

# Validation and calibration of AIRR-seq assays using synthetic and biological standards

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EMORY  
UNIVERSITY  
SCHOOL OF  
MEDICINE

LOWANCE CENTER FOR HUMAN  
IMMUNOLOGY



## Summary

- I. Use of synthetic standards
  - a. Primer bias
  - b. Internal assay control
  - c. Quantification
- II. Testing of clonal size identification
  - a. Use of immortalized cell line spike ins
- III. Biological replicates/considerations and clonal identification metrics

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# Synthetic Library Construction

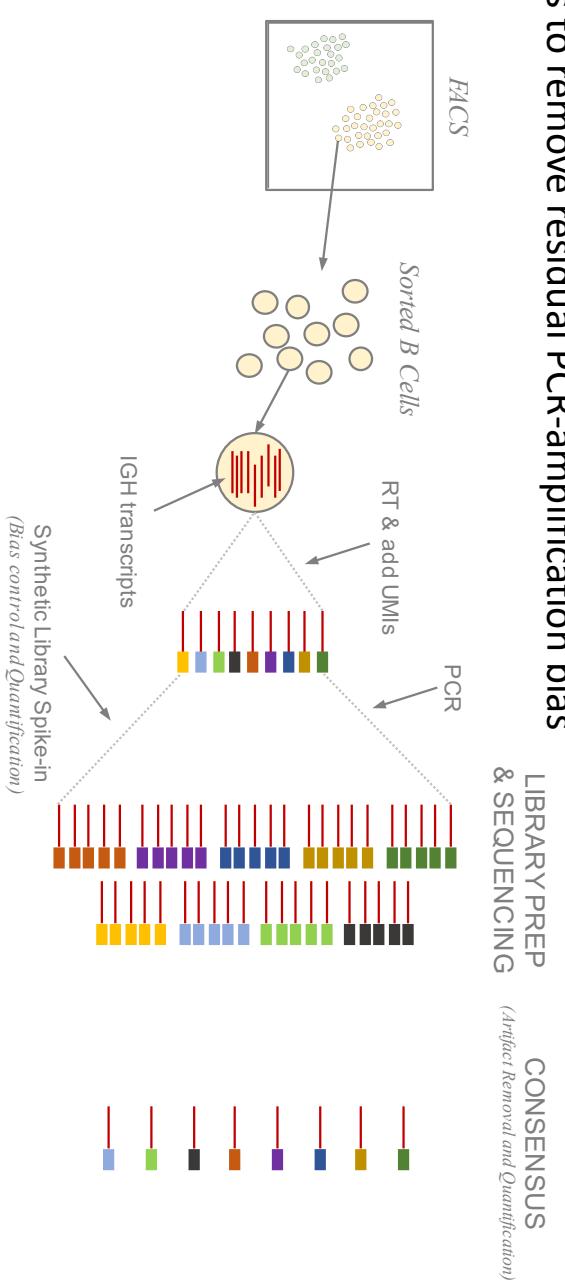
## Gene fragments as a synthetic library (Gblocks, GeneArt Strings, etc):

- Synthetic dsDNA, long molecule
- V+J+C genes
- Variable nucleotides in the design
  - 1.05 million unique sequences per template (10 nucleotides)

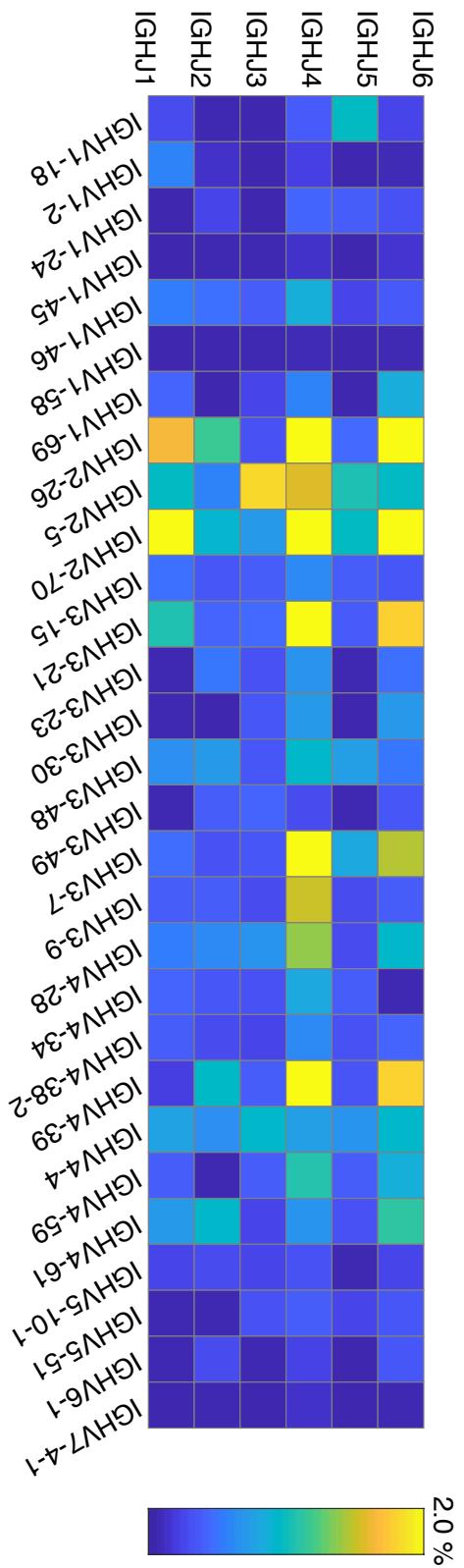


# Assay optimization using a synthetic library

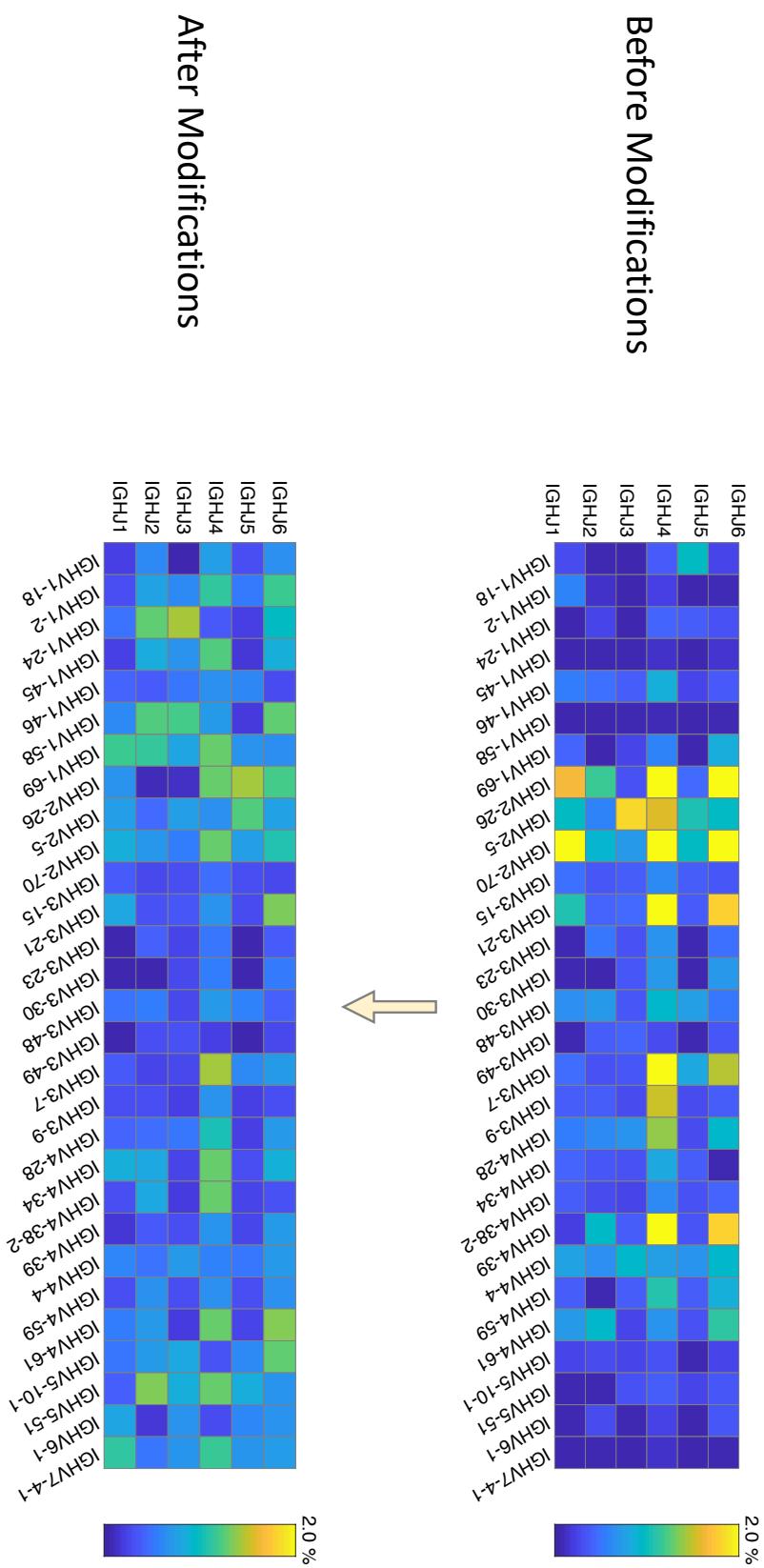
- As an internal control
  - Biologics can fail to amplify, but if the internal synthetic templates amplify, we know the assay worked
  - Unique libraries in separate samples can test for cross-contamination
- We can titrate primers, increasing amount of less efficient primers
- To measure and generate normalization factors to remove residual PCR-amplification bias
- To measure sequencing coverage for template quantification:
  - Cellular quantification with gDNA assuming 1 rearrangement
  - Template quantification with RNA



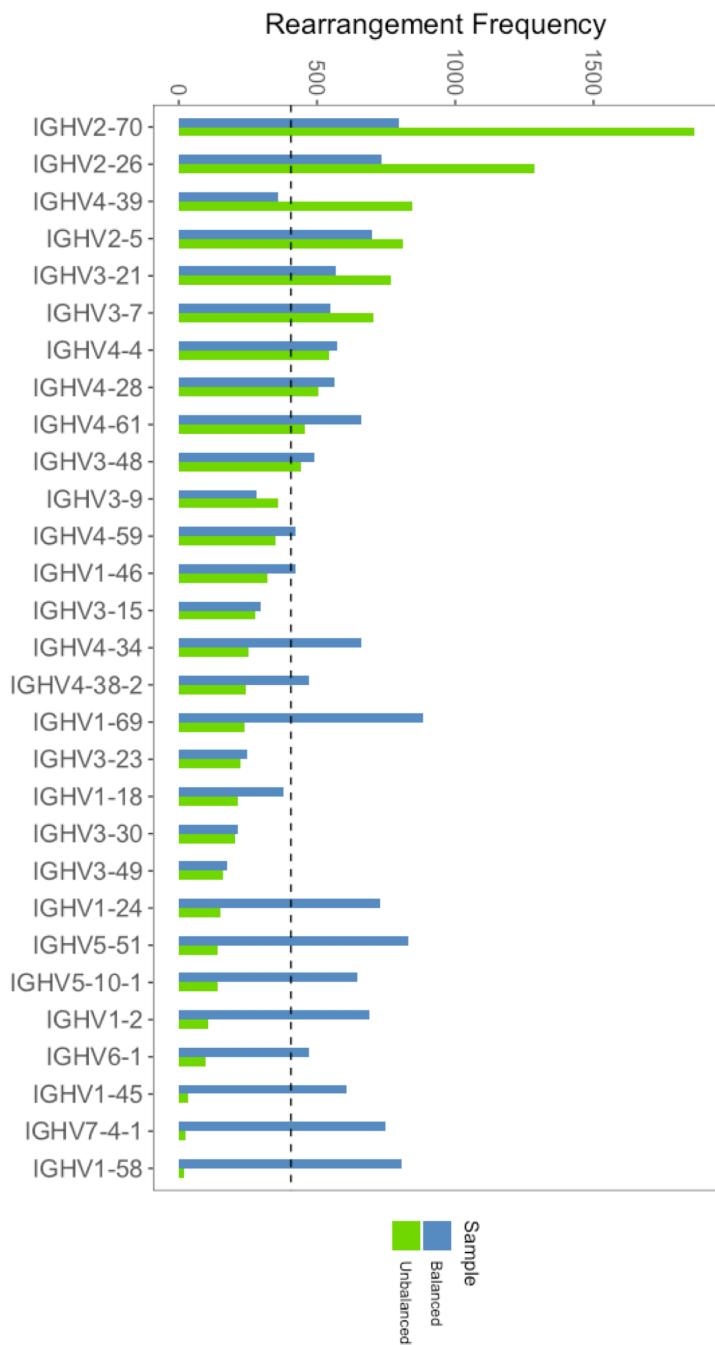
## Assay optimization using a synthetic library



# Assay optimization using a synthetic library



# Assay optimization using a synthetic library



# Considerations

## Synthetic Library

- Expensive to set up
- Only useful for multiplex PCR approach
- A lot of optimization required
- Sensitive to small measurement errors
- Non-specific amplification
- Need high depth of coverage
  - Template ID
    - Primer use – introduction of “false bias”
- SHM can cause problems

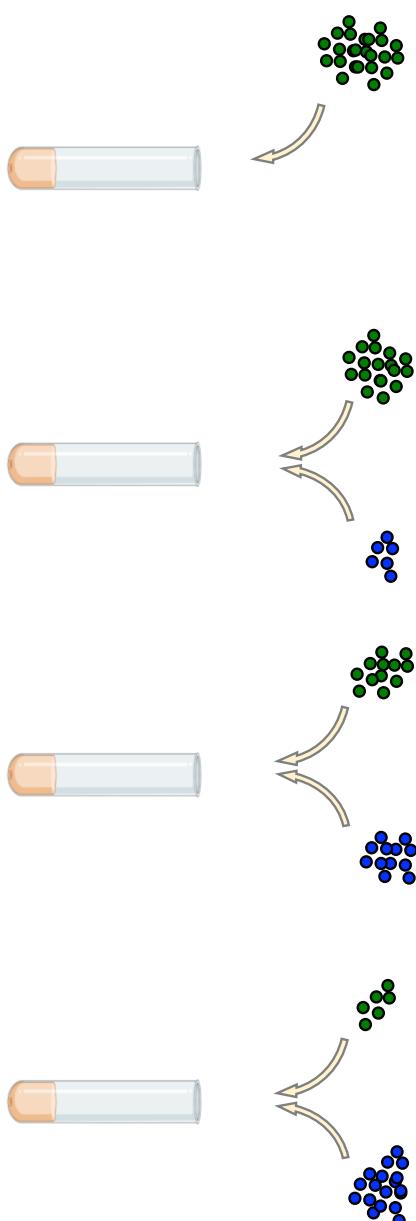
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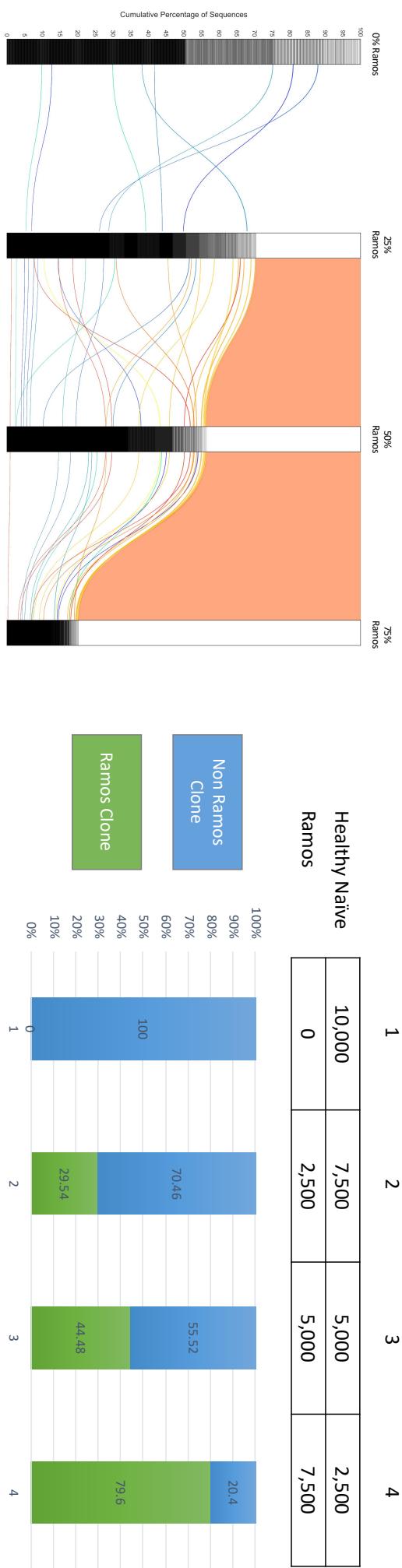
## Testing capabilities to accurately identify clonal size

Healthy Naïve  
Ramos cell line  
(Expresses VH4-34)

	1	2	3	4
10,000	7,500	5,000	2,500	
0	2,500	5,000	7,500	



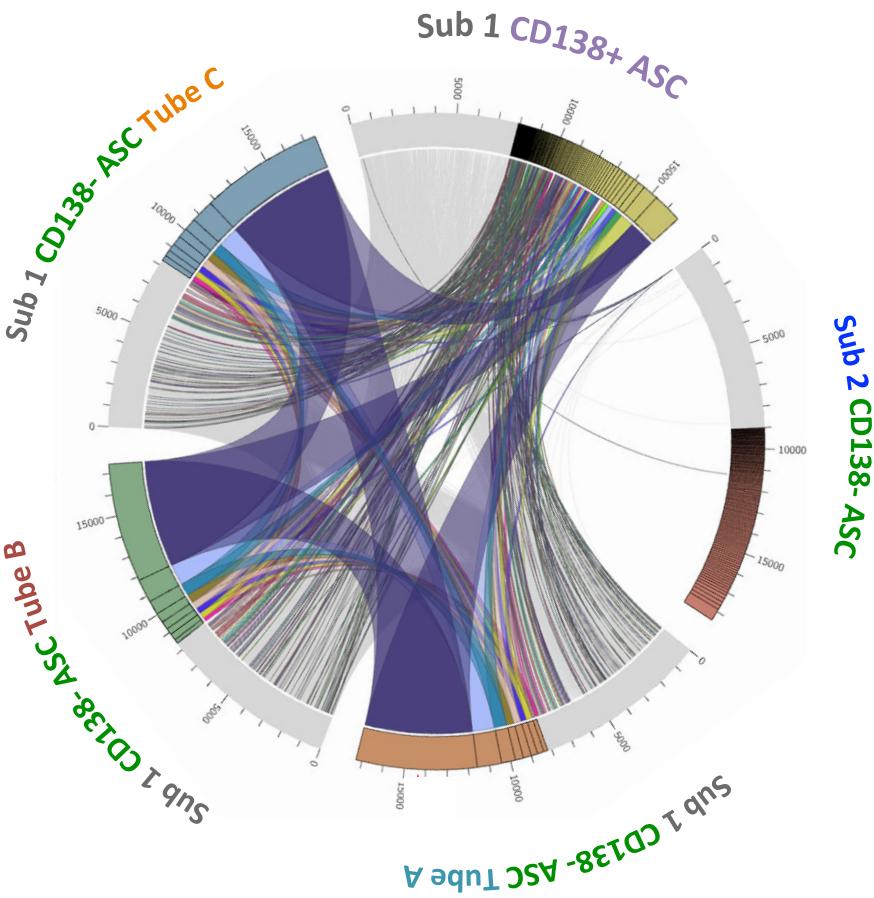
# Testing capabilities to accurately identify clonal size



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## Biological Validation



### Subject 1 (Flu vaccine – day 7)

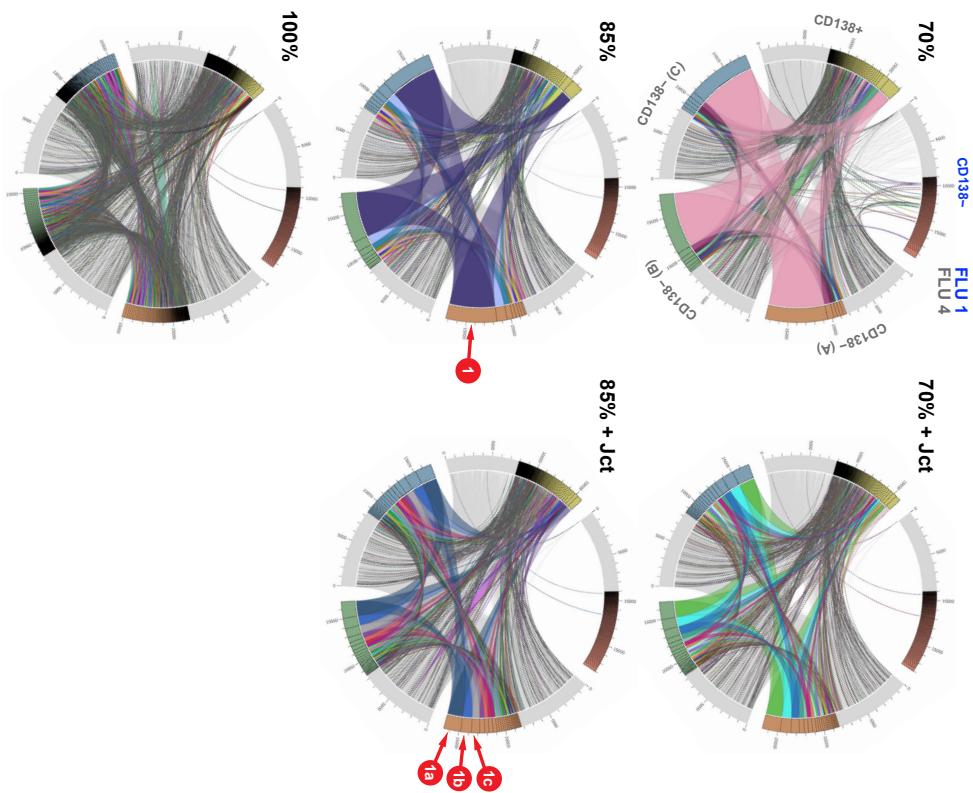
- 3 Tubes: CD138- ASC
- 1 Tube: CD138+ ASC

### Subject 2 (Flu vaccine – day 7)

- 1 Tube: CD138- ASC

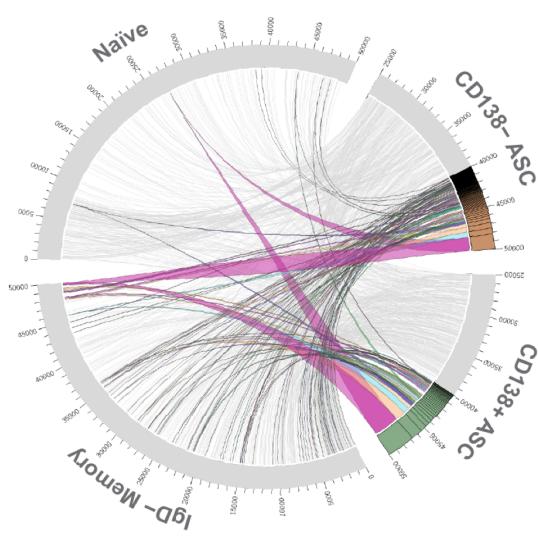
*30k cells were collected from each cell subset*

## Validation of Clone Identification Metric

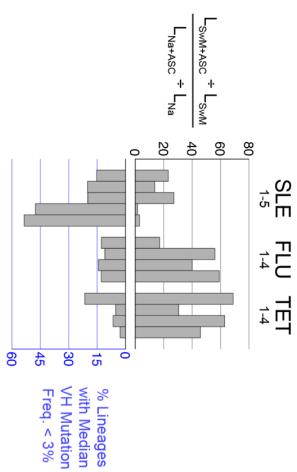
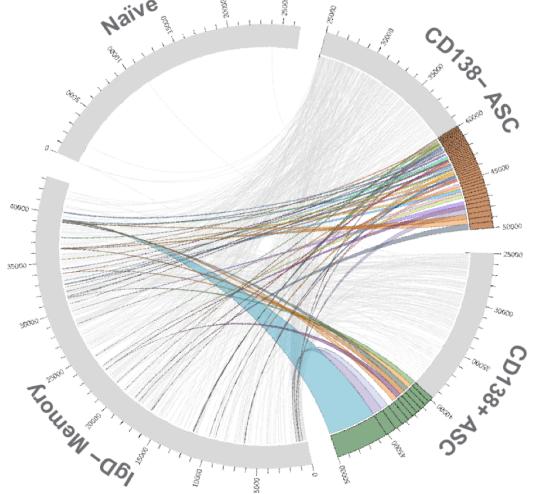


# Validation of Clone Identification Metric

## Systemic Lupus Erythematosus

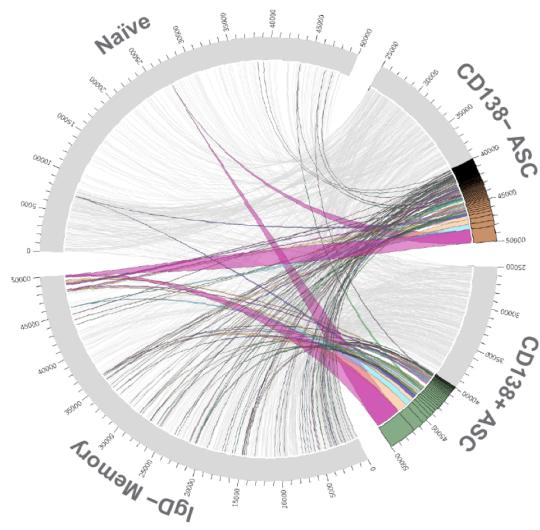


## Influenza Vaccine

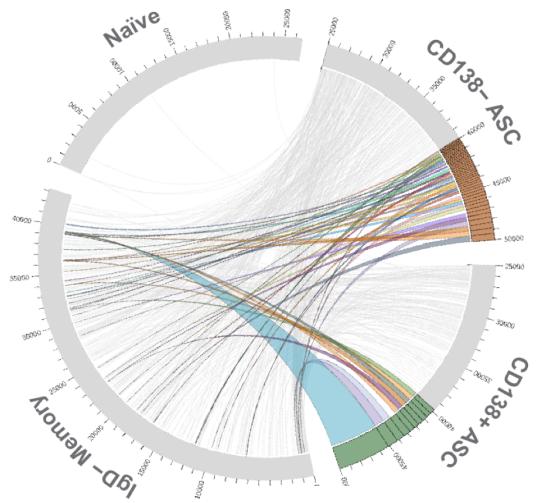


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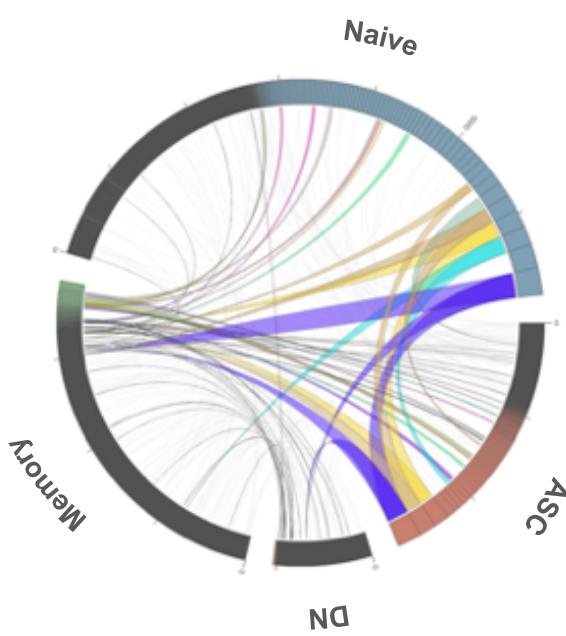
Systemic Lupus Erythematosus



Influenza Vaccine



COVID-19 (acute ICU patient)



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Questions?

## Acknowledgments

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