1 LOCAL CHROMATIN CONFORMATION

1.1 INTRODUCTION

The Hi-C assay provides a genome-wide overview of chromatin conformation, however this broad scope imposes resolution limits inherent to an all-vs-all assay. For a closer look at chromatin conformation within a region of interest, alternative C-based assays such as 3C, 4C and 5C can be employed alongside classical microscopy techniques like FISH.

Here I discuss two collaborative projects involving the use of 4C-seq and 5C data to "zoom in" on two well-studied regions related to limb development: the ZRS enhancer and HoxD gene cluster.

1.2 4C OF THE ZRS ENHANCER

Anterior-posterior patterning in the developing limb is regulated in mammals by *Sonic hedgehog* (SHH).[3] Specifically, the SHH gene is expressed within a confined region named the "zone of polarising activity". Its expression within this region is known to be regulated by a well-studied enhancer, the "zone of polarising activity regulatory sequence" or ZRS.[4] ZRS is located almost 1 Mb downstream of its target SHH promoter in humans, and is located in intronic regions of another gene, LMBR1, and is conserved across mammals and fish (Fig. 1).[4,5] Single point mutations and short insertions within this enhancer have been linked to various limb deformities, including pre- and post-axial polydactyly.[3,5,6] For example, a heritable point mutation in the ZRS enhancer is the cause of polydactyly in "Hemingway cats", a large group of domestic cats with extra toes that reside at the former home of Ernest Hemingway.[6]

Collaborators have developed a model system which allows inducible SHH expression in a non-expressing cell line named 14fp. Application of trichostatin A (TSA) then leads to detectable SHH expression, and increased levels of the histone activation mark H3K27ac at the ZRS (*unpublished data*). However, the question remains whether this TSA treatment is fundamentally altering local chromatin structure, that is, bring together the ZRS enhancer with its target SHH promoter, or whether ZRS and SHH are in contact in both the active and non-expressing cell lines and SHH expression is blocked through other means.

My part in this collaboration was to analyse 3C-seq (also known as 4C) data recorded by our collaboratoes for the SHH–ZRS region in mouse. Additionally, the 4C procedure was adapted for specific in-house sequencing instruments (IonTorrent™ as opposed to Illumina™) and as such required diagnostics to confirm the experimental data was accurate.

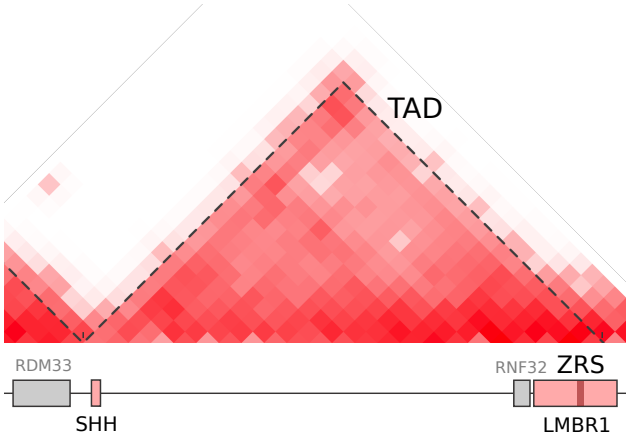

Figure 1: SHH–ZRS contacts occur within a stable TAD. An approximately 1 Mb region of the mouse genome is shown below a Hi-C contact map (derived from previously published data[1]). A clear TAD can be identified spanning from SHH to ZRS, dashed lines show TAD boundaries called by Dixon *et al.*[1]. This figure was generated for Anderson *et al.*[2].

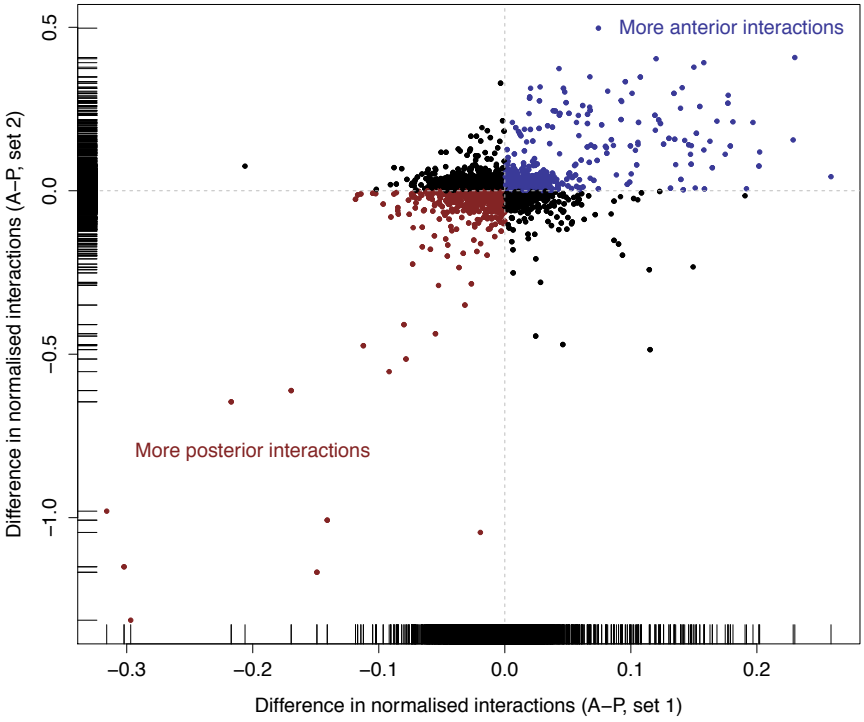

Figure 2: Will we use this stuff? Placeholder

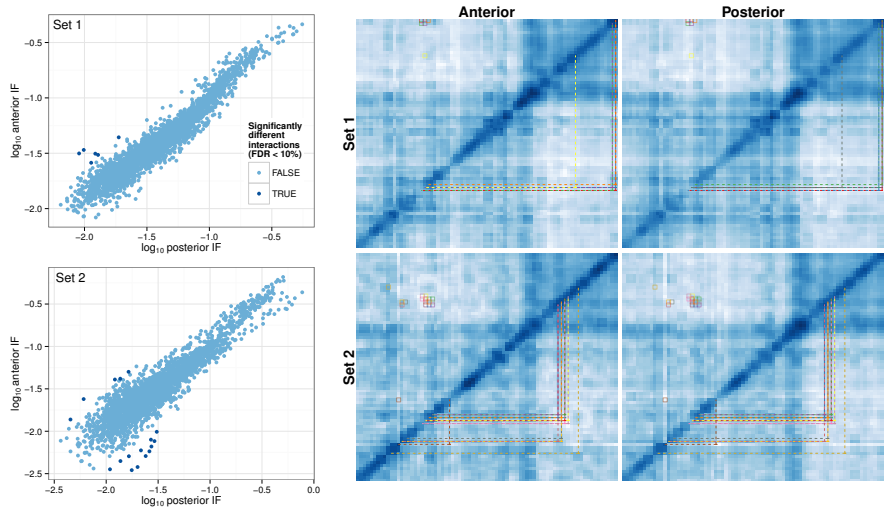

Figure 3: Will we use this stuff? Placeholder

1.2.1 Assay diagnostics

1.2.2 4C / Hi-C comparison

1.2.3 3D modelling

1.3 5C IN THE HOXD REGION

1.3.1 Differential contacts

1.3.2 5C / Hi-C comparison

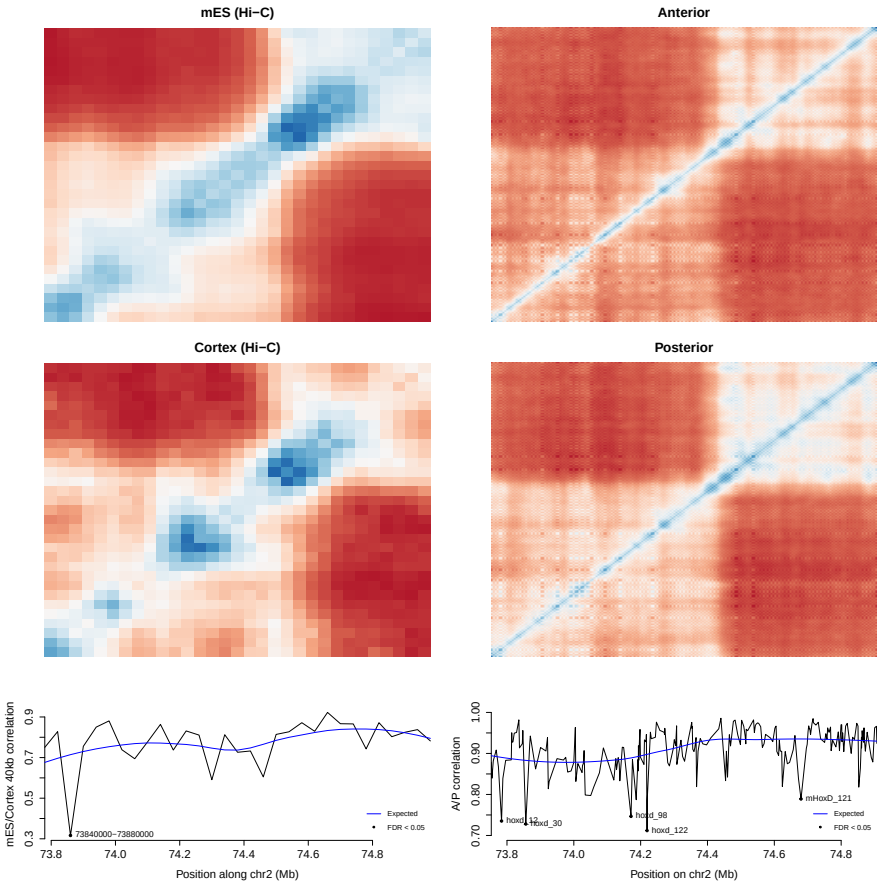

Figure 4: Will we use this stuff? Placeholder

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[1] Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, Hu M, Liu JS, Ren B (2012) Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature*, **485**(7398): 376–80.

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