Phytozome tutorial questions for cassava

Workshop questions part 1

- Use the Phytozome tutorial and help links on Phytozome and GenBank as well as google to get help on anything you need.
- Alpha amylase is an enzyme involved in starch mobilization by starting the degradation process.
- The gene has been cloned in cassava. (The sequence is published in the article PMID: 16297635). This workshop will use Phytozome to compare gene models in cassava to the cloned alpha amylase gene.
- The gene in cassava is MEamy2 and has the Genbank accession DQ011041. Find this gene record in the GenBank nucleotide database (http://www.ncbi.nlm.nih.gov/nuccore/).
- Get the sequence by changing the display to FASTA. Copy the sequence from GenBank and BLASTN against the masked cassava genome. Change the E-value cutoff to 1e-10 by clicking on the algorithm parameters link.
- How many hits are there? Which ones look reasonable and why? Can you tell the difference between the Feature scale and Target scale diagrams of the hits? Click on the features for the first hit to view in the browser.
- Zoom out by selecting Show 10 kb. In the Select Tracks menu, turn on the 454 EST tracks (PASA assembled EST and PASA aligned EST/cDNA) and click on update image.
- Try BLAT with max intron size 5 kb. Compare the results to BLASTN. Which exons are different?
Workshop questions part 2

- Although there is a good gene model in this genomic region we are looking at in the browser, how can we decide if this is the gene model that best matches the MEamy2 gene? Let's investigate whether we are looking at the correct gene region.
- Click on the gene in the region in the Transcript track. It is called cassava4.1_008754m.
- What Functional Annotations (or protein domains) are present in the protein? Do they match what you expect?
- If you click on the Peptide Homologs tab, what is the most similar protein in Phytozome to MEamy2?
- In the Gene Ancestry tab, click on the Viridiplantae family with 134 members. Turn on display of the cassava members in the Display Options tab. How many cassava paralogs are there?
- Display the peptide sequence of the gene model cassava4.1_008754m in the Sequences tab and BLASTP this against NCBI. Choose the nr/nt database at NCBI. What is the top hit? Does this convince you that cassava4.1_008754m corresponds to alpha amylase 2 (MEamy2)? How much of the protein sequence hits the sequence in NCBI? What is the rest of the sequence?

Background reference Cassava alpha amylase


Isolation and characterization of an alpha-amylase gene in cassava (Manihot esculenta). Tangphatsornruang S, Naconsie M, Thammarongtham C, Narangajavana J. National Center for Genetic Engineering and Biotechnology, 113 Phaholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand. sithichoke.tan@biotec.or.th

Abstract
The roots of cassava plants (Manihot esculenta Crantz) accumulate starch as their major form of carbohydrate reserve. Starch accumulation and properties are determined by a balance between starch biosynthesis and degradation processes. Alpha-amylases (EC 3.2.1.1) are alpha-1,4 endoglycosylcic enzymes, responsible for the mobilization of stored carbohydrate reserves by initiating the degradation process. Alpha-amylase genes have been shown to be differentially expressed at various developmental stages and environmental conditions through the action of plant hormones such as abscisic acid (ABA) and gibberellic acid (GA). In this study, we isolated an alpha-amylase gene from cassava tuberous roots (designated as MEamy2, GenBank accession number DQ011041). The deduced product of MEamy2 is 407 amino acid residues in length, with a calculated molecular mass of 46.7 kDa and an isoelectric point of 8.66. Southern blot analysis showed that the MEamy2 is present as a single copy in cassava genome. It shares the highest homology with AMY8 from apple fruit. The predicted structural model of MEamy2 contains three domains, active sites and starch-binding domain that are common with other plant alpha-amylases. RT-PCR analysis showed that the MEamy2 gene expression was induced in cassava roots within 2 hours after treatment with GA, but not ABA.
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