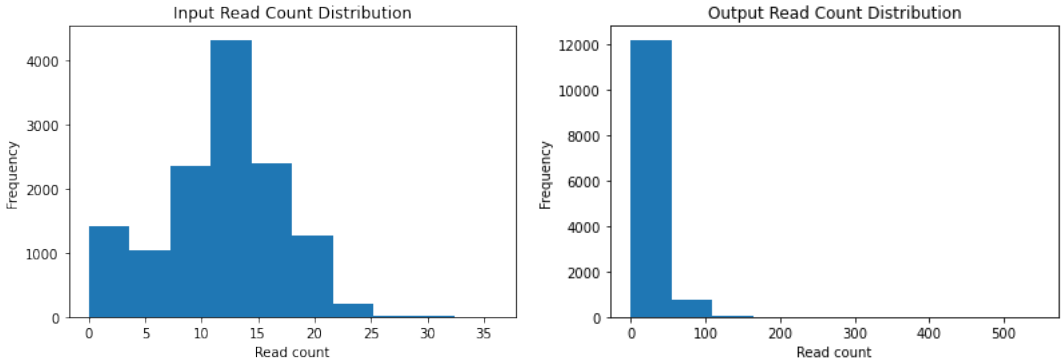
Variant library – binding affinity report

(Target: Homo sapiens clone HE1-34 circulating B cell antibody heavy chain variable region mRNA)

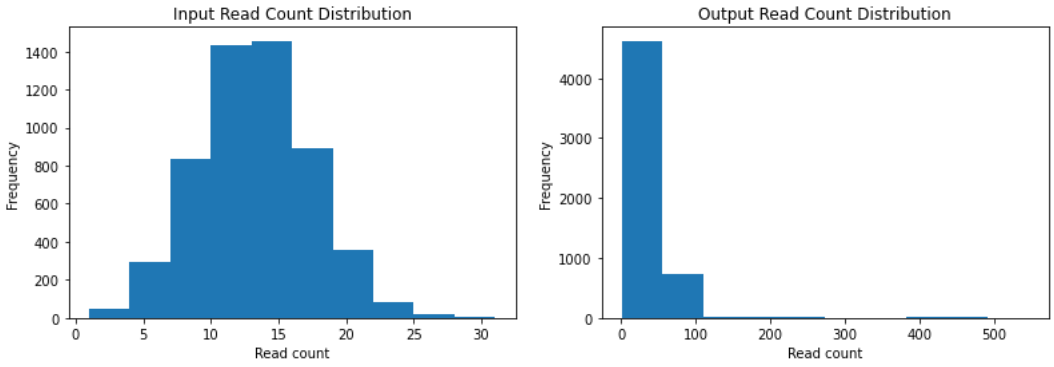
1) Distribution of reads

Analysis was performed on the numbers of read counts of the enriched sequences. Distributions of input and output sequence reads are shown below in the respective bar charts.



After removing those sequences with 0 reads, we observe the following distributions.

A normal distribution, with median of 13 reads is observed in the input read count data. A left-skewed distribution with a median of 30 is observed in the output read count data.

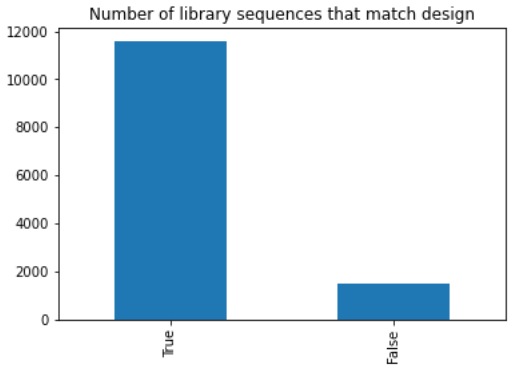


Sequences with 0 reads in their output (that have a positive number of reads in their input) would suggest that binding did not occur, this is to be expected. It is surprising to see the

considerable number of sequences with an input read count of 0. It would be worth investigating at which stage in the pipeline this issue occurred (design, PCR, NGS, filtering). We also see a number of sequences with no reads in the input but a positive number of reads in the output. It would also be worth investigating this further.

2) DNA sequence design

Total enriched sequences	13046	
Sequences that did not match design	1484	(11.4%)
Sequences that were longer than design	206	(1.58%)



1484 (11.4%) of sequences did not match the design. Of these, 206 were longer than the original design. 112 were 1 nucleotide longer, 63 were 2 nucleotides longer and 31 were 3 nucleotides longer.

3) Protein binding affinity

To assess protein binding affinity, the following metric was used:

Affinity = Read count in output library / Read count in input library

This ratio gives the fold change after the test for binding affinity. After removing sequences with 0 reads from the data, affinities range between 0.056 and 394. 99% of the data have an

affinity score of less than 16.5. The 50 sequences with the highest affinity scores range between 17.75 and 394. Interestingly, the highest scoring sequence deviated from our design. In order to verify this, it would be good to repeat the experiment for this sequence, to eliminate the possibility of error e.g. sample mix-up. It is possible that this is a spurious result as it has an affinity five time higher than the second highest sequence.
The three proteins with the highest affinity are:

Sequence 42, affinity 394.0

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CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTCTCCTGTGCAGC
CTCTGGATTCTCCCCCGCCTGCTACTACATGAGCTGGATCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGG
TTTCATACATTAGTGTCTTGGTCTCCACCATATACTACGCAGACTCTGTGAAGGGCCGATTCACCATCTCC
AGGGACAACGCCAAGAACTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTA
CTGTGCGAGAGA
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Sequence 1803, affinity 78.2

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CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTCTCCTGTGCAGC
CTCTGGATTCCGCGCGGCCTACTACTACATGAGCTGGATCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGG
TTTCATACATTAGTGGCTAGGAGTTCACCATATACTACGCAGACTCTGTGAAGGGCCGATTCACCATCTCC
AGGGACAACGCCAAGAACTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTA
CTGTGCGAGAGA
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Sequence 6713, affinity 52.0

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CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTCTCCTGTGCAGC
CTCTGGATTCGTCGACTGCTTCTACTACATGAGCTGGATCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGG
TTTCATACATTAGTGTCGGCTCCTGCACCATATACTACGCAGACTCTGTGAAGGGCCGATTCACCATCTCC
AGGGACAACGCCAAGAACTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTA
CTGTGCGAGAGA
```

4) Next steps

Going forward, it would be interesting to investigate the subset of sequences that do not match the design, to identify whether particular positions or regions have a higher prevalence of change and whether these errors are produced in a random or more systematic way. It would also be worth investigating this in the respective amino acid sequence, to investigate whether secondary or tertiary structures could be affecting the output at the PCR or NGS stages.

I aim to investigate further into the sequences with the highest affinity scores, these representing our best candidate proteins. Correlations between nucleotide position and affinity would be of interest.