

Grundlagen der Systembiologie und der Modellierung epigenetischer Prozesse

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Chromosome Conformation Capture Technology

- ▶ chromatin folding
- ▶ spatial arrangement of chromatin in the nuclear space
- ▶ protein-DNA interactions
- ▶ chromatin-chromatin interactions

Chromosome Conformation Capture (3C)

common experimental steps

1. formaldehyde cross-links proteins or protein to DNA
2. cross-linked chromatin is digested with a restriction enzyme
3. DNA ends of cross-linked DNA fragments are ligated
4. cross-links are reversed
5. quantification of DNA fragment ligations

Chromosome Conformation Capture (3C)

step 1

- ▶ blubb

Chromosome Conformation Capture (3C)

step 2



Chromosome Conformation Capture (3C)

step 3



Chromosome Conformation Capture (3C)

step 4



Chromosome Conformation Capture (3C)

step 5



Chromosome Conformation Capture Variants

3C

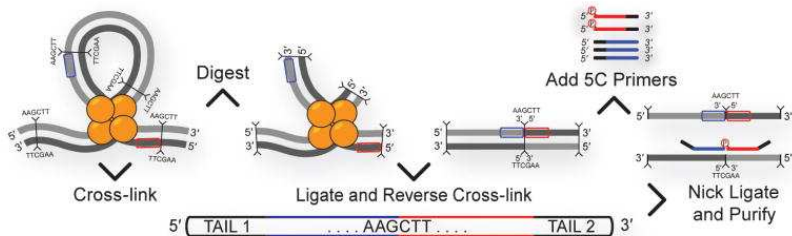
Name	Chromosome Conformation Capture
Question	are g_1 and g_2 neighbors in the nuclear space?
Size	6-600kb
Primers	F-primer against g_1 , R-primer against g_2
Analysis	quantification of the ligation frequency of g_1 and g_2

Chromosome Conformation Capture Variants

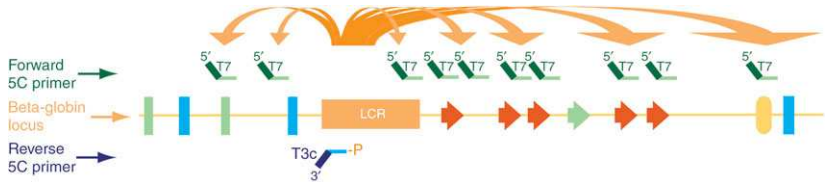
5C

Name	Carbon Copy Chromosome Conformation Capture
Question	are g_i and g_j neighbors in the nuclear space? for 100-10000 different g
Size	limited by the number of primers that can be used simultaneously
Primers	F-primer against g_i , R-primer against g_j right next to the restriction site
Analysis	quantification of the ligation frequency of g_i and g_j often by microarrays or sequencing

5C – Concept



5C – Primer Design

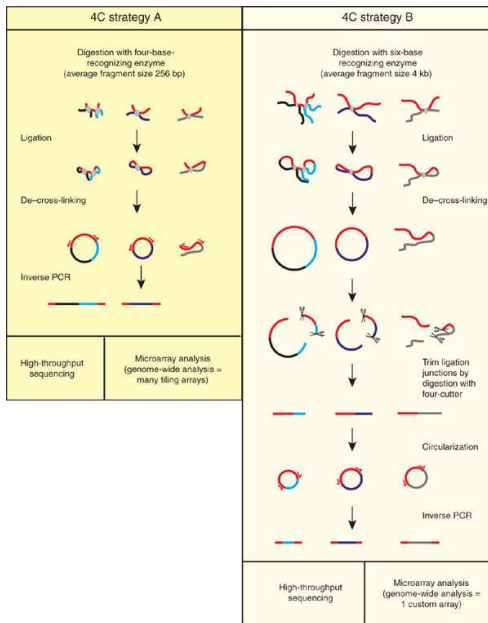


Chromosome Conformation Capture Variants

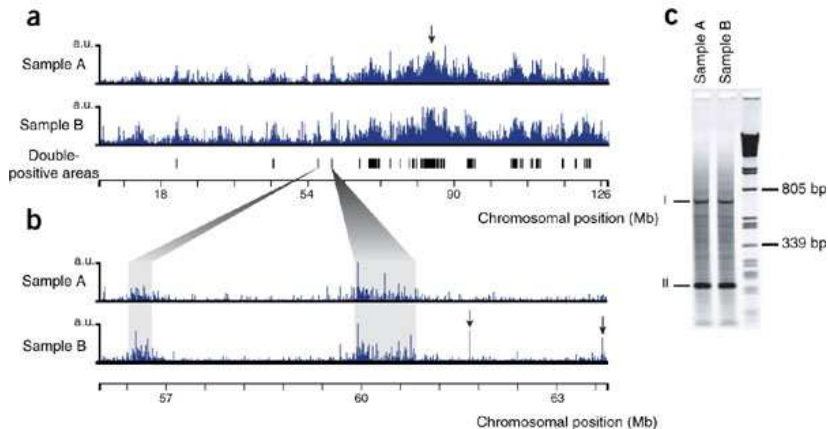
4C

Name	Circular Chromosome Conformation Capture
Question	to which sequences is g_1 (the “bait”) a neighbor in the nuclear space?
Size	genome size
Resolution	256bp - 7000bp
Primers	F-primer against g_1 , R-primer against g_1 pointing outwards
Analysis	sequencing of the sequence fragments ligated to g_1

4C – Two Concepts



4C – Results

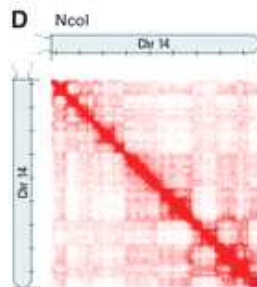
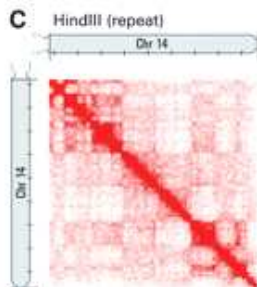
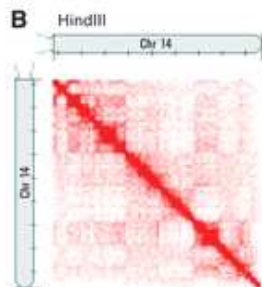
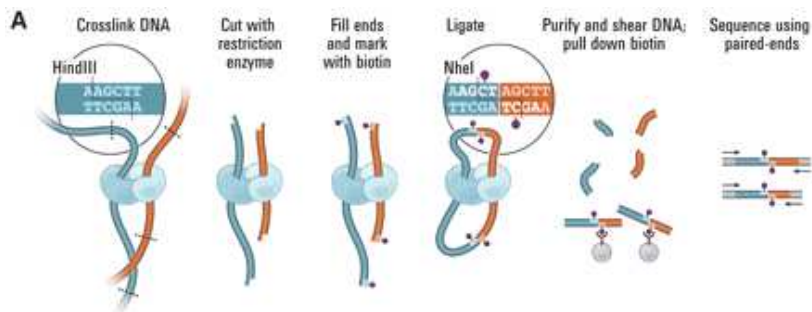


Chromosome Conformation Capture Variants

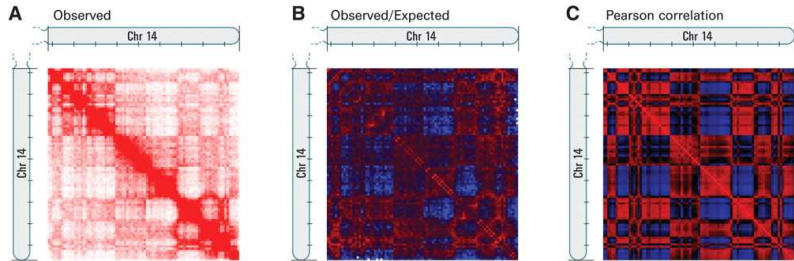
Hi-C

Name	Genome-wide Chromosome Conformation Capture
Question	which sequences are neighbors in nuclear space?
Size	genome size
Resolution	1Mb (possibly higher)
Primers	unspecific
Analysis	sequencing of the sequence fragments ligated together

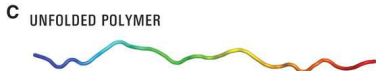
Hi-C – Concept



Hi-C – Data

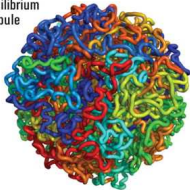


Architecture of the Human Nucleus

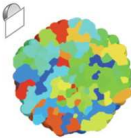


FOLDED POLYMER

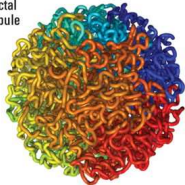
Equilibrium globule



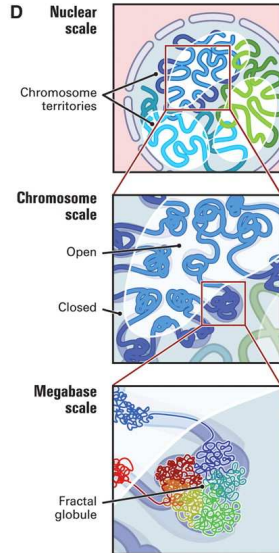
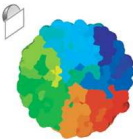
Cross-section view



Fractal globule



Cross-section view



References



[Lieberman-Aiden, 2009] Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragozy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO, Sandstrom R, Bernstein B, Bender MA, Groudine M, Gnirke A, Stamatoyannopoulos J, Mirny LA, Lander ES, Dekker J *Comprehensive mapping of long-range interactions reveals folding principles of the human genome*. Science. 2009 Oct 9; 326(5950): 289-93



[Simonis, 2007] Simonis M, Kooren J, de Laat W *An evaluation of 3C-based methods to capture DNA interactions*. Nat Methods. 2007 Nov ; 4(11): 895-901