Mice were infected with virus (TMEV) and immune cells were isolated and sequenced 7 day post infection. We know that ~70% of CD8+ T cells recognize a single antigen, VP2. However, each mouse appears to have a unique response at the TCR sequence level, despite ~70% each time binding to the same VP2 antigen. Could we observe ~70% of TCR sequences aligning closely in a single TCR family?? The purpose of creating a dendrogram of TCRs is to find functional families which might bind the same pMHC antigen but differ slightly in sequence.

TRUST4 takes RNAsequencing (expression) reads and pulls reads that map to:

* TCR (alpha/beta)
* TCR (gamma/delta)
* BCR (heavy/light)

The sampling depth of sequences will thus be much more limited than the scRepertoire, which uses a TCRa/b amplicon library and maps to the TCRa/b constructed transcriptome reference.

In scRepertoire\_outs, we have two analyses: stringent and relaxed. Relaxed is all TCRA and TCRB sequences attributed to a single barcode. Stringent requires that only one TCRA and one TCRB be attributed per barcode and chooses the more abundantly expressed one.

Samples:

CD45+ cell pool (contains ~50% T cells) – mouse 1

CD4/CD8+ pool (contains~95% T cells) – mouse 1

CD45+ cell pool treated with DMSO – mouse 2

CD45+ cell pool treated with Aak1i – mouse 3

We naively assumed that the VP2 response, because an overwhelming proportion of TCRs recognize this antigen, would be public. Public means that multiple individuals produce the same TCR sequence in response to an infection.