Gramene Exercises
Cereal Genomics Course
Cold Spring Harbor, New York, USA
October 19th, 2016

These exercises will illustrate the power of comparative plant genomics in research using the resources in Gramene.

Exercise 1. View a phylogenetic tree for a family of transcription factors

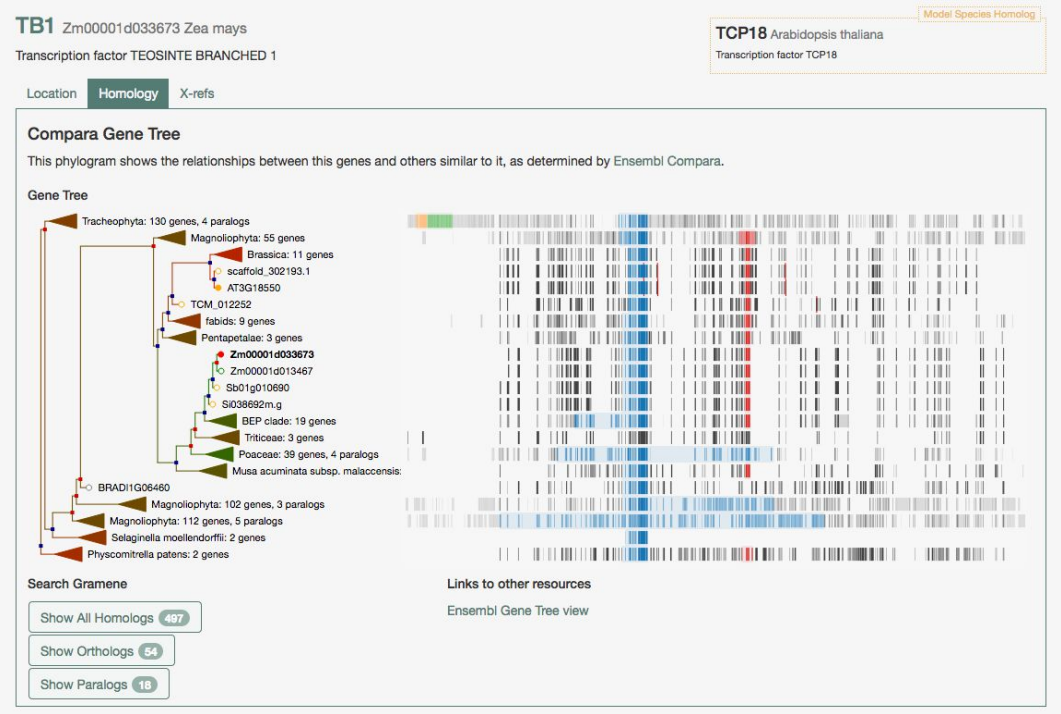
In this exercise, we will navigate a phylogenetic tree for plant genes in the TCP family of transcription factors (named after the first characterized protein members: maize TB1, snapdragon CYC, and rice PCF), highlight species-specific orthologs/paralogs with particular GO annotations in the tree. We will then proceed to generate lists of orthologs/paralogs and download both, images and tables with our results.

a. How many orthologs can you identify for maize TB1?

Hint: You may find the answer for this through different approaches. Gramene's new search will give you the quickest answer through a snapshot of the *tb1* (Zm00001d033673) gene tree. Other approaches are described in subsequent exercises.

1. Go to www.gramene.org. This is Gramene's homepage.
2. Enter TB1 in the search box. This will redirect you to search.gramene.org.
3. Find the maize *tb1*(Zm00001d033673) gene. Click on the "Homology" tab.

Answer: There are 54 orthologs of maize TB1 in the current Gramene build #51.



b. What is the most prominent TCP domain among members of the gene tree? How many maize genes have a TCP domain?

Note: By looking at the maize TB1 gene tree in Gramene's genome browser, 3 InterPro domains with TCP features appear to be shared among family members. IPR005333 is considered a "family" of protein domains as it encompasses TCP domains: IPR017887 and IPR017888.

Answer: Again, there are multiple ways to answer a question.

1) Via search.gramene.org:
    a) Go to the "Homology" tab of the Search results for maize *tb1* (see above).
    b) Click on the most prominent blue colored domain (IPR017887).
    c) Simple answer: IPR017887 - Transcription factor TCP subgroup. *Note: From a closer look, wheat genes in the tree appear to be lacking protein domain annotation even though their protein sequence lines up with TCP domains.*
2) Via the genome browser:
    a) From the "Homology" tab in your search results (see above), click on "Ensembl Gene Tree view" OR go to ensembl.gramene.org, search for maize TB1 and click on (Plant Compara) Gene Tree (EPlGT00820000103607).

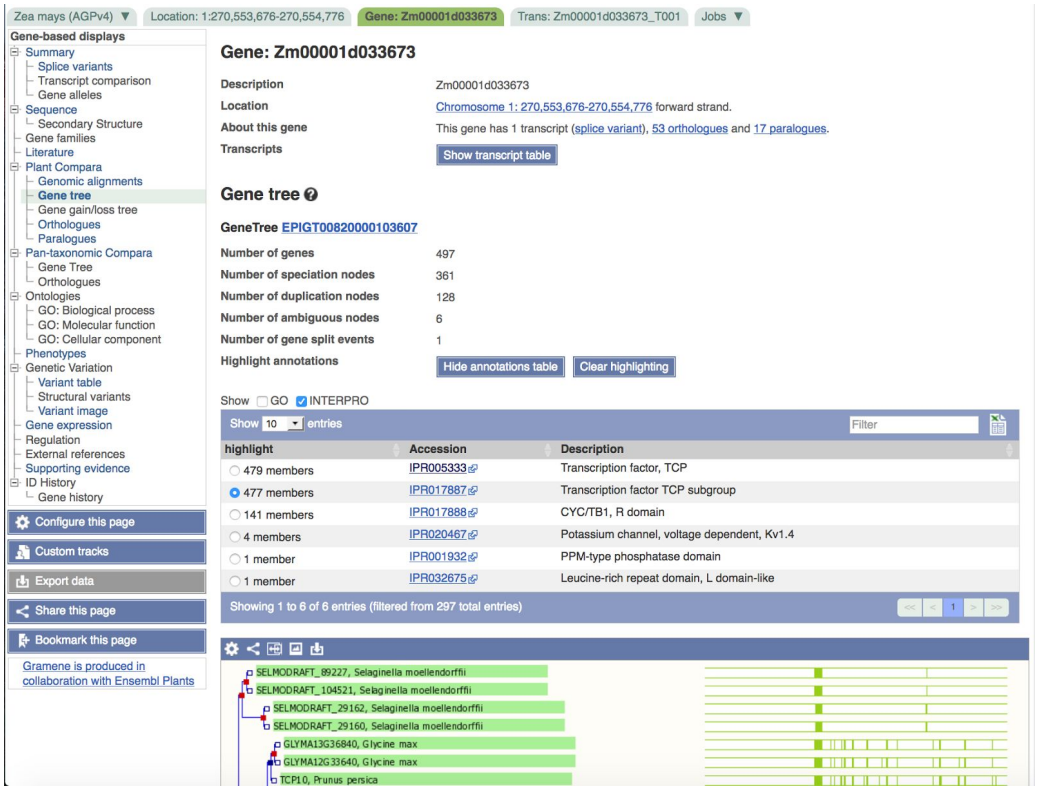
b) Select InterPro domains in the annotations table. By selecting an individual domain, all members that share it will be highlighted in the tree.

c) Detailed answer:

i) 479 members have IPR005333 Transcription factor, TCP.

ii) 477 members IPR017887 Transcription factor TCP subgroup

iii) 141 members IPR017888 CYC/TB1, R domain



3) Customized data dump: Using the BioMart utility.

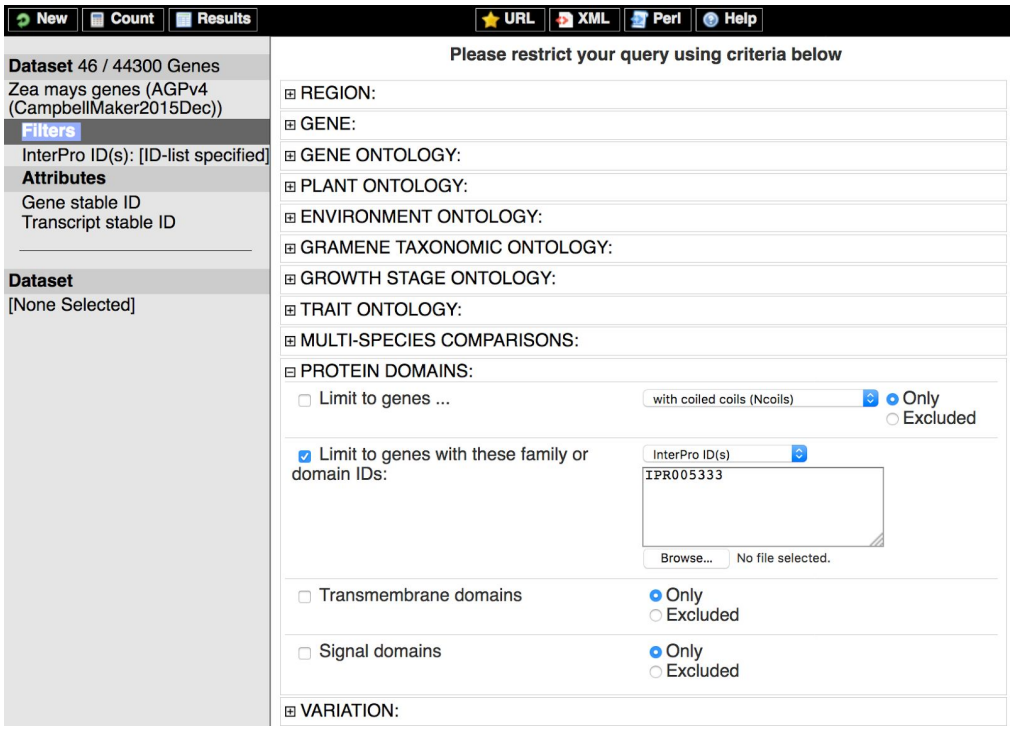
a) Go to http://ensembl.gramene.org/biomart/martview.

b) Select Database: “Plant Genes” and Dataset: “Zea mays genes”.

c) Under “Protein Domains”, select “Limit to genes with these family or (InterPro) domain IDs” and enter “IPR005333”, “IPR017887” or “IPR017888”.

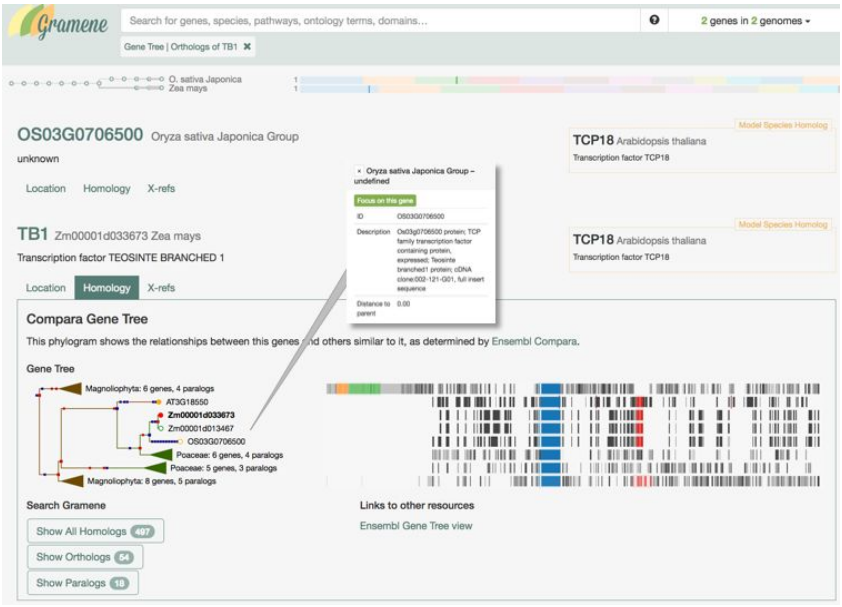
d) Click on “Count”. Alternatively, under “Attributes” select the associated data (e.g., gene or transcript ID, position, sequence, variants, GO terms, etc.) that you would like to download for these genes.

e) Answer: There are 46 maize genes with IPR005333, 45 with IPR017887 and 4 with IPR017888.

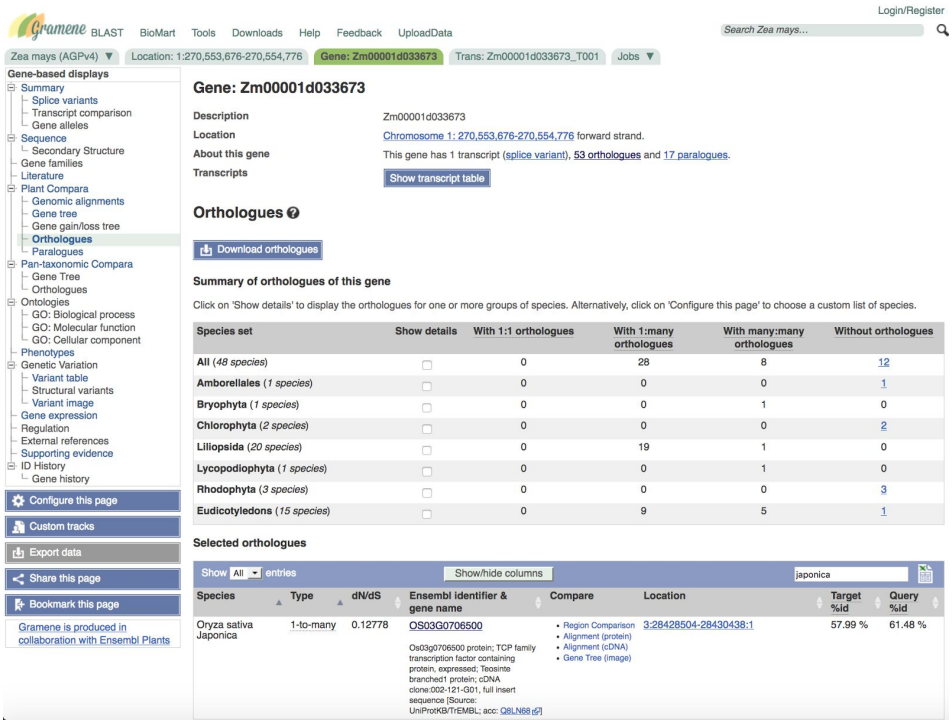


c. You have learned 3 ways to find orthologs for a given gene (via Search, Genome Browser and BioMart). Can you identify the (*Japonica*) rice ortholog of the maize *tb1* gene and highlight both genes in the TCP gene family tree?

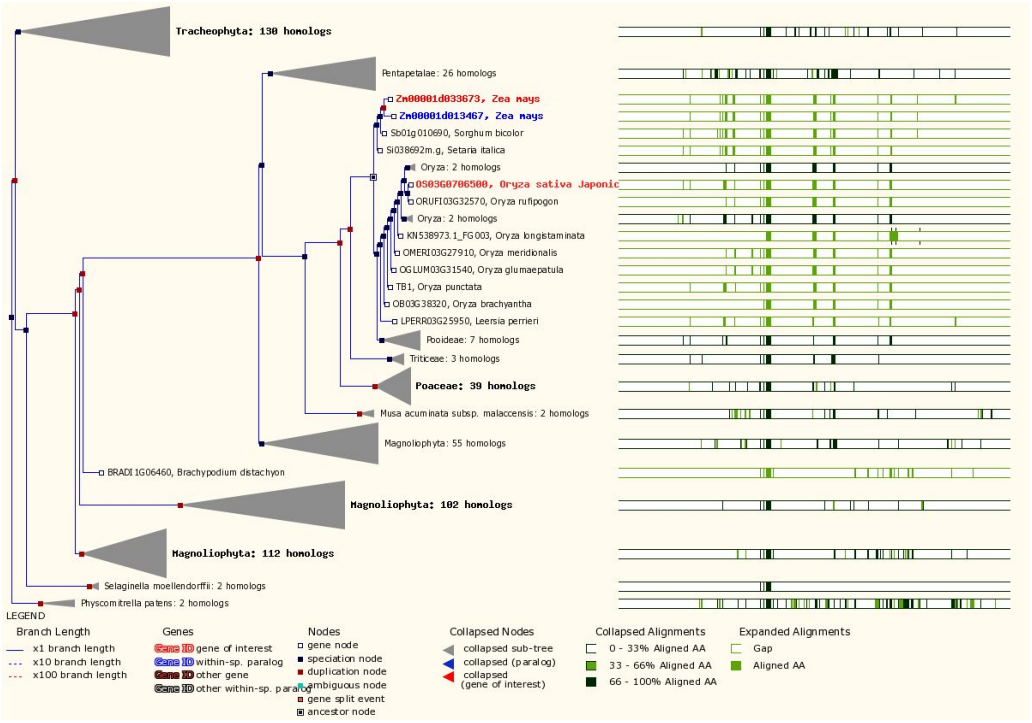
1) Via Search:
    a) From the “Homology” tab in Search results (see above), select *Zea mays* and *Oryza sativa japonica* from the drop-down menu on the top right of the Search page.
    b) Click on “Show Orthologs”.



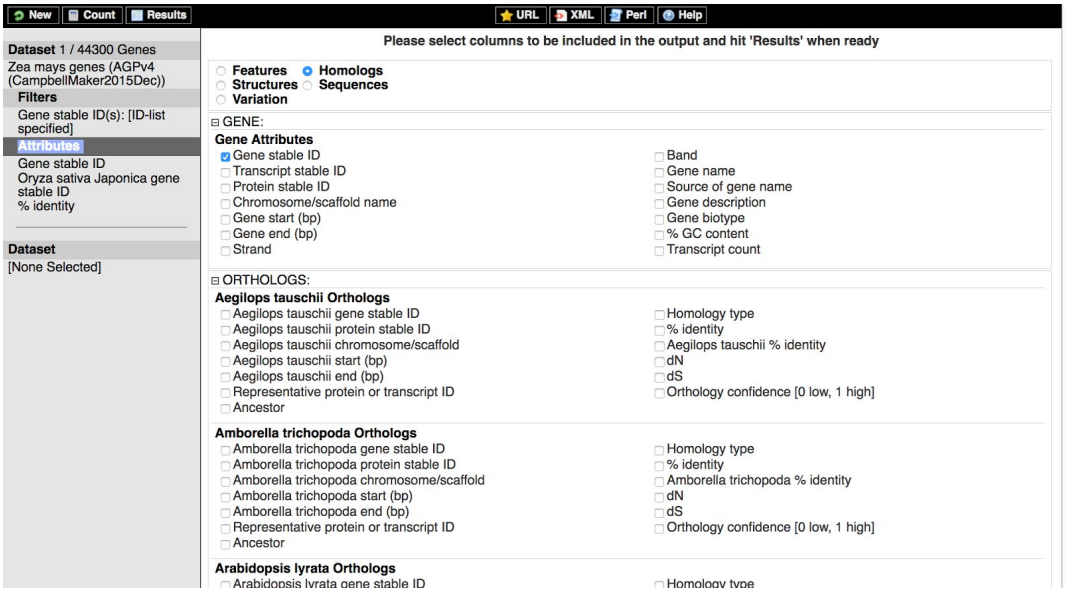
2) Via Genome Browser:
   a) From the left side menu of the Gene Summary page or the Plant Compara Gene Tree view (see above), select the (Plant Compara) "Orthologues" option
   b) Type "japonica" on the "Filter" box to select to show only rice orthologues in the results table.



   c) Click on the "Gene Tree (image) link" for the rice orthologue.



3) Via BioMart:
    a) From the "*Zea Mays* genes" data set in BioMart (see above), under the "Gene" filter, select "ID list limit".
    b) Enter "Zm00001d033673" as the "Gene stable ID" for maize *tb1*.
    c) Under "Attributes", select "Homologs".
    d) From the "Homologs" attributes form, under "Gene Attributes" select "Gene stable ID", and under "Orthologs" select "Oryza sativa Japonica gene stable ID" and any additional data desired (e.g., % identity).
    e) Click on "Results". Customize how to view and export your results.

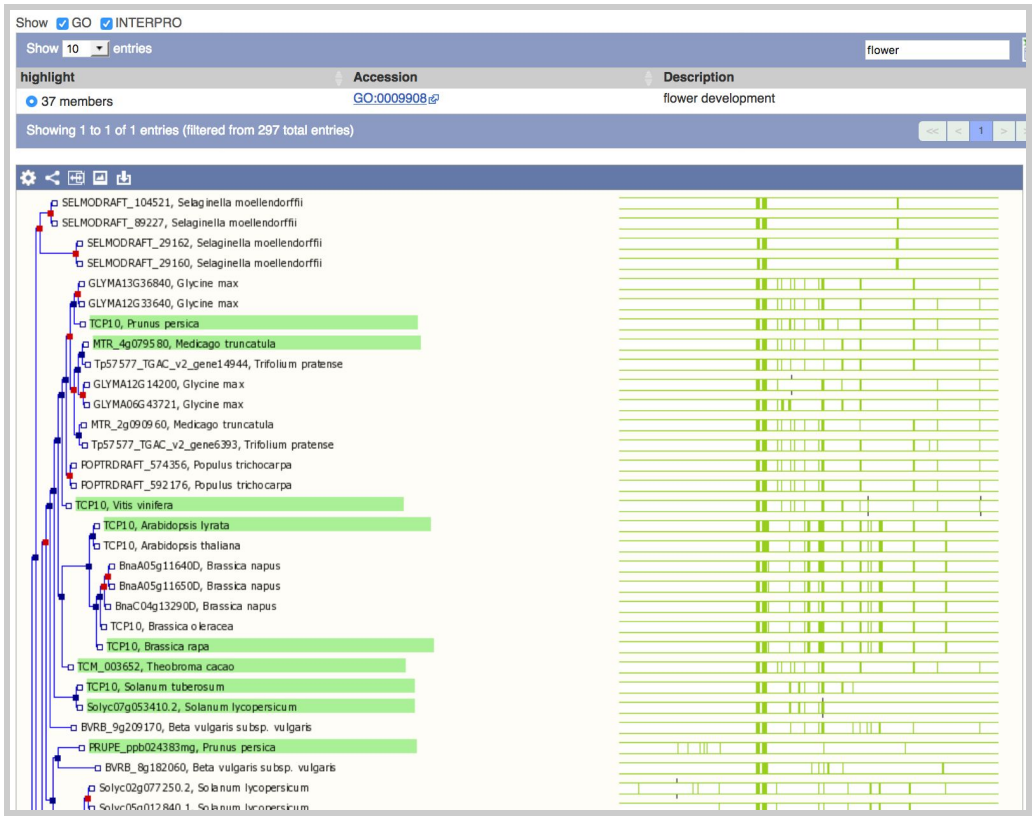


Answer: *OS03G0706500* (IRGSP1) or *LOC_Os03g49880* (MSU6).

d. Identify genes in the tree that have been associated with flower development.

Hint: GO:0009908 is the GO term identifier for “flower development”.

1. From the Plant Compara Gene Tree view (see above), enter the term “flower” in the Filter box to identify GO or InterPro term(s) for flower development.
2. Select GO:0009908



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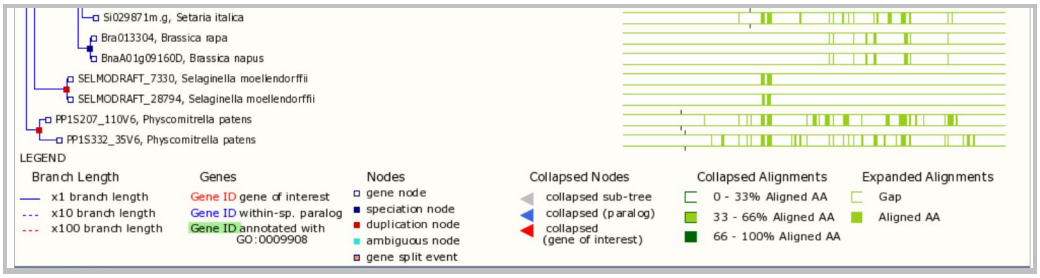


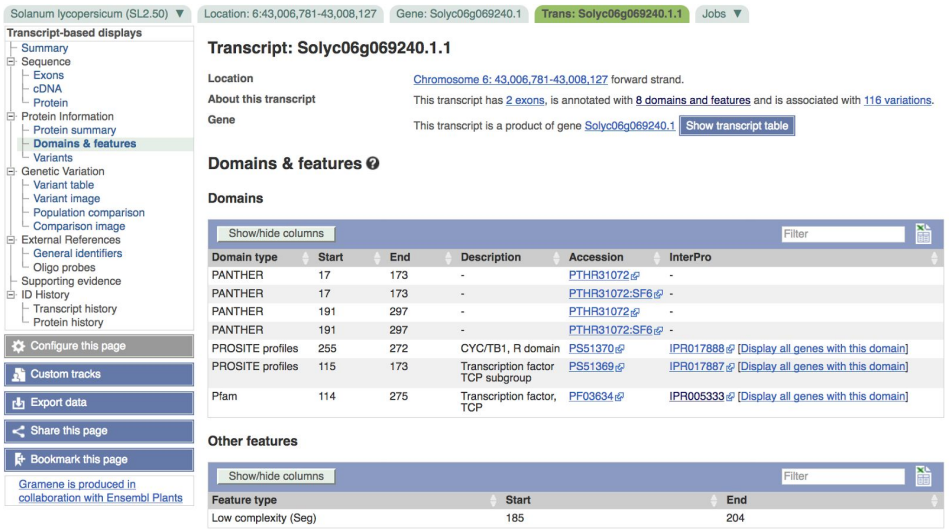
Exercise 2. Identify tomato transcription factors within the TCP gene family with a SNP that results in a truncated peptide.

Note: The SNP will introduce a stop codon(*) resulting in a truncated protein product.

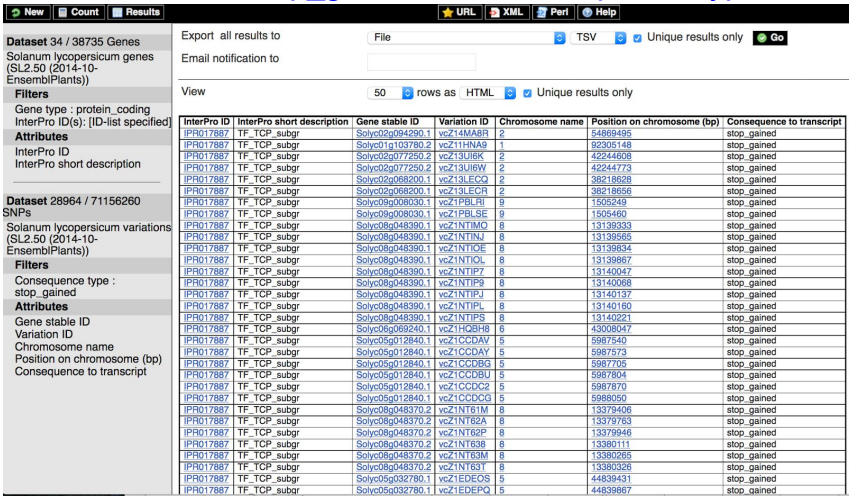
Hints:

1) Via Search and/or Genome Browser:
    a) From the Transcript page of Solyc06g069240.1, the tomato ortholog of maize *tb1* (proceed as above for the rice ortholog of TB1)
    b) Select “Domains & features” from the left side menu.

c) Find the IRP017887 domain and click on “Display all genes with this domain”.
d) Copy the resulting gene list and use it as input to mine for tomato variants with a “stop_gained” as functional consequence.



2) Via BioMart: First use IPR017887 (TCP domain) to Filter the tomato genes data set. Then select tomato variations as a second data set and under Filters, use “stop_gained” as “Consequence type”.



Exercise 3. Explore the genetic variation associated with a gene

We will now explore genetic variants along the Arabidopsis *cle18* gene to find 2 SNPs reported to have drastic functional consequences for the CLE18 peptide. CLE18 is a CLAVATA3/ESR-related (CLE) peptide with diverse roles in plant growth and development. Two functional consequences (Cao *et al*, 2011)[Nature Genetics].

a. Visualize the genetic variants for this gene
b. Are there any new stop codons introduced (nonsense variants) in this gene? Compare your findings with Supplementary Table 3
c. Are there any transcript-specific variants for this gene?
d. Download a subset of the variants (e.g., those that introduce an amino acid change in the protein)

Note: In addition to the Ensembl "Tools" for genomic analysis, other genetic analysis (e.g., Simple Sequence Repeat Identification Tool or SRIT) tools can be accessed through Gramene's archival Diversity pages at http://archive.gramene.org/diversity/tools.html

Exercise 4. Explore a metabolic pathway and compare it in two species

Let's now look at the carotenoid biosynthesis pathway in Gramene. You will notice that we currently offer two pathway platforms: Plant Reactome (rice reference pathways & orthology-based projections to 64 species) and BioCyc-based (10 plant species now served via Cyverse @ http://pathway.iplantcollaborative.org).

a. Search for "carotenoid biosynthesis"
b. Browse through results categories
c. Select annotations in the Plant Reactome (rice)
d. Download a list of proteins associated with this rice pathway in Reactome
e. Go back to the search results and look up for what species have annotated pathways in the BioCyc platform
f. Select rice and compare with maize
g. Download a list of all the genes associated with a carotenoid biosynthesis pathway in each, rice and maize
h. Check out other resources for maize pathways in MaizeGDB.Org

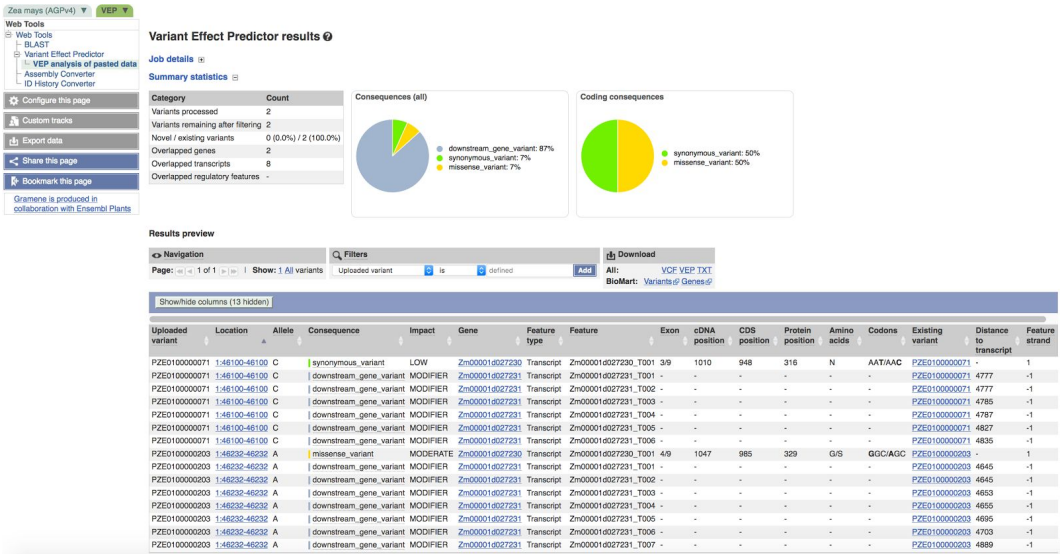
Exercise 5. Upload, visualize and share your own data into a new genome browser track

The Ensembl genome browser allows users to upload their own data and visualize it on a custom track. Data may be formatted in various file formats including GFF, GTF, BED, BAM, VCF, bedGraph, gbrowse, PSL, WIG, BigBed, BigWig, and TrackHub. Some data like GFF annotations may be directly uploaded from a local machine. Large data files like BED/BAM alignments or BigWig graphic display configurations need to be uploaded onto a local server that is accessible to the browser via an URL. Another way to share third-party data is via a DAS (Distributed Annotation System) registry, which would need to be set up by a software engineer.

The test data sets that we will upload and visualize for this exercise have been preloaded onto a local server that is publicly accessible: http://data.gramene.org/public/Zea_mays4m/methylome/. The data consists of BAM alignments and CpG methylation for B73 & Mo17 maize lines used in the study by Regulski *et al* (2013) [Genome Research 23:1651] and were used to create expression tracks in Gramene build 45.

Copy/paste the following sample VCF:

```
##fileformat=VCFv4.0
##fileDate=20161018
##source=MaizeHapMapMockUp
##reference=RefGenv4
##phasing=no
##INFO=<ID=MQ,Number=.,Type=Float,Description="RMS mapping quality">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT B73:MZ M97:MZ MKN009:MZ MKN010:MZ MKN011:MZ
1 46100 PZE0100000071 T C . PASS MQ=92 GT 0/0 0/0 0/0 0/0 0/0
1 46232 PZE0100000203 G A . PASS MQ=91 GT 0/0 0/0 0/0 0/0 0/0
```



Exercise 6. BLAST a sequence. Determine synteny for a genomic region. Convert coordinates between different genome assemblies.

In this exercise, we will identify orthologues of a species whose reference genome is not available in Gramene via BLASTX and find corresponding synteny blocks in other species.

a. Use the nucleotide sequence of the *Sorghum virgatum* Sh1 gene taken from Lin *et al* (2012) [Nature Genetics 44:720] to identify orthologous genes in maize, rice, and *Arabidopsis thaliana*.

Note: The corresponding gene in S. bicolor appears to be missing two exons.

b. Highlight the orthologs in two of those species in the tree as you learned in Exercise 1.

c. Download the genetic variation for each of the maize Sh1 orthologs as you learned in Exercise 2. How many nonsense substitutions can you find in each of these genes?

d. Lin *et al* (2012) also provide RefGen_v2 coordinates for maize shattering QTLs in Supplementary Table 5. Identify synteny blocks for the intervals at maize chromosomal regions (RefGen_v2) chr1: 259,223,260 - 261,622,457 and chr5: 15,806,322 - 16,428,681 in rice and sorghum. Download the synteny images that you generate.

Note: You will need to first use the Assembly converter tool to map the QTL intervals to RefGen_v3 and subsequently to RefGen_v4 coordinates. This will be publicly available in the upcoming Gramene build 52.

e. Download all the genes for a given synteny block. Can you identify a *Sh1* orthologous (YABBY-like) gene in it?

f. Compare your results with those in *Lin et al* (2012) [Nature Genetics 44:720]

>S. variegatum Sh1 CDS

ATGTCGGCCCAGCAGATCGCGCCGGTGCCGGAGCATGTGTGCTACGTGCACTGCAACTT
CTGCAATACAATTCTCGCGGTCAGTGTCCCGAGTCACAGCATGCTGAACATCGTGACAG
TCCGTTGTGGGCACTGCACTAGCCTGCTGTCAGTGAACTTGAGAGGACTCCTCCAATCA
CTCCCTGTCCAGAATCACTACTCGCAGGAGAATAATTTCAAGGTCCAAAATTTCAGCTT
TACTGAAAACTACCCTGAGTATGCACCTTCGTCTTCGAAATACCGCATGCCAACGATGT
TGTCAGCAAAAGGTGATCTGGATCATATGCTGCACGTGCGTGGTAAGCTCCAGAGAAGA
GGCAACGTGTTCCTTCAGCATATAACAGATTTATTAAGGAAGAGATACGAAGGATTAAA
GCAAGCAACCCAGACATAAGCCACAGGGAAGCCTTCAGCACTGCAGCAAAAAATTGGGC
ACATTTTCCAAACATTCATTTTGGACTAGGGCCCTATGAAAGTAGCAACAAGCTTGATG
AGGCCATTGGTGCAACGGGCCATCCCCAGAAAGTCCAAGATCTCTACTAA