BBL741 Assignment II

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How to create 64 possible zinc finger modules that can bind to 64 possible triplets?

Zinc finger proteins interact via their individual fingers to three base pair subsites on the target DNA.

The four key residue positions −1, 2, 3, and 6 on the alpha-helix of the zinc fingers have hydrogen bond interactions with the DNA.

Mutating these key residues enables the generation of many combinatorial possibilities that can bind to any DNA stretch of interest.

How to isolate 64 zinc fingers that specifically recognize each of the 64 possible DNA triplets?

GNN Binding triplets:
Segal, David J., et al. "Toward controlling gene expression at will: selection and design of zinc finger domains recognizing each of the 5′-GNN-3′ DNA target sequences." *Proceedings of the National Academy of Sciences* 96.6 (1999): 2758-2763.

ANN Binding triplets:
Dreier, Birgit, et al. "Development of zinc finger domains for recognition of the 5′-ANN-3′ family of DNA sequences and their use in the construction of artificial transcription factors." *Journal of Biological Chemistry* 276.31 (2001): 29466-29478.

CNN Binding triplets:
Dreier, Birgit, et al. "Development of zinc finger domains for recognition of the 5′-CNN-3′ family DNA sequences and their use in the construction of artificial transcription factors." *Journal of Biological Chemistry* 280.42 (2005): 35588-35597.

TNN Binding triplets:
Barbas, Carlos F. "*Zinc finger binding domains for TNN.*" U.S. Patent No. US11/564,141. 2010-11-16.

- For the creation of ZFs which are not yet available, we can use the sequences of ZFs for the available triplets.
- Predict the -1, 3, and 6 positions based on the data available and synthesis the ZFs
- Check for the binding specificity.

How will you use the above information to design a 9-base pair binding ZFP to a unique 9-base pair target site?

- ➢ Synthesise the nucleotide sequences for each of the three ZFs.
- ➢ Ligate them in the required sequence.
- ➢ Insert into the plasmid for expression.
- ➢ Express.

How to increase the sequence specificity of zinc fingers?

If there are two or more ZFs available that bind at the identical triplet, check for the affinity using the expression level of reporter proteins. Specificity can also be increased by the non-binding residues at positions 1,2, 4, and 5.