Benchmarking of T-cell receptor repertoire profiling methods reveals large systematic biases

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07/12/2020 – AIRR-C meeting

Biological resources WG pre-meeting

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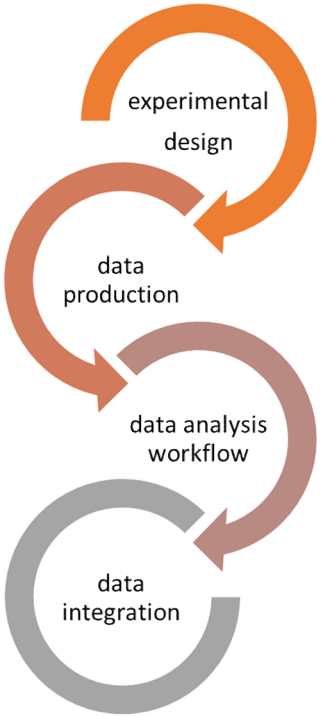
Next generation sequencing for AIRR study

Quantitative description of rearrangement sequences



- T-cell sorting **or** total T cell
- Tissue, organ **or** blood
- DNA **or** RNA

- Appropriate method to address the question
  - TCR RepSeq method *Rosati et al. 2017; Barennes et al. 2020*

- Alignment tools *Bolotin et al 2015 ; Gerritsen et al. 2016*
- Statistical analysis workflow *Shugay et al. 2015; Nazarov et al. 2015; Greiff et al, 2015;*
- Statistical modelling *Mariotti-Ferrandiz et al. 2016; Chaara et al. FI, 2017; Miho et al, 2019 ; Greiff et al, 2020*

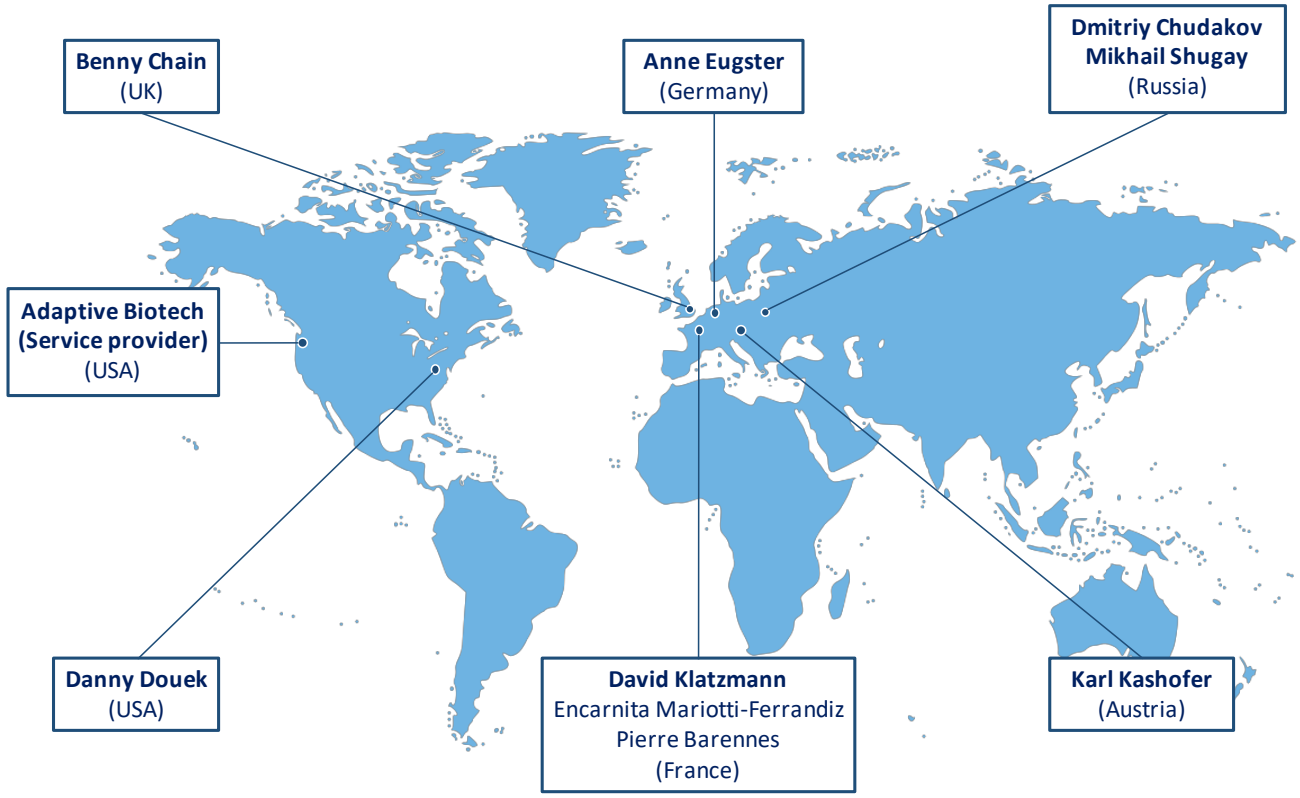
- TCR data sharing
- Standards from AIRR community *Rubelt et al. 2017; Breden et al. 2017*

Boudinot, P et al (2008) Molecular Immunology
Six, A et al. (2013) Frontiers in Immunology

Available methods

	template nature	*Amplification method*	*Applications*
Academic labs	RNA	RACE-PCR	bulk & single cell
	RNA/gDNA	Multiplex PCR VC/VJ	bulk & single cell
	RNA/gDNA	Multiplex-PCR VC/VJ	bulk
	gDNA	Multiplex-PCR VJ	bulk
	RNA	RACE-PCR	bulk
	RNA	RACE-PCR	Single cell
	RNA	RNASeq	Single cell
	RNA	RACE-PCR	Single cell

International collaboration



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ANALYSIS
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Check for updates

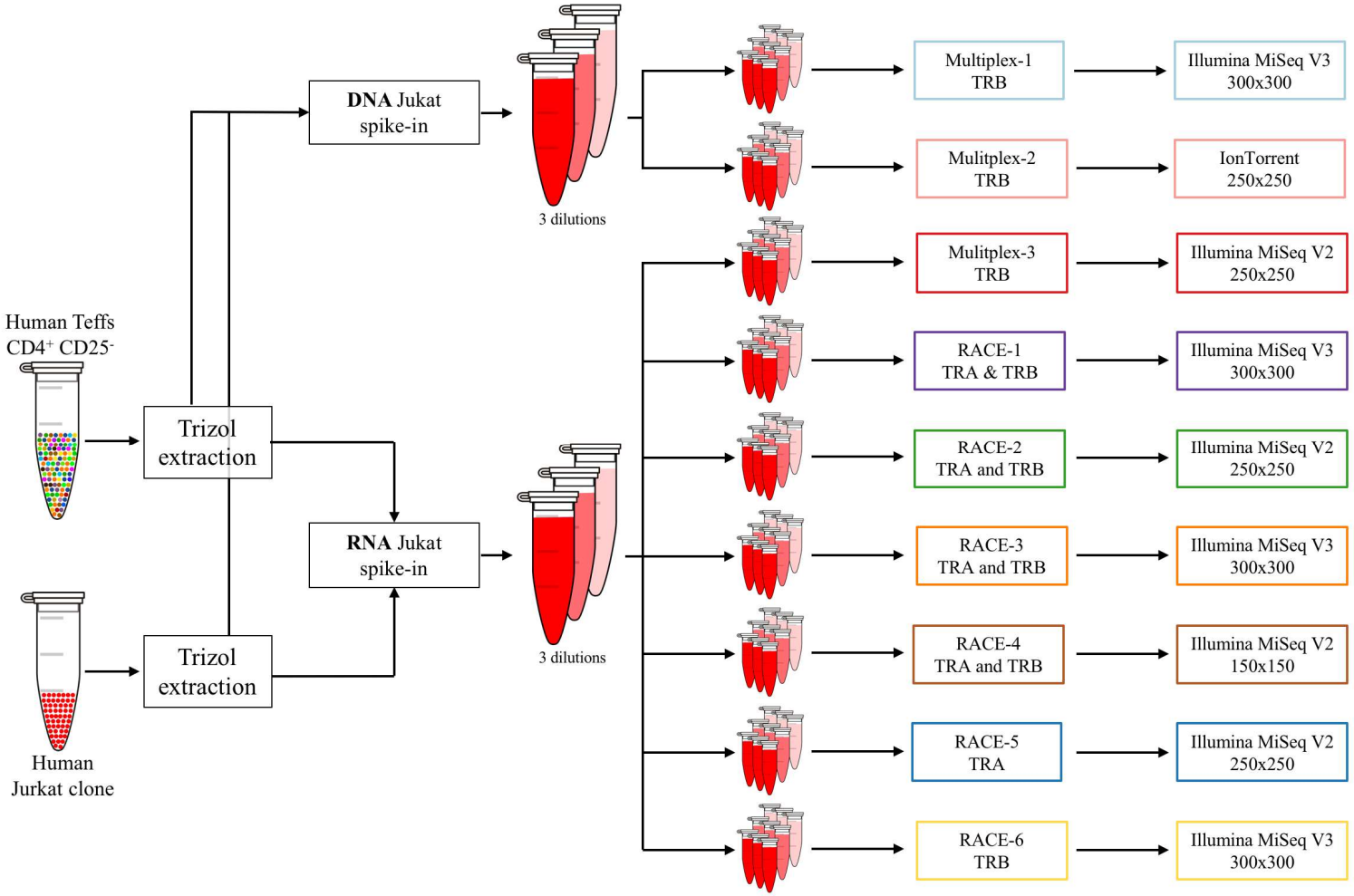
Benchmarking of T cell receptor repertoire profiling methods reveals large systematic biases

Pierre Barennes[1,2], Valentin Quiniou[1,2], Mikhail Shugay[3,4,5], Evgeniy S. Egorov[4], Alexey N. Davydov[6], Dmitriy M. Chudakov[3,4,5,6], Imran Uddin[7], Mazlina Ismail[7], Theres Oakes[7], Benny Chain[7], Anne Eugster[8], Karl Kashofer[9], Peter P. Rainer[10,11], Samuel Darko[12], Amy Ransier[12], Daniel C. Douek[12], David Klatzmann[1,2] and Encarnita Mariotti-Ferrandiz[1,2]✉

Pierre Barennes
PhD student

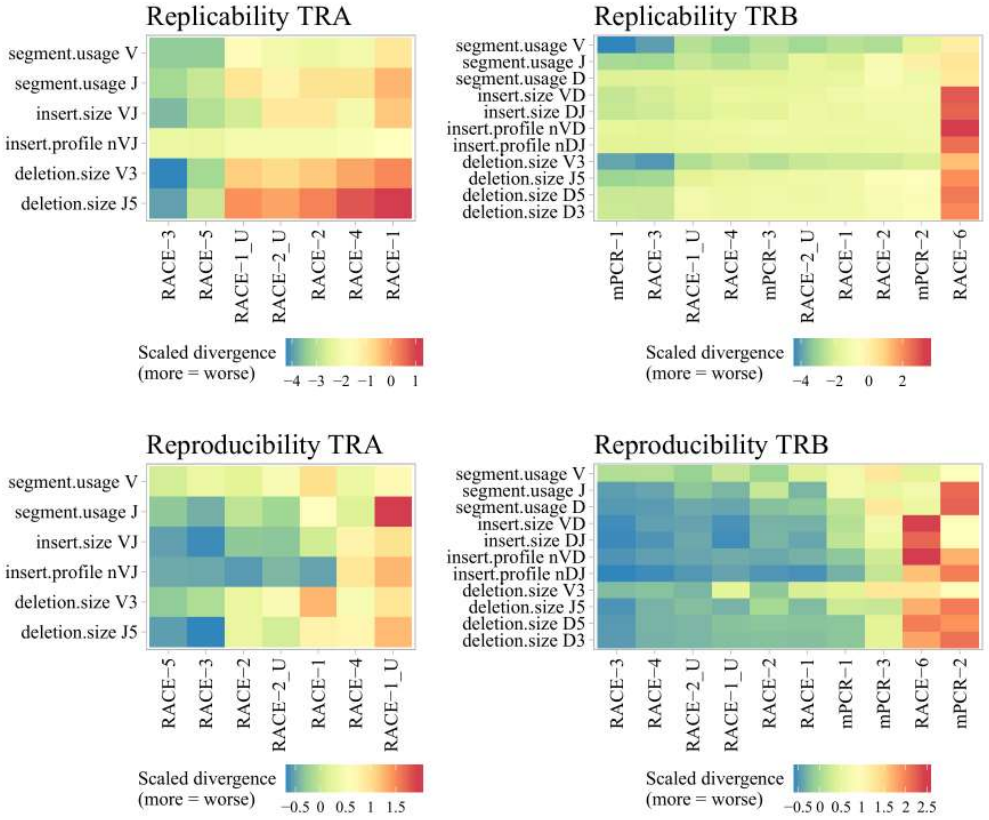
- Most used methods (publications)
- Voluntary based (challenge their method)
  - Academic labs
  - Commercially available kits
    - Service providers
    - Reagent providers

Experimental design



VDJ rearrangement

Replicability : similarity among data within the same method
Reproducibility : similarity among data between different methods

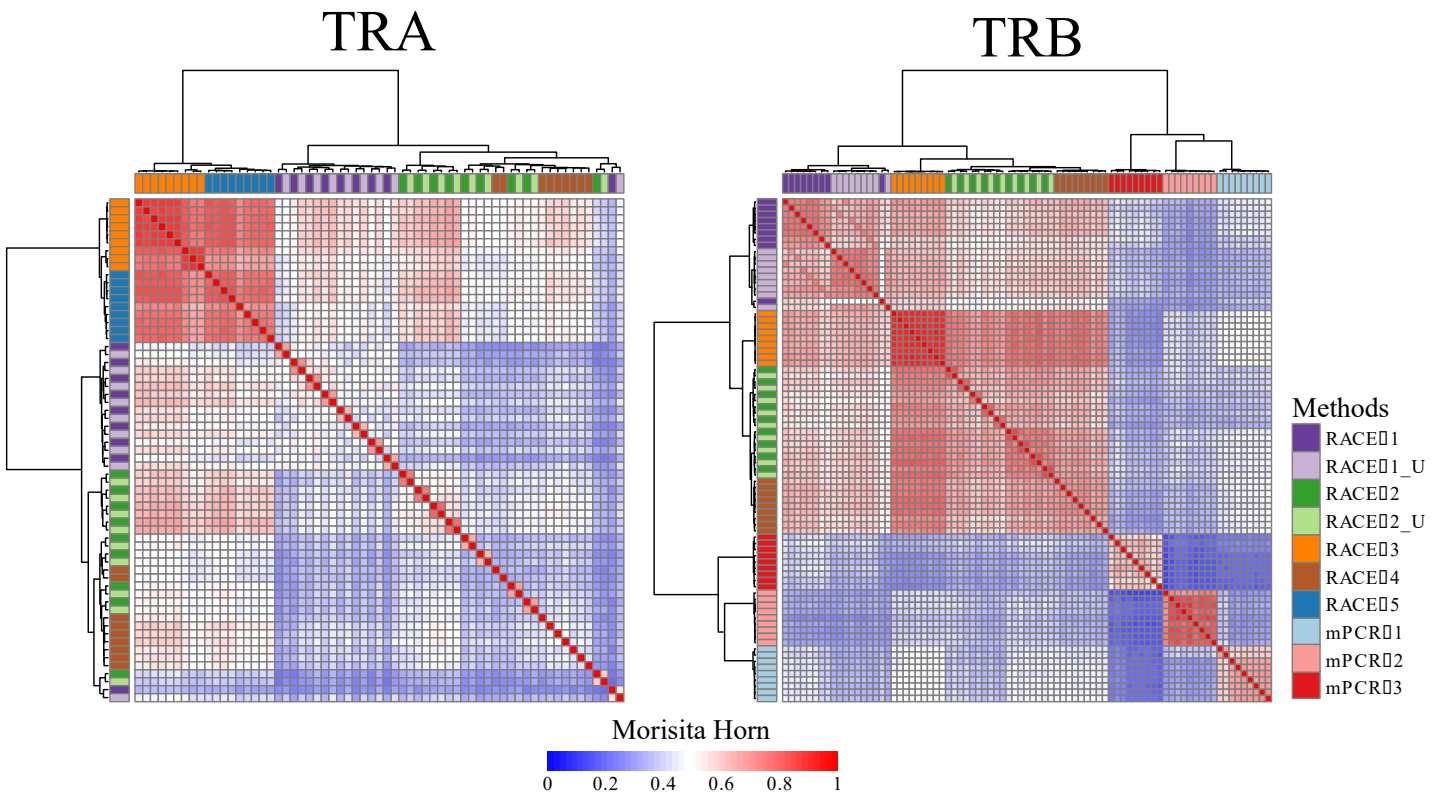


Methods differ between each other

- **basic parameters of the TCR repertoire**
- **inter-sample replicability and reproducibility**

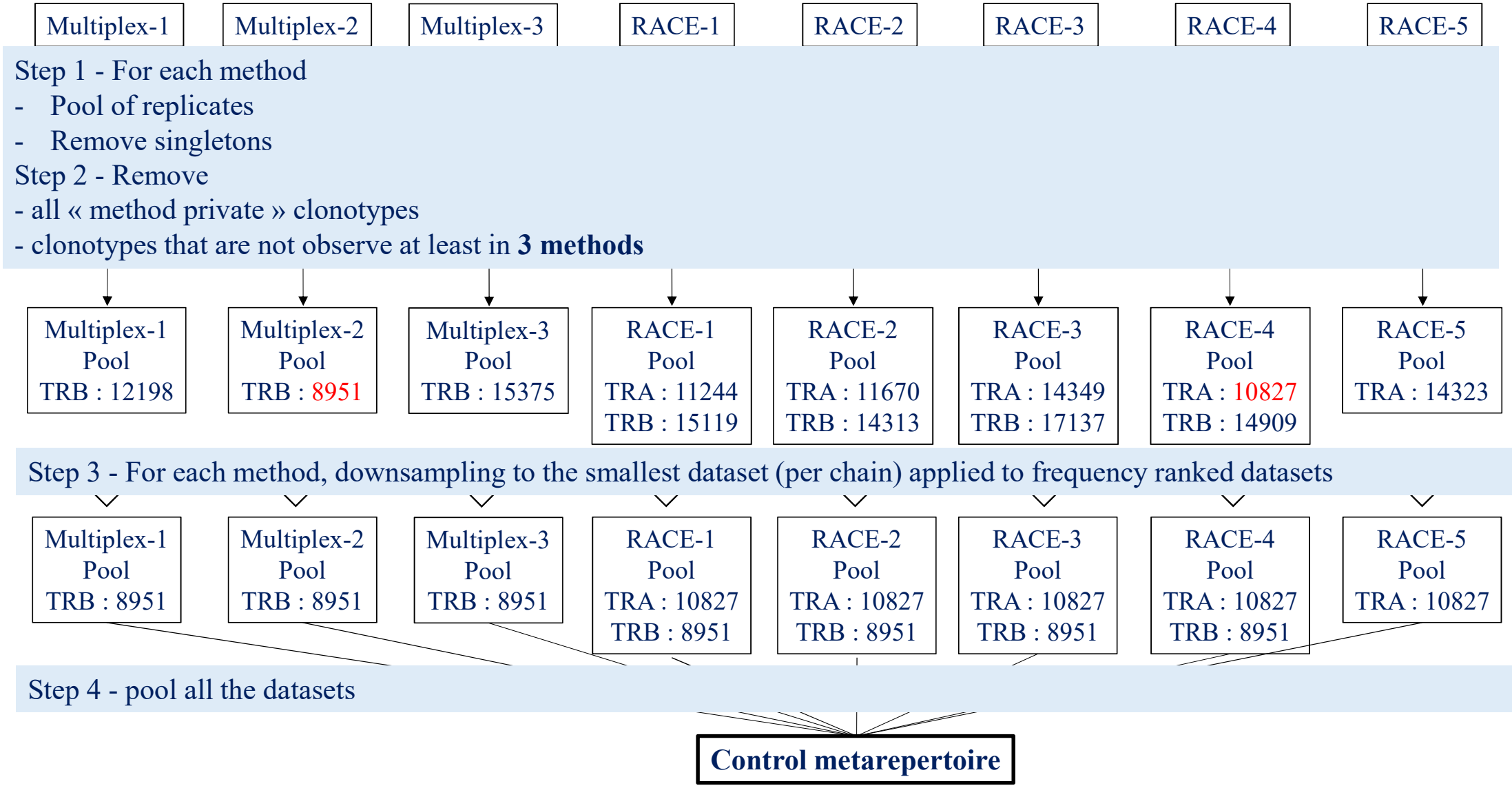
Columns are sorted according to the mean scaled distance (averaged over all rows) from the lowest (best replicability/reproducibility) to the highest (worst replicability/reproducibility)

Rearrangement similarity



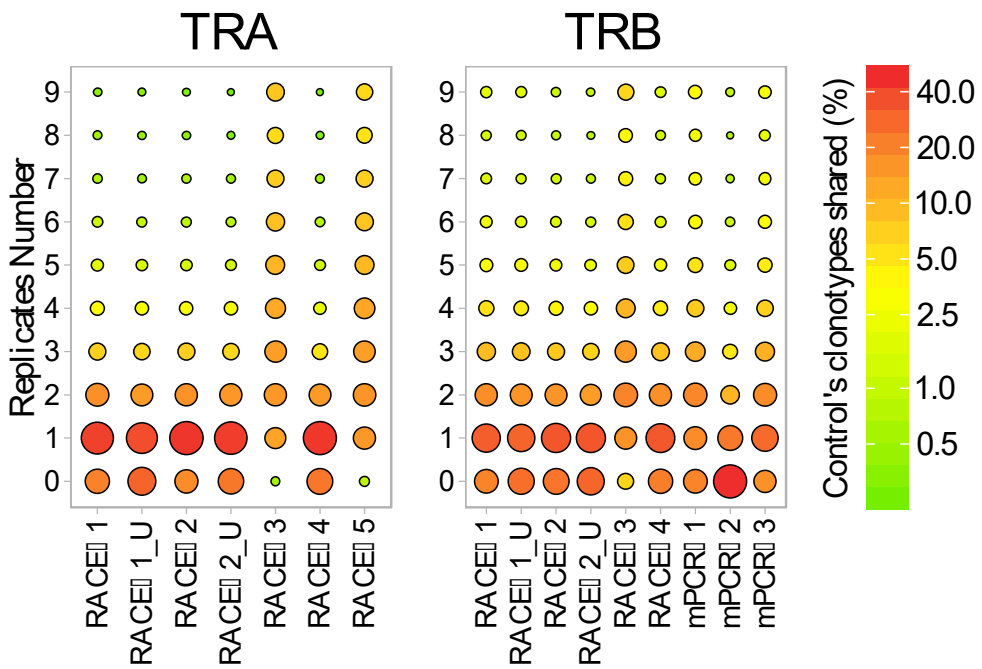
- In general, higher intra-method replicability for TRB than TRA chain, except RACE-3
- Major differences between 5'RACE and mPCR at the level of clonotype composition (same results using Jaccard distance)

Metarepertoire control to assess sensitivity & reproducibility



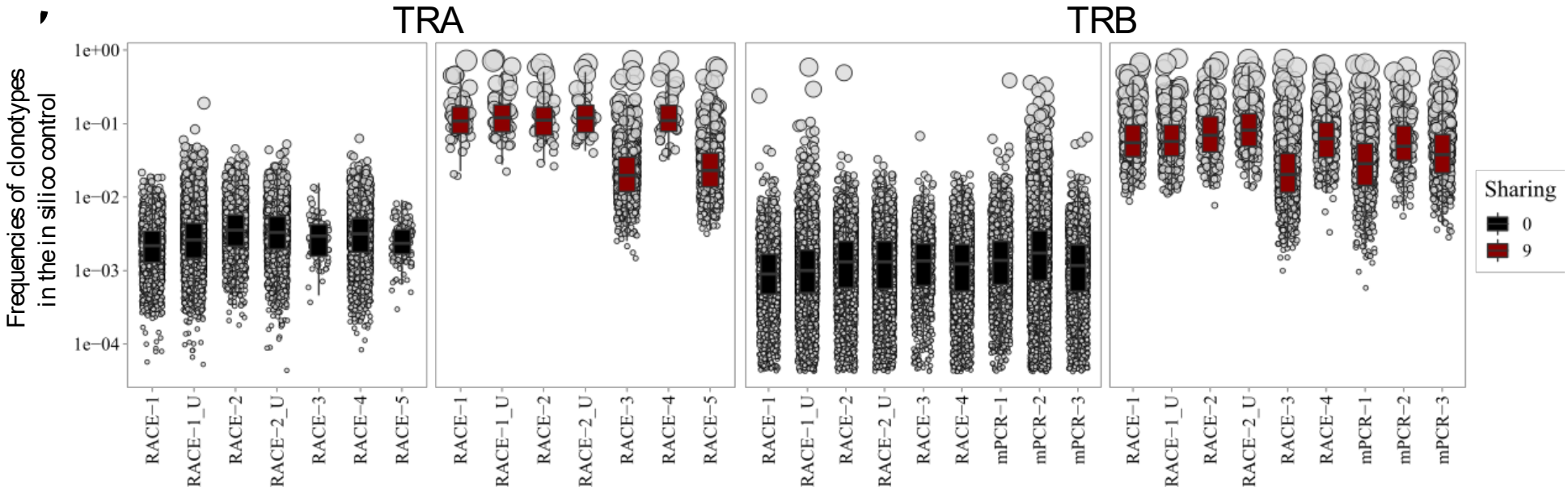
Replicability

% of clonotype from the metarepertoire control (MRC) found per method and per number of replicates (sharing)



- for TRA, RACE-3 and RACE-5 out-performed the other tested methods
  - capturing up to 20% of the MRCs in all 9 replicates (replicate number = 9)
  - missing (replicate number = 0) less than 1% of the MRCs
- for TRB, much less differences between the methods

Sensitivity



- RACE-3 and RACE-5 sensitivity are higher compared to all other methods

→ detect a larger proportion of clonotypes at lower abundances.

→ more obvious for TRA than for TRB

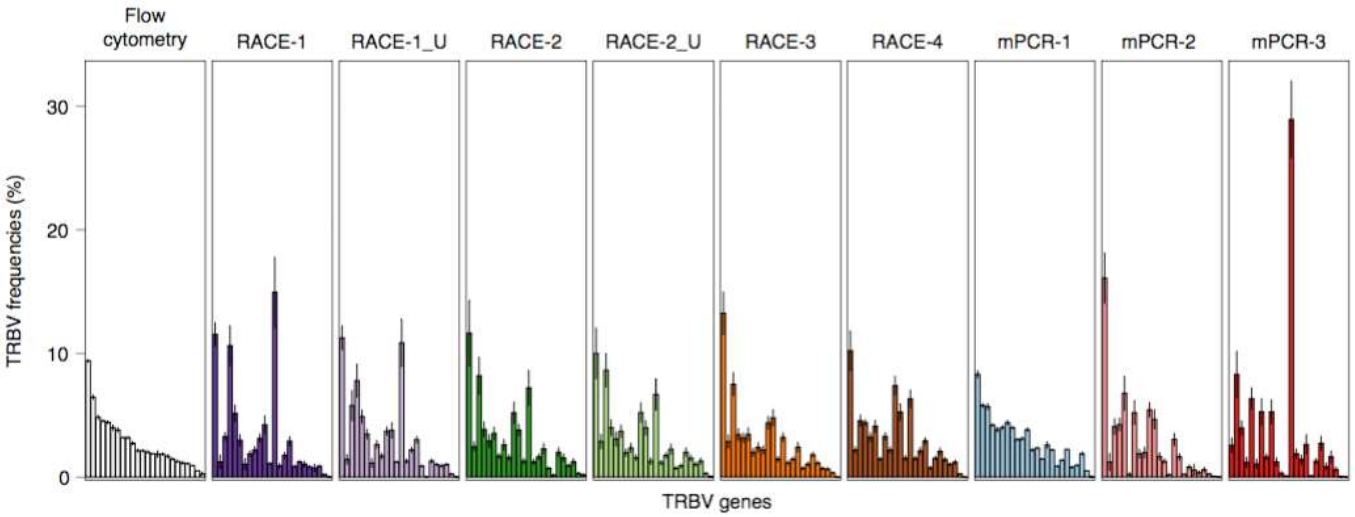
→ the other methods compared similarly between each other

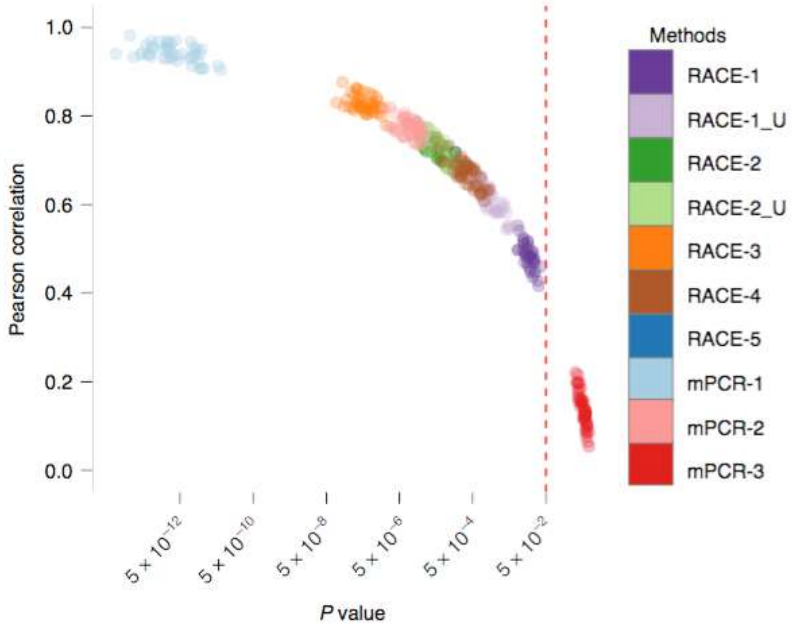
Summary

Table 1 | Comparative performance of the nine TCRseq molecular methods

TR chain	Method	Replicability	Reliability	Sensitivity	Cost per sample ($)	Controls and standards	Format type	fastq data availability
TRA	RACE-1	7	4	4	~230	-	Lab protocol	Yes
	RACE-1_U	4	5	4	~230	UMI	Lab protocol	Yes
	RACE-2	5	4	5	230–280	-	Service or kit	Yes
	RACE-2_U	4	5	5	230–280	UMI	Service or kit	Yes
	RACE-3	3	2	3	~150	-	Kit	Yes
	RACE-4	5	6	4	~150	-	Lab protocol	Yes
	RACE-5	2	3	3	~300	-	Lab protocol	Yes
TRB	mPCR-1	3	3	3	~350–550[a]	Synthetic TCRs	Service or kit	No
	mPCR-2	6	7	7	~25	-	Lab protocol	Yes
	mPCR-3	5	5	3	~350–550[a]	-	Service or kit	Yes
	RACE-1	6	5	4	~230	-	Lab protocol	Yes
	RACE-1_U	4	6	5	~230	UMI	Lab protocol	Yes
	RACE-2	6	6	6	230–280	-	Service or kit	Yes
	RACE-2_U	6	6	7	230–280	UMI	Service or kit	Yes
	RACE-3	2	2	3	~150	-	Kit	Yes
	RACE-4	3	5	4	~150	-	Lab protocol	Yes

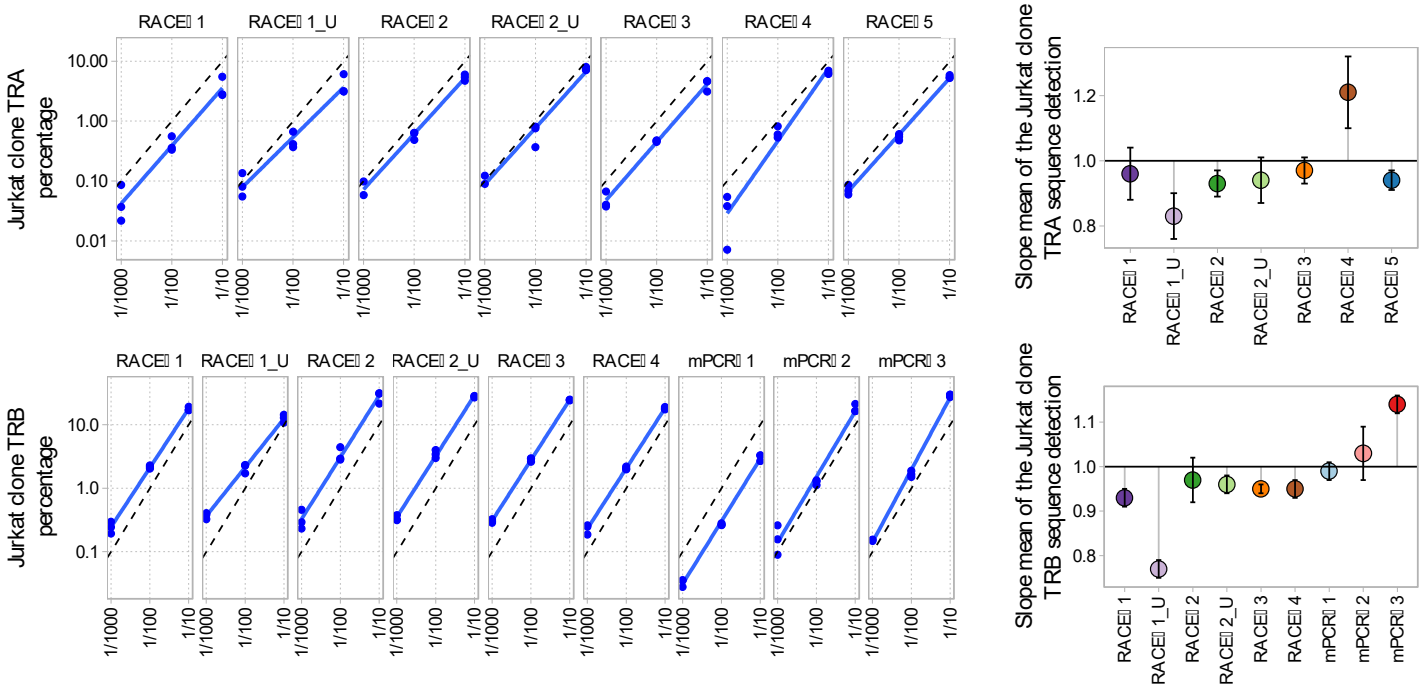
Controls - TRBV usage





- gDNA/mPCR methods have a good correlation with flow cytometry data
- RNA/RACE-based methods are variable, *r2* ranging from 0.4 to 0.8 ($P < 0.05$)
- mPCR-3 (RNA/mPCR-based) poorly correlates

Controls - clonal spike-in & detection sensitivity



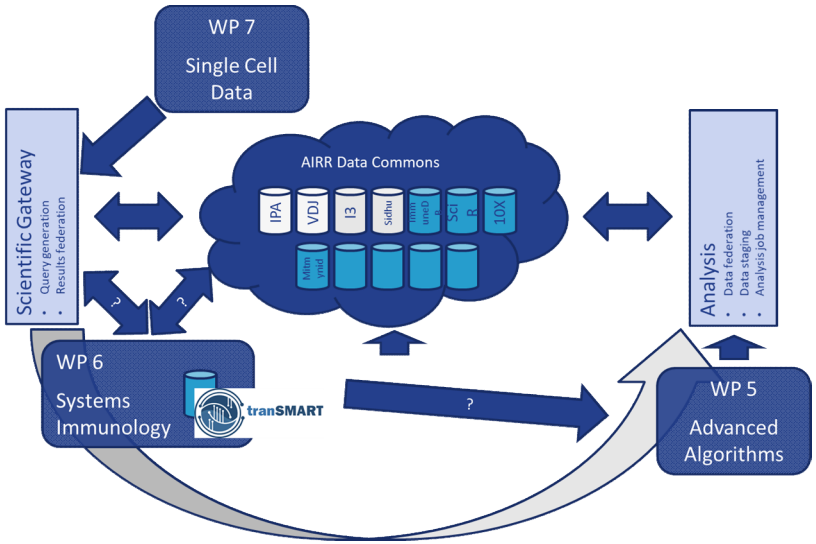
- All methods
  - TRA & TRB detected at all dilutions
  - TRA frequencies three times lower than expected
  - TRB frequencies three times higher than expected (opposite for mPCR-1)
  - Linearity between ratio maintained (except RACE-1_U, RACE-4 and mPCR-3)

Ongoing challenges

- We need federated tools to allow comparison and get valuable biological information out of those massive datasets

AIRR Community

iReceptor+ Sharing AIRR-seq data



Defining standards

- Data production
- Data storage & sharing
- Data exploration

Acknowledgments

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Nicolas Tchitchek

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DHU i2B

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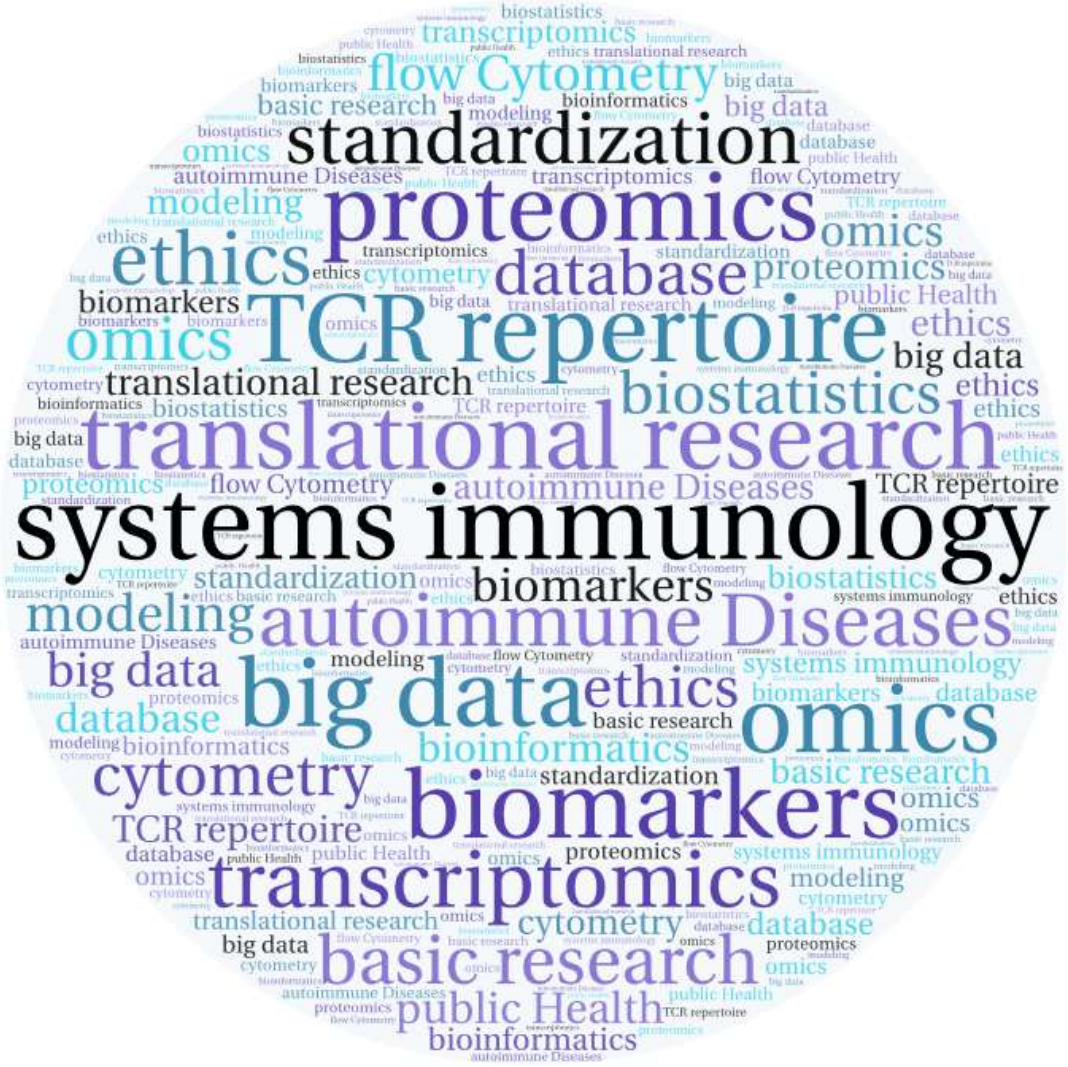
Felix Breden (SFU, Canada)

Brian Corrie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translational research
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proteomics
public Health
TCR repertoire
bioinformatics

AIRR Community

https://www.antibodysociety.org/the-airr-community/

AIRR Community Meeting V: "Zooming in to the AIRR Community"

AIRR Community Meeting V – Zooming in to the AIRR Community! will include three 4-hour virtual sessions on **December 8th-10th, 2020** and will include AIRR-C Working Group & Sub-committee presentations, scientific sessions, interactive poster sessions and software tool demonstrations and networking events.

In addition to the main meeting, there are two free pre-meetings. Be sure to check out the pre-meeting links below for agenda and registration/connection details:

1) **"AIRR-seq in the Pandemic"** co-hosted by the AIRR Community and Tsinghua University in China will take place on December 5th/6th and

2) **"AIRR-seq Biological Standards and Workflows"** hosted the Biological Resources Working Group on December 7th.