Role of Introns in Gene Regulation and Expression

ROP Biotechnology Program - Spring 2012

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May 22nd, 2012
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- Associate Project Scientist at UCD’s Molecular and Cellular Biology department

- Current research focus:
  *The role introns play in gene expression*

- Research applications:
  *Agricultural biotechnology, improving trait expression in GMOs*

- Latest publications:
  *Evidence for DNA-based mechanism for Intron Mediated Enhancement*

*Dr. Alan Rose*
Outline: Testing Effects of Introns on Gene Expression

1. Plasmid Construction
   - Identifying, designing, verifying

2. Transformation (Bacterial)
   - Inserting plasmid into plants

3. Identifying Single-Copy Lines
   - Look for 3:1 segregation, single inserts and homozygotes

4. Measuring Expression

5. Confirmation Tests

Agrobacterium
Plasmid Construction

- The intron is designed or identified and respective *primers* are ordered.
- PCR is used to *amplify intron* from the genomic code.
- The specific sequence is *cloned* into a plasmid with the exons.
- The intron + exon fragment is cloned into another plasmid to produce the *GUS fusion*.

Beta-glucuronidase gene - Analysis of gene expression in transformed plants
Finalized Plasmid and Verification

- *Restriction enzymes* are used to move fusion into the final plasmid (Later put into Agrobacterium)
- *PCR* is used to *verify* the insertion and presence of the specific intron:

Requirements for PCR analysis:

- DNA Polymerase enzyme- *Taq*
- Primer pair (upstream and downstream)
- *dNTPs*
- Buffer/Water
- The DNA template (in this case, the intron sequence)
1. Denature @ ~95°C
2. Anneal Primers @ ~65°C
3. Extension/Elongation @ ~72°C
4. Repeat 1-3 for amplification
Recovery and PCR Results

Checking recovery of ρAC 31, 32 & PNK 4, 5
PCR to verify identity of PNK 5, 6
Recovery and PCR Results
Repetition for Unsuccessful PCR
Verification of Intron Insertion

- Requirements: spin columns, buffers, collection tubes
- Membrane used: silica
- Procedure: bind, wash, elute

Purpose: Purification of DNA fragments for direct use in sequencing
- Contaminant free
- 8µl of purified PCR product
- 8µl of specific primers before and after the expected constructed sequence
Insertion of Plasmids into Plants

1. Putting the plasmid into the *Agrobacterium* using *Electroporation*:
   1. Competent cells are removed from -80°C
   2. Electroporation cuvettes are placed on ice
   3. Ligations are added to component cells
   4. Volts: 2.50, Resistance: 200 Ohms
   5. Cuvettes are placed in chamber
   6. 4.3-4.8 second time constant
### Transformation Efficiency (#Colonies/Plate)

<table>
<thead>
<tr>
<th>Amount Plated</th>
<th>Plasmid</th>
<th>#Colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>200µl</td>
<td>pAC 31</td>
<td>484</td>
</tr>
<tr>
<td>10µl</td>
<td>pAC 31</td>
<td>15</td>
</tr>
<tr>
<td>10µl</td>
<td>pNK 5 (+ Control)</td>
<td>53</td>
</tr>
<tr>
<td>200µl</td>
<td>EB (- Control)</td>
<td>0</td>
</tr>
<tr>
<td>10µl</td>
<td>pAC 32</td>
<td>0</td>
</tr>
<tr>
<td>200µl</td>
<td>pAC 32</td>
<td>4</td>
</tr>
</tbody>
</table>
T1 seeds from Transformed plants are collected and then plated on Kanamycin plates to identify Kan- resistant seedlings.
Transplanting to Soil

- 40 Kan-Resistant Seedlings are transplanted to soil to allow growth
- T2 seeds are harvested from the 40 T1 plants
Identifying Single-Copy Lines

- 100 seeds from each line are spotted on Kanamycin plates.

- Each line that segregates 3:1, i.e. ~75:25 are transplanted into soil.

- DNA is extracted from lines segregating with only one copy (3:1) and Southern Blots are used to determine number of inserts.
Continuing with only Single Inserts

- T3 seeds are harvested only from lines with single inserts

- Seedling that contained only one copy but at *multiple locations* are discarded

- Seeds from T3 are spotted to identify *homozygotes* (True breeders)
Measuring Expression

qRT-PCR: Real Time PCR

MUG Assay
**Conclusion**

- If an intron is present at the beginning base pairs of the translated region of a gene during transcription, then it is more likely for the gene to be expressed as gene regulation is increased. This was concluded on the basis of repeated tests with varying introns. Specific sequences within an intron have also proven to aid better in expression than other.