RESULTS AND DISCUSSION

1. Describe the purpose of each of the following steps or reagents used in DNA isolation (Part II):
   (Complete sentences)
   a. grinding with pestle.
   b. Edwards' buffer.
   c. boiling.
   d. tris-EDTA (TE) buffer with RNase A.

2. What is the purpose of performing each of the following PCR reactions: (Complete sentences)
   a. tubulin?
   b. wild-type soybean?
   c. Roundup Ready® soybean?

3. Which sample(s) in the gels pictured on the next page show the following banding patterns? Explain what each pattern means. (Foods 1 and 2 shown are two examples of expected results. Remember that your own samples can yield any of the combinations for tubulin and 35S in the table below.)

<table>
<thead>
<tr>
<th>187 bp (tubulin)</th>
<th>162 bp (35S)</th>
<th>Samples showing this pattern and explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>present</td>
<td>present</td>
<td>Explanation</td>
</tr>
<tr>
<td>present</td>
<td>absent</td>
<td></td>
</tr>
<tr>
<td>absent</td>
<td>absent</td>
<td></td>
</tr>
</tbody>
</table>

4. Observe the photograph of the stained gel containing your sample and those of other students. Orient the photograph with wells at the top. Interpret each lane of the gel.
   a. Scan across the photograph of your gel and others as well to get an impression of what you see in each lane. You should notice that virtually all experiment lanes contain one or two prominent bands.
   b. Now locate the lane containing the pBR322/BstNI marker on the left-hand side of the gel. Working from the well, locate the bands corresponding to each restriction fragment: 1857 bp, 1058 bp, 929 bp, 383 bp, and 121 bp (may be faint). Alternatively, locate the lane containing the 100-bp ladder, with the fastest migrating band equal to 100 bp and each successive band 100 bp larger (100, 200, 300, 400, etc.).
   c. The amplification products of the 35S promoter (162 bp) and of the tubulin gene (187 bp) should align between the 121-bp and 383-bp fragments of the pBR322/BstNI marker (or between the

4. Label all groups and samples (wells) on all photos.  
   a. For the 5 bands present, create a chart similar to the one above.

5. What did you learn about the scientific inquiry process from this experience? Give at least 3 detailed examples.
d. It is common to see one or two diffuse (fuzzy) bands of RNA and/or primer dimer at the bottom of the gel. RNA may be found at approximately the position of the 121-bp fragment of the pBR322/BstNI marker (or the 100-bp marker of the 100-bp ladder). RNA is the largest component of nucleic acid isolated from plant tissue, so some RNA may escape digestion by the RNase incorporated into the TE buffer in Part II of the experiment. Primer dimer is an artifact of the PCR reaction that results from two primers overlapping one another and amplifying themselves. Primer dimer is approximately 50 bp, and should be in a position ahead of the 121-bp marker fragment.

e. Additional faint bands, at other positions on the gel, occur when the primers bind to chromosomal loci other than 35S or tubulin,
RESULTS AND DISCUSSION

1. Describe the purpose of each of the following steps or reagents used in DNA isolation (Part II):
   a. grinding with pestle.  — break open cell walls  
   b. Edwards' buffer. — break open cell membrane lipids  
   c. boiling. — denature proteins, including DNAase which would damage DNA needed for PCR  
   d. tris-EDTA (TE) buffer with RNase A. — dissolves pellet of DNA, destroys RNA

What is the purpose of performing each of the following PCR reactions:
   a. tubulin? — present in all plants  + control for DNA  
   b. wild-type soybean? — control (no GM gene)  absence  
   c. Roundup Ready® soybean? — shows GM R2 gene  + presence of 35S promoter  

3. Which sample(s) in the gels pictured on the next page show the following banding patterns? Explain what each pattern means. (Foods 1 and 2 shown are two examples of expected results. Remember that your own samples can yield any of the combinations for tubulin and 35S in the table below.)

<table>
<thead>
<tr>
<th>187 bp (tubulin)</th>
<th>162 bp (35S)</th>
<th>Samples showing this pattern and explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>present</td>
<td>present</td>
<td>Food 2, transgenic</td>
</tr>
<tr>
<td>present</td>
<td>absent</td>
<td>WT, Food 1, nontemplate DNA</td>
</tr>
<tr>
<td>absent</td>
<td>present</td>
<td>Food 1, no PCR</td>
</tr>
<tr>
<td>absent</td>
<td>absent</td>
<td>No band</td>
</tr>
</tbody>
</table>

4. Observe the photograph of the stained gel containing your sample and those of other students. Orient the photograph with wells at the top. Interpret each lane of the gel.

a. Scan across the photograph of your gel and others as well to get an impression of what you see in each lane. You should notice that virtually all experiment lanes contain one or two prominent bands.

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e. Additional faint bands, at other positions on the gel, occur when the primers bind to chromosomal loci other than 35S or tubulin, giving rise to "nonspecific" amplification products.