Summary Table:

One of the nutrients that is extremely important for plant growth is phosphorus (P). Improving the efficiency of phosphorus use in plants is of utmost importance. In this study, we aimed to develop models based on actual RNA evidence with the hope of being able to target P deficiency in plants. Using the theoretical gene model of Zea mays (corn), which is one of the most important crops grown worldwide (Asim et al. 2017), we focused on the major problem affecting plant growth (Wu et al. 2016). Therefore, it is important to study P deficiency to understand the factors affecting it and to develop strategies to improve P use efficiency in plants.

Methods

 Genome model
- Identify gene models using (using genome and assembly version 5)
- Constructed a theoretical gene model using a curated 4 version gene model, RNA evidence from Mikkola (collapsed RNA sequencing data from six tissues), full-length cDNAs and isoform RNA evidence
- A region of interest was determined
- Primers were designed to be contained within constitutive regions to determine the splicing events

 Primer design was performed using Primer3 Plus. The half arrows indicate where primers bind in both transcripts.

 Results

 Figure 1: Transcripts Evidence for Gene Zm00001d030924. The transcripts were used to generate a collapsed map. The evidence includes RNA evidence from Mikkola (collapsed RNA sequencing data from six tissues), full-length cDNAs and isoform RNA evidence. The figure was taken from Apollo Instance of Corn Genome, version 5 (Ware, pess comm).

 Figure 2: Transcript evidence for Zm00001d030924 used to collapsed the gene map (Apollo version 4)

 Figure 3: Proposed Gene Structure of Gene Zm00001d030924. The boxes depict exons and the lines depict introns. The exons are numbered from E1 to E7 and the lines below each exon (eg. 1a, 1b) are different transcriptions of a particular exon. The white areas of the boxes are constitutive exons which are always included in mRNA. The grey areas of the boxes are alternative regions not always included in mRNA. There are four regions of interest (A,B,C,D, E). The structure was designed using RNA evidence from Apollo Instance of Corn Genome, version 5 (Ware, pess comm).

 Table 1: Comparison table for Zea mays genes involved in phosphorus uptake. Summary table displays list of 21 genes of phosphorus pathway. The table includes information of genome ID, name, location, number of known transcripts and number of exons for reference sequence.

<table>
<thead>
<tr>
<th>Genome ID</th>
<th>Name</th>
<th>Location</th>
<th>Number of Transcripts</th>
<th>Number of Exons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zm00001d030924</td>
<td></td>
<td></td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

 Figure 4: Transcript Comparison for Region of interest. The boxes are exons and the lines are introns. The region of interest includes exons 5 and 6 and the lines below each exon (eg. 6a, 6b) are different versions of a particular exon. The numbers are number of base pairs (bp) in an exon. Splicing patterns above can be used to generate two transcripts (isoform 1 and isoform 2). Primers to detect both transcripts were designed using Primer3 Plus. The half arrows indicate where primers bind in both transcripts.

 Figure 5: Proposed Gene Structure for gene Zm00001d030924. The boxes indicate exons and the lines indicate introns. The exons are numbered from E1 to Ed and the lines below each exon (eg. 4, 3b) are different version of a particular exon. The numbers are number of base pairs in an exon. Splicing pattern above can be generated two transcripts (isoform 1 and isoform 2). Primers to detect both were transcripts were designed using Primer3 Plus (Ricson et al. 2003) The half arrows are where the primers bind in both transcripts.

 Figure 6: The region of interest includes exon 3 and 4 and the lines below each exon (eg. 3b, 4b) are different version of a particular exon. The numbers are number of base pairs in an exon. Splicing pattern above can be generated two transcripts ( isoform 1 and isoform 2). Primers to detect both were transcripts were designed using Primer3 Plus (Ricson et al. 2003) The half arrows are where the primers bind in both transcripts.

 Figure 7: Reverse Transcription Polymerase Chain Reaction Test of RNA using Tubulin Primers. Agarose gel electrophoresis was used to separate products of RT-PCR using corn RNA samples as noted above the gels. The observed tubulin fragment size (195 bp) is noted at right. Expected size 195bp.

 Figure 8: The Gradient Gel to Find the Best Annealing Temperature for RT-PCR Gel Run. Six temperatures (57°C, 55.8°C, 53.9°C, 52°C, 47.8°C, 45°C) were tested for the best temperature for primer. The best annealing temperature for the primer was 51.2°C.

 Table 2: Isoform Comparison Table for Four isoforms Observed During Reverse Transcription.

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Transcript Evidence</th>
<th>Provides evidence for revision to gene model?</th>
<th>Expected and/or observed?</th>
<th>Domains likely missing?</th>
<th>Likely functional?</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>G2</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>

 Conclusions

- Region of interest is contained within a protein domain.
- Neural network model predicts two unexpected gene structures of 210 bp and 180 bp.
- Both RNA extraction and reverse transcription was successful.
- The best annealing temperature for the primer was 51.2°C.
- Both of the expected isoforms was observed. The expected isoform is not missing any domains and therefore both are functional.
- Both isoforms were observed. These domains did not lose any domain therefore it is functional.
- The expected size is 195bp (bp 252) and reverse transcription was successful.
- Predicted and observed RNA isoform (Plan, PANTHER, GeneID, CDD)
- The alternative protein that are made with functional for Peptide acid phosphatase-like, N-terminal.

 Future Direction

- The PCR tests should be repeated several times for more accurate result and to have more clear bands.
- Possible alternative splicing patterns that were not expected from the theoretical gene model can be used to modify the gene model.
- Any student who takes over the project can work on the isoforms that was not expected and include it in a modified gene model.

 References