDJ - BCR only |
| Human VDJ - TCR only |
| **Mouse VDJ - BCR and TCR** |
| Mouse VDJ - BCR only |
| Mouse VDJ - TCR only |

▾   Settings)

Subsample Sample Tags ❔

No value

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_Metrics Summary File

#Sequencing Quality#						
Total_Reads_ in_FASTQ	Pct_Reads_ Too_Short	Pct_Reads_Low_ Base_Quality	Pct_Reads_ High_SNF	Pct_Reads_ Filtered_Out	Total_Reads_After _Quality_Filtering	Library
48068574	1.1	6.98	6.08	11.2	42683736	BCR10k
61090421	0.71	5.81	3.55	9.16	55493393	TCR10k
14765915	0.11	11.51	5.66	15.59	12464008	mRNA10k
123924910	0.79	6.94	4.78	10.72	110641137	Combined_stats

#Library Quality#					
Total_Filtered _Reads	Pct_Contaminating_PhiX _Reads_in_Filtered_R2	Pct_Q30_Bases_ in_Filtered_R2	Pct_Assigned_ to_Cell_Labels	Pct_Cellular_Reads _Aligned_Uniquely _to_Amplicons	Library
42683736	0.03	62.61	84.39	79.76	BCR10k
55493393	0.07	64.58	82.17	76.58	TCR10k
12464008	0.01	66.32	90.6	86.88	mRNA10k
110641137	0.05	64.01	83.98	78.97	Combined_stats

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_VDJ_Metrics Summary File

#Overall VDJ Metrics#									
Reads_Cellular_Aligned_to_VDJ	Reads_CDR3_Valid_Unfiltered	Reads_CDR3_Valid_Putative	Pct_Reads_CDR3_Valid_from_Putative_Cells	Reads_CDR3_Valid_Putative_Corrected	Pct_Reads_CDR3_Valid_Corrected_from_Putative_Cells	Mean_Reads_CDR3_Valid_Corrected_per_Putative_Cell	Molecules_Unfiltered	Molecules_Corrected_Putative	Mean_Molecules_Corrected_per_Putative_Cell
63975808	48826575	38366518	78.58	36652359	75.07	45082.85	508537	23366	28.74

#Chain Type Metrics#									
Chain_Type	Reads_CDR3_Valid_Unfiltered	Reads_CDR3_Valid_Putative	Pct_Reads_CDR3_Valid_from_Putative_Cells	Reads_CDR3_Valid_Putative_Corrected	Pct_Reads_CDR3_Valid_Corrected_from_Putative_Cells	Mean_Reads_CDR3_Valid_Corrected_per_Putative_Cell	Molecules_Unfiltered	Molecules_Corrected_Putative	Mean_Molecules_Corrected_per_Putative_Cell
BCR_Heavy	15201582	12195119	80.22	11850057	77.95	14575.72	120649	6220	7.65
BCR_Kappa	4046534	2970188	73.4	2701916	66.77	3323.39	43742	2576	3.17
BCR_Lambda	4823527	4013658	83.21	3948324	81.86	4856.49	44895	2999	3.69
TCR_Alpha	11672066	8742143	74.9	8004555	68.58	9845.7	132640	4406	5.42
TCR_Beta	9801908	7541670	76.94	7420795	75.71	9127.67	125525	5588	6.87
TCR_Delta	808033	657997	81.43	657120	81.32	808.27	11159	356	0.44
TCR_Gamma	2472925	2245743	90.81	2069592	83.69	2545.62	29927	1221	1.5

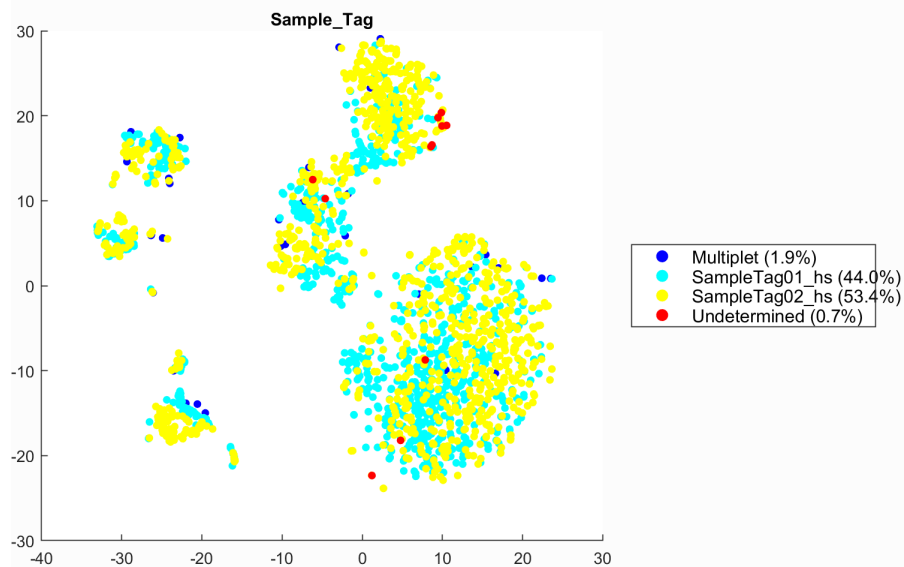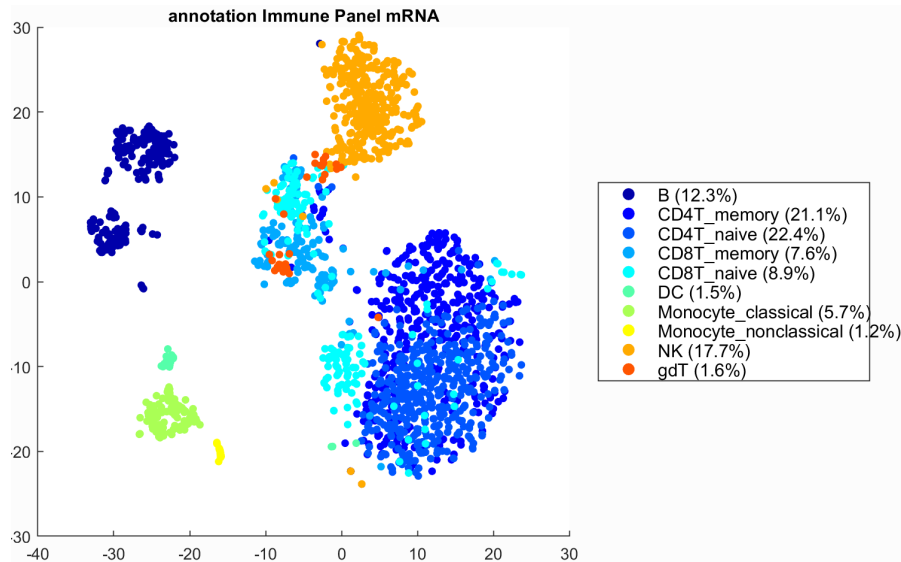| #Cell Type Metrics#									
Cell_Type_Experimental	Number_cells	BCR_Paired_Chains_Percent	TCR_Paired_Chains_Percent	BCR_Heavy_Percent_Cells_Positive	BCR_Heavy_Mean_Molecules_per_Cell	BCR_Kappa_Percent_Cells_Positive	BCR_Kappa_Mean_Molecules_per_Cell	BCR_Lambda_Percent_Cells_Positive	BCR_Lambda_Mean_Molecules_per_Cell
B	92	94.57	0	94.57	56.2	53.26	26.98	44.57	17.09
Dendritic	12	0	0	0	0	0	0	0	0
Monocyte_classical	128	0.78	0.78	0.78	0.14	0.78	0.59	0	0
Monocyte_nonclass	17	0	0	0	0	0	0	0	0
Natural_killer	210	1.43	3.33	2.86	4.74	1.43	0.09	2.38	6.69
T_CD4_memory	183	0	84.15	0.55	0.01	0	0	0	0
T_CD4_naive	46	0	84.78	2.17	0.04	2.17	0.02	0	0
T_CD8_memory	55	1.82	81.82	3.64	0.47	0	0	1.82	0.42
T_CD8_naive	55	0	89.09	1.82	0.13	0	0	0	0
T_gamma_delta	15	0	86.67	0	0	0	0	0	0

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_VDJ_perCell File

Cell_Index	Total_VDJ_ Read_Count	Total_VDJ_ Molecule_Count	BCR_Heavy_ V_gene_Dominant	BCR_Heavy_ D_gene_Dominant	BCR_Heavy_ J_gene_Dominant	BCR_Heavy_ C_gene_Dominant	BCR_Heavy_ CDR3_Nucleotide_ Dominant	BCR_Heavy_ CDR3_Translation_ Dominant	BCR_Heavy_ Read_Count	BCR_Heavy_ Molecule_Count
195260	46123	96	IGHV4-34*01	IGHD6-19*01	IGHJ5*02	human_IGHG	GCGAGAGGAGGA(	ARGGGYRSGWYGA	16914	27
275040	29983	82	IGHV4-39*01	IGHD3-16*01	IGHJ5*02	human_IGHM	GCGAGACGTGATC	ARRDRLGS	24035	58
790251	22513	87	IGHV3-30*02	IGHD3-3*01	IGHJ4*02	human_IGHM	GCGAAAGATTGGA	AKDWSAFDY	346	16
856783	4172	24							0	0
565345	15494	40	IGHV3-15*01	IGHD4-17*01	IGHJ6*02	human_IGHD	ACCACAGAGCTCG	TTELDYGVGGYYYY	11283	24
512464	0	0							0	0
251681	0	0							0	0
558698	22957	59	IGHV4-4*06	IGHD1-7*01	IGHJ5*02	human_IGHM	GCGAGAGTTCATA	ARVHNWNYPADDN	18124	45
11337	0	0							0	0
777579	26444	83	IGHV4-31*02	IGHD2-2*01	IGHJ3*02	human_IGHM	GCGAGGTATTGTA	ARYCSSPSCSGAFD	18452	38
734829	44185	121	IGHV1-2*02	IGHD6-19*01	IGHJ4*02	human_IGHM	GCGAGTGGTGGC1	ASGGWYHY	33403	91
853307	18633	70	IGHV5-51*01	IGHD4-17*01	IGHJ3*02	human_IGHA	GCGAGACTCTCGT	ARLSSPDYDLPLGRI	13215	43
831417	0	0							0	0
6245	0	0							0	0
695148	31532	75	IGHV3-30*01	IGHD6-19*01	IGHJ4*02	human_IGHM	GCGAGATTGTATA	ARLYSSGWGHVDY	13888	43
688461	0	0							0	0
673277	51375	94	IGHV1-8*01	IGHD3-16*01	IGHJ5*02	human_IGHM	GCGAGAGGCGCC(	ARGALLSGAHWGY\	40231	63
813557	27089	62	IGHV1-18*01	IGHD5-12*01	IGHJ4*02	human_IGHM	GCGAGACCGGGG(	ARPGGDYSGYDYG\	21345	39
592931	25211	71	IGHV1-2*02	IGHD2-2*02	IGHJ6*02	human_IGHG	GCGAGGGAAGCT(	AREAVVLPAAIRHFC	7106	15

BD

_VDJ_perCell File

Cell_Index	Total_VDJ_Read_Count	Total_VDJ_Molecule_Count	BCR_Heavy_V_gene_Dominant	BCR_Heavy_D_gene_Dominant	BCR_Heavy_J_gene_Dominant	BCR_Heavy_C_gene_Dominant	Cell_Index	BCR_Paired_Chains	TCR_Paired_Chains	Cell_Type_Experimental	_Heavy_cule_Count
195260	46123	96	IGHV4-34*01	IGHD6-19*01	IGHJ5*02	human_IGHG	195260	TRUE	FALSE	B	27
275040	29983	82	IGHV4-39*01	IGHD3-16*01	IGHJ5*02	human_IGHM	275040	TRUE	FALSE	B	58
790251	22513	87	IGHV3-30*02	IGHD3-3*01	IGHJ4*02	human_IGHM	790251	TRUE	FALSE	B	16
856783	4172	24					856783	FALSE	TRUE	T_CD8_memory	0
565345	15494	40	IGHV3-15*01	IGHD4-17*01	IGHJ6*02	human_IGHD	565345	TRUE	FALSE	B	24
512464	0	0					512464	FALSE	FALSE	Natural_killer	0
251681	0	0					251681	FALSE	FALSE	Natural_killer	0
558698	22957	59	IGHV4-4*06	IGHD1-7*01	IGHJ5*02	human_IGHM	558698	TRUE	FALSE	B	45
11337	0	0					11337	FALSE	FALSE	Natural_killer	0
777579	26444	83	IGHV4-31*02	IGHD2-2*01	IGHJ3*02	human_IGHM	777579	TRUE	FALSE	B	38
734829	44185	121	IGHV1-2*02	IGHD6-19*01	IGHJ4*02	human_IGHM	734829	TRUE	FALSE	B	91
853307	18633	70	IGHV5-51*01	IGHD4-17*01	IGHJ3*02	human_IGHA	853307	TRUE	FALSE	B	43
831417	0	0					831417	FALSE	FALSE	Monocyte_classical	0
6245	0	0					6245	FALSE	FALSE	Monocyte_classical	0
695148	31532	75	IGHV3-30*01	IGHD6-19*01	IGHJ4*02	human_IGHM	695148	TRUE	FALSE	B	43
688461	0	0					688461	FALSE	FALSE	Natural_killer	0
673277	51375	94	IGHV1-8*01	IGHD3-16*01	IGHJ5*02	human_IGHM	673277	TRUE	FALSE	B	63
813557	27089	62	IGHV1-18*01	IGHD5-12*01	IGHJ4*02	human_IGHM	813557	TRUE	FALSE	B	39
592931	25211	71	IGHV1-2*02	IGHD2-2*02	IGHJ6*02	human_IGHG	592931	TRUE	FALSE	B	15

VDJ + SMK

An overview of VDJ + SMK performance data performed using 2 donor samples

BD

Sample Tag Metrics

annotation Immune Panel mRNA

- B (12.3%)
- CD4T_memory (21.1%)
- CD4T_naive (22.4%)
- CD8T_memory (7.6%)
- CD8T_naive (8.9%)
- DC (1.5%)
- Monocyte_classical (5.7%)
- Monocyte_nonclassical (1.2%)
- NK (17.7%)
- gdT (1.6%)

Sample_Tag

- Multiplet (1.9%)
- SampleTag01_hs (44.0%)
- SampleTag02_hs (53.4%)
- Undetermined (0.7%)

Sensitivity – 99.33%

Specificity

B	99.16
CD4T_memory	99.76
CD4T_naive	99.76
CD8T_memory	100
CD8T_naive	98.85
DC	100
Monocyte_classical	100
Monocyte_nonclassical	100
NK	97.95
gdT	100

BCR Performance – Sensitivity
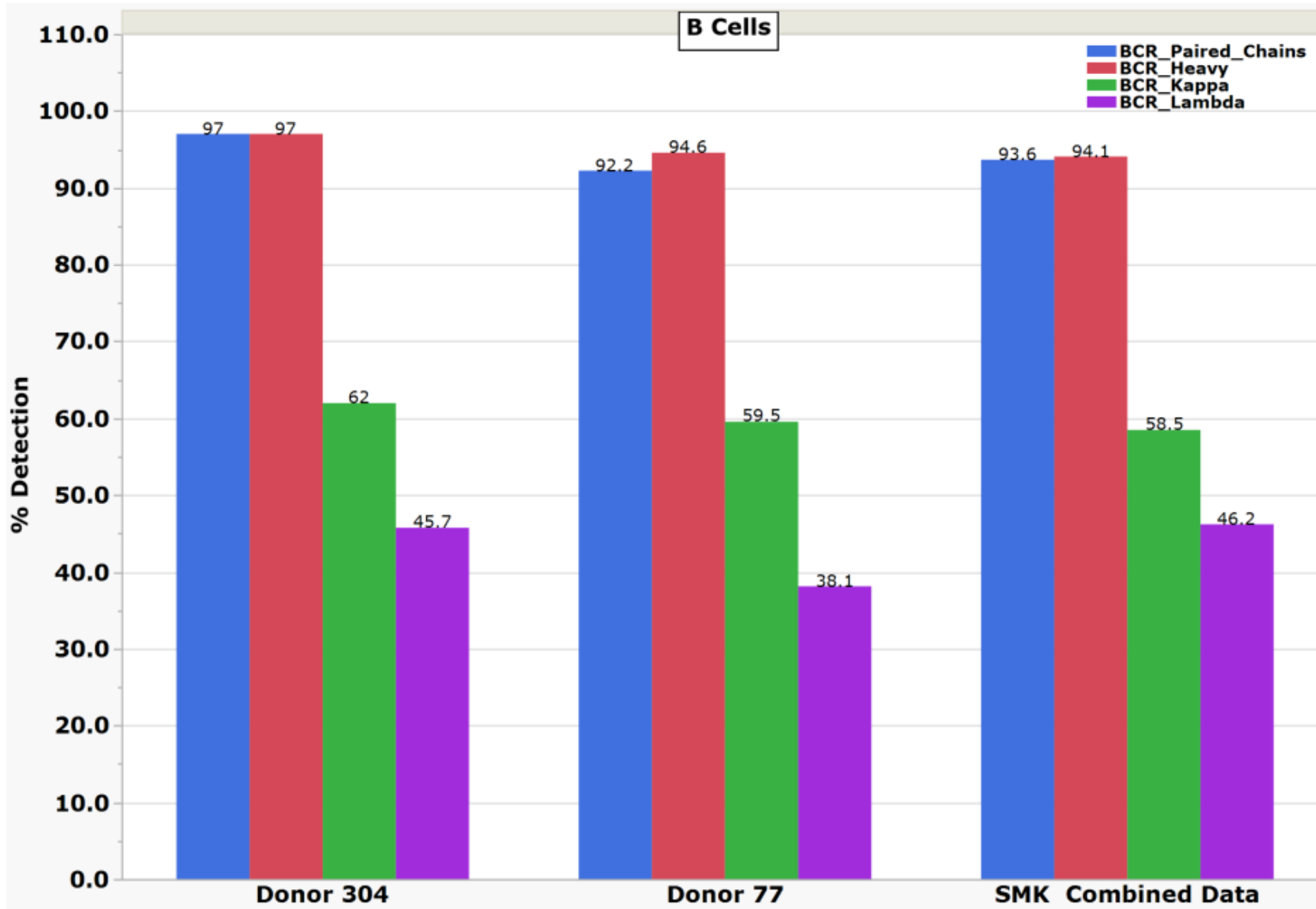
BCR Performance – Specificity
(% non-B cells with BCR CDR3 chain sequences)
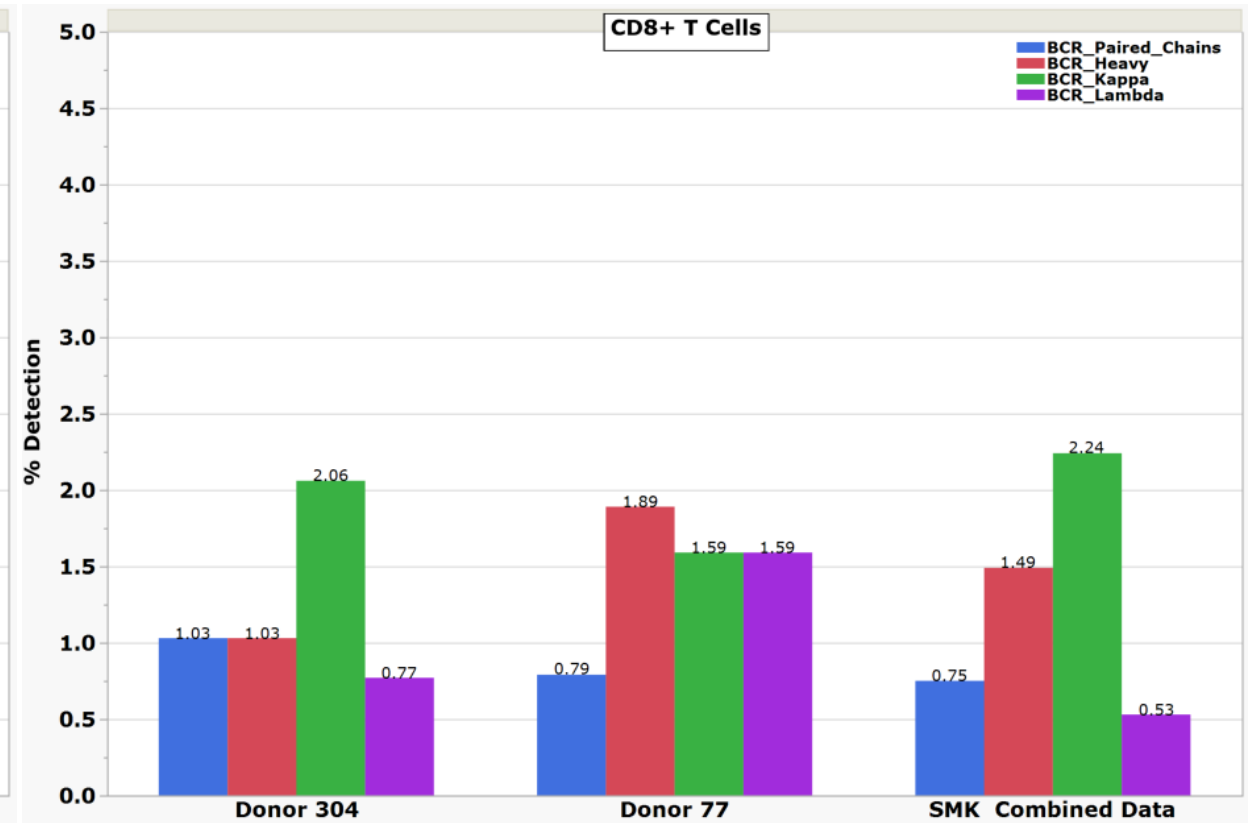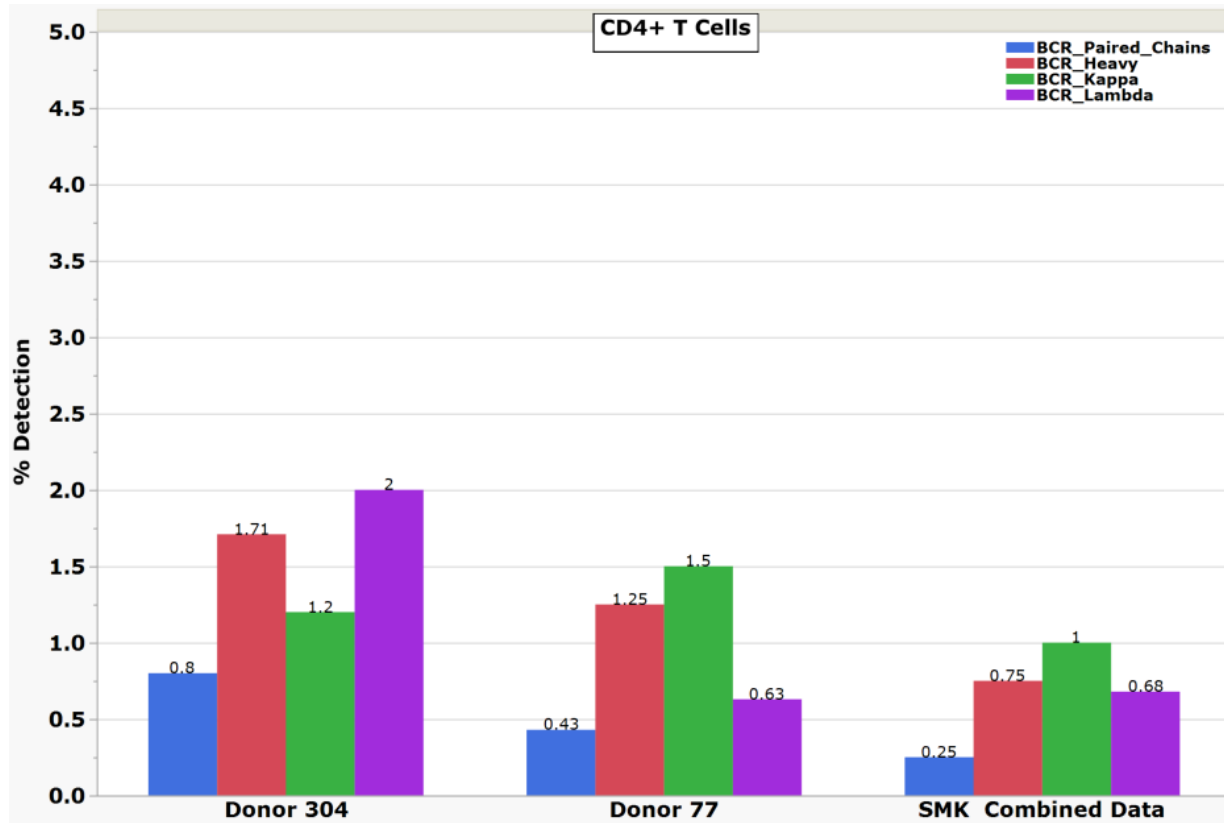
BCR Performance – Specificity
(% non-B cells with BCR CDR3 chain sequences)
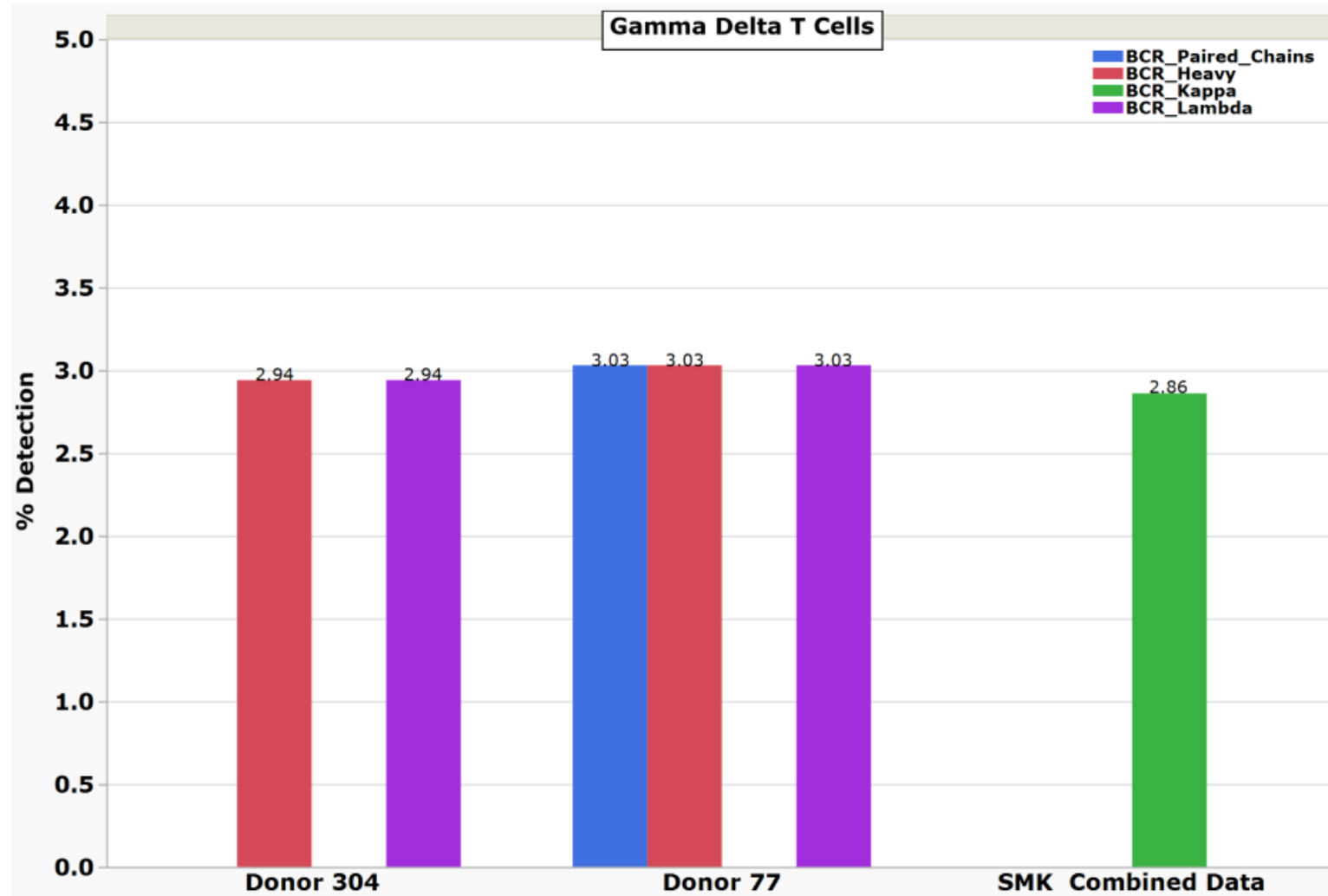
TCR Performance – Sensitivity
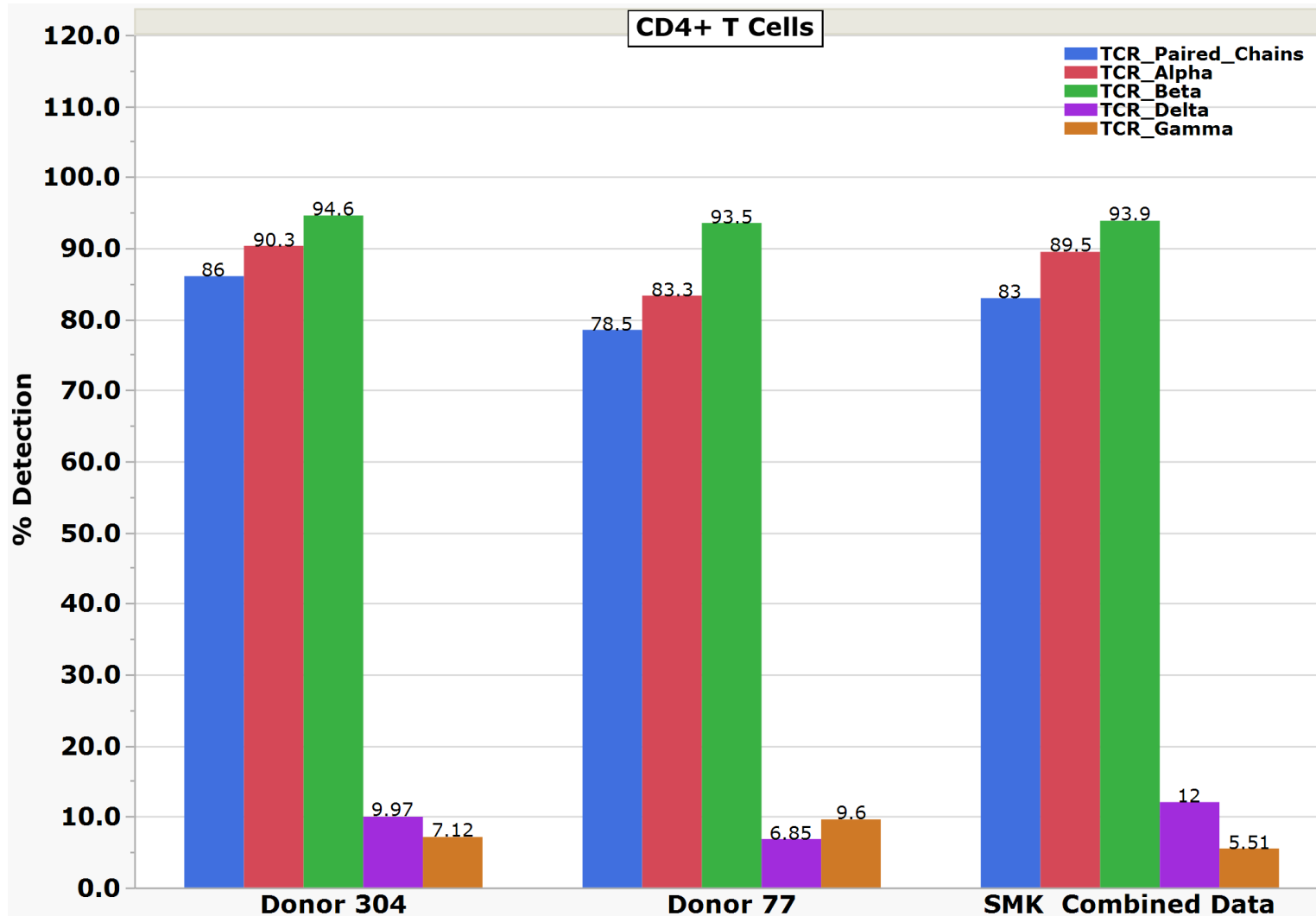
TCR Performance – Sensitivity
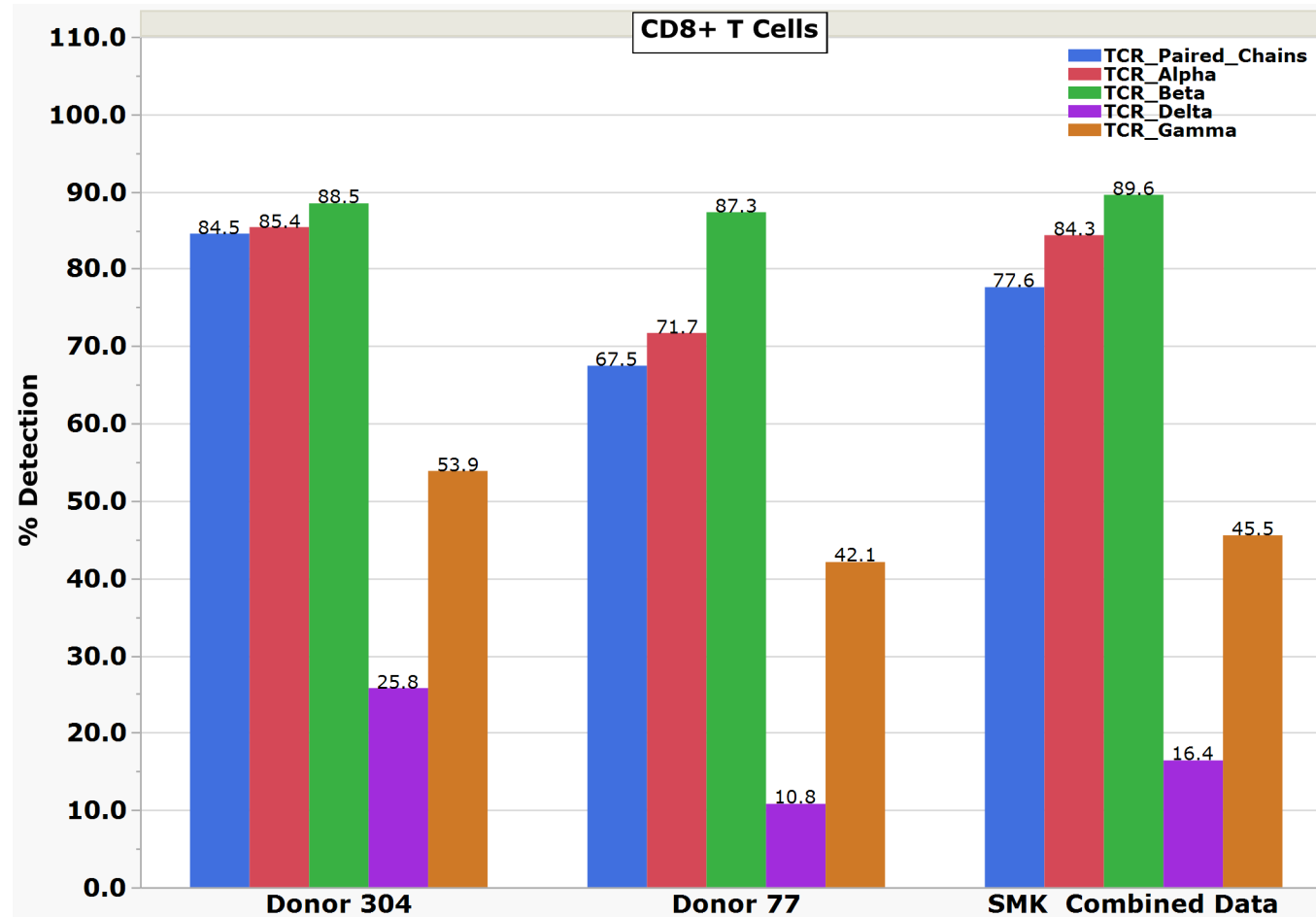
TCR Performance – Sensitivity
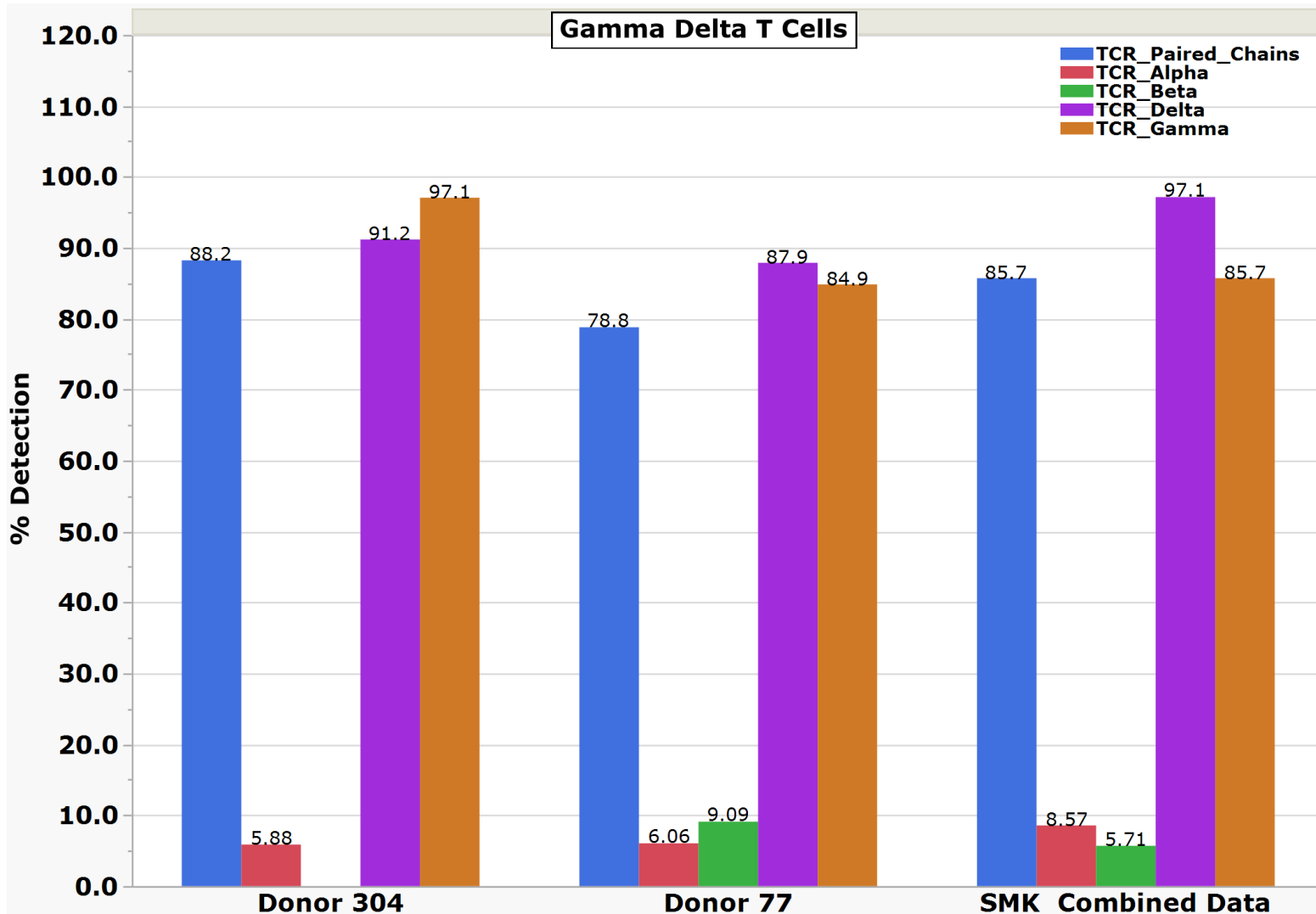
TCR Performance – Specificity
(% non-T cells with TCR CDR3 chain sequences)
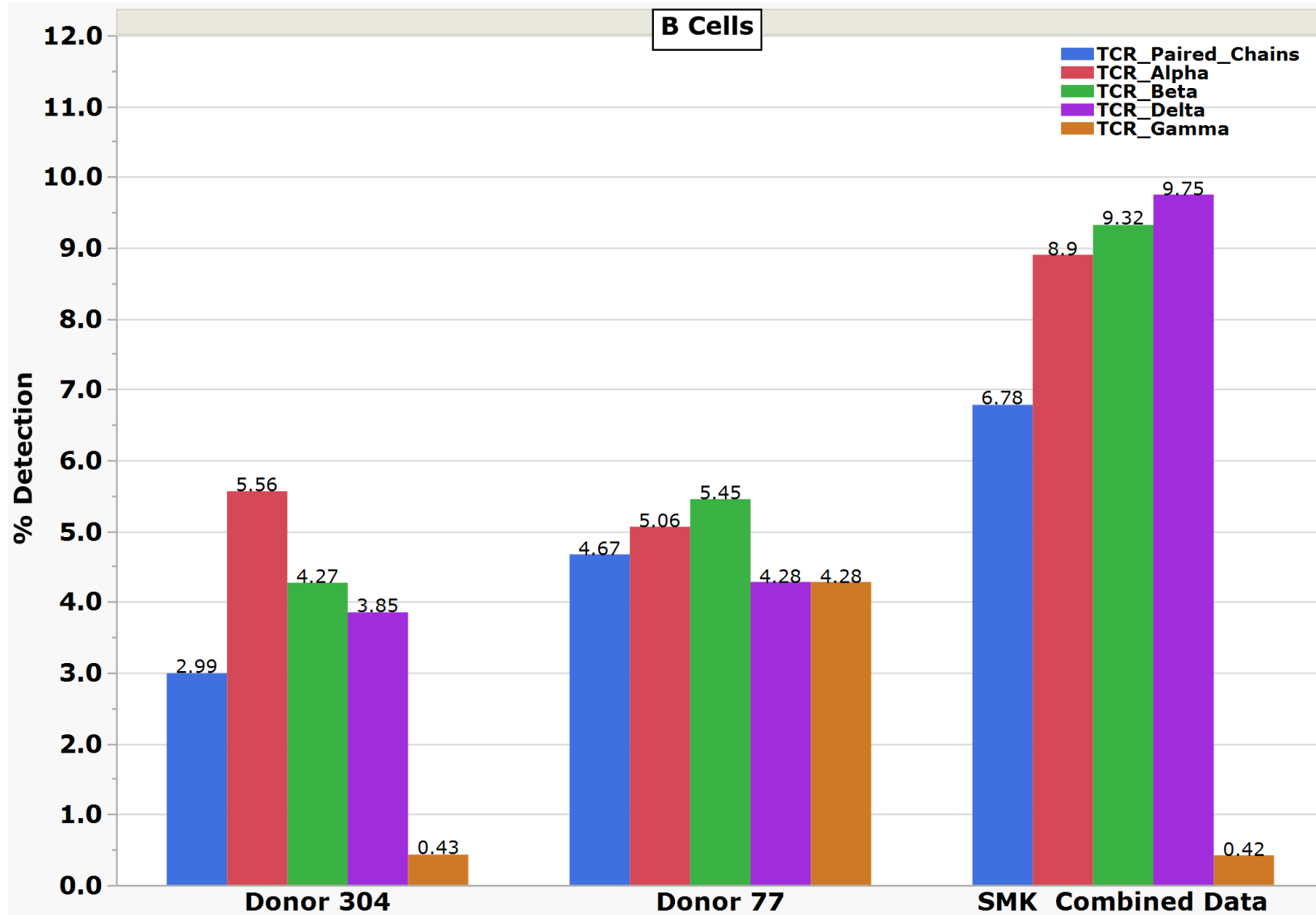
VDJ + BD® AbSeq

An overview of VDJ + AbSeq performance data

BD AbSeq Marker Detection in B Cells
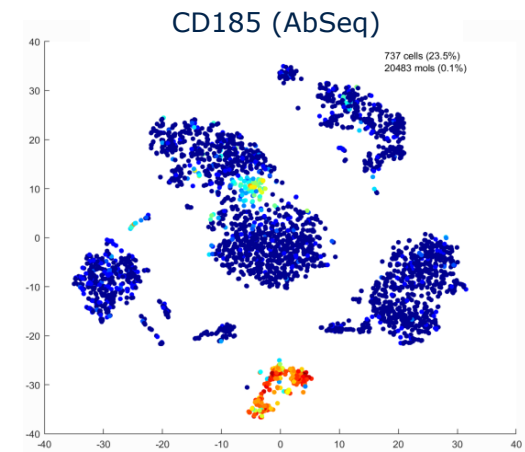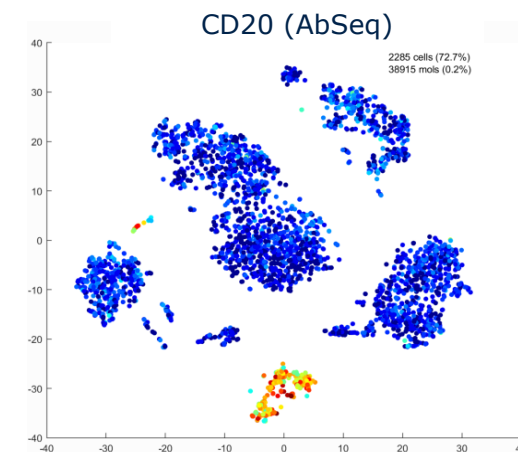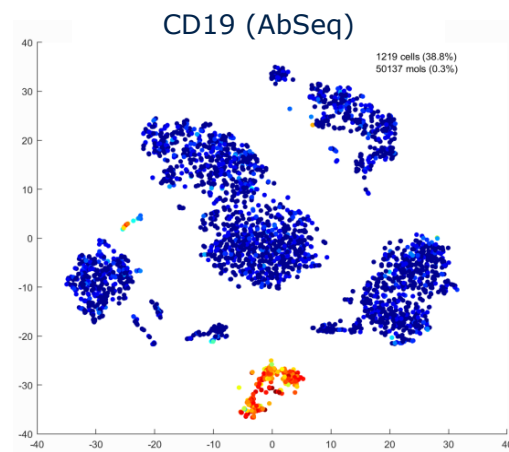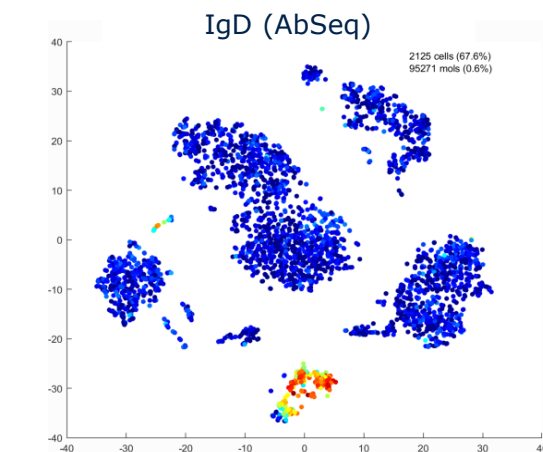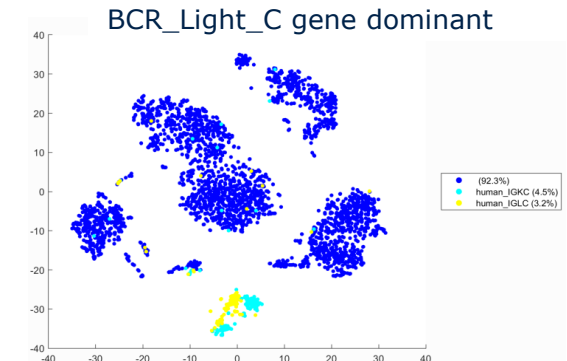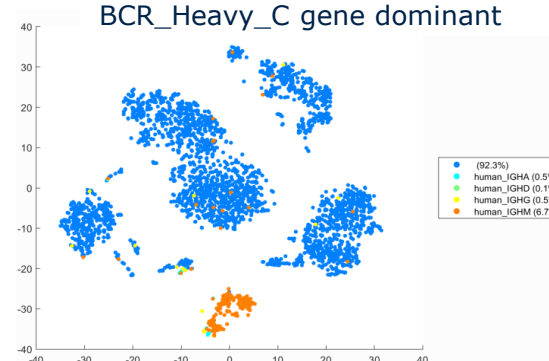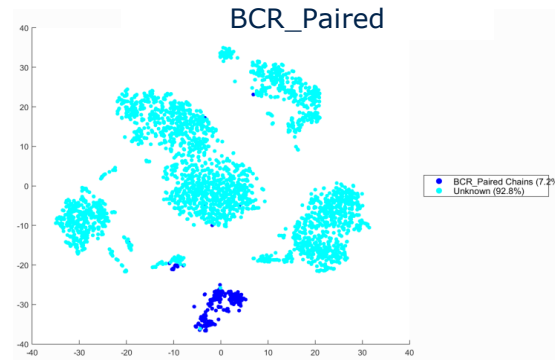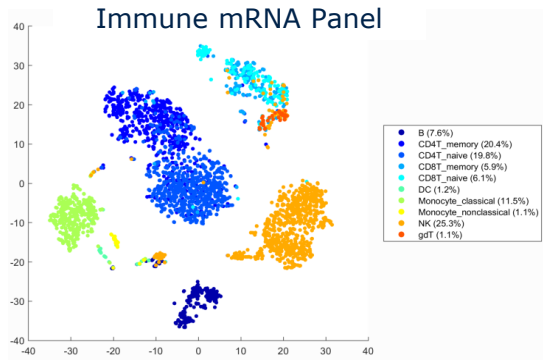
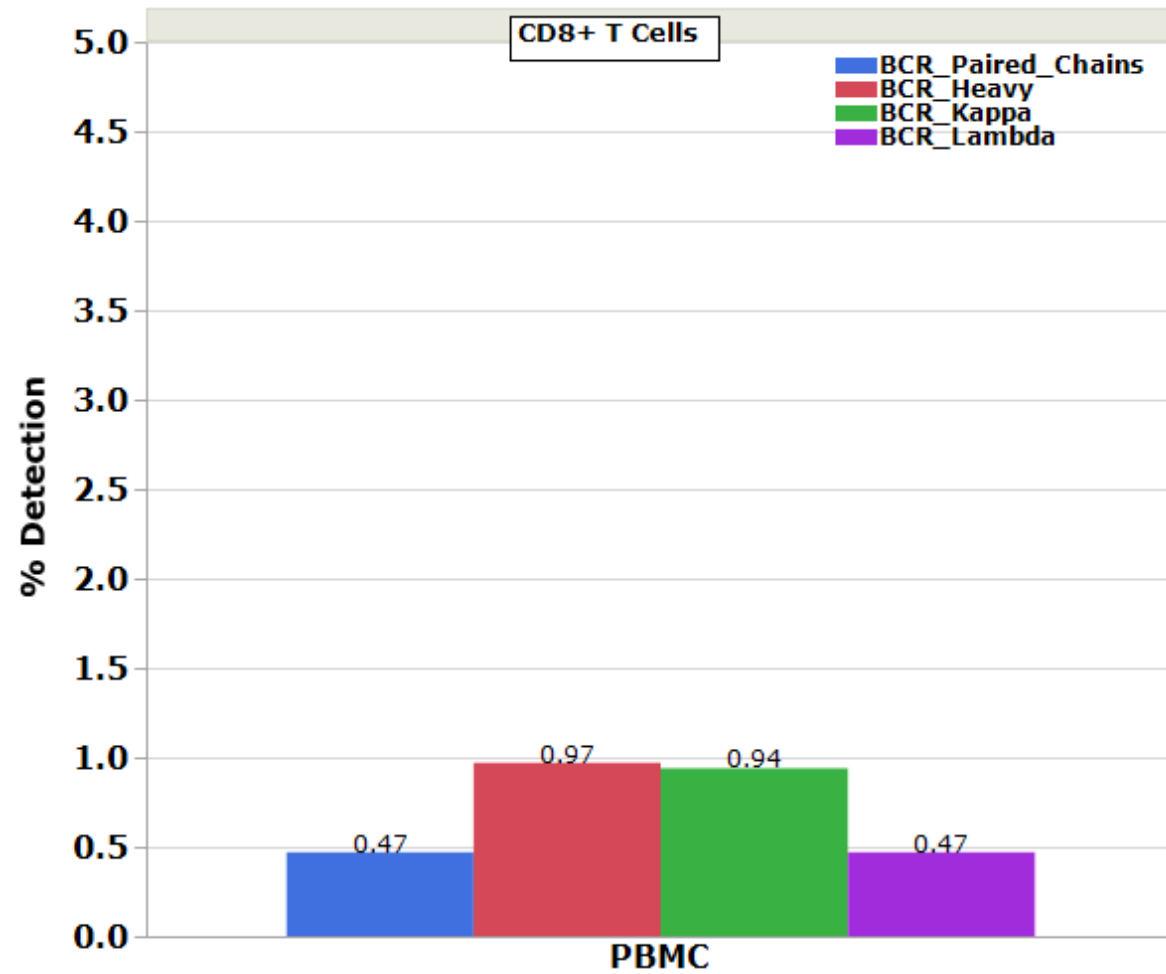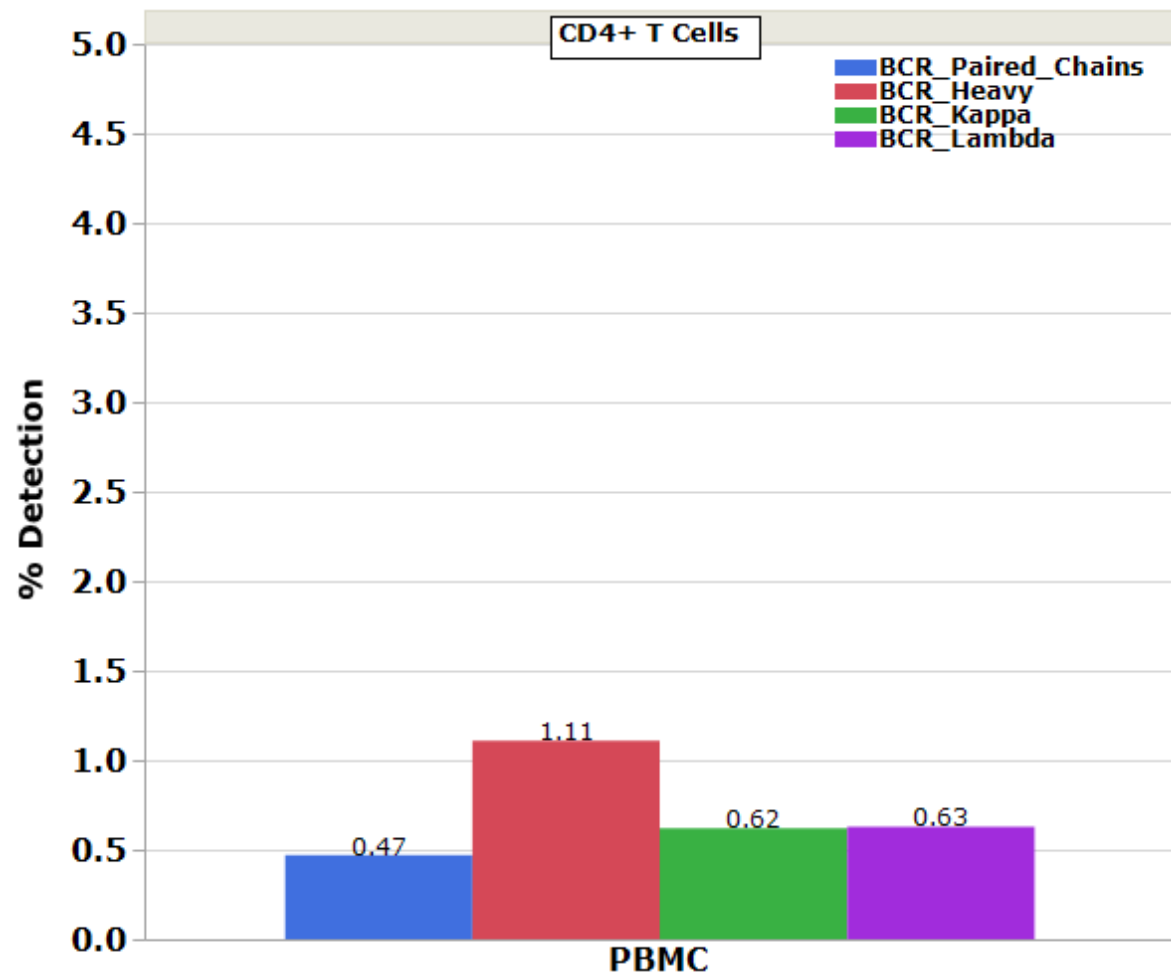# BD AbSeq Marker Detection in T Cells
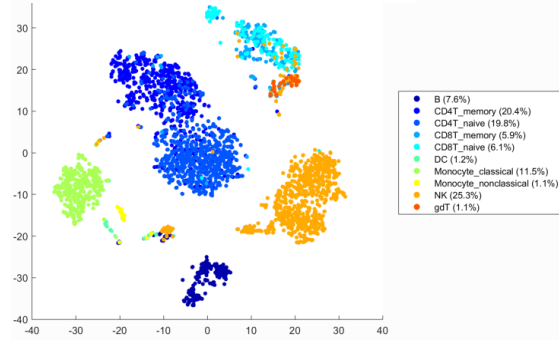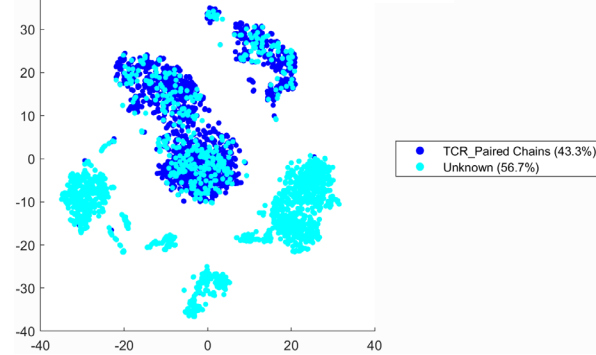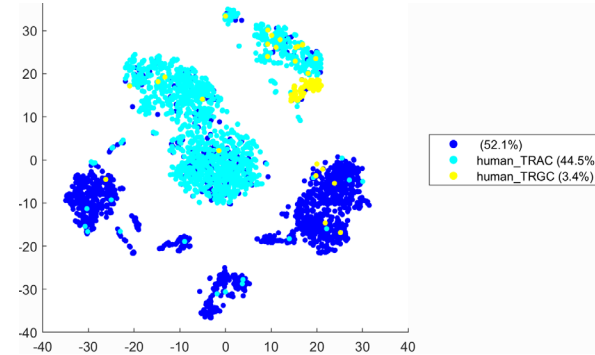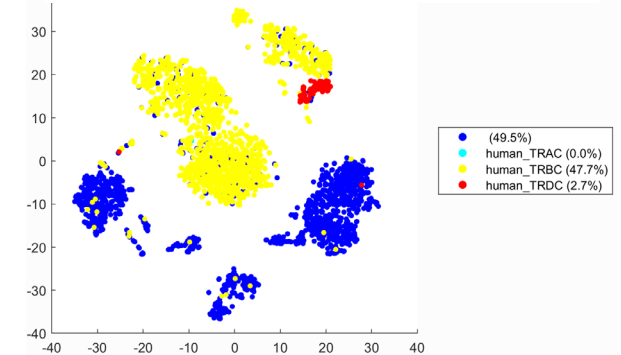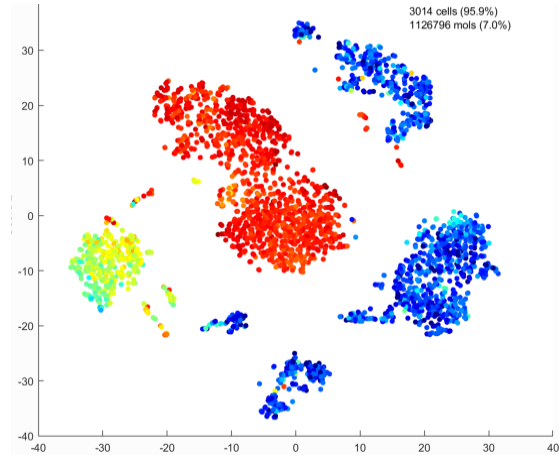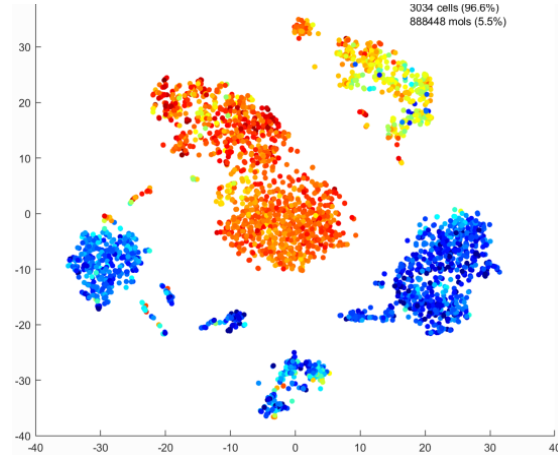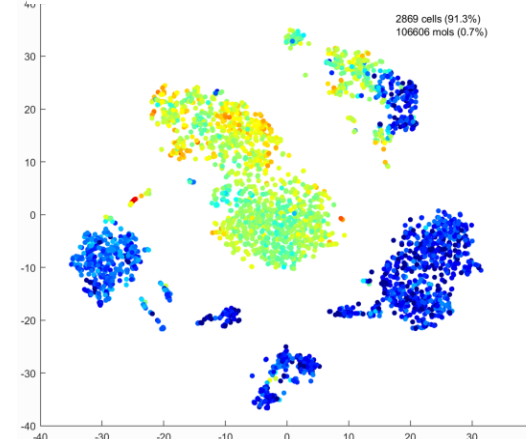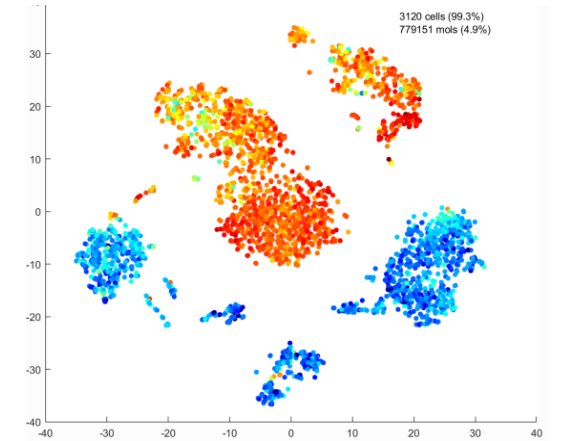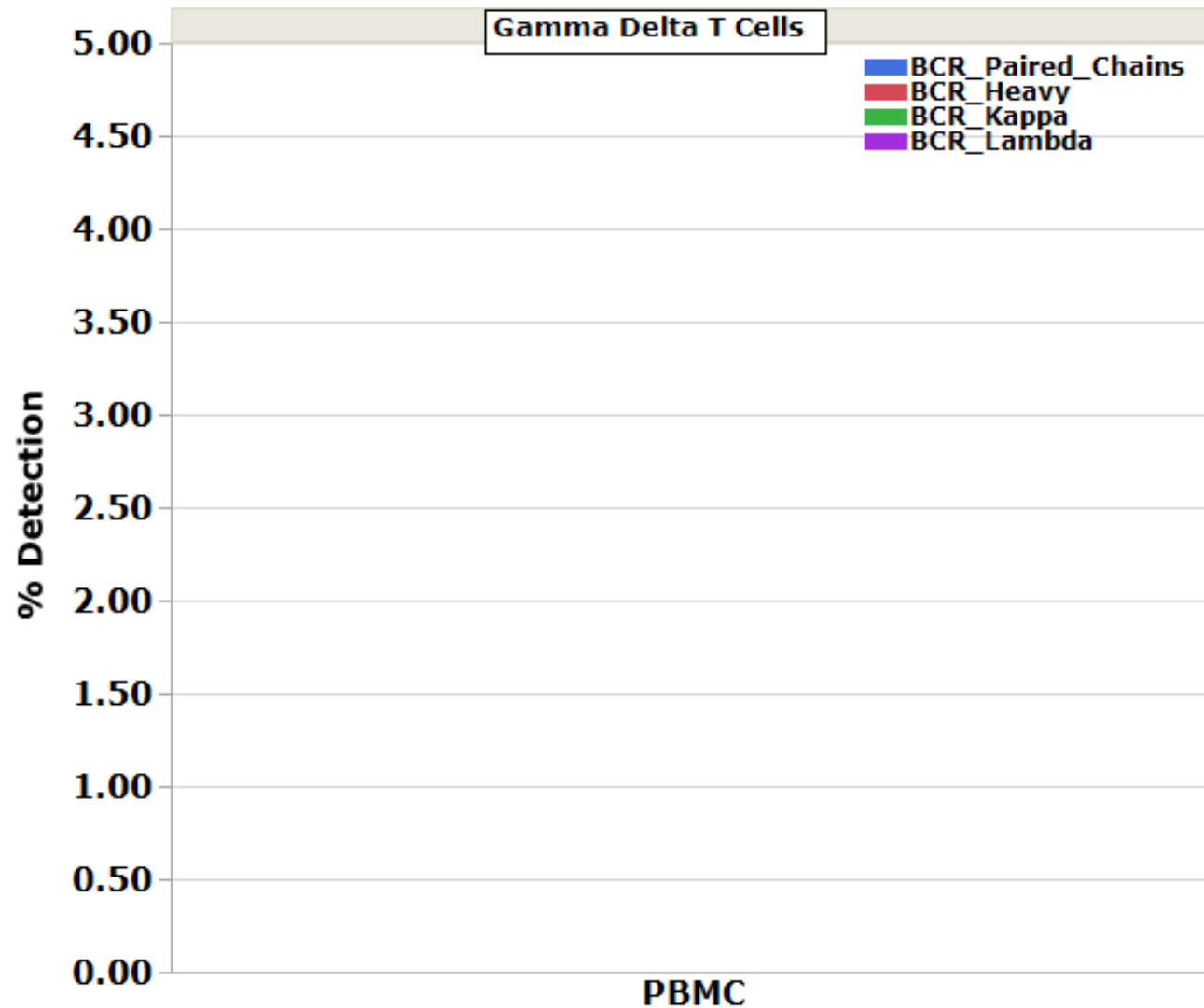
BCR Performance – Sensitivity
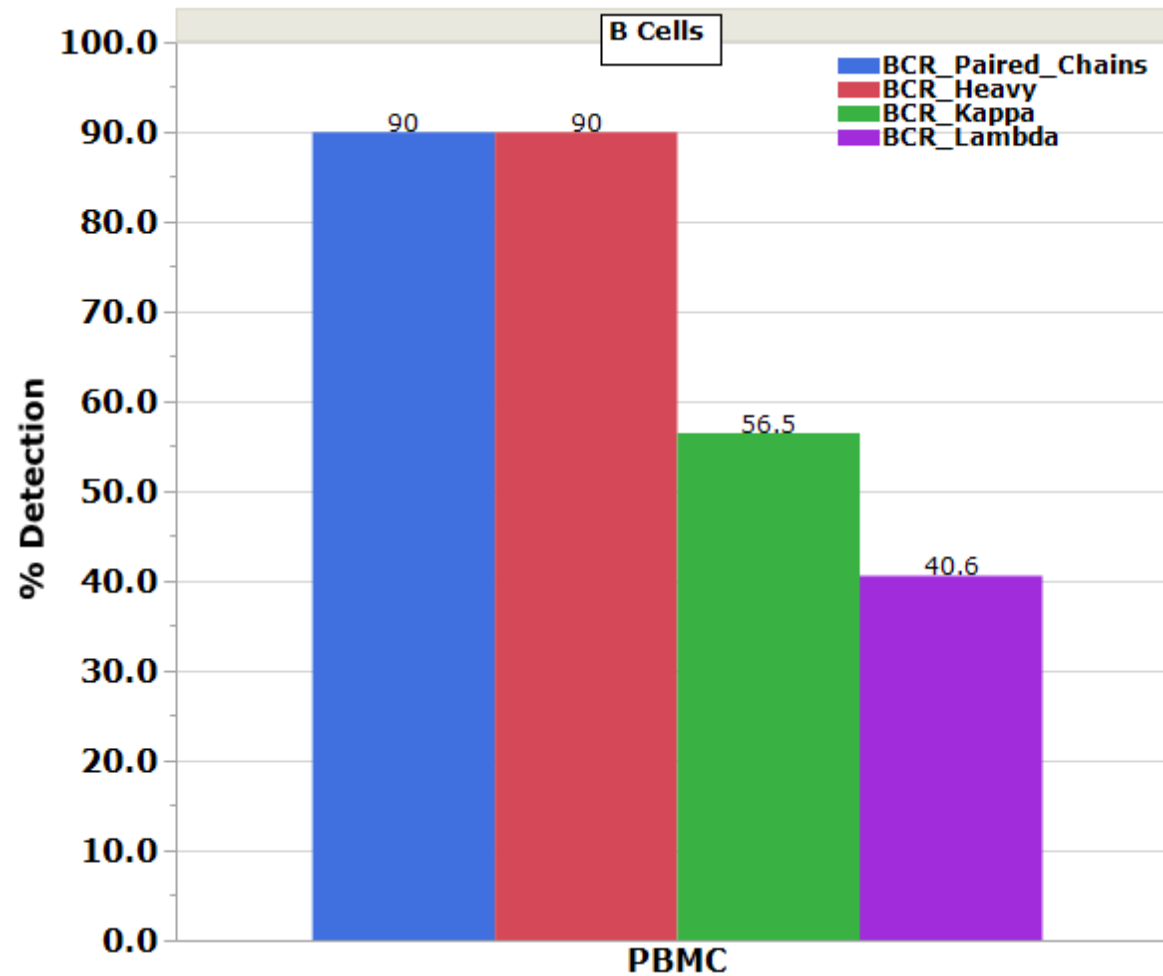
BCR Performance – Specificity
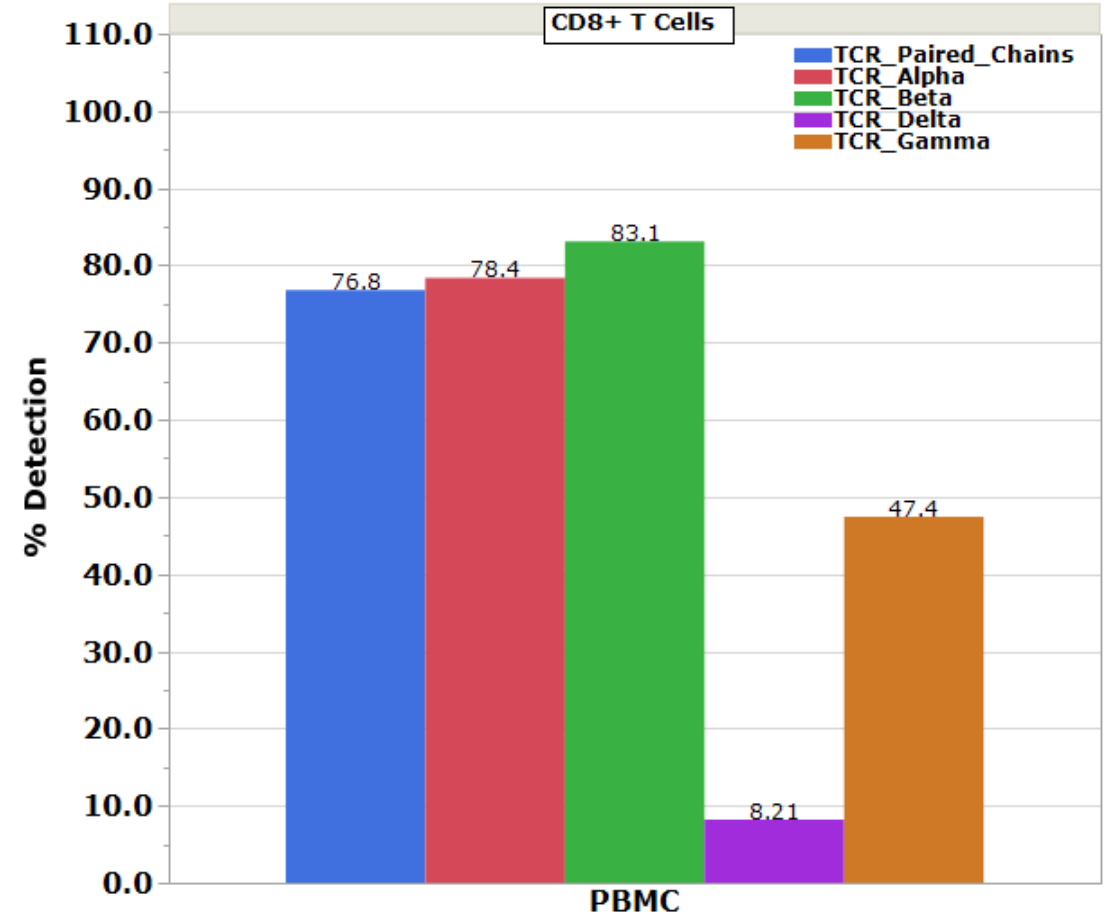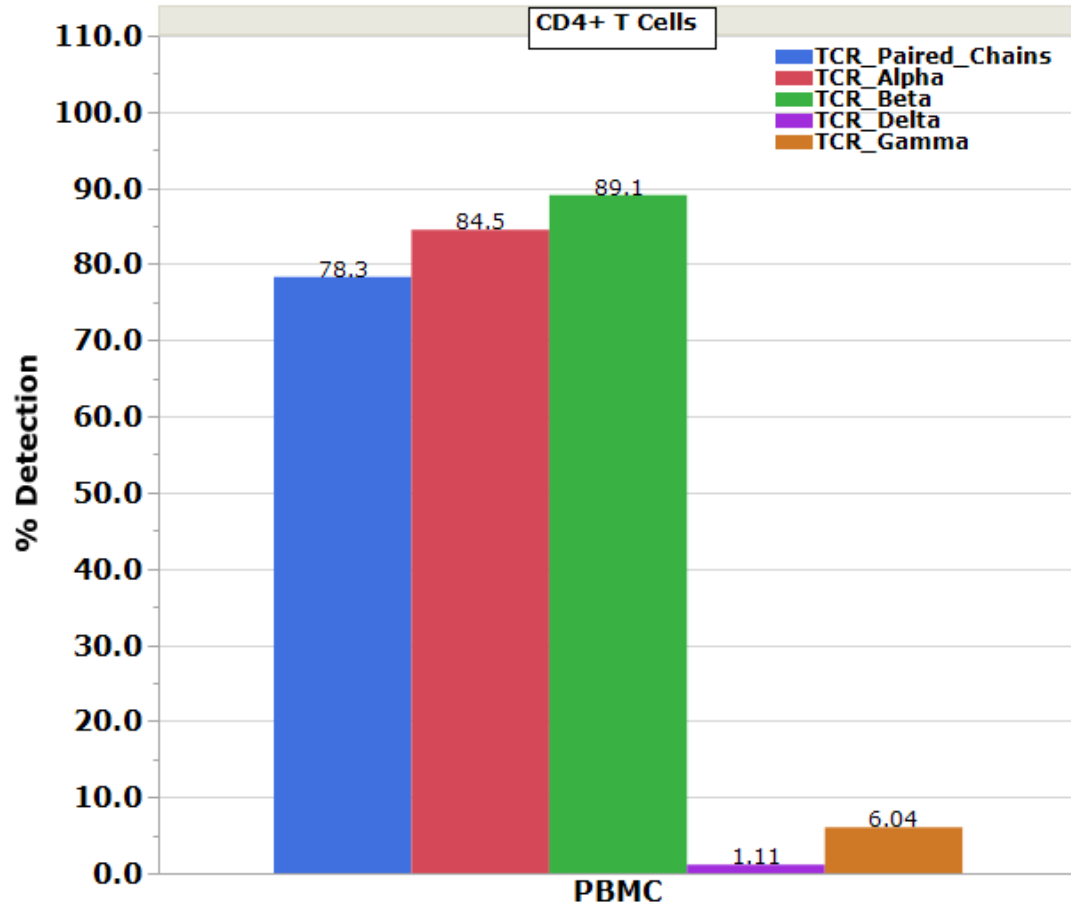## (% non-B cells with BCR CDR3 chain sequences)

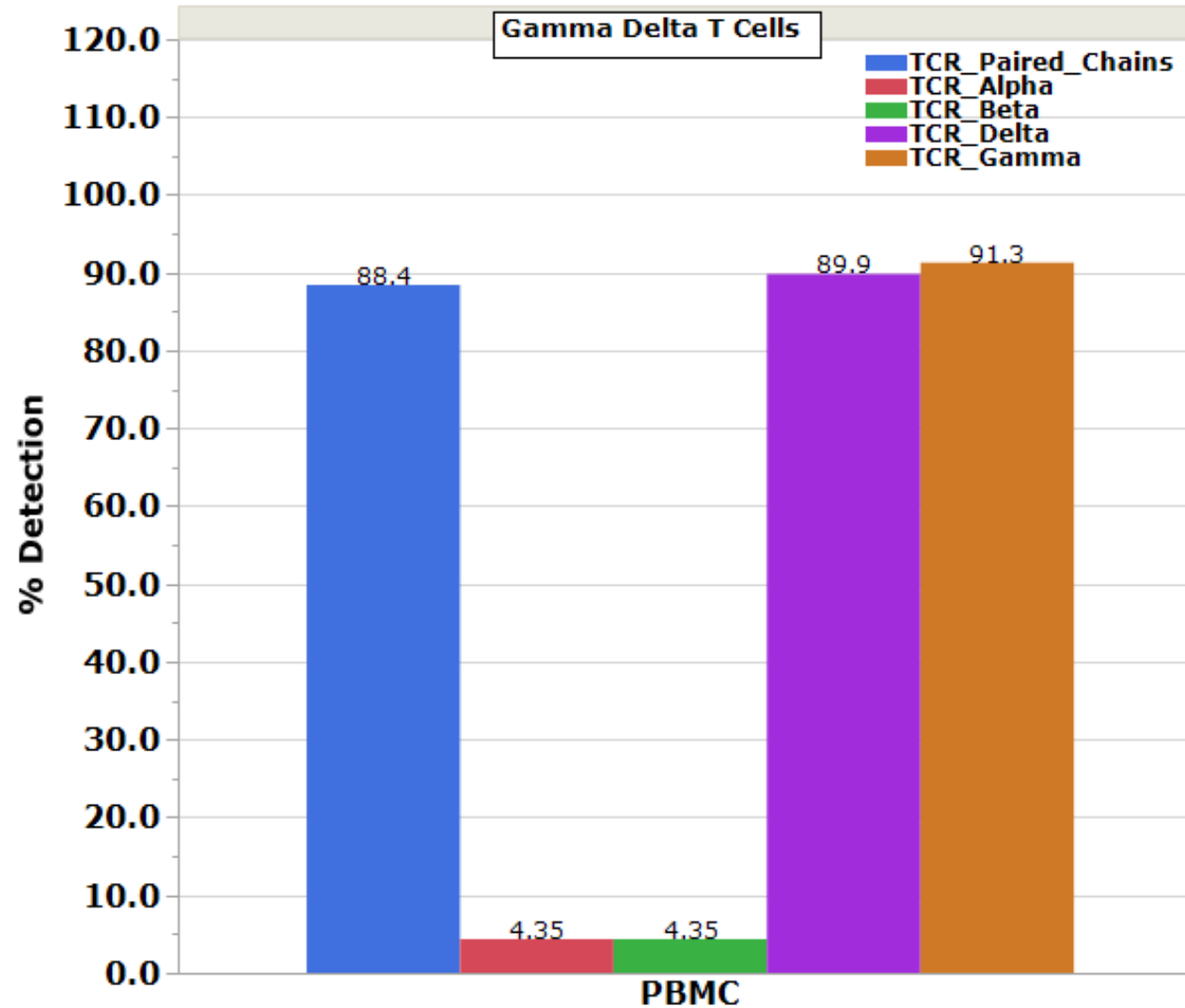# BCR Performance – Specificity
(% non-B cells with BCR CDR3 chain sequences)
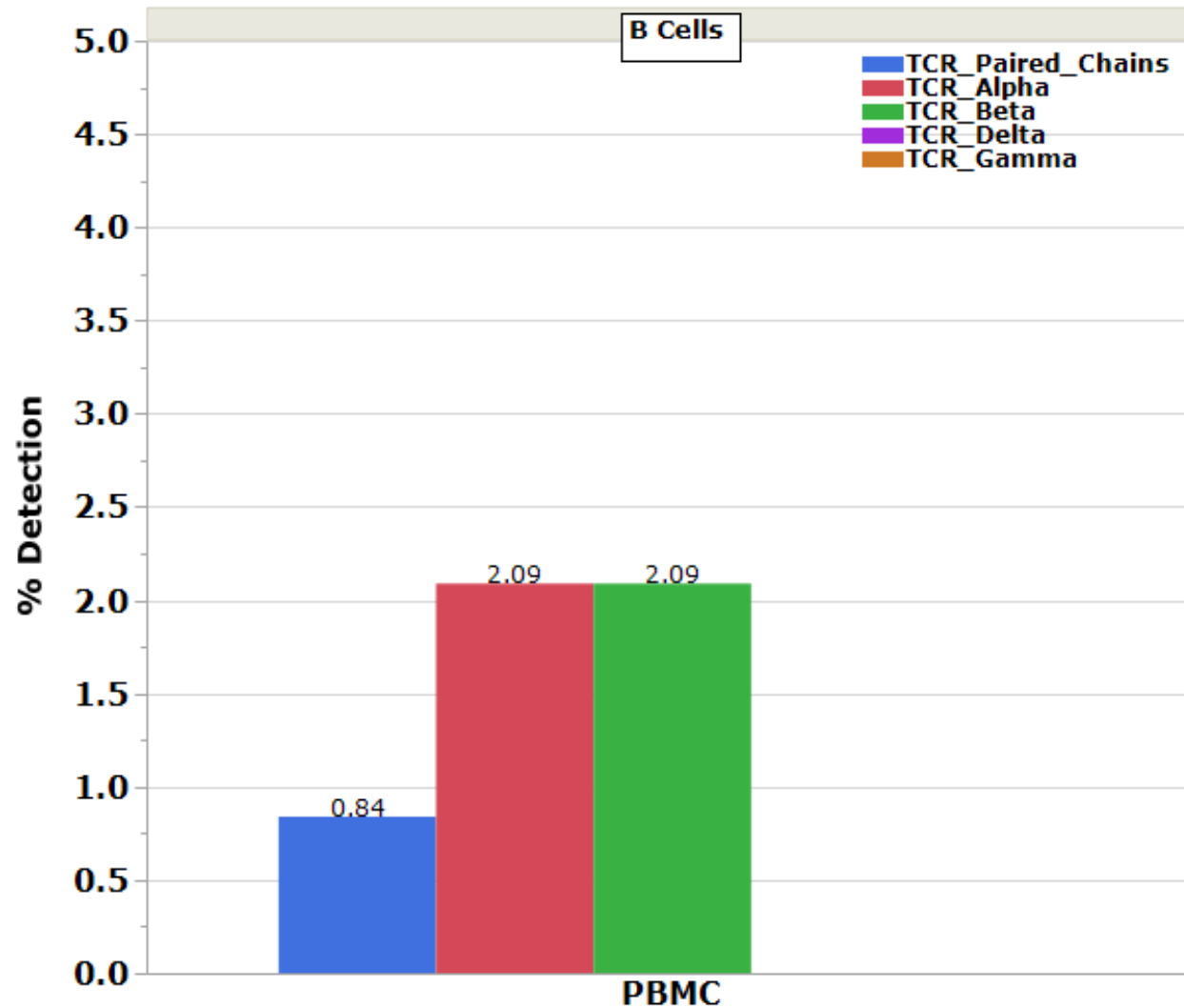
TCR Performance – Sensitivity

TCR Performance – Sensitivity

TCR Performance – Specificity
(% non-T cells with TCR CDR3 chain sequences)

Mouse Primer Panel

TCR Primer Panel

Mouse T cell PCR1 Primers

Primer Names	IDT Primer Stock Conc. (µM)	Vol/Primer Required (µl)	Total Vol of IDTE (µl)	Final Vol (µl)	Sequence
TRAC N1					TTTTCGGCACATTGATTTGGGAG
TRBC N1					CTCAGGCAGTAGCTATAATTGCT
TRDC N1	200	12.5	437.5	500	CAATCTTCTTGGATGATCTGAGACT
TRGC1-TRGC2 N1					GGAAAGAACTTTTCAAGGAGACAAAGG
TRGC4 N1					CATCCTTTTCTTTCCAATACACCC

Mouse T cell PCR2 Primers

TRAC N2					CAGACGTGTGCTCTTCCGATCTAGGTTCTGGGTTCTGGATGT
TRBC N2					CAGACGTGTGCTCTTCCGATCTCAATCTCTGCTTTTGATGGCTC
TRDC N2	200	12.5	437.5	500	CAGACGTGTGCTCTTCCGATCTGTAGAAATCTTTCACCAGACAAGC
TRGC1-TRGC2 N2					CAGACGTGTGCTCTTCCGATCTTTGGGGGAAATGTCTGCA
TRGC4 N2					CAGACGTGTGCTCTTCCGATCTAATAGTAGGCTTGGGAGAAAGTCTG

BCR Primer Panel

Mouse B cell PCR1 Primers

Primer Names	IDT Primer Stock Conc. (µM)	Vol/Primer Required (µl)	Total Vol of IDTE (µl)	Final Vol (µl)	Sequence
IGHA N1					AACTGGCTGCTCATGGTGTA
IGHD N1					AAGTGTGGTTGAGGTTCAGTTCTG
IGHE N1					GAAGTTCACAGTGCTCATGTTC
IGHG1 N1					CAGAGTGTAGAGGTCAGACT
IGHG2A-IGHG2C N1					TCGAGGTTACAGTCACTGAG
IGHG2B N1	200	7.5	442.5	525	GATCCAGAGTTCCAAGTCACAG
IGHG3 N1					TACGTTGCAGATGACAGTCT
IGHM N1					TGGATGACTTCAGTGTTGTTCTG
IGKC N1					TGTAGGTGCTGTCTTTGCTG
IGLC1 N1					CTGTAACTGCTATGCCTTTCCC
IGLC2-IGLC3 N1					TTGGTGGGATTTGAAGTGTCC

Mouse B cell PCR2 Primers

Primer Names	IDT Primer Stock Conc. (µM)	Vol/Primer Required (µl)	Total Vol of IDTE (µl)	Final Vol (µl)	Sequence
IGHA N2					CAGACGTGTGCTCTTCCGATCTTGTCAGTGGGTAGATGGTGG
IGHD N2					CAGACGTGTGCTCTTCCGATCTCTGACTTCCAATTACTAAACAGCC
IGHE N2					CAGACGTGTGCTCTTCCGATCTTAGAGCTGAGGGTTCCTGATAG
IGHG1 N2					CAGACGTGTGCTCTTCCGATCTCAGTGGATAGACAGATGGGGGT
IGHG2A-IGHG2C N2					CAGACGTGTGCTCTTCCGATCTATGGGGCTGTTGTTTTGG
IGHG2B N2	200	39.0	91.0	520	CAGACGTGTGCTCTTCCGATCTGTGGATAGACTGATGGGGGTGTT
IGHG3 N2					CAGACGTGTGCTCTTCCGATCTAGGGAAGTAGCCTTTGACAAG
IGHM N2					CAGACGTGTGCTCTTCCGATCTGACATTTGGGAAGGACTGACTC
IGKC N2					CAGACGTGTGCTCTTCCGATCTAGATGTTAACTGCTCACTGGATG
IGLC1 N2					CAGACGTGTGCTCTTCCGATCTGTTAGTCTCGAGCTCTTCAGA
IGLC2-IGLC3 N2					CAGACGTGTGCTCTTCCGATCTCAGTGTGGCTTTGTTTTCCT