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Recombinant DNA Technique

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Cloning tps1 Gene in *Citrus sinensis*

In the following weeks our group would like to establish the Cstps1 gene from the organism Citrus Sinensis (Orange) into a Biobrick compatible vector. The vector will then be inserted into compatible strain of *E. coli*. The *E. coli* is expected to create the proteins that this gene codes for and produce the processing enzyme of CVS. The CVS is then expected to react with the FPP in the media to radiate a distinct citrus scent commonly affiliated with Valencia Oranges.

Our technique to isolate the DNA in the Valencia Orange (*Citrus sinensis*) we will use the process found in the article An Efficient Protocol for Genomic DNA Extraction From Citrus Species. This protocol calls for many interesting materials including liquid nitrogen and chloroform (we’re excited!). After a series of solution manipulations we precipitate a pellet using isopropanol, washing the pellet, loving the pellet, and air drying the pellet. After adjusting concentrations within the DNA and other solutions, we will lead into our PCR process.

Our PCR process will rely heavily on the primers we have made for our gene. This may be a problem because in the process of researching our primers we have found out that there is a possibility of self-dimers which makes it hard to attach on to our DNA strand. Assuming the primers work smoothly we will then amplify the gene sequence to a workable amount to insert our gene into differently qualified vectors. Before the vector insertion step can happen our group has to do an extra step and resequence our gene in a determined search to find the pesky introns.

In the following process we hope to find no introns, 1647 base pairs, because then our gene would not need to create more primers. However, there is a possibility that the gene will have introns. In the abstract steps that follow we hope to locate any necessary removable introns. After running a PCR, we will resequence our gene to check for introns because during our gene search we were only able to find our mRNA code for the proteins and the cDNA. If the new sequence has more than 1647 base pairs then we need to find our introns. If introns exist we will create two more primers to splice the intron out. Once our gene is properly spliced, another PCR process is in order. The primers (Figure 1) will be the same to amplify our refined gene that was needed to locate our whole gene within the organism’s genome.

Primers

19\_orange1\_F: atgtcgtctggagaaacatt

19\_orange2\_R: tcaaaatggaacgtggtctc

19\_orange1a\_F: gaattcgcggccgcttctagatgtcgtctggagaaacatt

19\_orange2a\_R: tactagtagcggccgctgcagtcaaaatggaacgtggtctc

**Figure 1:** 1\_F is the forward primer for our gene, 2\_R is the reverse, 1a\_F is forward Biobrick-compatible primer, and 2a\_R is the reverse Biobrick-compatible primer

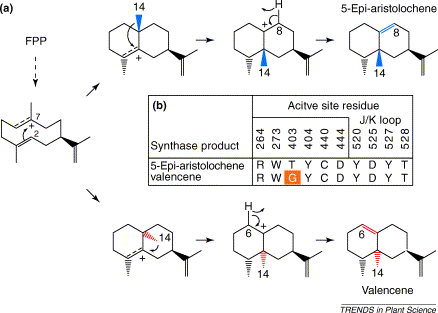
Promoters

[BBa\_I13453](http://partsregistry.org/wiki/index.php?title=Part:BBa_I13453)  
[BBa\_I0500](http://partsregistry.org/wiki/index.php?title=Part:BBa_I0500)  
[BBa\_I765001](http://partsregistry.org/wiki/index.php?title=Part:BBa_I765001)

**Figure 2:** BBa\_I13453 is a pBad induced promoter without AraC, BBa\_I0500 is induced by L-arabinose and repressed by AraC, BBa\_I765001 is an experimental UV induced promoter. All of these promoters are used in *E. coli* with Biobrick compatibility.

Furthermore we will then take the next step and insert our gene into the selected vectors. In our initial research we have established that the non-Biobrick-compatible primers may be easiest to work with resulting in an ultimate decision to use a T-vector as a transfer vector. Once implanted, our gene/vector will then be injected into the bacteria *E. coli*. This step is mostly necessary as a checkpoint to see if our gene/vector is properly inserted and the *E. coli* can sustain life with the gene/vector. Assuming our T-vector works we then splice out our gene via the EcoR1 and Spe1 restriction enzymes, only to ligate the spliced gene into our Biobrick-compatible vectors. The Biobrick-compatible vectors will have our selected promoters (Figure 2) already installed. Using the Biobrick-compatible vector we will transfer our gene into another set of *E. coli*. We will test and check this *E. coli* via nostrils. In our expected outcome the gene will be expressed and the CVS enzyme will be excreted into the media containing our FPP resulting in a citrus scent.

The citrus scent is a reaction, or process, that the CVS enzyme reacts with FPP that gives the Valencia Orange its smell. The farnesyl diphosphate (FPP) is the starting chemical until the CVS enzyme breaks it down to Valencene. Valencene is the actual chemical behind the citrus smell in oranges. As Figure 3 shows below, one can see how the FPP reacts and the synthesis process then forms two different molecules. The products, 5-Epi-aristolochene and Valencene, are similar shaped chemicals with Valencene having a *cis* carbon 14 and a double bond on carbon 6, whereas the 5-Epi-aristolochene has a *trans* carbon 14 and a double bond on carbon 8.



**Figure 3:** The enantiomers Valencene and 5-Epi-aristolochene are the result of the CVS enzyme and FPP.

In conclusion, our plan is to produce *E. coli* that has the citrus scent. We will first follow protocol in isolating the DNA of the *Citrus sinensis*. Next, we will amplify the gene from our DNA via PCR method and our researched primers. In addition to selecting this gene we need to resequence the gene to check for introns. If introns are present they will be spliced out with new primers and the PCR with our original primers will be redone with the refined gene. Finally the gene will be placed into the T-vector as a checkpoint, to make sure our gene does not affect the livelihood of the *E. coli*. After this is confirmed we will then splice the T-vector and the Biobrick-compatible vector and ligate our gene into the Biobrick-compatible vector. This is our final step before our final test of Biobrick-compatible *E. coli* that produces the CVS enzyme within the FPP media to emit a citrus scent.

References

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