Grundlagen der Systembiologie und der Modellierung epigenetischer Prozesse

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January 3, 2011
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

<table>
<thead>
<tr>
<th>Name</th>
<th>Chromosome Conformation Capture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Question</td>
<td>are $g_1$ and $g_2$ neighbors in the nuclear space?</td>
</tr>
<tr>
<td>Size</td>
<td>6-600kb</td>
</tr>
<tr>
<td>Primers</td>
<td>F-primer against $g_1$, R-primer against $g_2$</td>
</tr>
<tr>
<td>Analysis</td>
<td>quantification of the ligation frequency of $g_1$ and $g_2$</td>
</tr>
</tbody>
</table>
## Chromosome Conformation Capture Variants

### 5C

<table>
<thead>
<tr>
<th>Name</th>
<th>Carbon Copy Chromosome Conformation Capture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Question</td>
<td>are $g_i$ and $g_j$ neighbors in the nuclear space? for 100-10000 different $g$</td>
</tr>
<tr>
<td>Size</td>
<td>limited by the number of primers that can be used simultaneously</td>
</tr>
<tr>
<td>Primers</td>
<td>F-primer against $g_i$, R-primer against $g_i$ right next to the restriction site</td>
</tr>
<tr>
<td>Analysis</td>
<td>quantification of the ligation frequency of $g_i$ and $g_j$ often by microarrays or sequencing</td>
</tr>
</tbody>
</table>
5C – Concept

Cross-link → Digest → Ligate and Reverse Cross-link → Add 5C Primers → Nick Ligate and Purify

5' TAIL 1 . . . AAGCTT . . . TAIL 2 3'

Juniorprof. Dr. Prohaska
Sysbio
5C – Primer Design

- Forward 5C primer
- Beta-globin locus
- Reverse 5C primer
- LCR
- T3c
- 3'
- T7
- 5'
- T7
Chromosome Conformation Capture Variants

4C

<table>
<thead>
<tr>
<th>Name</th>
<th>Circular Chromosome Conformation Capture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Question</td>
<td>to which sequences is $g_1$ (the “bait”) a neighbor in the nuclear space?</td>
</tr>
<tr>
<td>Size</td>
<td>genome size</td>
</tr>
<tr>
<td>Resolution</td>
<td>256bp - 7000bp</td>
</tr>
<tr>
<td>Primers</td>
<td>F-primer against $g_1$, R-primer against $g_1$ pointing outwards</td>
</tr>
<tr>
<td>Analysis</td>
<td>sequencing of the sequence fragments ligated to $g_1$</td>
</tr>
</tbody>
</table>
## 4C – Two Concepts

<table>
<thead>
<tr>
<th>4C strategy A</th>
<th>4C strategy B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Digestion with four-base-recognizing enzyme</strong>&lt;br&gt;(average fragment size 256 bp)</td>
<td><strong>Digestion with six-base-recognizing enzyme</strong>&lt;br&gt;(average fragment size 4 kb)</td>
</tr>
<tr>
<td><img src="image1" alt="Diagram of 4C strategy A" /></td>
<td><img src="image2" alt="Diagram of 4C strategy B" /></td>
</tr>
<tr>
<td><strong>Ligation</strong></td>
<td><strong>Ligation</strong></td>
</tr>
<tr>
<td><strong>De-cross-linking</strong></td>
<td><strong>De-cross-linking</strong></td>
</tr>
<tr>
<td><strong>Inverse PCR</strong></td>
<td><strong>Trim ligation junctions by digestion with four-cutters</strong></td>
</tr>
<tr>
<td><img src="image3" alt="High-throughput sequencing" /></td>
<td><img src="image4" alt="High-throughput sequencing" /></td>
</tr>
<tr>
<td><img src="image5" alt="Microarray analysis (genome-wide analysis = many tiling arrays)" /></td>
<td><img src="image6" alt="Microarray analysis (genome-wide analysis = 1 custom array)" /></td>
</tr>
</tbody>
</table>
4C – Results

(a) Sample A
(b) Sample B
(c) Double-positive areas
(d) Chromosomal position (Mb)
(e) Sample A
(f) Sample B
(g) 805 bp
(h) 339 bp
# Chromosome Conformation Capture Capture Variants

## Hi-C

<table>
<thead>
<tr>
<th>Name</th>
<th>Genome-wide Chromosome Conformation Capture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Question</td>
<td>which sequences are neighbors in nuclear space?</td>
</tr>
<tr>
<td>Size</td>
<td>genome size</td>
</tr>
<tr>
<td>Resolution</td>
<td>1Mb (possibly higher)</td>
</tr>
<tr>
<td>Primers</td>
<td>unspecific</td>
</tr>
</tbody>
</table>

**Analysis**

sequencing of the sequence fragments ligated together
Hi-C – Concept

A. Crosslink DNA
   - HindIII
   - AAGCTT TTGAGA
   - Cut with restriction enzyme
   - Fill ends and mark with biotin
   - Ligate
   - Purify and shear DNA, pull down biotin
   - Sequence using paired-ends

B. HindIII
   - Chr 14

C. HindIII (repeat)
   - Chr 14

D. Ncol
   - Chr 14
Hi-C – Data
Architecture of the Human Nucleus

C UNFOLDED POLYMER

FOLDED POLYMER
Equilibrium globule

Cross-section view

Fractal globule

Cross-section view

D Nuclear scale

Chromosome territories

Chromosome scale
Open
Closed

Megabase scale
Fractal globule