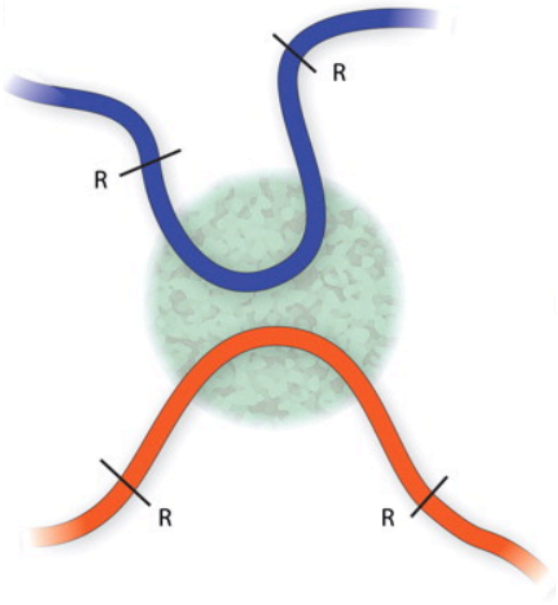
3C:
Chromosome Conformation Capture

Method objective:

To establish a representation of the 3D organization of the genomic DNA in an organism

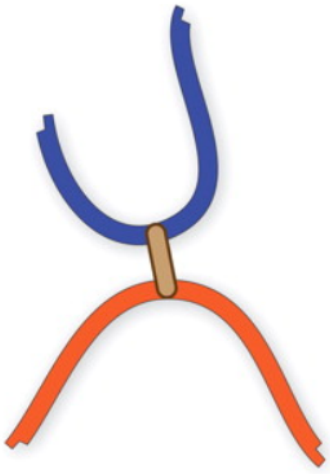
Proteins mediate interactions between segments of DNA such as enhancers interacting with promoters.



1.Cross-linking

This chromatin conformation is captured by linking the interacting chromatin together.

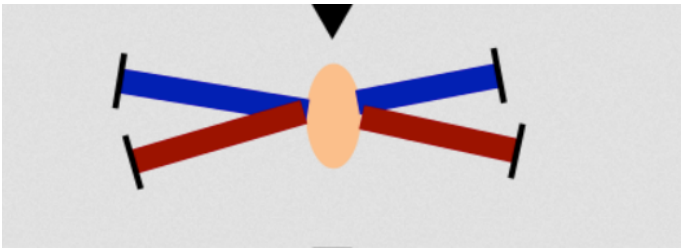
Fixative reagent: formaldehyde



2. Digestion

The fixed chromatin is cut with a restriction enzyme recognizing 6 bp.

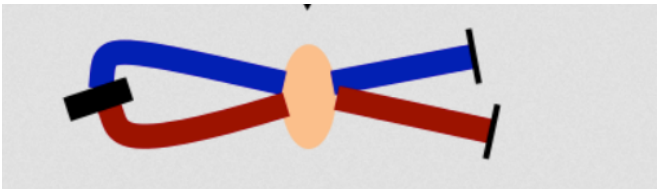
Important: the restriction enzyme has to be appropriate for the digestion desired.



3. Religation

The sticky ends are religated under dilute conditions to promote religation of intramolecular fragments.

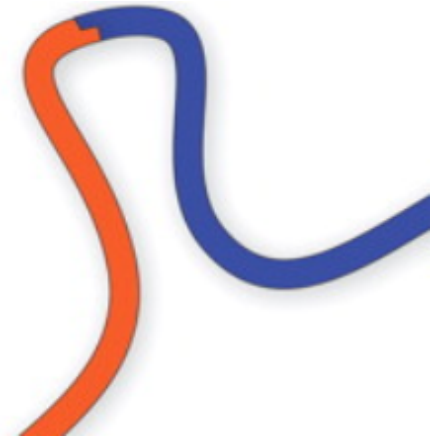
Creates a single fragment of DNA that interact but may be far away on the linear template.



4. Reverse cross-linking

To reverse the cross-link, the temperature is raised.

The result is a 1D print of the 3D nuclear structure.

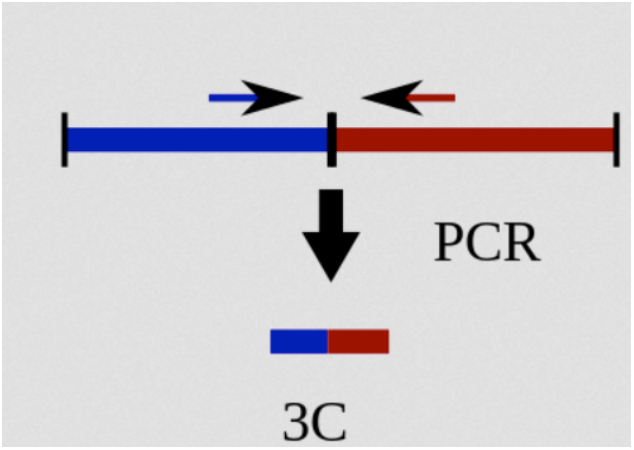


5. Quantification

Quantitative PCR (qPCR) - use fluorescent reporter, which permits detection after primer hybridizes to sequence of interest

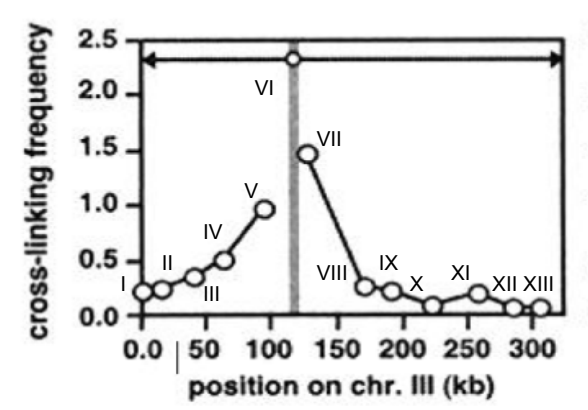
Measure amount of ligation product over linear qPCR product formation using the fluorescent probe

Primers designed near and toward the ends of the restriction site of interest.



6. Comparison

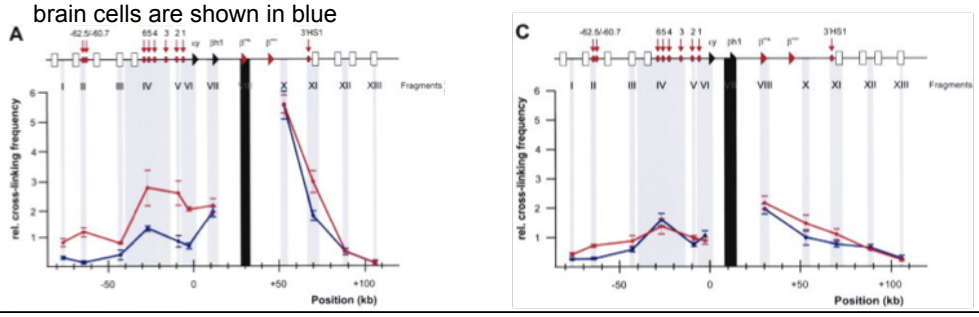
- Perform quantitative PCR (qPCR) on multiple ligated restriction fragments
- Divide these qPCR results by those obtained in the control experiments to obtain relative cross-linking frequency
- Plot these relative cross-linking frequencies against a map of the chromosome or segment in question



Background for Question

Depiction of the ß-globin locus in mice

- The black arrows correspond to the Ɛγ and ßh1 genes, which are inactive in fetal brain and liver cells , while the red arrows correspond to the $ß^{maj}$ and $ß^{min}$ genes, which are active in fetal liver cells, but inactive in fetal brain cells
- The shaded regions containing a given roman numeral represent restriction fragments
- The red dots correspond to DNaseI hypersensitivity (HS) sites. HS sites 1-6 represent the ß-globin locus control region (LCR), which is known to enhance the expression of active ß-globin genes
- Relative cross-linking frequencies observed in fetal liver cells are shown in red, while those in fetal brain cells are shown in blue



Question

With all of the relevant information given, decide which of the following statements are true.

1. If we wanted to examine the interaction of the HS sites in the LCR with the ß-globin genes more precisely, we would need to use restriction enzymes that cut this section of the ß-globin locus more frequently.

2. If relative cross-linking frequencies are roughly monotone decreasing as we move from adjacent fragments on the chromosome to more distant fragments on the chromosome, this suggests a linear chromosome conformation. For this reason, the ß-globin locus in brain cells appears to have a roughly linear conformation.

3. The LCR (fragments IV-VI), a known enhancer, is in closer spatial proximity to active ß-globin genes than inactive ß-globin genes.

4. If fragments II and IV had relative cross-linking frequencies greater than 1, this would imply that they were in closer spatial proximity to one another compared to if the ß-globin locus were linear in conformation.

5. The fact that fragment III has similar relative cross-linking frequencies in both fetal liver and brain cells with both inactive and active b-globin genes implies that it is not in close spatial proximity to such genes via loop formation.

Names and contributions of group members:
Sebastian Munk: Preliminary research, powerpoint, and "questions that can be addressed with this technique"
Nolan Shelley: Preliminary research, powerpoint, and question.
Mikaela Nevin: Preliminary research and writeup

Technique chosen: Chromosome conformation capture (3C)

What does this technique 'do'?
3C provides information about the frequency with which any two sites in the genome are in close proximity to one another.

What applications is this technique employed for?

- Most frequently, 3C is used for the detection and analysis of the interactions that occur between genes and distant *cis*-regulatory elements (i.e. Intrachromosomal interactions)
- Elucidating the roles of various proteins and other factors in facilitating these interactions. Factors can be removed or added (e.g. by knocking them out or overexpressing them) and 3C can be used to analyse what effect, if any, the change has on interactions between your sequences of interest.
- 3C can be used to detect interactions between functional elements on different chromosomes
- 3C has been used to identify novel enhancers for a particular gene (Gheldof *et al.* 2002)

What questions relating to gene regulation and/or development can be addressed using this technique? Provide two examples (peer-reviewed papers) that use this technique.

1. 3C can be applied in further studies of how the chromatin loop is formed during interaction of genes and their regulatory elements, and the role of the loop in gene expression (Kim *et al.* 2015). Though some factors involved in the chromatin loop formation have been identified there is still different models that have not been examined as thoroughly as some of the others. One example would be that 3C could be applied to examine the role of eRNA in chromatin loop formation.

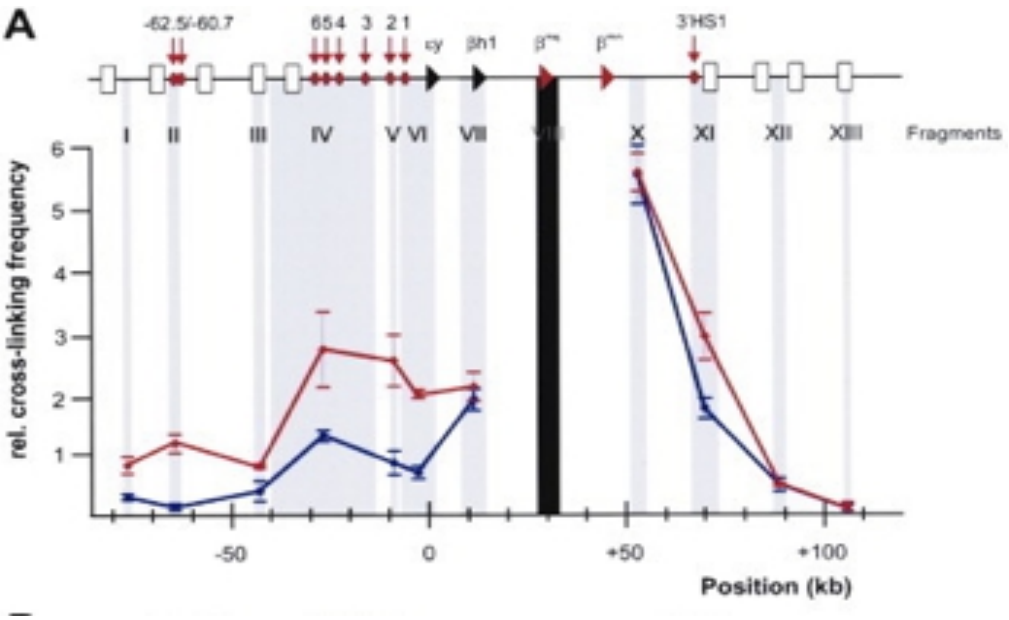
2. 3C can be applied to study interchromosomal interactions involved in the differentiation of naive CD4+ T cells into a specific cell type of T helper cells (Spilianakis *et al.* 2005). This technique can be used to examine the interactions of particular genomic regions as LCRs. It can help determine whether a LCR is involved in regulation of adjacent genes or if the LCR is acting as a regulator of genes on other chromosomes through interchromosomal interactions.

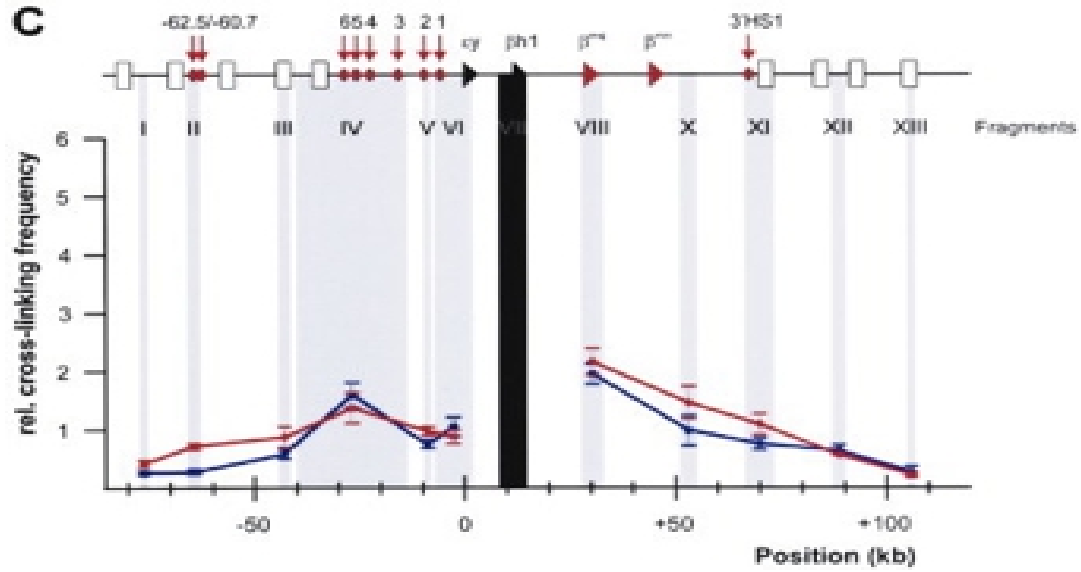
What critical reagents are required to use this technique?
All reagents required for cell preparation and nuclei extraction.
Formaldehyde to cross-link DNA
Appropriate restriction enzyme (one that cuts efficiently through the region of genomic DNA being analyzed)
DNA ligase
All reagents required for semi-quantitative PCR
important note about the PCR step: You need a control template that contains all possible ligation products in equimolar amounts. This allows correction for variation in primer efficiency. You also need to determine the range of template over which PCR product formation is linear. Measuring

amount of product beyond this range, at the plateau stage of the reaction, will give unreliable results because it is sensitive to small variations in initial template concentration.

What critical information is required to be able to employ this technique?
In order to perform 3C, you must know the sequence near the central restriction site of both regions whose interaction you are investigating, so that you can design PCR primers that can anneal to this region.

Figures used for question, from Tolhuis *et al.* 2002





References:

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Gheldof, Nele, et al. "Cell-type-specific long-range looping interactions identify distant regulatory elements of the CFTR gene." *Nucleic acids research* (2010): gkq175.

Spilianakis, Charalampos G., et al. "Interchromosomal associations between alternatively expressed loci." *Nature* 435.7042 (2005): 637-645.

Tolhuis, Bas, et al. "Looping and interaction between hypersensitive sites in the active β-globin locus." *Molecular cell* 10.6 (2002): 1453-1465.

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