Development of Genes for Keratin Transgenesis

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How much of the flanking DNA region is required?

- Gene expression relies upon DNA sequences outside the protein-coding region.
  - Testing of gene constructs in...
    - cell culture
    - via mouse transgenesis
  - Approach: progressively delete flanking DNA until gene expression is lost or becomes non-specific.
Determining the region of control of transgene expression

- Sequential deletion of 5’ flanking region

<table>
<thead>
<tr>
<th>Region</th>
<th>Length (bp)</th>
<th>Coding Region</th>
<th>3' flanking Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>5' flanking</td>
<td>2800</td>
<td>K2.10</td>
<td>Yes</td>
</tr>
<tr>
<td>region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400</td>
<td></td>
<td>K2.10</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>K2.10</td>
<td>No</td>
</tr>
</tbody>
</table>
Identification of the sequences that are critical for correct gene expression

- **Approach**: Mutate specific DNA sequences one at a time or in combination and test gene expression level.

- **Example**: Transgenesis in mice after mutation of the LEF-1 sequence in the K2.10 gene promoter.
The effect on fibre properties if extra copies of active follicle keratin genes are present.

• Mouse transgenesis with the K2.10 gene.
  – Transgenic mouse produces hair which is brittle and breaks easily