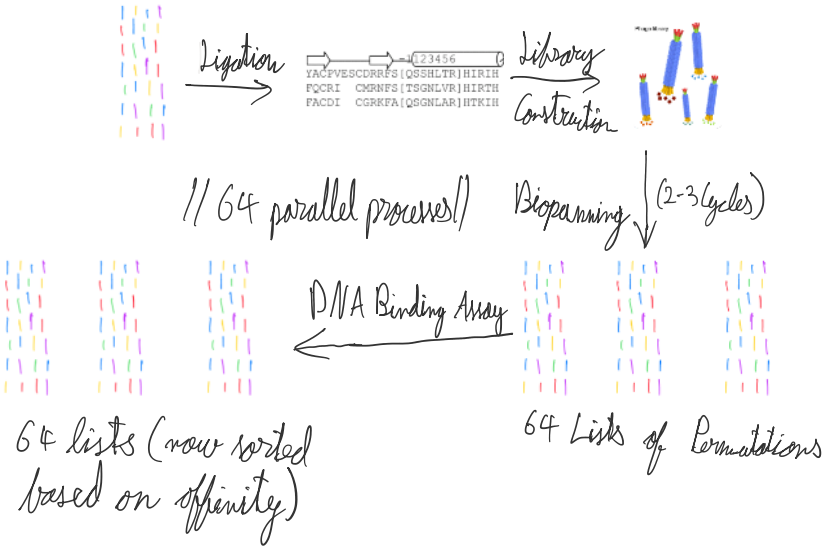
# BBL 741 Term Paper

It has been observed that the amino acid residues responsible for binding to nucleotides lie in the alpha helix region of the ZFN from -1 to 6 position.

► So we create a library with all amino acid residues randomised in that region for phage display




Now we need to find the best permutation from each list. They are characterised by:

► Affinity above a certain threshold (high enough to trigger promoter in validation task)

► Affinity towards other 3 bp targets should be as minimal

We first find threshold affinity for each list by performing binary search based on reporter expression (or) gel shift analysis


We then score every permutation based on the following function

$$f(p) = K \sum_{i=1}^{64} \frac{1}{r_{p,i}}$$

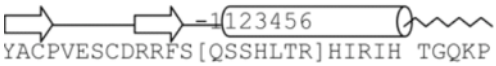
[where $r_{p,i}$ is the rank of 'p' in 'i'th 3 bp targets sorted list; $r_{p,i} = \infty$ if p is absent in $i^{th}$ list]

► Make another list where these permutations are ranked based on this new score (say 'Ls')

► Now, the least ranked permutation with affinity over the estimated threshold is our desired ZFP!

## Part 2

To our ZFP scaffolding, we attach a TGQKP and TGEKP linkers between ZF 1 and 2 and 2 and 3 respectively and ligate appropriate sequences from the lookup table we created in the -1 to 6 region

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YACPVESCDRRFS[QSSHLTR]HIRIH TGQKP
FQCRI  CMRNFS[TSGNLVR]HIRTH TGEKP
FACDI  CGRKFA[QSGNLAR]HTKIH
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