Identification, isolation and functional characterization of terpene transferases in *Cannabis sativa*

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Theoretical background

Secondary plant metabolites are pharmaceutically interesting compounds. The diversity of these compounds depends considerably on the aromatic prenylation reactions. In general the enzymes which catalyze these reactions are called terpene or prenyltransferases. Identification of new terpene transferases with similar function from already known enzymes depends on creation of a cDNA library of the referring plant and mapping it towards already known gene sequences of other transferases.
Cannabis sativa possesses therapeutically relevant compounds. Besides the most recognized cannabinoid Δ^9-Tetrahydrocannabinol (Δ9-THC) there are others with anti-inflammatory, anti-fungal and anti-microbial properties. The goal of this project is to identify new promiscuous candidate genes involved in prenylation reactions in the metabolic pathway of cannabinoids. Furthermore, screening for pharmaceutically and therapeutically important compounds should be performed among the end-products of the selected enzymes.

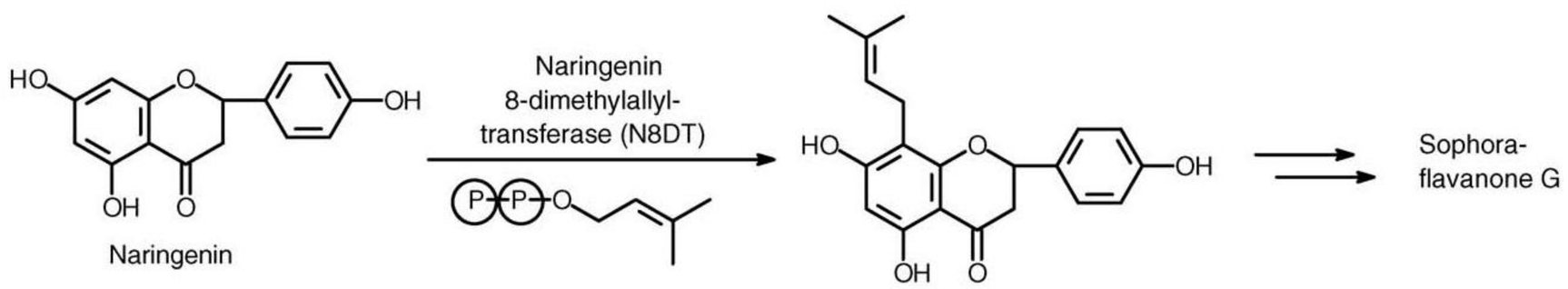
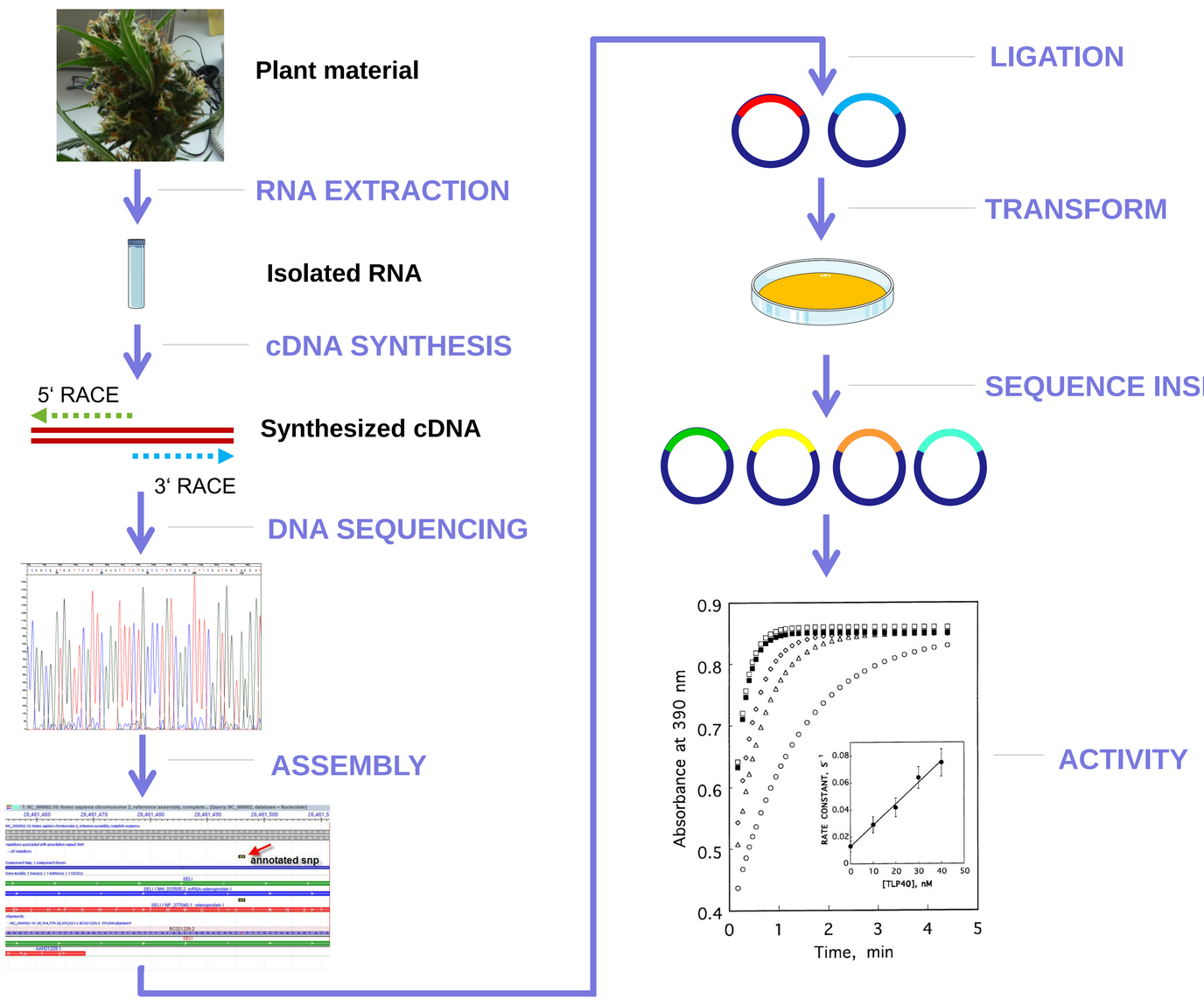


Fig. 1: Enzyme reaction of a prenyl transferase from *Sophora flavescens* [1]

An example of working on identification of new terpene transferases in plant-secondary metabolism is the attempt to clone a naringenin 8-dimethylallyltransferase (N8DT) from *Sophora flavescens* (*Fabaceae*). This enzyme is a membrane-bound prenyltransferase localized in the plastids which is involved in the prenylflavonoid and prenylisoflavonoid biosynthesis. A cDNA library was generated from a cell culture and the search comprised among other things an aspartate-rich motif. This motif serves as possible binding site for prenyl diphosphates.

Project structure



Generating a cDNA library from trichomes from *Cannabis sativa*

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Checking literature for sequences of known terpene transferases involved in biosynthesis of Cannabinoids

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Assembly of reference sequences against generated cDNA library

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Cloning of candidate genes in microorganims such as *E.coli* or *S.cerevisiae*

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Analysis of protein expression, activity and characteristics

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Large scale production of pharmaceutically relevant compound

Fig. 2: Schematic illustration of project structure; pictures modified from reference [2]

References
[1] Heide L (2009) Curr Opin in Chem Biol 13: 171-179.
[2] www.acgtinc.com, http://www.nature.com/emboj/journal/v17/n6/images/7590872f6.jpg, http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/img/transcript_diff_snp.png, http://coastal.er.usgs.gov/coral-microbes/images/Figure3LG.gif
[3] Sirikantaramas S, Taura F, Morimoto S, Shoyama Y (2007) Curr Pharm Biotechnol 8: 237-243.