

August 2014

## Metabolic Modeling of Secondary Metabolism in Plant Systems

Lisa M. Leone  
*University of Massachusetts Amherst*

Follow this and additional works at: [https://scholarworks.umass.edu/masters\\_theses\\_2](https://scholarworks.umass.edu/masters_theses_2)



Part of the [Biochemical and Biomolecular Engineering Commons](#), and the [Molecular, Cellular, and Tissue Engineering Commons](#)

---

### Recommended Citation

Leone, Lisa M., "Metabolic Modeling of Secondary Metabolism in Plant Systems" (2014). *Masters Theses*. 27.

[https://scholarworks.umass.edu/masters\\_theses\\_2/27](https://scholarworks.umass.edu/masters_theses_2/27)

This Open Access Thesis is brought to you for free and open access by the Dissertations and Theses at ScholarWorks@UMass Amherst. It has been accepted for inclusion in Masters Theses by an authorized administrator of ScholarWorks@UMass Amherst. For more information, please contact [scholarworks@library.umass.edu](mailto:scholarworks@library.umass.edu).

**METABOLIC MODELING OF SECONDARY METABOLISM IN PLANT SYSTEMS**

A Thesis Presented

by

LISA M. LEONE

Submitted to the Graduate School of the

University of Massachusetts Amherst in partial fulfillment

of the requirements for the degree of

**MASTER OF SCIENCE IN CHEMICAL ENGINEERING**

May 2014

Department of Chemical Engineering

METABOLIC MODELING OF SECONDARY METABOLISM IN PLANT SYSTEMS

A Thesis Presented

By

LISA M. LEONE

Approved as to style and content by:

---

Susan Roberts , Chair

---

Michael Henson, Member

---

Jeffrey Blanchard, Member

---

T. J. Mountziaris, Department Head  
Chemical Engineering

## **DEDICATION**

This thesis is dedicated to the memory of Babur Z. Khalique, whose passing was more absurd and untimely than my own.

## EPIGRAPH

Myself when young did eagerly frequent  
Doctor and Saint, and heard great Argument  
About it and about: but evermore  
Came out of the same Door as in I went.

With them the Seed of Wisdom did I sow,  
And with my own hand labour'd it to grow:  
And this was all the Harvest that I reap'd—  
“I came like Water, and like Wind I go.”

- Omar Khayyam

## **ACKNOWLEDGMENTS**

I would like to thank my advisors, Susan Roberts and Mike Henson, for supervising my work and teaching me so much about what academic science is about.

I would like to thank my department head Lakis, and also Marie Wallace, for helping me navigate the bureaucracy necessary to complete my degree.

I would like to thank my labmates in the Roberts lab (Sarah, Rohan, Whitney and Marty) as well as my colleagues in the Chemical Engineering department, for making the grad school experience a little more bearable.

I would like to thank my parents, Robert and Ellen Leone, for supporting me in all my decisions, including my decision to leave graduate school. I would also like to thank my future in-laws, Nan Jacobson Wise and John Wise, for being supportive of me during my transitive period.

Most of all I would like to thank my fiancée, Adam Wise, for helping me to be brave enough to leave a program that was making me miserable, for helping me in every way imaginable to transition to the working world, and for being the most amazing person I've ever met.

## ABSTRACT

### METABOLIC MODELING OF SECONDARY METABOLISM IN PLANT SYSTEMS

MAY 2014

LISA M. LEONE, B.S., DREXEL UNIVERSITY

M.S.Ch.E, UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Susan C. Roberts

In the first part of this research, we constructed a Genome scale Metabolic Model (GEM) of *Taxus cuspidata*, a medicinal plant used to produce paclitaxel (Taxol®). The construction of the *T. cuspidata* GEM was predicated on recent acquisition of a transcriptome of *T. cuspidata* metabolism under methyl jasmonate (MJ) elicited conditions (when paclitaxel is produced) and unelicited conditions (when paclitaxel is not produced). Construction of the draft model, in which transcriptomic data from elicited and unelicited conditions were included, utilized tools including the ModelSEED developed by Argonne National Laboratory. Although a model was successfully created and gapfilled by ModelSEED using their software, we were not able to reproduce their results using COBRA, a widely accepted FBA software package. Further work needs to be done to figure out how to run ModelSEED models on commonly available software.

In the second part of this research, we modeled the MJ elicited/defense response phenotype in *Arabidopsis thaliana*. Previously published models of *A. thaliana* were tested for suitability in modeling the MJ elicited phenotype using publicly available computation tools. MJ elicited and unelicited datasets were compared to ascertain differences in metabolism between these two phenotypes. The MJ elicited and unelicited datasets were significantly different in many respects, including the expression levels of many genes associated with secondary metabolism. However, it was found that the expression of genes related to growth and central metabolism were not

generally significantly different for the MJ+ and MJ- datasets, the pathways associated with secondary metabolism were incomplete and could not be modeled, and FBA methods did not show the difference in growth that was expected. These results suggest that behavior associated with the MJ+ phenotype such as slow growth and secondary metabolite production may be controlled by factors not easily modeled with transcriptome data alone.

Additional research was performed in the area of cryosectioning and immunostaining of fixed *Taxus* aggregates. Protocols developed for this work can be found in Appendix B.



## TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS .....	v
ABSTRACT.....	vi
LIST OF TABLES .....	xi
LIST OF FIGURES .....	xii
CHAPTER	
1. PLANT SECONDARY METABOLISM.....	1
Paradigms of Anti-Cancer Drug Discovery .....	1
Natural Product Diversity and Production.....	2
Natural Harvest.....	5
Chemical Synthesis.....	5
Heterologous Synthesis in Microbes .....	6
Plant Cell Culture.....	7
Plant Cell Suspension Culture .....	8
Hairy Root Culture.....	10

Yield Enhancement Strategies .....	11
The Paclitaxel Story .....	14
Research Objectives .....	17
2. METABOLIC MODEL OF <i>TAXUS CUSPIDATA</i> .....	18
Background .....	18
Flux Balance Analysis .....	18
Creation of Metabolic Networks .....	19
Results .....	20
Illumina Data Translation .....	20
Draft Model .....	21
Discussion .....	25
3. GENOME SCALE METABOLIC MODELING OF <i>ARABIDOPSIS THALIANA</i> UNDER METHYL JASMONATE ELICITED CONDITIONS .....	27
Introduction .....	27
Materials and Methods .....	30
Model .....	30
Data .....	30
GIMME and Essential Genes .....	32
FBA and Phenotype Simulations .....	32

Results .....	33
GIMME.....	33
E-Flux .....	38
Discussion.....	44
Gene Essentiality .....	44
Phenotype Simulation (GIMME and E-Flux).....	46
APPENDICES	
A. TABLES FOR GENOME SCALE METABOLIC MODELING OF <i>ARABIDOPSIS</i>	
<i>THALIANA</i> UNDER METHYL JASMONATE ELICITED CONDITIONS.....	51
B. CRYOSECTIONING AND IMMUNOFLUORESCENCE PROTOCOLS .....	145
BIBLIOGRAPHY .....	152

## LIST OF TABLES

Table	Page
Table 1 – Media components and bounds in <i>T. cuspidata</i> .....	23
Table 2. Draft GEM model statistics. ....	23
Table 3. Lowly expressed genes in MJ+ and MJ- conditions. ....	36
Table 4 Growth in E-Flux simulations vs. FBA. Growth was calculated as the flux through the “biomass” reaction.....	39
Table 5 – List of 15 genes that were found to be significantly responsive to MJ in (Pauwels et al. 2008) and also carried non-zero fluxes in 17 reactions in the FBA model of <i>A. thaliana</i> metabolism. Many of the genes (e.g. Sinapyl alcohol NADP oxidoreductase, p coumaroyl CoA caffeoyl CoA 3 hydroxylase) are associated with the lignin pathway (Humphreys & Chapple 2002). All of the genes were positively up regulated at the 6 hour time point. ....	44
Table 6 – List of genes included in <i>A. thaliana</i> model and relative expression values (from (Pauwels et al. 2008)). Values of “n/a” indicate that the gene was not in the expression dataset for two possible reasons, one being that the gene was not included on the ATH1 chip, and the other being that the probe corresponding to the gene was discarded from the analysis because of technical issues with the microarray. ....	81
Table 7 – Table of upper and lower bounds for MJ- and MJ+ conditions in E-Flux simulations. ....	144

## LIST OF FIGURES

Figure	Page
Figure 1 – Anti-cancer natural products derived from plants. ....	2
Figure 2 – Biosynthetic pathways of paclitaxel and Vinca alkaloids in <i>Taxus</i> and <i>C. roseus</i> plants respectively. ....	4
Figure 3 - Relationship between biomass growth rate (g dry weight/L/Day) and sucrose uptake rate (g/L/Day) from experiment (points) and draft model .....	24
Figure 4 Analysis of <i>A. thaliana</i> metabolic network requirements. (a) Histogram of essential genes required to produce biomass using 100 randomly generated expression sets. The red asterisk represents the number of essential genes in MJ+ and MJ- datasets with same analysis. (b) Histogram of total number of reactions carrying flux in aforementioned simulations. ....	34
Figure 5. Essential vs. non-essential genes. Blue bars represent MJ- (unelicited control), red bars represent MJ+ (elicited). Expression levels of essential genes have higher expression levels (>10), while non-essential genes have lower expression levels (<5). ....	37
Figure 6. E-flux comparison of fluxes from MJ- to MJ+ (a) MJ- (x-axis) E-flux derived fluxes plotted against MJ+ (y-axis) E-flux derived fluxes. The sets of fluxes are well correlated ( $R^2 = 0.962$ ), although experimentally these conditions exhibited divergent behavior. (b) MJ- (blue <b>cross</b> ) and MJ+ (red <b>x</b> ) e-flux derived fluxes plotted against FBA derived fluxes (x-axis). MJ- and MJ+ are not well correlated with FBA fluxes ( $R^2 = 0.412$ and $R^2 = 0.4028$ respectively) but the E-flux derived datasets are both extremely similar to each other. Both trendlines are right on top of each other. ....	40
Figure 7. Relative gene expression values of MJ- (blue cross) and MJ+ (red x) plotted against FBA derived fluxes. Many reactions with high expression are not included in the model, and it can be seen that of the highly expressed genes that exhibit high positive or negative flux in the model, there are not significant differences in MJ- vs. MJ+ datasets.....	41
Figure 8 – Example of a cryosectioned <i>Taxus</i> aggregate stained with calcufluor (blue) to designate cell wall and propidium iodide (red) to denote the nucleus. This aggregate was sliced into 30 $\mu\text{m}$ slices.....	151

# CHAPTER 1

## PLANT SECONDARY METABOLISM

Much of this text was taken from (Leone & Roberts 2013) with permission from Springer.

### **Paradigms of Anti-Cancer Drug Discovery**

From 1981 to 2002, 74% of the anticancer agents approved by the FDA were inspired by, derived from, or true natural products (NPs) (Wilson & Danishefsky, 2006). Biologically-derived compounds often exhibit “privileged structures” in terms of biological activity (Evans et al. 1988). Unfortunately, these structures are often chirally complex and pose unique challenges for large-scale commercial production. For a significant number of NPs, a route to chemical synthesis does not exist, and for the vast majority of NPs, total chemical synthesis methods are not commercially viable due to structural complexity, low yields and environmental concerns (Kolewe et al. 2008). In the 1970s and 1980s, a lack of immediate production strategies for some NPs entering clinical trials led to a perceived “supply crisis” for certain drugs, including the anti-cancer compounds paclitaxel and camptothecin. These situations led to reluctance in the pharmaceutical industry for investment in NPs.

The ease of chemical synthesis in simple, non-NP-derived molecules is perhaps one reason why synthetic combinatorial libraries became popular in drug development (Ortholand & Ganesan 2004). The total chemical synthesis of a synthetic combinatorial library’s components as a starting point ensures that every compound will have a relatively simple (and known) route for eventual large-scale production. A disappointing result of this cost-saving methodology is that the systematic exploration of organic chemistry space has been limited to known and easily synthesizable structures, which are not necessarily biologically relevant (Lipkus et al. 2008). Among the millions of compounds screened in academia and industry, only a relatively small

number of compounds from high-throughput screens have been shown to exhibit activity against drug targets, and even fewer have overcome toxicity issues and moved to clinical trials (Newman & Cragg 2007). Because of these difficulties, synthetic combinatorial libraries have shifted over the past decade from large collections of simple compounds to a diversity-centered exploration of a smaller chemistry space, aided by computational technologies (Schnur et al. 2011).

With this new focus, the drug industry has reverted back to a paradigm in which natural organisms have unique advantages over a chemist's bench. Plants, marine organisms and microbes still represent an extremely diverse and relatively untapped space for lead discovery. The NP research of the past 30 years has made rapid progress in alleviating possible supply issues through a variety of advanced technologies, including plant cell culture (PCC) and heterologous expression in microbial systems, as discussed below.

### Natural Product Diversity and Production

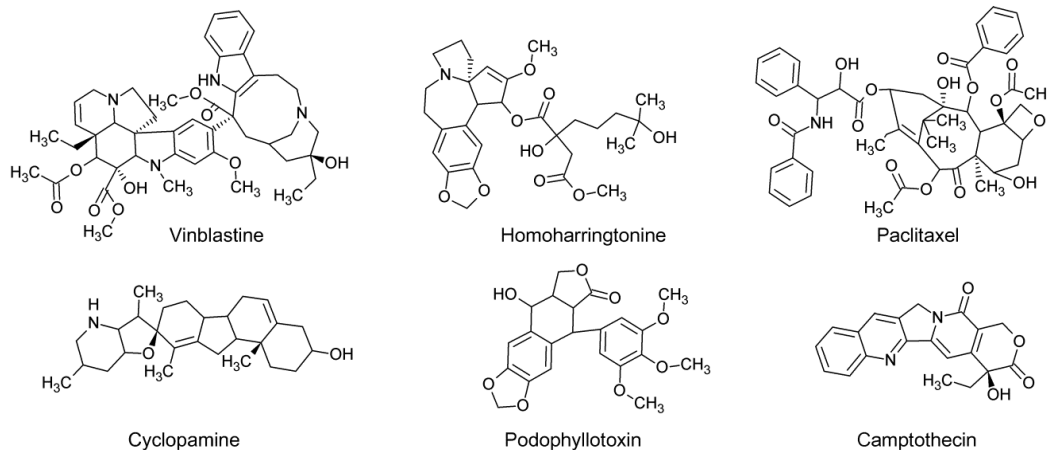


Figure 1 – Anti-cancer natural products derived from plants.

Naturally occurring plants, microbes and fungi have been the source of a staggering amount of NP diversity throughout the history of drug discovery. In the case of plants, over 10,000 alkaloids and 23,000 terpenoids have been characterized, and these most certainly are an underestimation

of the capabilities of plants, of which only a small portion of the world's species are estimated to have been sampled (Cheng et al. 2007). Many of these NPs (Figure 1) have medicinal qualities.

Most plant NPs fall into the class of secondary metabolites (SMs), so called because they do not necessarily serve primary metabolic functions in the growth and maintenance of their native plant. Biosynthesis of plant SMs is complex, involving many different precursors from primary metabolism. Some of these pathways are interconnected, for example, all of the compounds within the alkaloid and terpenoid classes of NPs originate in part from the common precursor isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyldiphosphate (DMAPP).

Biosynthesis of many SMs often requires dozens of enzymes acting in concert, and as a result is under strict metabolic control. The abbreviated biosynthetic pathways of paclitaxel and the Vinca alkaloids in *Taxus* spp. and *Catharanthus roseus*, respectively, are shown in Figure 2. While SMs do not play a role in primary metabolism in that they do not directly pertain to plant growth or maintenance, many plant NPs have shown activity in nature as insecticides and anti-fungals, and are thought to confer evolutionary advantages to their native plant (Hartmann 2007).



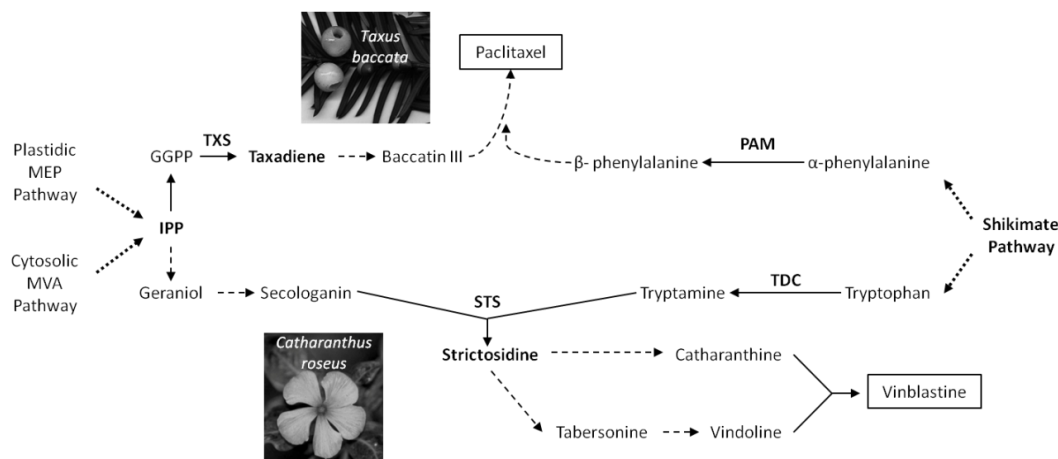


Figure 2 – Biosynthetic pathways of paclitaxel and Vinca alkaloids in *Taxus* and *C. roseus* plants respectively.

The “screening” hypothesis of NP diversity postulates that the chances of any single NP possessing a potent bioactivity, as a fungicide or insecticide for example, is very low. Therefore, it is advantageous for plants to produce many slightly different metabolites in small concentrations, in the hopes that at least one will be useful against an external stressor, such as herbivorous insects (Firn & Jones 2003). In this perspective, a single plant can be compared to a naturally occurring drug discovery program, in which many compounds with slightly different functionalities are produced from simple starting structures. Plants accomplish this incredible diversity at minimal biosynthetic cost by employing promiscuous enzymes, using branched and matrix biosynthetic pathways, and generally producing minute quantities of these SMs (Fischbach & Clardy 2007).

Different amounts of pure product are needed at different stages of development. After a promising compound has been identified, milligram quantities are needed for structural determination, multiple grams are needed for clinical development, and multiple kilograms are needed for initial clinical trials (Cragg, Boyd, Grever, Schepartz, & Grever, 1995; Koehn & Carter, 2005). If a novel compound is found to be effective in clinical trials and approved for use,

supply will need to quickly ramp up to many kilograms for commercialization. As shown in the story of paclitaxel (see below), supply issues can be a major hurdle for NPs. Supply issues are especially untenable in the treatment of terminal diseases such as cancer, where patients cannot wait months or years for a supply route to be established. Fortunately, the field of NP synthesis has matured since the paclitaxel supply crisis of the 1990s and there now exist many options for supply depending on the properties of the NP and plant source.

### **Natural Harvest**

Natural harvest is the method by which many plant NPs have initially been collected, and for intermediate to long term production it is an option when 1) the plant producing the compound of interest is fast growing and easily cultivable, as in the case of *C. roseus*, or 2) when the product of interest is produced in relatively large quantities *in planta*, as in the case of podophyllotoxin in *Podophyllum hexandrum* rhizomes (Farkya et al. 2004). For many naturally occurring compounds neither of these conditions is met, and environmental concerns can also arise when a plant is endangered or vital to a local ecosystem. Additionally, in the case of novel NPs found through strategies such as combinatorial biosynthesis, the native plant will generally not produce the compound at all.

### **Chemical Synthesis**

Generally a default for the chemical industry, chemical synthesis is an option when the compound of interest is relatively simple and easy to synthesize, as in the case of flavopiridol, an anti-cancer NP in clinical trials that is currently supplied via total synthesis (Naik et al. 1988). Complete chemical syntheses have been published for many NPs of interest, such as paclitaxel (Nicolau et al. 1994; Holton et al. 1994), but they often do not represent a viable, scaleable production method due to low yields, complexity and number of steps, as well as the use of environmentally

unfriendly chemicals and solvents. Chemical semi-synthesis can be used to create NP derivatives with more favorable pharmacological properties, as in the case of the camptothecin derivatives irinotecan and topotecan, and in some cases, can be used to derive a NP from precursors that occur naturally at high levels. For example, a paclitaxel intermediate, 10-deacetylbaccatin III, was chemically modified to produce a semi-synthetic version of paclitaxel in the 1990s (Denis et al. 1988) and was used as the primary supply route for paclitaxel by Bristol-Myers Squibb for over a decade.

### **Heterologous Synthesis in Microbes**

Transfer of plant NP pathways to microbial hosts has advanced rapidly in the past decade. The driving force behind this development is the perceived advantage of microbes, such as *Escherichia coli* and *Saccharomyces cerevisiae*, as production organisms for fermentation, because they are well-studied model systems; additionally, a mature infrastructure exists for the fermentation of these microbes at large scales. One of the first success stories in this field was heterologous expression in yeast (Ro et al. 2006) and *E. coli* (Dietrich et al. 2009) of enzymes to synthesize precursors to artemisinin, an anti-malarial agent from the plant *Artemisia annua*. Under a tripartite partnership of Amyris Biotechnologies, the Institute for One World Health and the pharmaceutical company Sanofi-Aventis, the fermentation for artemisinin is currently moving into the commercial phase and is expected to begin production in 2012 (Hale et al. 2007; Solomon 2011). Efforts to transfer metabolic pathways for isoflavones (Leonard et al. 2008), taxanes (Ajikumar et al. 2010), and alkaloids (Nakagawa et al. 2011) are currently underway. However, major hurdles to microbial biosynthesis still exist, including incomplete NP biosynthesis pathway knowledge and access to cloned genes, as well as the difficulty in expressing plant enzymes in microbial systems.

Despite much research, knowledge of the biosynthetic pathways of many important plant NPs remain incomplete *in planta*. For example, in the paclitaxel pathway there are thought to be approximately 19 steps, of which several remain completely unknown, and among the known steps, enzyme identification is incomplete (Croteau et al. 2006). The formation of the oxetane ring in particular is essential to the clinical function of paclitaxel (Kingston 1994), but enzyme candidates for this reaction have not yet been found. A special difficulty in identifying biosynthesis genes is that genes in the same biosynthetic pathway are not clustered in plants as in microbial systems, making identification difficult unless the entire plant genome is sequenced (Schäfer & Wink 2009). Next-generation sequencing techniques are starting to alleviate this challenge, but in the present and immediate future, lack of plant genetic knowledge and tools has been a major bottleneck in the transfer of plant pathways to microbes.

Another challenge that must be addressed on a case by case basis for microbial transfer is enzyme engineering. Adapting enzymes for optimal function in a non-native host can be difficult, particularly for plant cytochrome P450s (CYP450s). CYP450s and their associated reductase partners are heme-thiolate enzymes constituting one of the largest super families of proteins, and are found in both prokaryotes and eukaryotes. In eukaryotes, CYP450s are membrane bound, while in prokaryotes they are soluble (Werck-Reichhart & Feyereisen 2000). CYP450s are responsible for many of the unusual oxidative reactions seen in the biosynthesis of plant SMs, making them essential for heterologous expression of many plant NPs. Numerous challenges exist in the expression of plant CYP450s, including: improper folding, lack of an appropriate membrane binding site in prokaryotes, and difficulties in maintaining adequate NADPH pools for reductase activity. These difficulties can be overcome on a case by case basis via protein engineering and related strategies, and multiple functioning plant CYP450s have been successfully expressed in *E. coli* (Chemler & Koffas 2008).

## **Plant Cell Culture**

Heterologous production of plant NPs in microbes will continue to develop for the foreseeable future, but it is important to situate these breakthroughs in the context of current pharmaceutical supply. Microbial fermentation has been touted as a cost-effective route for large-scale production of high value plant NPs, but as mentioned above, it is not possible for certain NPs, particularly for those with undefined biosynthetic routes. Plant cell culture (PCC) is an attractive option for immediate production of plant NPs when heterologous production is infeasible.

Environmentally-friendly PCC has been used industrially for plant NP supply since 1984, when Mitsui Petrochemical Industries, Ltd. began using a cell suspension of *Lithospermum erythrorhizon* to produce the pigment shikonin on a 750-L bioreactor scale (Georgiev et al. 2009). Today, PCC is utilized commercially to produce a wide variety of pharmaceuticals, pigments and food additives at scales up to 75,000-L, as in the case of Phyton Biotech, Inc.'s paclitaxel process.

Plant SMs, which form the majority of plant NPs, are often under strict and specific metabolic control; for example, some plant NPs are synthesized in response to exogenously applied elicitors such as methyl jasmonate, salicylic acid or nitric oxide, which are known to be key signaling molecules in the plant defense response (Zhao et al. 2005). Some plant NPs require specialized cell types to synthesize the desired NP; for example, the biosynthesis of vindoline, one intermediate in the vinblastine and vincristine pathway (Figure 2) appears to require three different mature cell types and multiple intracellular compartments (Kutchan 2005). Overcoming limitations imposed by metabolic control is a major area of research, with efforts placed towards activating inherent secondary metabolism via elicitation, as well as metabolic engineering and expression of transcription factors to rationally control plant metabolism.

### **Plant Cell Suspension Culture**

Plant cell suspension culture (PCSC) is a subset of PCC in which cells from an intact plant or plant embryo are “de-differentiated” using phytohormones and grown in liquid media. Plant cells are totipotent, in that given correct signals they can differentiate and develop into any plant cell type or organ; this also gives them the ability to be maintained in a de-differentiated state. Given their constant growth, PCSCs must be subcultured into fresh media every 1-4 weeks depending on their specific growth rate. Importantly, cryopreservation techniques have been developed for a number of medicinally-relevant plant species to preserve vitality without repeated subculture (Mustafa et al. 2011). However, recovery of PCSCs from cryopreserved cultures is a time-consuming process and does not always result in a predictable performance (Harding 2004). As a result of incomplete separation after cell division, plant cells in suspension grow as aggregates ranging in size from a few cells (100 µm diameter) to thousands of cells (2 mm diameter) (Kolewe et al. 2008). In addition, plant cells are more sensitive to shear than microbial cells, owing to their large, water filled vacuoles. A number of novel impeller configurations and bioreactor types have been developed to meet the unique needs of PCSCs – most notably the recent invention of disposable, gas-permeable-bag wave-type bioreactors – although many plant cell types have been grown successfully in commonly available stirred tank bioreactors at culture volumes up to 70 m<sup>3</sup> (Eibl & Eibl 2007).

A major area of research in PCSC is the variability in metabolite accumulation of de-differentiated plant cells that have been subcultured repeatedly over a period of years. It is known that the callus culture method by which de-differentiated PCSCs are formed generally creates a heterogeneous population of cell types in suspension (Senger et al. 2006). The de-differentiated state itself sometimes leads to genomic instability over repeated subculture (Baebler et al. 2005). Additionally, the aggregated nature of plant cells in suspension may cause epigenetic changes, affecting product accumulation through unknown mechanisms (Patil et al. 2011). The relationship between aggregate size and NP accumulation has been studied in a number of different plant

systems, but results are varied, in part due to inconsistent experimental methodologies. In a recent study using a more accurate whole-culture measure of metabolite synthesis, paclitaxel accumulation in de-differentiated PCSC increased as aggregate sized decreased, suggesting aggregation as a process parameter that should be optimized (Kolewe et al. 2011).

A recent development in PCSC is the suspension culture of cambial meristematic cells (CMCs). *In planta*, cells in the cambial meristem layer of tissue can grow indefinitely and become any cell type. Because of this feature they have been likened to plant stem cells and defined as “un-differentiated” in contrast to the “de-differentiated” cells discussed previously. Suspension cultures of *Taxus* CMCs were made by creating callus culture from isolated *Taxus* cambial meristem tissue. The CMC cultures grew faster, formed smaller aggregates and displayed greater genetic stability than de-differentiated cultures (Lee et al. 2010).

### **Hairy Root Culture**

CMCs are an attractive new option for plant cell cultures; however, some plant NPs are produced preferentially in more differentiated cell types and may be produced in small quantities, or not at all, in truly un-differentiated cells (Roberts & Kolewe, 2010). Hairy root culture (HRC) is another subset of PCC in which root tissue from a plant of interest is genetically transformed by the soil bacterium *Agrobacterium rhizogenes*, leading to branched “hairy roots” that can grow indefinitely without exogenously supplied phytohormones needed for PCSCs. Because HRCs are differentiated tissue, they frequently have the ability to produce SMs that de-differentiated PCSCs cannot, and in some cases can even produce plant NPs that are not normally found in roots of intact plants. For instance, HRCs of *A. annua* accumulated artemisinin, whereas in whole plants artemisinin is produced only in the aerial sections (Kim et al. 2002). HRCs also appear to be more genetically stable than de-differentiated cultures and do not lose biosynthetic potential upon repeated subculture (Georgiev, Ludwig-Muller, & Bley, 2010).

Despite their proven biosynthetic capabilities, HRCs have not yet been used commercially for production of any plant NPs, mostly due to the lack of proven, large-scale bioreactors. The branched and corporeal nature of HRCs make them difficult to culture in stirred tank bioreactors, and nutrient transport limitations result in slow growth and low productivity. Novel bioreactor designs may improve HRC, and among the most promising are mist bioreactors. This novel bioreactor type sprays exposed roots with a nutrient solution and it has been shown to increase growth and metabolite productivity at high root densities (Weathers et al. 2008).

### **Yield Enhancement Strategies**

Apart from variability in product accumulation, another major area of research in PCC is enhancement of biosynthetic capabilities. This goal is realized through a number of strategies, including chemical elicitation of secondary metabolism, selection of elite cell lines, and metabolic engineering. While plant NPs are generally produced in extremely small quantities under normal conditions, in some cases SMs may constitute 20-60% of a plant's dry weight (Verpoorte et al. 1999). Activation of secondary metabolism using elicitors such as methyl jasmonate has been shown to significantly increase production of plant NPs, almost 50-fold in the case of paclitaxel in *T. baccata* PCSC (Yukimune et al. 1996). Elicitation pathways are highly conserved in plants, and elicitors such as methyl jasmonate have been shown to activate secondary metabolism in a wide variety of plants through similar pathways (Gundlach et al. 1992; van der Fits & Memelink 2000).

A useful technique in cell culture to improve yields is to select individual cells that have high biosynthetic productivity and culture them as "elite" cell lines. This technique is generally difficult to accomplish with plant cell lines, as cultures are composed of genetically heterogeneous aggregates that may respond differently to selection pressures. Furthermore, due to the unstable nature of de-differentiated cells, elite cell lines may lose their increased capacity over



time. A successful example of using selection pressure to create stable, elite cell lines is establishment of *Lavendula vera* cultures producing high amounts of the phenolic plant NP rosmarinic acid. Selection was accomplished by feeding toxic amino acid analogues in the media with the expectation that only cells expressing high amounts of an enzyme common to both amino acid and rosmarinic acid biosynthesis would be able to detoxify the amino acid analogues and remain viable (Georgiev, Pavlov, & Ilieva, 2006). This approach was effective in creating genetically stable, high-producing cell lines, but is limited to cases in which an effective screening procedure exists. Additionally, the heterogeneous and aggregated nature of PCSCs may dilute the effect of small numbers of high-producing cells because they are not separated from large numbers of low-producing cells. In cases where it is possible to disassociate individual cells from aggregates, labeling of the plant NP of interest and flow cytometric cell sorting is a more robust method for isolating and re-culturing elite cell lines (Naill & Roberts 2005b; Naill & Roberts 2005a)

Manipulation of biosynthetic pathways at a genetic level offers significant potential for increasing yields of plant NPs in PCC. Recent technological developments, such as 454 pyrosequencing, have lowered the barriers to identifying genes in biosynthetic pathways, and deliver the promise of making “non-model” organisms amenable to metabolic analysis (Bräutigam & Gowik 2010). Once biosynthetic pathway genes are known, *Agrobacterium tumefaciens* transformation can be used to stably introduce or silence genes in plants, although some types of plants such as trees and crop plants can be recalcitrant to *Agrobacterium* transformation. Engineering of *Agrobacterium* strains for increased virulence as well as manipulation of plant culture conditions have resulted in a wider applicability of this technique (Gelvin 2003). Transcription factors are also targets of metabolic engineering efforts. For example, simultaneous up-regulation of the gene *G10H* and the *ORCA3* transcription factor in HRCs of *C. roseus* resulted in a 6.5 fold increase in catharanthine, a precursor of vinblastine (Ni et al. 2011). In many cases, however, transcription factor

engineering is not effective at increasing production of the desired NP, and new models of plant metabolism may better our understanding of how to manipulate metabolism on a systems level (Stitt et al. 2010).

One promising technology to better understand plant metabolism and ways to improve it are genome scale metabolic models (GEMs). These models take into account all known metabolic reactions within a cell and apply optimization conditions (usually maximization of growth) to simulate fluxes for the metabolic reactions. GEMs have been applied successfully in the metabolic engineering of microbes such as *E.coli* for the production of chemicals such as succinic acid (Lee et al., 2005). Using a GEM, researchers can model fluxes for all biosynthetic reactions and can identify bottlenecks in pathways or competing pathways that reduce flux to the target molecule. Genetic engineering techniques can then be used to upregulate or knockout genes of interest and improve overall flux to the target molecule. GEMs have been created for multiple plant species including the model plant species *Arabidopsis thaliana*, as well as food crops such as corn and rice (Saha et al. 2011; Poolman et al. 2013).

Creating GEMs that can be used for genetic engineering of plant secondary metabolites is more complicated than for bacterial systems. First, plant secondary metabolic pathways are incomplete and poorly studied, so it may be difficult in many cases to create an accurate metabolic model that will include secondary metabolic pathways. Second, plants are highly compartmentalized and require more complex models than prokaryotes to accurately model fluxes. Third, because plants are multi-cellular and different cell types may have different functions, optimization conditions may not be as simple as maximization of growth. For optimization of secondary metabolites in particular, some molecules are only produced in specialized cell types or under stress conditions, in which optimization of growth would not give accurate accounting of fluxes.

Because so little is known about fluxes in plant cells, especially for specialized cell types and cells undergoing stress responses, measurement of fluxes using Metabolic Flux Analysis (MFA) is a strong component of the plant metabolic engineering toolbox (O'Grady et al. 2012). MFA is used to experimentally measure certain fluxes in central metabolism by taking advantage of patterns in protein labeling using isotopically labeled carbon substrates. This technique has been used in the medicinal plant *C. roseus* to discern central carbon fluxes using NMR (Sriram et al. 2007). Limitations of MFA include expense of labeled substrates and the difficulty of interpreting results via NMR or GC/MS, especially for compartmentalized organisms and photosynthesizing organisms (Niklas et al. 2010).

### **The Paclitaxel Story**

Paclitaxel is one of the most successful drugs in the history of chemotherapeutics, with annual reported sales in 2000 exceeding 1.5 billion USD (Expósito et al. 2009); however, the early history of paclitaxel was fraught with complications, and at many points it was almost discarded as a drug lead. The story of paclitaxel (summarized in Table 2) began in 1962, when a sample of bark from the Pacific yew, *T. brevifolia*, was collected by USDA workers. An extract tested positive for activity in the KB cytotoxicity assay. Following the positive result, Dr. Monroe Wall fractionated the sample and isolated the cytotoxic agent, paclitaxel, in 1971. Paclitaxel had favorable but unremarkable cytotoxic activity against common cell-based cytotoxicity screens; however, interest in paclitaxel was ignited in 1979, when Dr. Susan Horwitz showed that paclitaxel had a unique mechanism of action against tumor cells. Whereas previous spindle poisons (e.g., the vinca alkaloids vinblastine and vincristine) act by rapidly depolymerizing microtubules and preventing spindle formation in mitosis, paclitaxel instead stabilizes the tubulin polymers, preventing the cell from properly assembling its spindle and continuing through mitosis.

Paclitaxel was a poor drug lead due to low aqueous solubility, structural complexity precluding easy chemical synthesis, and the lack of a large and renewable supply of the compound.

However, interest from the research community, due to its novel mode of action, helped to move it past major difficulties during its initial development, including several deaths in Phase I clinical trials due to allergic reactions with Cremophor, an emulsifying agent in its formulation. Although at many points in paclitaxel's history the chances of it emerging as a successful chemotherapeutic appeared slim, clinical trials continued after the formulation was re-worked, and extremely promising Phase II results were obtained against refractory ovarian cancer (Cragg, Schepartz, Suffness, & Grever, 1993).

Even as paclitaxel enjoyed success in the clinic, there was still a major hurdle to its development as a large-scale chemotherapeutic. While early research and initial clinical trials had relied on collection and extraction of bark from wild-growing *T. brevifolia*, the collection and extraction processes were environmentally damaging. *T. brevifolia*'s properties as a SM producer were a perfect storm of unfortunate specifications: the tree was slow growing, paclitaxel accumulated only in the bark, and yields were very low. In contrast to harvest of the vinca alkaloids from fast-growing *C. roseus*, natural harvest of paclitaxel was unsustainable – 16,000 lbs of bark from approximately 2000 yew trees were required to produce 1 kg of paclitaxel (Cragg & Snader 1991). In the early 1990s the paclitaxel supply problem was finally recognized as a crisis. In 1991 the NCI entered into a Cooperative Research and Development Agreement (CRADA) with Bristol-Myers Squibb (BMS) to heavily fund research into alternate supply routes. Meanwhile, the destruction of trees became a high profile environmental issue and in 1992 federal legislation (The Pacific Yew Act) was passed to manage the survival of the *T. brevifolia*. Concurrently in 1992, paclitaxel was approved by the FDA to treat ovarian cancer and demand for the drug sharply accelerated. By 1993 NCI was supporting 35 grants on paclitaxel research, with funding of \$4.6 million, in addition to BMS's independent research (Cragg et al., 1993).

The most immediate relief for the supply issues was a semi-synthetic method that was first developed in 1986 and later re-worked to be more efficient (Denis et al. 1988). 10-deacetylbaccatin III was extracted from the needles of various *Taxus* species and converted to paclitaxel via chemical methods. Needle harvest was more environmentally friendly than bark harvest, leaving the tree viable and intact, and in 1994 the semi-synthetic method was approved by the FDA to supply paclitaxel, now trademarked Taxol®. However, the semi-synthesis method was expensive and environmentally unfriendly due to a number of harsh chemical solvents used. Research into production alternatives continued to be a hot topic during the 1990s, and in 1994 a total synthesis of paclitaxel was first reported (Nicolau et al. 1994; Holton et al. 1994). Paclitaxel is a complex molecule containing over 11 chiral centers and a unique oxetane ring chemistry, and total organic syntheses have still not resulted in any cost-effective or industrially-viable processes.

Plant cell culture (PCC) was first funded by the NCI as a supply route for plant-derived anti-cancer agents as early as 1977, but the contracts were terminated prematurely in 1980 due to lack of interest at the time (Cragg et al., 1993). A number of PCC projects were funded by the NCI in response to the paclitaxel crisis, and the first patent for plant cell suspension culture of *T. brevifolia* for paclitaxel production was issued in 1991, with reported yields of 1-3 mg/L (Christen et al. 1991). Phyton Biotech, Inc. was formed in 1990 near Cornell University (Ithaca, NY) in response to a renewed interest in PCC technologies to supply paclitaxel. The company grew quickly and in 1993 acquired Phyton GmbH and a 75,000 L cGMP PCC facility in Heidelberg, DE. Phyton Biotech licensed their PCC process to BMS in 1995, and continued to improve the paclitaxel process, recently filing a patent for strategies to increase broth titers to 900 mg/L. (Bringi et al. 2007). The current worldwide supply of paclitaxel is provided by Phyton Biotech, which is now a subsidiary of DFB Pharmaceuticals, Inc. Recent research in *Taxus* PCC

focuses on improving paclitaxel yield and minimizing production variability by better understanding paclitaxel biosynthesis and regulation on a genetic level, as well as the influence of key process variables such as aggregation (Wilson & Roberts, 2011).

### **Research Objectives**

The work in this thesis is an exploration of the use of genome scale metabolic models in understanding plant secondary metabolism, with an aim towards future genetic manipulations of secondary metabolite producing plants to improve yields. In Chapter 2, a general overview of genome scale models is given, and the process of creating a metabolic model from a transcriptome for a plant species of interest (*T. cuspidata*) is outlined. In Chapter 3, a published genome scale metabolic model of *A. thaliana* is used in conjunction with transcriptome data under varying conditions to model changes in metabolism. Different methods for integrating transcriptome data with metabolic models are explored and discussed.

## CHAPTER 2

### METABOLIC MODEL OF *TAXUS CUSPIDATA*

#### Background

##### **Flux Balance Analysis**

Genome-scale metabolic models (GEMs), also referred to as metabolic reconstructions, represent the current start-of-the-art for capturing whole cell metabolism by quantifying steady-state fluxes through a network of enzymatic reactions. This technique has been used to model systems with a range of complexity from *Escherichia coli* (Edwards & Palsson 2000) to *Homo sapiens* (Bordbar & Palsson 2012). The primary element of a GEM is the reaction network described by a stoichiometric matrix relating the individual reactions and their participating metabolites. The term “genome-scale” describes the process by which the stoichiometric matrix is obtained, usually from an annotated genome of the organism (Borodina & Nielsen 2005). The stoichiometric matrix is invariably underdetermined with more unknown reaction fluxes than metabolite balances, meaning that the solution space is infinite. To determine unique solutions, the stoichiometric matrix is combined with a suitably chosen objective function that represents the “cellular objective” and presumably captures various unmodeled regulatory processes. The resulting optimization problem is a classic linear program, and the process of solving the model is termed Flux Balance Analysis (FBA). The default objective function is maximization of the growth rate, which requires that the biomass composition be specified in terms of the reaction fluxes to the biomass precursors (Feist & Palsson 2010). This objective function has been successfully applied to numerous microbial models (Edwards & Palsson 2000; Duarte et al. 2004).

To date, curated GEMs exist for over 80 organisms and draft models exist for thousands of other organisms. Once available, a GEM can be used to analyze and engineer metabolism through a set of computational methods collectively termed “constraint-based modeling”(Orth et al. 2010) and available through software platforms such as COBRA (Becker et al. 2007).

### **Creation of Metabolic Networks**

Before FBA can be performed, a metabolic network must be constructed to provide the stoichiometric matrix. In practice, many of these metabolic networks have been constructed from existing databases of curated biochemical and genomic data, such as AraCyc for Arabidopsis (Poolman et al. 2009) and RiceCyc for rice (Poolman et al. 2013). The Kyoto Encyclopedia of Genes and Genomes (KEGG) database(Kanehisa & Goto 2000) is also a useful resource in model building, as it provides information on pathway directionality and GPR correlations for many organisms that may not possess curated databases. The ability to create a GEM for any organism relies on the ability to accurately recognize enzymes encoded in the genome or transcriptome, annotate those enzymes with correct metabolic functionality, and build a complete enzymatic reaction, including cofactors.

Application of genome-scale modeling to plant species has proven to be difficult for a number of reasons, most notably the lack of genomic information and the complexity of plant metabolic pathways. Despite these challenges, GEMs have recently been developed for several plant species including *A. thaliana* (Poolman et al. 2009; de Oliveira Dal’Molin et al. 2010; Mintz-Oron et al. 2011) and important food and biofuel crops such as maize (Saha et al. 2011), rice (Poolman et al. 2013), and the bioenergy crops sugarcane and sorghum (Dal’Molin et al. 2010). Although plants are complex multi-cellular systems that have disparate tissues and organ functions, these models typically apply the maximum growth objective for resolving unknown reaction fluxes. Existing plant GEMs have been used to answer basic questions about plant biology or to facilitate



improvement of crop species, and as yet there have been no models created for medicinal plants such as *T. cuspidata*.

## **Results**

### **Illumina Data Translation**

In previous work with collaborating groups, a comprehensive transcriptome for *T. cuspidata* cultured cells using the 454 and Illumina sequencing technologies was developed. For 454 sequencing, cells were MJ elicited or unelicited and harvested every 24 hours post-elicitation over a time period spanning 22 days of the culture period. Equal amounts of RNA from each culture were then pooled and submitted as a single sample for sequencing. A base transcriptome library was generated by sequencing the pooled and fragmented RNA sample on one full PicoTiterPlate (PTP) using the 454 Genome Sequencer FLX Titanium System™ (Roche, Branford, CT) (Patil 2013).

In addition, MJ elicited and unelicited *T. cuspidata* RNA isolated from cultures at time points of 18 and 72 hours were created for Illumina sequencing. The Illumina HiSeq 2000 platform (Illumina, Inc. San Diego, CA) was used for these samples. Contigs were generated using both the 454 and Illumina sequencing libraries. CLC genomics workbench (CLC Bio, Aarhus, Denmark) was used to generate contigs using the A, C, G, T voting method to resolve conflicts (Patil 2013). 48,614 contigs with >200 bp and >50x coverage were generated. Annotation was performed using Blast2GO default parameters (Conesa et al. 2005).

This transcriptome represents the full biosynthetic capabilities of *T. cuspidata* in both the MJ elicited and unelicited state, and was used to develop the metabolic model. A Matlab script was developed to translate the transcriptome. This script had four steps:

1. The sequence of the contig was sent to BLAST and a BLAST report was received. The reading frame from the top BLAST hit was used in subsequent steps.

2. The transcriptome derived DNA contig was translated into amino acid sequence using functions from the Matlab bioinformatics toolbox and the reading frame from the top BLAST hit.
3. Because the contig sequence often had beginning or ending ‘nonsense’ sequences, the correct portion of the amino acid sequence was determined via homology with the top hit of the BLAST report.
4. The annotations associated with each contig from Blast2GO as previously described were added to the contig in FASTA format.

This script was applied to the 29,000 contigs from the *Taxus* sequencing project that had significant homology with previously annotated proteins and thus had BLAST reports.

### **Draft Model**

A draft GEM of *T. cuspidata* primary metabolism was created by the ModelSEED using transcriptomics data from 454 sequencing, as described above, and recently developed plant modeling tools available in the ModelSEED metabolic reconstruction pipeline (Henry et al. 2010). A key novelty of the *Taxus* model is that its future use was intended to be a study of secondary metabolism. Most GEMs (especially in higher organisms such as plants) do not include secondary metabolism, largely due to lack of genomic information. For example, in the diterpenoid paclitaxel biosynthetic pathway of *Taxus*, multiple enzymes catalyzing reactions in the latter half of the 19 step pathway remain unknown (Hampel et al. 2009). The intended focus of this research was on increasing the synthesis of precursors to general terpenoid metabolism that are included in the model, such as isopentenyl diphosphate (IPP), its allylic isomer dimethylallyl diphosphate (DMAPP), and the first committed metabolite in the diterpenoid (paclitaxel) pathway, geranylgeranyl diphosphate (GGPP) (Gräwert et al. 2011; Roberts 2007). By determining the regulatory points in primary metabolism to increase precursor concentration and combining with targeted gene manipulations within secondary metabolism (e.g., regulatory

steps in the paclitaxel biosynthetic pathway (Nims et al. 2006), increases in yields to paclitaxel may be achievable.

The data used for draft metabolic model reconstruction was a combination of transcriptomes from MJ elicited and unelicited cultures. As described above, of the roughly 40,000 contigs generated from sequencing, 29,000 were found to have

statistically significant BLAST hits, allowing preliminary annotation. These annotated sequences were translated into amino acid sequences using the open reading frame (ORF) associated with top BLAST hits. The resulting annotated protein fragments were used by the ModelSEED to automatically generate Gene-Protein-Reaction (GPR) associations for all contigs corresponding to enzymes recognized in the ModelSEED database. A total of 924 reactions were generated directly from the annotated contigs by the ModelSEED. Many of these reactions referenced multiple contigs, which may be a result of multiple isoforms or subunits of a particular enzyme.

The ModelSEED software was used to gap-fill the draft model by adding reactions necessary to achieve *in silico* growth. Prior to gap-filling, growth was not possible due to dead-end reactions in the metabolic network and the inability to synthesize all biomass components. The gap-filling procedure required specification of the biomass equation and definition of the culture media. The biomass equation used in our draft model was that previously developed for an *A. thaliana* GEM (Poolman et al. 2009). A standard heterotrophic plant cell culture media (Table 1) was included in the model by adding bounded exchange reactions for available media components.

Chemical	Bounds (mmol/gDW/hr)
H2O	-100
O2	-100
CO2	-100

Phosphate	-100
NH3	-100
Sulfate	-100
H+	-100
Nitrate	-100
Sucrose	-100
Mg	-100

Table 1 – Media components and bounds in *T. cuspidata*

Using a mixed-integer linear programming methodology to minimize the number of gap-filled reactions, the ModelSEED software predicted that growth could be achieved by adding 37 noncontig-associated reactions to the model (Table 2). The gap-filled draft model contained 1001 reactions and 948 metabolites. By performing FBA with a maximum growth objective, successful prediction of the biomass growth rate as a function of the sucrose uptake rate was achieved using ModelSEED software (Figure 3). The model data were provided by Argonne National Laboratory using their software.

<i>Total Reactions</i>	1001
Contig-associated	924
Gapfilled	37
Transport/exchange	40
<i>Metabolites</i>	948

Table 2. Draft GEM model statistics.

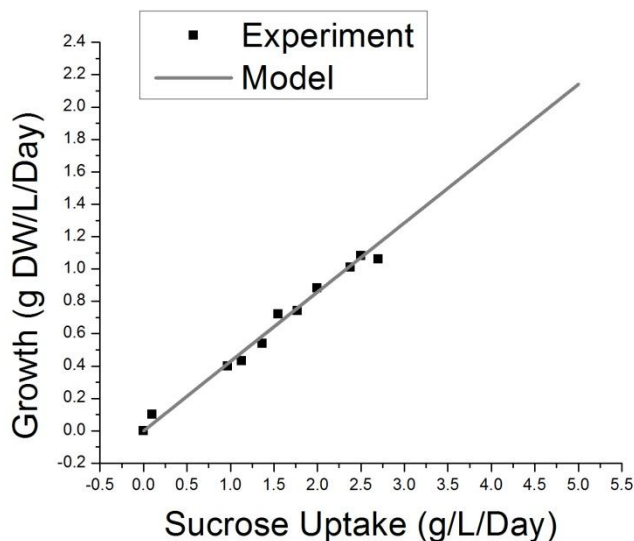


Figure 3 - Relationship between biomass growth rate (g dry weight/L/Day) and sucrose uptake rate (g/L/Day) from experiment (points) and draft model

Unfortunately, at the time of this research, the software that ModelSEED made publicly available was not useable. At that time, ModelSEED distributed beta versions of their Linux-based software that were accessible on Windows systems and internet interfaces such as Kbase. The software could not predict growth on “full” media, which was defined to contain all possible building blocks a cell would need. This “full” media contained more chemical components than the media used to culture *T. cuspidata* experimentally and should have been more than sufficient to enable model growth. The lack of growth using the new software distributions from ModelSEED suggested problems with the software and not with the model.

It was attempted to convert the model, which was stored in SBML format, to COBRA format and run it in Matlab using the well-known COBRA toolbox (Becker et al. 2007). COBRA has built-in functions to facilitate translation of models stored in SBML, ‘translate\_sbml.m’ and ‘convertsbmltocobra.m’. These functions in the past had required adjustment to avoid errors, e.g., removing code from translate\_sbml.m that tried to access features in a model that were not present, such as notes. It is important to remember that the softwares involved in FBA are

generally written by overworked biology graduate students, not computer scientists, and as such they are full of bugs and not robust. The COBRA ‘translate\_sbml.m’ function worked on the ModelSEED SBML without issue to create a Matlab structure for the model, and COBRA’s function ‘convertSBMLToCobra.m’ also worked without issue to create a COBRA model in Matlab. However, when it was attempted to run FBA on the model in COBRA using the ‘optimizeCbModel.m’ function, the model would not produce growth. It was found that other models downloaded in SBML format from the ModelSEED repository and converted to COBRA format would similarly not produce biomass. For example, the *E. coli* model iJR904 (Reed et al. 2003) would successfully produce biomass when downloaded from the website of the Palsson group in SBML format, converted to COBRA and ran with ‘optimizeCbModel.m’, but when iJR904 was downloaded in SBML format from the ModelSEED website, it would experience the same problem as the *Taxus* model, in that it would successfully convert to a COBRA model, but would not produce biomass when ‘optimizeCbModel.m’ was run. The SBML codes of the original iJR904 model and the ModelSEED version were compared, but the problem with the ModelSEED version could not be diagnosed. The fact that the iJR904 model from the ModelSEED repository experienced the same problem as the *Taxus* model suggests that the root cause may be an inability of ModelSEED models to be accurately converted to COBRA format.

## **Discussion**

In this study, an automated software was used in conjunction with transcriptome data to create a metabolic model of a plant. To our knowledge, this was the first time that a metabolic model was created using a transcriptome instead of a genome, and this was also the first genome-scale model of a medicinal plant, as opposed to a model plant such as *A. thaliana* or a crop species such as *Zea mays*. The use of a transcriptome from next-generation sequencing significantly reduced the barrier to entry for genome scale metabolic models of non-model species. However, despite automation, genome-scale metabolic models still require significant manual refinement, and the

lack of actual experimental knowledge of *T. cuspidata* hampered efforts to make the model more accurate. Additionally, because much of the software is built by labs for their own use, it is still quite difficult to use existing software packages such as those put out by the ModelSEED. The COBRA toolbox represents an attempt to standardize FBA and make it more widely available, but it is probably that available software will still be sub-par until commercial packages become available. As it stands, the *Taxus* metabolic model requires significant improvement to become useful.

## CHAPTER 3

# GENOME SCALE METABOLIC MODELING OF ARABIDOPSIS THALIANA UNDER METHYL JASMONATE ELICITED CONDITIONS

### Introduction

Many important plant-derived compounds fall under the category of secondary metabolites in that they are not essential for the normal functioning of the native plant. Secondary metabolites are largely thought to participate in plant defense, protecting plants from external threats such as fungal diseases and insect herbivory (Bourgau 2001). Secondary metabolism is activated by key compounds produced endogenously by the plant, termed elicitors. The study of elicitation and the plant defense response is currently an extremely active area of research in plant biology (De Geyter et al. 2012). Of the known compounds involved in elicitation, jasmonic acid and structurally similar compounds, collectively referred to as jasmonates, are major players in the most dynamic defense response pathway. Methyl jasmonate (MJ) is a stable esterified jasmonate that is present in nature and often applied exogenously *in vitro* to elicit the defense response in plants. MJ itself does not induce the defense response, but must be conjugated to isoleucine *in planta* to bind to transcription factors, such as JAZ (Fonseca et al. 2009). Elicited plants or cell cultures exhibit changes in diverse metabolic pathways, including decreases in ATP production related to mitochondrial membrane perturbation (Ruiz-May et al. 2011), decreased participation in cell cycle (Swiatek et al. 2002), and increased production of secondary metabolites (Gundlach et al. 1992; Mueller et al. 1993). An elicited plant can be thought of as having an altered phenotype while it is exhibiting the defense response.



Because jasmonates significantly induce secondary metabolite formation, they are a typical component of bioprocesses involving cultured plant cells. Paclitaxel accumulation in particular is greatly enhanced upon MJ elicitation (Yukimune et al. 1996). Previous studies have shown that MJ treatment results in upregulation of paclitaxel biosynthesis genes in *Taxus cuspidata* cultured cells (Nims et al. 2006). Other studies in model systems such as *Arabidopsis thaliana* have focused on transcriptional regulation of the jasmonate response at a systems level (Pauwels et al. 2008), but there have been no systematic studies on how metabolic flux is affected by MJ elicitation.

Genome-scale metabolic models (GEMs), also referred to as metabolic reconstructions, are tools used to quantify steady-state fluxes through a network of enzymatic reactions for a particular organism. Because they can model systemic changes in an organism's metabolism, they are an ideal tool to study MJ elicitation. In general, GEMs have an inherent advantage over time-dependent models (kinetic models) in that knowledge of rate parameters for each reaction is not necessary – only the stoichiometry of each reaction must be obtained. After a GEM is constructed, it can be used to analyze and engineer metabolism through a set of computational methods collectively termed “constraint-based modeling” (Orth et al. 2010). Flux Balance Analysis (FBA) is currently the most popular of these GEM methods. Given a metabolic network and a “cellular objective function,” FBA methods tune flux through each reaction until optimal flux through the objective function is achieved. Common objective functions for FBA have included maximization of cellular growth, minimization of ATP usage, production of secondary metabolites, or combinations of the afore-mentioned functions. An active area of research in the field is the integration of ‘omics data (transcriptomics, metabolomics and proteomics) into FBA methods to increase accuracy and to model complex cellular programs where the cell's regulatory logic may be unknown. This application, referred to here as “phenotype simulation” was of most interest to us in modeling the MJ elicited state in the model plant species *A. thaliana*. *A. thaliana*

was chosen for our study because there exist multiple, continually improved GEMs of *A. thaliana* metabolism (Poolman et al. 2009; de Oliveira Dal'Molin et al. 2010; Saha et al. 2011; Mintz-Oron et al. 2011). Despite progress and improvement on *A. thaliana* GEMs, there problems still exist with plant GEMs that do not necessarily apply to microbial systems. Plants are complex and multi-cellular systems that may have disparate tissue and organ functions. The most common objective function (unlimited growth) may not apply to plant tissues under many circumstances, especially under circumstances like MJ elicitation where experimental evidence shows that growth is inhibited.

Phenotype simulation is useful in this circumstance because transcriptomic data from MJ elicited *A. thaliana* cultures (Pauwels et al. 2008) can be used to augment FBA predictions and model MJ elicitation phenomena such as growth inhibition and secondary metabolite production if these phenomena are reflected in gene expression changes. The phenotype simulation algorithms that were chosen in this case to model MJ elicitation were GIMME and E-Flux. GIMME can be thought of as the simplest method of phenotype simulation. A cellular objective is set, and reactions are assigned Boolean ON/OFF values based on the expression level of the gene corresponding to that reaction compared to a threshold expression value (Becker & Palsson 2008a). FBA is run on the augmented matrix, and if a reaction necessary for the objective function has been turned off, GIMME may turn it back on. E-Flux operates differently in that instead of turning reactions ON or OFF, it changes the upper and lower bounds of each reaction in accordance with its expression value (Colijn et al. 2009). Highly expressed reactions will have larger upper and lower bounds, allowing more flux through if the objective function demands it. Lowly expressed reactions will not be able to produce large amounts of flux, even if it would assist in the optimization of the objective function.

In applying GIMME and E-flux to MJ elicitation modeling, our goal was that these phenotype simulation methods would capture the growth inhibition and secondary metabolite production

that are experimentally observed in *A. thaliana* (Pauwels et al. 2009). If growth-associated genes had low expression, they would be turned OFF in GIMME or have small bounds in E-flux, and the modeled growth activity would be lessened. If genes related to secondary metabolism were increased, flux would be shifted to these pathways, resulting in both secondary metabolism flux and growth inhibition.

## **Materials and Methods**

### **Model**

The previously published *A. thaliana* iRS1597 model containing 1234 genes, 1591 reactions, and 1916 metabolites was used in this analysis (Saha et al. 2011). The model includes reactions for secondary metabolism. The model was obtained in SBML format and converted subsequently to COBRA and TIGER compliant models using those respective software toolboxes (Becker et al. 2007; Jensen et al. 2011).

### **Data**

Previously published data on MJ elicitation were used in this analysis (Pauwels et al. 2008). Suspension cultures of *A. thaliana* (L.) Heynh. (ecotype Columbia-0) were grown for 7 days, diluted 10-fold in fresh medium and at 16 hours were either elicited with 50  $\mu$ M MJ or mock-elicited with equal volume of DMSO. Data were collected at 0.5, 2, and 6 hours post-treatment. Data used in this analysis were from MJ+ and MJ- cultures 6 h after treatment. Normalized data were obtained from the ArrayExpress database ([www.ebi.ac.uk/arrayexpress](http://www.ebi.ac.uk/arrayexpress); accession no. E-ATMX-13). For each condition, two independent experiments were used to generate transcripts for microarray profiling, and one biological repeat underwent RT-qPCR with multiple reference gene normalization on 4 reference genes to validate the data. The reference genes At1g69280, At4g17300,

At3g25800, and At1g04300 were of comparable intensity to the genes of interest and exhibited minimal variability in the RT-qPCR analysis. The data were background corrected and normalized using the BioConductory Affy Package in the following manner: “The Affy mas5calls function assigned probe sets present, absent, or marginally detectable. Probe sets with fewer than two present calls during the time course were considered not reliably detected and were removed from further analysis. With the BioConductor Limma package, probe sets were selected with a significantly altered intensity value at one or more time points after MeJA elicitation. The MeJA-treated and mock-treated cultures were compared pairwise at the three time points (30 min, 2 h, and 6 h). The decideTests function assigned significance to ttests while correcting for multiple testing of probe sets and contrasts with the false discovery rate method. The corrected P value threshold was set to 0.05.” (Pauwels et al., 2008, SI). Only processed expression data corresponding to genes included in the *A. thaliana* model were considered in our analysis. In the study, 598 of the 22,746 probe sets were found to be differentially regulated by MJ. Of these genes, 75 were differentially regulated in the first 0.5 hour, rising to 495 at the 6 hour time point. Of the 328 genes expressed only at the 6 hour time point, 60% were down regulated in the MJ+ condition. Genes were clustered according to up or down regulation at different time points and further clustered according to function or class using MAPMAN software (Nagel et al. 2005). The cluster analysis revealed that genes associated with the phenylpropanoid pathway were up regulated by MJ addition, while genes associated with mitosis and cell cycle progression were down regulated. Following the cluster analysis, additional experiments were performed to explore the findings. One result of interest from this paper is that MJ

addition to *A. thaliana* cell cultures increased flux through the monolignol pathway (related to the phenylpropanoid pathway), which was confirmed via HPLC detection of oligolignols, including lignin. Another result of interest was that MJ inhibited the growth cycle and stopped cell cycle progression in the G<sub>2</sub> phase, which was confirmed via flow cytometry (Pauwels et al. 2008).

### **GIMME and Essential Genes**

The TIGER toolbox for Matlab (Jensen et al. 2011) was used to implement GIMME (Becker & Palsson 2008a). TIGER's implementation of GIMME requires a metabolic model containing an objective function, a set of expression values corresponding to the model's Gene-Protein-Reaction (GPR) matrix, and a threshold value for expression. Because a biologically relevant "threshold value" for GIMME could not be determined, this algorithm was used instead to determine "essential genes" in the MJ elicited and unelicited states. This was accomplished by setting a threshold higher than any one expression value, thus setting all reactions OFF, and then determining which genes GIMME deemed necessary to turn back on. All genes turned ON by GIMME were deemed "essential." This process was dependent on the particular profile of an expression dataset, leading to a GIMME-determined "essential gene set" for each condition tested. To test the bounds of which genes could be "essential" or "non-essential" 100 sets of gene expression data were created containing randomized values for each gene. Using the Gurobi5 solver and TIGER toolbox, GIMME was applied to each set, and the numbers of "essential" and "non-essential" genes were collected for each case, as well as the number of reactions carrying flux in each case.

### **FBA and Phenotype Simulations**

To perform FBA, we utilized the *GIMME.m* command in the TIGER toolbox for GIMME, and the *optimizeCbModel.m* command in the COBRA toolbox for E-Flux and all other instances of

FBA. The Gurobi5 solver was used in all cases. Elicited (MJ+) and unelicited (MJ-) data were integrated with the *A. thaliana* GEM using the GIMME and E-Flux algorithms. In the case of GIMME, phenotype simulation was carried out using the method described above, in which MJ+ and MJ- expression sets were given thresholds exceeding values in either data-set, and “essential” genes were collected for both data-sets. The E-Flux algorithm (Colijn et al. 2009) was implemented using Matlab scripts and Excel worksheets that set bounds for each reaction based on the expression value of the gene corresponding to that reaction. The highest bounds were 1000 mmol/gDW/hr and assigned to the highest expression value, while lower expression values were given bounds corresponding to their ratio with the highest expression value. Bounds for MJ+ and MJ- E-Flux simulations can be found in Appendix A. When multiple genes were acting on a reaction, the appropriate expression value was determined following the Boolean logic rules. In the case of AND logic (more than one gene is needed for the reaction), the minimum expression value was used, and in the case of OR logic (more than one gene is available for the reaction, but only one is needed), the maximum expression value was used.

## **Results**

### **GIMME**

Using the GIMME algorithm, the capabilities of the *A. thaliana* GEM were explored. For 100 randomly generated expression data-sets, the number of essential genes and reactions carrying flux were determined in a GIMME simulation with a growth objective function. We found that in all cases, a core set of 116 genes were activated, while an additional set of 121 genes were turned on in at least one condition. The average number of genes activated was 153, and the average number of reactions carrying flux was 543. There was not a significant relationship between number of genes activated and number of reactions carrying flux (Figure 4). This is because there are promiscuous relationships among both genes and reactions, i.e., one reaction may be

influenced by multiple genes, and one gene may influence multiple reactions. These results served to establish the bounds of the *A. thaliana* modeling system.

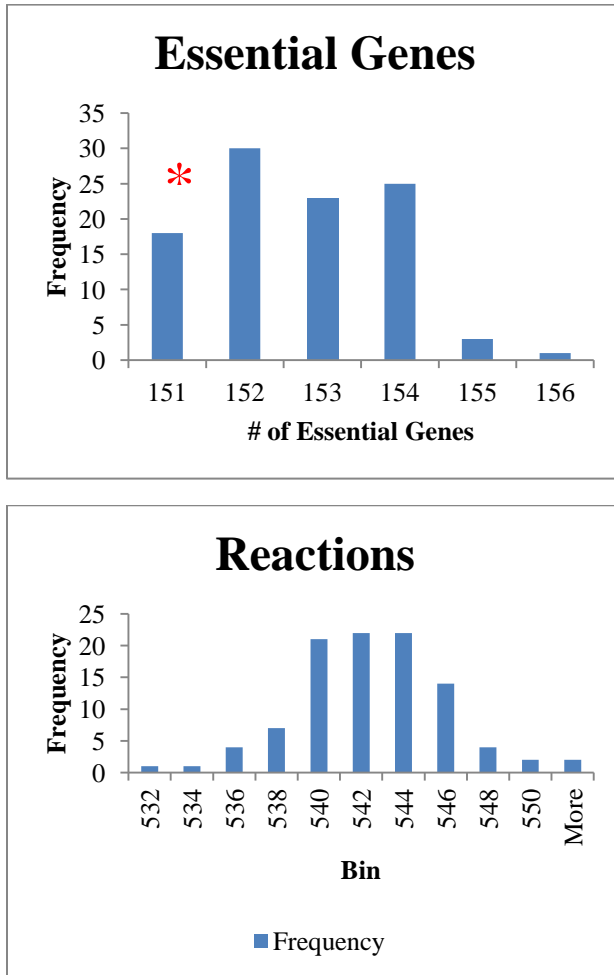


Figure 4 Analysis of *A. thaliana* metabolic network requirements. (a) Histogram of essential genes required to produce biomass using 100 randomly generated expression sets. The red asterisk represents the number of essential genes in MJ+ and MJ- datasets with same analysis. (b) Histogram of total number of reactions carrying flux in aforementioned simulations.

To model MJ- phenomena using GIMME, we initially performed simulations with our control dataset, MJ-, to determine what a biologically relevant threshold would be. We thought that there might be a threshold at which many genes not essential for growth would be turned OFF.

However, we found that over 15% of genes in both datasets had expression levels below the means of the two expression datasets, which were 6.40 for MJ- and 6.39 for MJ+. Averages were

calculated using the entire microarray dataset, not just genes included in the metabolic model. One of the lowly expressed essential genes, AT1G32780, was in the bottom 10% of the distribution, precluding a meaningful threshold value. A full list of MJ- and MJ+ expression values for each gene can be found in Appendix A. A list of essential genes below mean expression values can be found in Table 2. These lowly expressed genes may represent problems with the model, the dataset, or our algorithmic approach. This initial modeling with our control dataset suggested that a biologically relevant threshold for GIMME could not be determined for our dataset and model.

Gene Name	MJ+ Exp	MJ- Exp	Description of Function	E.C. Number
AT1G32780	3.64	3.88	alcohol dehydrogenase	1.1.1.1
AT3G22740	3.87	4.09	homocysteine S-methyltransferase	2.1.1.10
AT4G26770	3.96	4.23	phosphatidate cytidyltransferase	2.7.7.41
AT4G14090	4.11	4.19	anthocyanidin 3-O-glucoside 5-O-glucosyltransferase	2.4.1.298
AT1G04610	4.32	4.49	indole-3-pyruvate monooxygenase	1.14.13.168
AT1G36160	4.71	4.47	acetyl-CoA carboxylase / biotin carboxylase	6.4.1.26.3.4.14
AT4G26520	4.74	4.66	fructose-bisphosphate aldolase, class I	4.1.2.13
AT2G30770	4.81	4.49	cytochrome P450 (indoleacetaldoxime dehydratase)	4.99.1.6
AT3G49160	4.94	4.75	pyruvate kinase	2.7.1.40



AT5G52100	4.97	5.14	chloroplast NAD(P)H dehydrogenase	1.6.99.-
AT5G42250	6.09	6.46	S-(hydroxymethyl)glutathione dehydrogenase / alcohol dehydrogenase	1.1.1.284 1.1.1. 1
AT1G67550	6.17	6.39	urease	3.5.1.5
AT4G29890	6.29	6.25	choline monooxygenase	1.14.15.7
AT1G17050	6.29	5.96	all-trans-nonaprenyl-diphosphate synthase	2.5.1.84

Table 3. Lowly expressed genes in MJ+ and MJ- conditions.

Because our random phenotypic modeling had shown that determination of “essential” genes proceeded in a path dependent manner dependent on the expression data-set, we postulated that there might be differences in the number or type of essential genes in the MJ- and MJ+ dataset. We performed GIMME simulations using MJ- and MJ+ datasets, with a threshold set to initially turn OFF all genes. After essential genes were determined, we binned essential and non-essential genes according to their expression level in each dataset (Figure 5).

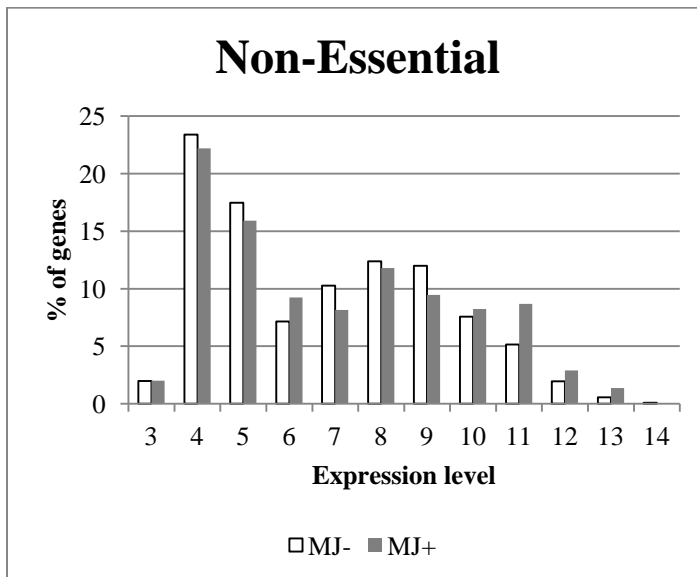
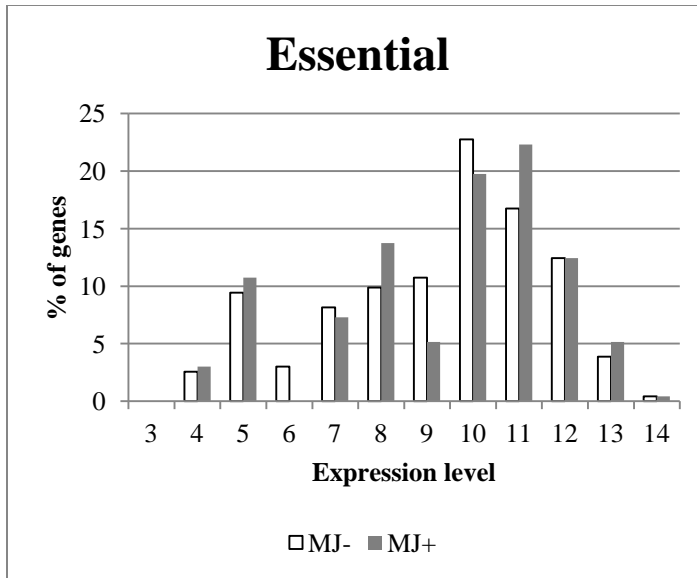


Figure 5. Essential vs. non-essential genes. Blue bars represent MJ- (unelicited control), red bars represent MJ+ (elicited). Expression levels of essential genes have higher expression levels (>10), while non-essential genes have lower expression levels (<5).

It was hypothesized that because higher growth is observed in MJ- cultures experimentally, essential genes in MJ- cultures would have higher expression values and be represented in those bins (Figure 5a). However, we did not observe that trend, in fact the MJ+ expression set had generally higher expression among essential genes. It was also hypothesized that because MJ+

cultures are experimentally observed to produce secondary metabolites that are not connected to growth, that there would be a higher proportion of highly expressed genes (expression values above 10) in MJ+ cultures, and this was in fact the case (Figure 5b).

### **E-Flux**

GIMME operates with a simplistic methodology that genes are OFF or ON, but in reality low gene expression does not necessarily correlate to an absence of a gene, just low amounts. In our next round of modeling, E-Flux was used to better simulate more fine-grained behavior. E-Flux operates by widening or narrowing the bounds a particular reaction can carry flux through depending on the expression of the gene controlling that reaction. In this way, a more nuanced result pertaining to cellular growth becomes apparent. In the determination of essential fluxes using GIMME, reactions carrying small amounts of flux to one essential precursor are given the same amount of weight as central reactions that carry large amounts of flux to multiple essential precursors, even though the latter would necessitate much more expression than the former during growth conditions. It was postulated that the growth inhibition observed in MJ+ cultures could be due to inhibition of a few key enzymes especially essential for growth. E-Flux can help simulate whether some genes are important in carrying large amounts of flux.

Using this algorithm, it is possible that if a few genes were especially important bottlenecks to growth, and more highly expressed in the MJ- dataset, that growth flux would be higher in the MJ- set compared to the MJ+ set. FBA was performed using a growth objective function, and resulting fluxes from MJ- and MJ+ datasets were compared to fluxes from both normal FBA and to each other. In the reaction pertaining to biomass formation (growth flux), there was very little difference between MJ- and MJ+ conditions (Table 4).

Condition	Growth (mmol/gDW/hr)
FBA	52.49
MJ- E-flux	51.91
MJ+ E-flux	52.49

Table 4 Growth in E-Flux simulations vs. FBA. Growth was calculated as the flux through the “biomass” reaction

In terms of overall flux distributions, the MJ+ and MJ- flux distributions are well correlated ( $R^2 = 0.9629$ ) and are in fact nearly identical. These results are contrary to experimental data showing that the MJ- condition grew faster than the MJ+ condition. When the MJ+ flux distribution was plotted against the MJ- flux distribution, the trendline had a slope of 1.01, showing that the flux distributions are well correlated with each other (Figure 6). In contrast, when these same MJ- and MJ+ flux distributions were individually plotted against the flux distribution from an FBA simulation, they were poorly correlated, with trendlines had slopes of 0.45 and 0.46 for MJ- and MJ+ respectively, and  $R^2$  values of 0.412 and 0.403, respectively (Figure 6).

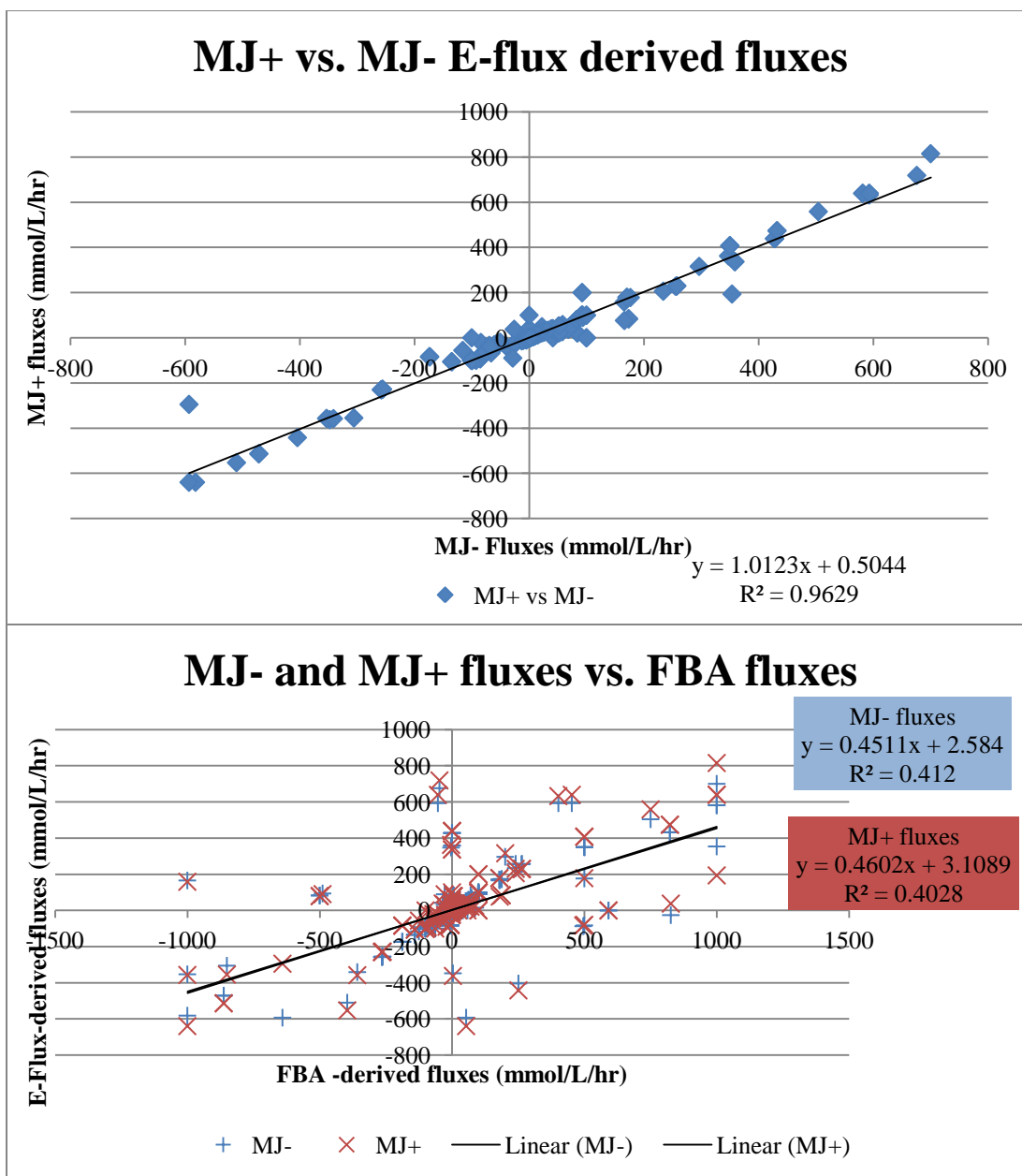


Figure 6. E-flux comparison of fluxes from MJ- to MJ+ (a) MJ- (x-axis) E-flux derived fluxes plotted against MJ+ (y-axis) E-flux derived fluxes. The sets of fluxes are well correlated ( $R^2 = 0.962$ ), although experimentally these conditions exhibited divergent behavior. (b) MJ- (blue cross) and MJ+ (red x) e-flux derived fluxes plotted against FBA derived fluxes (x-axis). MJ- and MJ+ are not well correlated with FBA fluxes ( $R^2 = 0.412$  and  $R^2 = 0.4028$  respectively) but the E-flux derived datasets are both extremely similar to each other. Both trendlines are right on top of each other.

Because the E-flux simulations did not yield divergent results as hypothesized, we further considered the expression values in MJ- vs. MJ+ datasets. The “controlling” relative expression value for each reaction in the MJ- and MJ+ conditions is plotted against the FBA-derived fluxes in Figure 7. As can be seen, the differences in MJ- vs. MJ+ datasets are very small in every reaction that carries flux in FBA simulation. In most cases the MJ+ values are equal to or higher than the MJ- values, suggesting that MJ- expression levels are not higher for growth-associated genes although MJ- cultures exhibited higher growth experimentally.

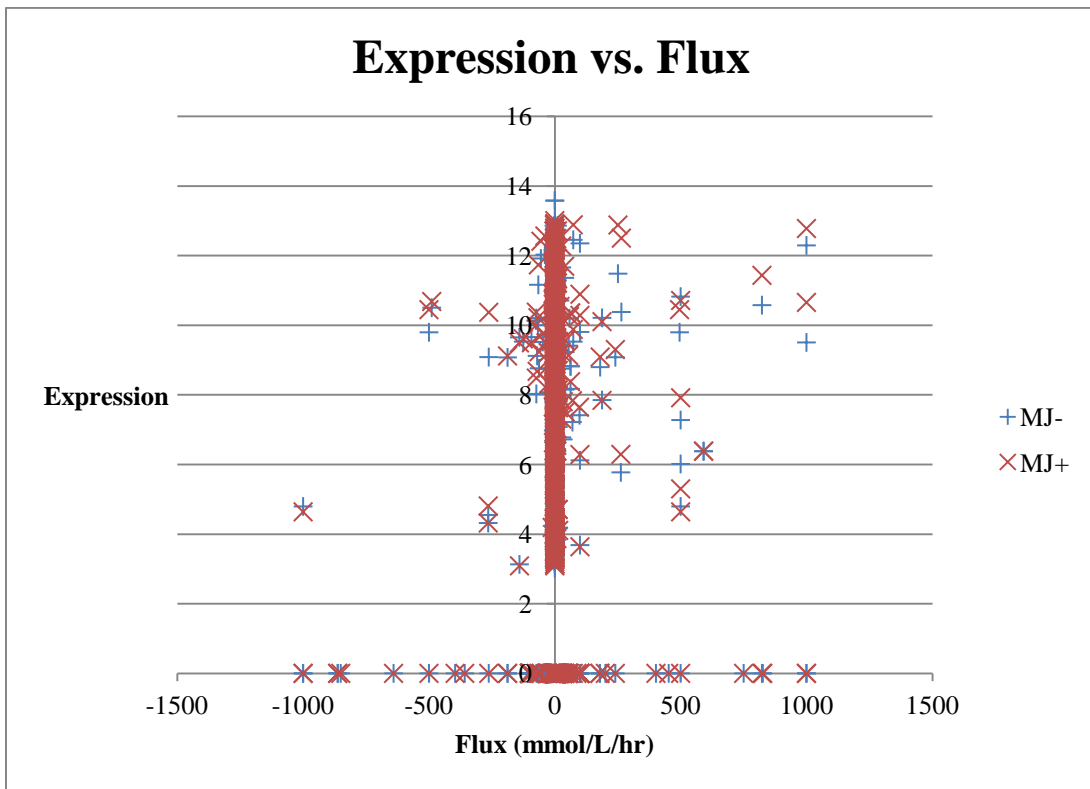


Figure 7. Relative gene expression values of MJ- (blue cross) and MJ+ (red x) plotted against FBA derived fluxes. Many reactions with high expression are not included in the model, and it can be seen that of the highly expressed genes that exhibit high positive or negative flux in the model, there are not significant differences in MJ- vs. MJ+ datasets.

To explore whether these results are statistically significant, we cross-referenced the statistically significantly up or down regulated genes in (Pauwels et al. 2008). Of the 22,000 genes included

on the chip, 581 were up or down regulated with a p-value below 0.05. Of these 581 genes, 51 were included in our model. Of these 51, 15 participated in 17 reactions that carried flux above or below zero in the FBA simulation (Table 5).

Locus	MJ-Expression	MJ + Expression	Reaction name in model	FBA Flux (mmol/g DW/hr)	TAIR annotation
AT5G36220	5.26	9.41	ascorbate acceptor oxidoreductase	1000.00	cytochrome P450 81D1
AT5G22300	5.08	7.93	Indoleacetonitrile aminohydrolase	264.56	nitrilase 4
AT3G17820	11.77	12.88	L Glutamate ammonia ligase ADP forming	250.99	glutamine synthetase (GS1)
AT3G02360	10.50	11.38	R01528 p	187.73	6-phosphogluconate dehydrogenase family protein
AT2G45290	9.28	10.35	R01641 p	62.58	transketolase, putative
AT2G45290	9.28	10.35	R01830 p	62.58	transketolase, putative
AT2G40890	10.05	11.92	p coumaroyl CoA caffeoyl CoA 3 hydroxylase	9.97	cytochrome P450 98A3, putative
AT3G21240	6.56	9.09	trans Cinnamate CoA ligase AMP forming	7.87	4-coumarate--CoA ligase 2 / 4-coumaroyl-CoA synthase 2
AT3G53260	6.88	9.59	L phenylalanine ammonia lyase trans cinnamate forming	7.87	phenylalanine ammonia-lyase 2
AT1G18500	8.73	9.62	acetyl CoA 3 methyl 2 oxobutanoate C acetyltransferase	7.35	2-isopropylmalate synthase, putative
AT1G20510	7.20	8.70	perillic acid CoA ligase ADP forming	3.56	4-coumarate--CoA ligase family protein contains Pfam AMP-binding enzyme domain PF00500



AT1G20510	7.20	8.70	perillic acid CoA ligase AMP forming	-3.56	4-coumarate--CoA ligase family protein contains Pfam AMP-binding enzyme domain PF00501
AT3G19450	6.79	8.26	Sinapyl alcohol NADP oxidoreductas e	2.94	cinnamyl-alcohol dehydrogenase
AT3G19451	6.79	8.26	4 coumaryl alcohol NADP oxidoreductas e	2.10	cinnamyl-alcohol dehydrogenase
AT3G29200	8.24	9.36	Chorismate pyruvatemutas e	2.10	chorismate mutase
AT4G35830	11.43	12.01	R01324 m	-39.37	aconitate hydratase, cytoplasmic
AT3G54640	9.37	10.37	L serine hydro lyase	-262.47	tryptophan synthase, alpha subunit

Table 5 – List of 15 genes that were found to be significantly responsive to MJ in (Pauwels et al. 2008) and also carried non-zero fluxes in 17 reactions in the FBA model of *A. thaliana* metabolism. Many of the genes (e.g. Sinapyl alcohol NADP oxidoreductase, p coumaroyl CoA caffeoyl CoA 3 hydroxylase) are associated with the lignin pathway (Humphreys & Chapple 2002). All of the genes were positively up regulated at the 6 hour time point.

## **Discussion**

### **Gene Essentiality**

Experimental studies of gene essentiality are often used to validate *in silico* metabolic networks of microbial metabolism. However, there are difficulties common to all metabolic networks in determining which genes are essential or non-essential. In a study comparing *in silico* determined essential genes to experimentally determined essential genes in multiple microbes, it was found that a few factors contributed strongly to false negative predictions of essential genes for growth, i.e., a gene is predicted to be non-essential, but it really is essential (Becker & Palsson 2008b).

Incorrectly predicted essential genes tend to be less well connected in the model, implying that there is incomplete knowledge of their multiple functions. They are more likely to be blocked reactions, suggesting that there is incomplete knowledge of metabolically proximal genes. They are also less likely to be connected to important overcoupled metabolites, such as ATP. This study suggests that these incorrectly predicted genes may be involved in metabolic processes that have not yet been completely characterized or defined (Becker & Palsson 2008b).

It is very easy to create large populations of mutant cell lines in an organism such as *E. coli* and experimentally determine viability under many different conditions. It is much harder to do so in a polyploid, multi-cellular organism that undergoes multiple growth stages, such as *A. thaliana* and all plants in general. The first difficulty is that plants are notoriously difficult to genetically transform, even in a model species such as *A. thaliana*, due to time consuming regeneration procedures. Stable genetic transformations of plant species only became widely used in the late 1990's, in contrast to bacterial transformations that have been common since the 1970's (Chang et al. 1994). Another key difficulty in plant species is their complicated growth cycle and multicellularity. A mutation that would be classified experimentally as "essential" could just affect the gametophyte or embryo and not be truly metabolically lethal (Lloyd & Meinke 2012). Most plant genetics studies occur in many different types of systems within Arabidopsis, for example, genetic studies can be performed on full grown plants, seedlings and embryos. Very few studies of essential genes occur in plant cell culture, although they are perhaps the most straightforward system where distinctions could be made in purely metabolic gene essentiality, rather than embryo, gametophyte, or seedling specific mutations. This makes it difficult to establish whether a mutation is actually metabolically lethal or just lethal to embryos or seedlings. Another complication is the general redundancy of plant genetics. There are duplicate or triplicate genes for the same genetic process that may be expressed in different growth stages or tissues, or all in the same cell (Hanada et al. 2009).

In this work we have determined a core set of 116 genes (Appendix A) that are predicted to be essential under all conditions. The natural continuation of this work would be to knock-out these genes in *A. thaliana* suspension cell culture and see whether these mutations result in lethality. This would suggest that these genes are metabolically essential, because in cell culture there is no ambiguity between growth phases and cell type – there is only one type of cell, and only undifferentiated growth.

### **Phenotype Simulation (GIMME and E-Flux)**

Our initial results with GIMME suggested that binary thresholding using expression data was not sufficient to model growth in *A. thaliana*, even in the absence of complicated behaviors due to elicitation. The presence of even a few essential genes with low expression did not allow growth. This behavior, though unfortunate for the purpose of modeling MJ elicitation, is good for improving our understanding of plant metabolism. If the paradigm of transcriptome/GEM integration is that high expression leads to high flux of controlled reactions, then there is something wrong with our model or something wrong with our paradigm.

Model inaccuracies could include incorrect annotation of genes or incorrect annotation of reactions. If the genes predicted to perform an essential function in reality perform another, non-essential function, that would explain why they have low expression in the system. If a reaction is mapped to a certain gene with low expression, but one gene with high expression is key to its function, and is left out, then the reaction will show that it is only controlled by the gene with low expression, and model dysfunction will result. Our list of lowly expressed but essential genes (Table 3) found by applying GIMME to the MJ- (control) dataset may point in the direction of model improvement by pinpointing reactions that have low expression but are nonetheless essential to the function of the current model.

Recent research suggests that inaccuracies in the transcriptome/GEM integration paradigm may also be likely. Studies in mouse fibroblasts showed that the amount of accumulated protein was more dependent on the rate of translation than the amount of transcripts (Schwanhäusser et al. 2011). The relationship of transcript abundance vs. translation of protein can also be very slow in plants (up to several days to replace significant amounts of proteins), so that the steady state assumption of FBA may be violated in many systems (Piques et al. 2009). Degradation rates are another potent variable that is entirely unmodeled by FBA mixed with transcriptomic data integration. In plant suspension cultures, rates of protein degradation may vary by as much as 60 fold, depending on variables such as protein function and location (Li et al. 2012). The emerging paradigm in plants is that signaling pathways pertaining to features such as circadian rhythms and light abundance have much more rapid turnover than general proteins pertaining to metabolism, which helps to reduce expenditure of proteins on rapidly fluctuating/cycling environments (Stitt 2013).

In the specific case of methyl jasmonate elicitation, our comparison of MJ- and MJ+ expression values against FBA fluxes (Figure 6) suggests that our approach, based on differences between MJ- and MJ+ expression for important growth regulating genes, may need modification. A major difficulty with the E-Flux model is the complexity of the rules governing the Gene-Protein-Reaction (GPR) matrix. Consider the example of AT5G36220, a gene that was found to be significantly upregulated upon MJ elicitation (Table 5). AT5G36220 corresponds to the reaction “ascorbate acceptor oxidoreductase” in our model. 70 other genes also correspond to “ascorbate acceptor oxidoreductase” in the model. The Boolean rules for this reaction dictate that any of these genes can drive the reaction. In the MJ- condition, AT5G36220 has a relative expression value of 5.26, while in the MJ+ condition AT5G36220 has a relative expression value of 9.41. The 71 genes that correspond to “ascorbate acceptor oxidoreductase” have expression values ranging from a minimum of 2.76 and 2.77 in the MJ- and MJ+ datasets respectively, and

maximum values of 9.51 and 10.66 the MJ- and MJ+ datasets respectively. The maximum values were used to determine the E-Flux bounds, as given by the Boolean rules. This means that the significant MJ responsiveness of AT5G36220 is overshadowed by 70 other reactions that can drive the reaction. This is a challenging problem with no simple solution. In this case, the complexity and vagueness of the model obscured the effect of MJ on this reaction. Further improvements to the model that may help alleviate this would be more specificity connecting genes to reactions, and fewer non-relevant associations.

An additional problem with the dataset itself is that the study we took it from only collected data until 6 hours post elicitation. This may represent only an initial inductive, wave of transcription that involves signaling proteins but not metabolically involved enzymes. Datasets that include later time points post-elicitation may give more divergent results. Another difficulty is the incompleteness of our model, especially in reactions pertaining to secondary metabolism. The authors of the paper whose dataset we used in our analysis noted that MJ “primarily triggered gene activation,” and that only a small fraction of the genes having differential expression were down regulated (Pauwels et al. 2008). Our analysis shows that even genes strongly associated with growth metabolism are not down regulated by MJ elicitation, in that genes with high fluxes (of positive and negative magnitude) in our model do not show significant differences comparing MJ- to MJ+. It is also noted in the original paper, and in subsequent work in the Roberts lab, that MJ has a strong inhibitory effect on the plant growth cycle, and results in the arrest of cells in G2 phase (Patil et al. 2012). It was our thought that this growth arrest would lead to reduced transcription of enzymes involved in synthesis of biomass components. However, it appears that either transcriptional control of growth associated enzymes does not begin until later in the MJ induced cascade, or that the levels of these proteins are controlled by mechanisms other than transcription. A proteomic investigation of enzymes in a longer time-study of MJ elicitation might help to elucidate the mechanism by which MJ induction controls growth metabolism.

Another drawback to our current modeling approach was that most of the genes strongly induced by MJ even at the 6 hour time point are secondary metabolites. It is thought that the secondary metabolism activation may divert flux from growth specific enzymes and depress growth without needing to down regulate transcription of those enzymes. Unfortunately, secondary metabolism related enzymes are not well represented in our current metabolic network. Enzymes pertaining to secondary metabolism are variable and species specific, and are not as well studied as genes pertaining to phenomena such as growth and reproduction. Their pathways are in many cases incomplete, resulting in either blocked reactions in our metabolic network, or lack of transcriptomic information for pathway genes that are represented. If these secondary metabolism genes were better studied, and included in our model, it is possible that forcing flux through them using an approach like E-Flux might yield better modeling of the growth inhibition of MJ+ cultures and also allow us to better study how this flux is diverted.

## APPENDICES

## APPENDIX A

### TABLES FOR GENOME SCALE METABOLIC MODELING OF *ARABIDOPSIS THALIANA* UNDER METHYL JASMONATE ELICITED CONDITIONS

Gene Locus	MJ- Relative Expression	MJ+ Relative Expression
AAM67233	n/a	n/a
ARTHCP025	n/a	n/a
ARTHCP026	n/a	n/a
ARTHCP027	n/a	n/a
ARTHCP030	n/a	n/a
ARTHCP068	n/a	n/a
ARTHCP071	n/a	n/a
ARTHCP074	n/a	n/a
ARTHCP076	n/a	n/a
ARTHCP077	n/a	n/a
ARTHCP078	n/a	n/a
ARTHCP079	n/a	n/a
ARTHCP080	n/a	n/a
ARTHMP006	n/a	n/a
ARTHMP007	n/a	n/a
ARTHMP024	n/a	n/a
ARTHMP026	n/a	n/a
ARTHMP035	n/a	n/a
ARTHMP043	n/a	n/a
ARTHMP051	n/a	n/a
ARTHMP058	n/a	n/a
ARTHMP086	n/a	n/a
ARTHMP098	n/a	n/a
AT1G01050	10.6624517	10.441234
AT1G01090	10.36131475	10.27603897
AT1G01190	5.294331658	4.971469731
AT1G01390	3.220415312	3.265237505
AT1G01600	4.610141912	4.480648225
AT1G01710	7.114827093	7.550653746
AT1G02050	5.560205009	5.443617382



AT1G02640	3.965411229	3.721669628
AT1G02790	3.604335235	3.59328989
AT1G02920	12.32676017	12.69709481
AT1G02940	6.30921425	5.718943136
AT1G02950	3.735232927	3.700569514
AT1G03190	7.517293743	7.575593331
AT1G03475	8.131400109	8.085668142
AT1G04610	4.490124116	4.320831707
AT1G04640	6.930047603	7.038432588
AT1G04710	9.131616299	9.327752867
AT1G05260	4.746311037	4.801844627
AT1G05530	3.431432388	3.138131912
AT1G05570	8.248005156	7.814843419
AT1G05590	7.172665685	7.295770672
AT1G05610	3.667953312	3.40331519
AT1G06030	3.878344256	3.858120702
AT1G06290	10.45995286	10.67263346
AT1G06310	4.881749747	4.787449544
AT1G06410	10.09358712	10.07040592
AT1G06570	8.38032038	9.173361696
AT1G06820	8.228174855	8.429170676
AT1G07110	9.002057243	9.031836211
AT1G07230	7.800926162	7.725515734
AT1G07420	9.054894633	9.129751722
AT1G07450	3.961265338	3.968119214
AT1G07780	8.794528366	8.919773389
AT1G08065	3.863150058	3.917234748
AT1G08080	4.024576806	3.811487287
AT1G08510	8.870299786	8.632051284
AT1G08550	4.891481935	5.300732413
AT1G08840	5.930015498	5.970434419
AT1G09240	4.157072294	4.238304273
AT1G09420	8.01869452	8.103000405
AT1G09430	9.42774324	9.486319468
AT1G09795	7.606617052	7.361465739
AT1G09830	8.424000025	8.452476718
AT1G10070	4.340183031	4.230940253
AT1G10670	10.0303833	9.993147277
AT1G10900	6.54194649	6.712527591
AT1G10930	n/a	n/a
AT1G11590	3.11254917	2.984583942

AT1G11600	4.726023274	4.455753518
AT1G11610	3.291932234	3.131896544
AT1G11680	9.600879729	9.57245695
AT1G11860	10.12665717	10.44888723
AT1G11880	6.59742166	6.59234505
AT1G12000	11.97408179	12.109658
AT1G12050	8.881852492	9.359571395
AT1G12200	8.986812875	10.4952867
AT1G12230	9.264002708	9.238672273
AT1G12240	5.20656181	6.227027317
AT1G12350	7.524476951	7.794745473
AT1G12780	8.174022318	7.946655448
AT1G13090	5.358735953	5.47452795
AT1G13100	3.251191139	3.288092251
AT1G13210	5.92037399	5.988319697
AT1G13440	12.72488774	13.084719
AT1G13560	10.10967073	9.840553602
AT1G13700	6.242790242	5.772107212
AT1G13710	4.895899329	4.746523009
AT1G14030	5.966171073	6.02689575
AT1G14070	3.681809198	3.676192101
AT1G14080	3.73597367	3.613378457
AT1G14520	n/a	n/a
AT1G14540	3.443920376	3.287677629
AT1G14550	4.641515564	4.475341877
AT1G14610	10.04109956	9.85859798
AT1G14700	4.294048555	4.090805798
AT1G14810	11.38541432	11.51679523
AT1G15550	5.802150519	5.827727676
AT1G15950	9.426926386	9.757886781
AT1G16340	7.601260101	7.6811177
AT1G16350	9.191012144	9.392047641
AT1G16570	8.010616322	7.923043213
AT1G16700	11.1767099	11.14233937
AT1G16900	7.687654728	7.442272493
AT1G16980	3.534011634	3.436206466
AT1G17000	3.012876428	3.139072554
AT1G17050	5.968244857	6.291523492
AT1G17160	7.505744117	7.625238895
AT1G17260	3.746193215	3.664649477
AT1G17420	5.195010196	6.440613787

AT1G17500	6.590811424	6.499331411
AT1G17745	9.373355321	10.81128893
AT1G17890	6.468991881	6.413509972
AT1G18270	5.125748909	5.479665592
AT1G18440	6.585465863	6.465899811
AT1G18500	8.734339084	9.616382013
AT1G18870	3.395790229	3.380216049
AT1G19640	3.662049054	3.299161254
AT1G19670	4.147721828	4.955340262
AT1G20020	8.414578612	8.934829309
AT1G20050	10.75333487	10.65541725
AT1G20480	6.435877191	6.454424215
AT1G20510	7.195527423	8.700006217
AT1G20575	8.737271222	8.650477582
AT1G20620	9.555751552	10.07272978
AT1G20630	8.730594589	9.178952828
AT1G20950	10.59792609	10.5165792
AT1G20960	9.564731724	9.489566365
AT1G21400	3.377147889	3.323455519
AT1G21980	7.714611405	7.631582548
AT1G22020	6.945341148	6.781050217
AT1G22400	5.131986382	5.156466844
AT1G22410	10.29636969	11.00942415
AT1G22430	3.409263096	3.511742891
AT1G22440	8.640758624	8.931786541
AT1G22940	7.775890615	7.701469664
AT1G23360	7.046887626	7.089077964
AT1G23730	3.956126112	3.742412983
AT1G23800	8.513683542	9.075432831
AT1G23820	10.10524359	10.84028043
AT1G24100	6.633589397	6.931350397
AT1G24110	4.248479704	4.147245088
AT1G24280	9.169165333	8.9218641
AT1G24320	3.427918529	3.486018203
AT1G24735	5.098655998	4.968652628
AT1G24807	8.2440986	9.243245788
AT1G24909	8.2440986	9.243245788
AT1G25220	8.2440986	9.243245788
AT1G25350	9.828797861	9.602691156
AT1G25410	3.955331606	3.95079411
AT1G26130	6.443958417	6.076416044

AT1G26560	3.683822437	3.829495668
AT1G26570	6.14097513	5.654317407
AT1G27450	11.4591508	11.35183546
AT1G27680	7.314104963	7.49377715
AT1G27880	7.358319142	7.024792689
AT1G27980	8.197256877	8.236211546
AT1G28090	6.457756935	6.230019543
AT1G28120	8.583935563	8.704967006
AT1G29410	8.794528366	8.919773389
AT1G29870	3.585387023	3.433092596
AT1G29880	9.630660979	9.500116312
AT1G29900	10.09952441	10.15614796
AT1G30000	6.189681158	6.129812768
AT1G30100	3.560606006	3.349225184
AT1G30110	5.64127575	5.823719542
AT1G30120	9.442640878	9.524532284
AT1G30510	10.22653468	9.532476859
AT1G30530	5.736698858	6.1729201
AT1G30620	8.212915493	8.264537336
AT1G30870	4.342406733	3.898451408
AT1G31070	8.022664111	7.877839333
AT1G31180	10.33396862	10.25641075
AT1G31230	8.25047591	8.498471002
AT1G31360	6.869176003	6.86838454
AT1G31860	9.829520362	9.742465837
AT1G31910	8.171265746	8.574036167
AT1G32060	8.308041698	8.769754079
AT1G32210	10.63836768	10.42609821
AT1G32380	8.339041224	8.628242069
AT1G32440	9.561595654	9.338723647
AT1G32780	3.883207967	3.637320689
AT1G34430	9.553619466	9.367721696
AT1G34510	3.475610166	3.53613043
AT1G35530	7.007840796	6.95317496
AT1G36160	4.471549198	4.710625135
AT1G36280	8.434329379	8.88924465
AT1G36370	9.941855773	8.316838452
AT1G43670	10.27315192	10.39062873
AT1G43710	10.95887482	10.79086197
AT1G43800	9.256656139	9.18725293
AT1G44180	6.967182341	7.201489762

AT1G44318	4.454668274	4.414134438
AT1G44446	7.652054254	7.831946625
AT1G44820	6.967182341	7.201489762
AT1G44970	3.515297083	3.700659387
AT1G47840	5.431248761	5.274761383
AT1G48030	11.22460544	11.18316543
AT1G48130	4.216534089	4.162175183
AT1G48470	4.781274565	4.780501536
AT1G48850	10.28458972	11.06035363
AT1G48860	11.22414611	12.09130112
AT1G49140	11.12050169	11.46177753
AT1G49340	7.894930233	7.72644524
AT1G49430	3.447184673	3.392698616
AT1G49570	4.750387126	4.591674553
AT1G50000	6.701987322	6.424943588
AT1G50090	3.824280623	3.617042751
AT1G50110	8.137090908	8.10667483
AT1G50200	10.39326243	10.45883934
AT1G50430	10.63815196	10.74436412
AT1G50480	9.840832395	10.55359251
AT1G51260	4.586082529	4.69735255
AT1G51680	10.33390295	12.49070525
AT1G53000	9.416161595	9.115542603
AT1G53310	10.25352888	10.21801935
AT1G53830	4.159036763	3.504068484
AT1G54100	9.120447693	10.25223687
AT1G54220	7.981214042	8.050630699
AT1G54280	4.283128156	4.291234624
AT1G54340	7.041005106	7.354492985
AT1G55020	5.142298959	5.008363363
AT1G55090	8.157797412	8.132681017
AT1G55180	4.434684983	4.269763467
AT1G55510	6.943612172	7.525950757
AT1G55880	7.994521255	7.812247218
AT1G55920	6.648786951	9.428034721
AT1G58180	6.352388447	7.060124936
AT1G59820	9.320635159	8.919331061
AT1G59900	6.771066104	7.015149288
AT1G60490	7.374210075	7.311698356
AT1G60550	7.846576288	8.162508788
AT1G60600	6.623712636	6.623310633

AT1G60810	8.459640081	8.177569459
AT1G60890	6.894653652	7.017507605
AT1G61720	4.832560942	4.640340586
AT1G62430	7.595459313	7.633593831
AT1G62640	8.083627336	8.182029957
AT1G62660	3.671301379	4.867613333
AT1G62800	6.44906651	6.632860911
AT1G62940	3.783557152	3.934573616
AT1G63000	10.08805217	10.25306159
AT1G63180	4.991589806	4.597541764
AT1G63290	9.463048876	9.870900077
AT1G63380	6.281845246	5.912466528
AT1G63460	6.072885837	6.19035653
AT1G63660	11.30973061	10.93905393
AT1G63970	9.53847307	9.65529752
AT1G64190	7.900770988	7.843122903
AT1G64400	4.45134368	4.284740237
AT1G64440	7.334787062	7.337645601
AT1G64710	5.833127159	5.622950175
AT1G64970	7.393821813	7.861820166
AT1G65060	4.508214883	4.709112463
AT1G65560	7.108553241	7.033325301
AT1G65820	10.11424798	10.75810012
AT1G65930	10.95708369	11.43546763
AT1G66430	7.282269925	6.519521606
AT1G66520	5.240185656	5.182760785
AT1G66530	9.217217022	9.183392697
AT1G67070	4.602054483	4.624841622
AT1G67090	12.38552704	12.34864195
AT1G67110	3.98489359	3.870618975
AT1G67440	7.72780407	8.044289937
AT1G67550	6.385403732	6.17299516
AT1G67560	7.917185921	8.184776114
AT1G68460	6.320270874	6.383297599
AT1G68710	6.186409784	6.198334683
AT1G68750	3.994630323	3.790738949
AT1G68850	3.30669502	3.181394498
AT1G68890	5.235180005	5.412264687
AT1G69370	8.717704484	9.108576747
AT1G69740	10.20320641	10.2928406
AT1G69770	10.74655131	10.41417842

AT1G70310	10.22230339	10.59861009
AT1G70410	8.725562673	7.393091155
AT1G70580	7.308995218	7.646859085
AT1G70980	10.60178249	10.57475026
AT1G71010	8.105784117	7.786834524
AT1G71100	8.392391513	8.380649149
AT1G71220	n/a	n/a
AT1G71230	8.232022101	8.227010893
AT1G71695	3.471376798	5.491107888
AT1G71750	7.265712671	7.343628815
AT1G71920	9.872803286	9.902812923
AT1G71990	7.001432614	7.189915354
AT1G72520	5.073097701	5.933505166
AT1G72550	10.9072917	10.78110721
AT1G72700	7.991429302	8.14336332
AT1G72810	7.940659226	8.603705295
AT1G72880	n/a	n/a
AT1G72990	6.671760519	6.553537759
AT1G73050	4.88787092	4.620948922
AT1G73250	9.014711448	8.622482999
AT1G73740	6.286626765	6.624099721
AT1G74010	7.524764486	8.341222132
AT1G74020	9.011605089	10.30547528
AT1G74030	10.10605514	10.54388168
AT1G74040	8.576248411	8.552577436
AT1G74260	10.32297426	10.20212159
AT1G74470	11.55970304	11.44744161
AT1G74540	4.071118534	4.363691979
AT1G74550	4.937211261	4.975530849
AT1G74710	7.397801988	6.963655566
AT1G74920	9.990942744	10.05719036
AT1G74960	8.856548915	8.623502614
AT1G75280	7.97802469	9.301821024
AT1G75330	10.47042414	10.71828907
AT1G76400	10.14643121	10.01376086
AT1G76490	9.767189374	9.688838186
AT1G76550	10.29022388	10.38138163
AT1G76680	7.806042545	11.6648262
AT1G76730	7.652768178	7.87729685
AT1G77120	10.07015678	10.32564318
AT1G77590	9.845571712	9.76412318

AT1G77670	9.72052336	9.497672653
AT1G77720	7.245567478	7.320717585
AT1G77740	6.974202104	7.017399021
AT1G78440	3.591746455	3.540791752
AT1G78510	6.666471488	6.782073281
AT1G78570	8.720282071	8.996025659
AT1G78580	5.338928243	5.142028077
AT1G78670	8.712839789	9.795571768
AT1G78680	7.957533771	8.440907556
AT1G78800	7.664004323	7.568590729
AT1G78960	6.935491317	6.768549322
AT1G79460	6.392863674	5.919406775
AT1G79470	10.27901383	10.10759463
AT1G79530	9.685416507	9.879971554
AT1G79550	12.63382841	12.73465897
AT1G79890	7.603283868	7.65829219
AT1G80050	8.138961567	7.811877749
AT1G80340	5.588517771	5.530464328
AT1G80350	7.832220098	7.67370252
AT1G80560	10.95714781	10.90427805
AT1G80600	10.25070805	10.23433699
AT1G80660	3.910585819	3.859692033
AT1G80820	5.976433549	7.460132127
AT1G80950	7.968761322	8.061340877
AT2G01140	12.23437806	12.41487196
AT2G01290	9.457510138	9.519962757
AT2G01350	10.35137491	10.62838359
AT2G01440	7.235602982	7.273109092
AT2G02000	5.247845432	5.473161509
AT2G02050	11.62714978	11.5690561
AT2G02500	8.766322081	8.696129238
AT2G02930	8.481866731	10.60844184
AT2G03210	4.70127639	4.807839721
AT2G03220	8.151620019	8.299938715
AT2G03760	6.438322639	7.52728221
AT2G04450	3.959835942	3.963138483
AT2G04540	6.712330667	6.62849821
AT2G05710	12.32919902	12.56632344
AT2G07050	9.413470161	9.534619283
AT2G07560	3.896519497	3.846542122
AT2G07751	6.550363444	6.528769983



AT2G07785	n/a	n/a
AT2G11810	4.345359529	4.486160651
AT2G11890	8.924021958	8.743169435
AT2G13370	8.329520737	8.315545268
AT2G13680	3.972509108	3.828516537
AT2G14170	7.802222007	7.797997306
AT2G15350	4.070270851	4.051743501
AT2G15370	4.070270851	4.051743501
AT2G15620	11.52136271	10.89185518
AT2G16370	7.863226652	8.202790291
AT2G16500	9.396581083	9.596848505
AT2G16570	12.24291346	12.15070913
AT2G16790	7.105090027	8.041852401
AT2G17265	9.012061955	9.206775124
AT2G17370	8.375021216	8.160707703
AT2G17630	10.94518128	11.21707703
AT2G18140	5.091927756	4.926646715
AT2G18150	5.091927756	4.926646715
AT2G18230	9.567992859	9.417774367
AT2G18950	6.944582525	7.763826372
AT2G18960	12.53975215	12.77997636
AT2G18980	4.447869693	4.394795018
AT2G19570	8.072940125	7.937753916
AT2G19670	11.02872423	10.96782423
AT2G19800	4.632402641	4.139984654
AT2G19860	7.973302759	8.12809002
AT2G19940	8.917445342	9.048490891
AT2G20340	5.496683295	6.106572515
AT2G20360	10.3410099	10.37578177
AT2G20420	12.56699092	12.5149007
AT2G20690	9.548396453	9.668555417
AT2G20860	9.853958342	10.00209483
AT2G21170	11.46583043	11.73498389
AT2G21260	10.35201014	10.52127941
AT2G21330	10.38930587	10.46155178
AT2G21790	10.86240157	10.78317725
AT2G21940	7.093749082	7.734421272
AT2G22230	9.696855161	9.727408104
AT2G22240	7.821770041	8.327757106
AT2G22420	7.043330296	7.479998495
AT2G22450	8.361653901	8.317607624

AT2G22480	9.309340446	9.131612291
AT2G22810	5.290833518	5.483387257
AT2G22990	3.456089543	3.268738533
AT2G23410	3.638719216	3.501674193
AT2G23420	8.408184074	8.884247384
AT2G23600	8.885902267	9.768589974
AT2G23610	4.095450603	4.761765045
AT2G23800	4.097128102	3.954499546
AT2G24200	11.3173195	11.68954698
AT2G24210	3.82057854	3.698140419
AT2G24520	5.271146976	5.389310602
AT2G24580	3.823758711	4.397332217
AT2G24800	3.386525548	3.306802826
AT2G25080	8.815343543	8.968455651
AT2G26080	10.40659429	10.35385159
AT2G26230	8.502131694	8.527541735
AT2G26420	3.518644481	3.460836348
AT2G26800	8.25252289	8.26607668
AT2G26930	8.173376317	8.293092847
AT2G27150	7.020420637	7.490888259
AT2G27490	7.752419956	7.731252476
AT2G28210	n/a	n/a
AT2G28860	4.091637039	4.07828681
AT2G28880	5.692406316	5.871306174
AT2G29150	3.848331237	3.665402088
AT2G29260	7.467039006	7.413194084
AT2G29290	3.270471914	3.237365988
AT2G29300	3.738814981	3.691869357
AT2G29310	4.315148137	4.364448178
AT2G29320	5.00692879	5.21874256
AT2G29330	3.424029136	3.141752392
AT2G29340	8.07023905	8.928413991
AT2G29360	4.651789026	4.832407879
AT2G29370	4.192473601	4.332664402
AT2G29440	10.11078386	10.93897304
AT2G29460	6.263272504	8.408117048
AT2G29480	3.747348927	6.446957736
AT2G29490	4.83114423	10.99060459
AT2G29630	8.05255638	8.360553895
AT2G29690	8.210959499	8.023443398
AT2G29990	7.753231435	8.032931916

AT2G30490	9.856649935	11.2289161
AT2G30670	4.121663319	4.063488488
AT2G30750	3.051053364	3.076575293
AT2G30770	4.492573877	4.810305171
AT2G30860	9.556791995	11.85456549
AT2G30920	7.303013627	7.341316698
AT2G30970	10.14352499	10.26781145
AT2G31170	8.219180731	8.313918321
AT2G31390	10.28598819	10.71026909
AT2G31570	7.909604413	8.005766663
AT2G31580	7.747459237	7.701249809
AT2G31810	9.207289304	8.700229528
AT2G32440	5.569931956	5.571662769
AT2G32520	11.1511407	11.29882642
AT2G33150	10.50975636	10.81359444
AT2G33220	7.180749859	7.158540701
AT2G33730	9.494809852	9.448426864
AT2G33840	9.598664171	9.521242168
AT2G34060	6.450688208	5.901638667
AT2G34490	3.456860213	3.60387113
AT2G34500	4.818266637	5.033920984
AT2G34555	4.305913997	4.017968689
AT2G34590	9.585430974	9.695815213
AT2G34850	4.077543477	4.063769698
AT2G35040	11.39389356	11.39004915
AT2G35390	6.313299628	6.415659736
AT2G35690	6.914657431	6.919652099
AT2G36190	5.268322621	4.941342762
AT2G36230	9.062573936	8.961079198
AT2G36390	8.405832465	8.615203608
AT2G36460	12.54650516	12.75432304
AT2G36530	12.78807726	12.88669727
AT2G36750	3.685657693	3.742552783
AT2G36800	4.279024692	7.009681476
AT2G36880	11.59559007	12.78765279
AT2G37040	5.86291697	8.79811914
AT2G37130	4.925375615	5.028353926
AT2G37500	9.055541542	9.133141019
AT2G37690	10.34203642	10.1753927
AT2G37840	6.117944031	6.190074986
AT2G38040	9.064898086	8.662048387

AT2G38050	7.195039869	7.625473064
AT2G38380	3.926250926	3.738942631
AT2G38390	3.926250926	3.738942631
AT2G38670	11.12537629	10.86735793
AT2G38700	9.57903551	9.401385263
AT2G39040	4.891287661	4.747285252
AT2G39290	9.804022923	9.623441036
AT2G39630	9.288220914	9.216220702
AT2G39770	10.93967182	11.18153314
AT2G40190	n/a	n/a
AT2G40840	10.17618803	10.63148812
AT2G40890	10.04985404	11.92259716
AT2G40930	8.81002702	8.715063943
AT2G41210	4.85613234	4.709978658
AT2G41220	9.600370042	9.960349257
AT2G41480	11.03102756	10.59741212
AT2G41490	8.146702661	8.230623552
AT2G42010	8.248911109	8.254672187
AT2G42490	8.863885313	8.892288077
AT2G42600	9.954946765	10.03166864
AT2G43090	12.25008057	12.21443105
AT2G43360	10.40524629	11.19997562
AT2G43480	3.685870364	3.871076443
AT2G44050	11.9194776	11.94274574
AT2G44160	10.09980178	10.72279456
AT2G44450	12.66822778	12.17739477
AT2G44460	9.672185901	9.437461513
AT2G44470	4.399228826	4.598778565
AT2G44480	3.253010287	3.396147421
AT2G44490	5.820352424	6.470545608
AT2G44520	8.834835498	8.965815671
AT2G44530	9.17741633	8.823406023
AT2G44660	8.5093971	8.48901722
AT2G45220	4.63566803	4.608125262
AT2G45290	9.279480829	10.35453721
AT2G45440	11.54939134	11.55524728
AT2G45790	10.83751235	10.96770144
AT2G46860	4.106926328	4.02614786
AT2G47030	3.774943281	3.447713468
AT2G47420	9.911158773	9.772366286
AT2G47510	10.72314153	10.91080248

AT2G47690	8.552270027	8.611697085
AT2G47730	9.736242536	11.38163542
AT2G48150	5.149996991	4.983140871
AT3G01120	10.57576184	10.43054595
AT3G01190	4.202942737	4.02096879
AT3G01500	4.462446237	4.262363769
AT3G01850	8.1246264	8.294361663
AT3G02230	13.07708005	12.9069644
AT3G02260	8.709451896	8.744580739
AT3G02350	9.112868751	8.63153124
AT3G02360	10.50141299	11.38457855
AT3G02470	11.42174317	11.32863907
AT3G02570	7.133676911	7.149579929
AT3G02580	8.858042287	9.042143448
AT3G02610	5.300229909	5.268113338
AT3G02620	5.300229909	5.268113338
AT3G02630	9.072523083	9.022861611
AT3G02660	6.874489502	6.804188215
AT3G02760	9.092783095	9.070045007
AT3G02780	9.358245787	9.59815295
AT3G02870	10.35042207	10.27125474
AT3G02875	7.984648627	9.436722289
AT3G03070	10.84148855	10.86964966
AT3G03190	3.856467973	3.851308107
AT3G03330	8.472139035	8.563177351
AT3G03670	4.909539122	4.846895711
AT3G03980	8.270324711	8.399335371
AT3G04000	7.756562328	8.53184985
AT3G04050	4.903964659	4.637884934
AT3G04080	7.799486748	7.367128785
AT3G04120	13.47887342	13.81866839
AT3G04520	8.285156202	9.471117157
AT3G04600	8.666130979	8.79278718
AT3G04790	10.13771171	10.43325253
AT3G04870	6.869463255	7.281374985
AT3G04940	7.108517129	7.023018832
AT3G05630	6.566474641	6.51870873
AT3G05970	8.97016092	9.096864518
AT3G06200	7.775568404	7.940301685
AT3G06310	8.539429035	8.524798128
AT3G06580	9.045338912	9.020286632

AT3G06650	9.331815448	9.580529715
AT3G06810	8.282016897	8.525512383
AT3G06850	7.416943481	7.364820453
AT3G06860	9.68497741	9.952571448
AT3G07270	8.004711437	8.166801148
AT3G07420	5.193603289	5.35656368
AT3G07800	9.591269087	9.510422136
AT3G07850	4.751204141	4.424738103
AT3G07960	4.640126905	4.618970355
AT3G07990	5.619913748	5.12058697
AT3G08610	11.2938367	11.34853084
AT3G09150	5.721873908	5.804724376
AT3G09920	6.144985707	6.080701839
AT3G10050	10.29712463	10.6317915
AT3G10230	7.369189903	7.590144112
AT3G10340	3.902432832	3.833599123
AT3G10700	8.0089545	7.6125712
AT3G11710	12.45726977	12.35118086
AT3G11750	6.849053192	6.72654982
AT3G12260	11.71314958	11.66074827
AT3G12290	9.85287916	10.07069369
AT3G12780	10.2048326	10.37701174
AT3G12800	9.89838997	10.66841438
AT3G13180	7.28492262	7.176415964
AT3G13450	6.283403437	6.550642374
AT3G13490	7.704667791	7.644750186
AT3G13730	4.710659793	5.100161342
AT3G13790	4.224710073	4.883523372
AT3G13900	4.558757489	4.425021296
AT3G13930	9.664580064	9.842462644
AT3G14270	6.228756417	6.30419631
AT3G14390	11.21484031	11.30495767
AT3G14415	6.144435911	6.314412876
AT3G14940	6.583413082	6.423976426
AT3G15290	7.446215956	7.407369237
AT3G15730	10.02116251	9.64114079
AT3G16950	10.89683675	10.81247009
AT3G17070	3.661393521	3.670985722
AT3G17240	11.36436083	11.69590998
AT3G17390	13.14850789	12.59300605
AT3G17760	3.442773543	3.293925729

AT3G17820	11.77120979	12.88071776
AT3G17940	8.843852553	9.051580431
AT3G18080	3.9928485	4.02026949
AT3G18410	11.12050169	11.46177753
AT3G19160	4.52457745	4.719178424
AT3G19210	n/a	n/a
AT3G19280	4.916279562	5.15309459
AT3G19420	8.453478378	8.311565385
AT3G19450	6.786898017	8.258364659
AT3G19480	6.909206558	6.689100723
AT3G19710	4.137914181	4.095741071
AT3G19820	11.07360174	10.7661357
AT3G20020	8.022297306	7.864771706
AT3G21110	9.946631287	9.433250196
AT3G21230	4.209814655	4.60217716
AT3G21240	6.56326994	9.089347037
AT3G21500	4.807938596	4.640937813
AT3G21560	4.1839746	4.128255776
AT3G21770	4.958545018	4.931078563
AT3G22400	3.857187642	3.931651444
AT3G22460	5.982492582	5.931843149
AT3G22740	4.089585774	3.871746552
AT3G23490	9.360483739	9.445047298
AT3G23580	8.335680777	9.156234336
AT3G23630	3.52642743	3.524063123
AT3G23810	9.966055993	10.34698486
AT3G23940	11.34330428	11.2117428
AT3G24030	8.252253961	7.846610763
AT3G24090	7.204454128	7.364179868
AT3G24200	8.982977495	9.017781319
AT3G24503	5.380747865	5.637238263
AT3G25110	8.042282059	7.753498804
AT3G25140	11.00163556	10.41338637
AT3G25220	9.146644389	9.212712061
AT3G25570	3.643817657	3.795240646
AT3G25610	8.704622934	9.371678441
AT3G25820	4.049701642	3.986614158
AT3G25830	4.049701642	3.986614158
AT3G25960	4.309399958	4.150404889
AT3G26150	3.147766706	3.191255796
AT3G26160	3.746539246	3.686896232

AT3G26170	5.489440558	5.484185528
AT3G26190	3.378724844	3.382265432
AT3G26200	4.911369119	4.914459137
AT3G26210	3.241176963	3.169841765
AT3G26220	2.955402809	3.023610938
AT3G26230	3.2027576	3.192655547
AT3G26270	3.737793336	3.612242285
AT3G26280	3.399411822	3.247584564
AT3G26290	3.314272059	3.307748438
AT3G26300	3.16619904	3.051015223
AT3G26310	3.377835147	3.221510531
AT3G26320	3.091508313	3.305973121
AT3G26830	9.215162726	10.65702897
AT3G27060	11.30267856	11.39756946
AT3G27180	n/a	n/a
AT3G27300	6.915713209	7.440587003
AT3G27740	10.92851468	10.93961635
AT3G27870	3.596964892	3.569694367
AT3G28200	8.206977442	7.73178221
AT3G28480	8.781819851	8.801946369
AT3G29200	8.236018687	9.359307044
AT3G29320	7.891303129	8.2828829
AT3G29360	11.45837321	11.03209193
AT3G30180	3.755823997	3.659029418
AT3G42640	7.2535536	6.994979802
AT3G43190	5.419844452	5.440008548
AT3G43270	6.835687102	6.767768697
AT3G43670	6.582744733	6.539831592
AT3G43800	5.674436065	5.577521569
AT3G44250	4.513516664	4.218535416
AT3G44300	10.91466078	12.50297518
AT3G44320	7.033703627	8.102546906
AT3G44880	7.630272966	8.03138016
AT3G45100	7.405447393	7.412855162
AT3G45130	4.572085154	4.597143131
AT3G45140	3.566059673	3.715350658
AT3G45940	3.870373014	3.779674221
AT3G46100	8.343608797	8.522395528
AT3G46440	9.660665275	9.493271853
AT3G46970	8.848693743	9.211682993
AT3G47000	8.563039575	8.794908904



AT3G47050	6.240945019	6.101939723
AT3G47340	3.688787123	3.617662028
AT3G47350	n/a	n/a
AT3G47390	7.238097462	7.16164828
AT3G47800	3.733473988	4.051084751
AT3G47930	9.105637649	9.034708698
AT3G47950	7.488199777	6.660660092
AT3G48170	9.762081783	10.18581339
AT3G48360	3.603381401	3.695163807
AT3G48540	n/a	n/a
AT3G48560	11.4082398	11.33454798
AT3G48730	9.90687262	9.835491976
AT3G48780	8.003064291	7.911799122
AT3G48790	8.003064291	7.911799122
AT3G49110	8.543096651	10.01482556
AT3G49120	8.543096651	10.01482556
AT3G49160	4.749580128	4.936204859
AT3G49360	5.832697473	5.213590856
AT3G49600	7.219214278	7.138852207
AT3G49640	7.646648051	7.253078407
AT3G49680	9.375650459	9.772648619
AT3G49700	4.931174714	4.917758177
AT3G49960	3.71042012	3.548827154
AT3G50110	6.943428174	6.755760371
AT3G50520	8.331055918	8.482839781
AT3G50660	5.410045145	4.370481417
AT3G50740	3.727644112	3.856211841
AT3G51160	9.850672958	9.44634649
AT3G51240	4.18117553	4.280367397
AT3G51820	9.402771948	9.422120342
AT3G51840	8.992371045	9.309434571
AT3G52200	9.699561135	9.759162104
AT3G52720	3.917566846	3.875933513
AT3G52840	4.183321348	4.154813693
AT3G52930	13.01993179	13.0064711
AT3G52940	8.180210628	8.231653319
AT3G52970	3.796558056	3.876839181
AT3G52990	10.12063228	10.44424559
AT3G53030	7.545333153	7.485084628
AT3G53110	10.13361086	10.24640311
AT3G53130	7.643428024	7.893839749

AT3G53260	6.884510773	9.58821189
AT3G53280	3.73521875	3.737022452
AT3G53290	3.352903143	3.178612292
AT3G53300	3.148752314	3.111046231
AT3G53520	8.672955758	8.342842113
AT3G53580	10.1689938	10.184374
AT3G53620	8.766965118	7.530960235
AT3G54050	7.364257507	7.672073187
AT3G54250	7.902461227	7.476354829
AT3G54440	n/a	n/a
AT3G54470	n/a	n/a
AT3G54640	9.368732632	10.37242334
AT3G55030	8.195688175	7.780746911
AT3G55120	4.64955362	5.913349624
AT3G55260	8.091181241	7.750241303
AT3G55360	9.15353544	9.016127261
AT3G55400	8.690031942	8.941289707
AT3G55410	10.4527495	10.65303309
AT3G55440	12.46602258	12.67059886
AT3G55590	6.816539831	6.691886111
AT3G55610	9.039414674	9.175752415
AT3G55650	3.282792994	3.088315863
AT3G55810	3.282792994	3.088315863
AT3G55870	4.153243923	4.232009779
AT3G56300	4.683700811	4.725463165
AT3G56310	9.800390861	10.06244947
AT3G56630	6.736468717	6.991094153
AT3G56940	9.603440874	9.904093362
AT3G56960	5.778449147	5.665659826
AT3G57050	9.181507097	9.304580227
AT3G57220	8.053479449	7.902977761
AT3G57610	11.312456	11.27953885
AT3G58140	8.666726434	8.671539335
AT3G58610	12.77849384	12.71203602
AT3G59410	8.443041332	8.361614487
AT3G59480	4.805978898	4.577217788
AT3G59970	10.09980178	10.72279456
AT3G60330	5.330303827	5.111544635
AT3G60750	11.71871121	11.8338379
AT3G61440	10.55112699	11.65592752
AT3G61510	4.504323067	4.544692023

AT3G62790	9.2192379	9.456375742
AT3G63080	8.117711787	7.920628236
AT3G63110	5.30906329	5.057828616
AT4G00520	4.095297443	4.437321696
AT4G01480	10.08781076	9.949221836
AT4G01690	9.68647556	9.611968021
AT4G02580	10.23902173	10.10429973
AT4G02610	7.440913045	7.541222302
AT4G02780	3.822424892	3.835230076
AT4G04040	7.47081761	7.475553128
AT4G04880	5.955143089	6.220084066
AT4G04910	9.39205765	9.216195829
AT4G04930	3.429120607	3.391547531
AT4G04955	5.652104062	5.846820804
AT4G05160	8.121102015	8.542504929
AT4G05390	9.692075034	9.995919316
AT4G05530	8.465336009	8.770674472
AT4G08770	8.485881908	11.36327666
AT4G08780	3.842328451	5.351036609
AT4G08900	7.464880248	7.654538424
AT4G08990	6.854237484	7.398849161
AT4G09520	8.385844038	8.324246017
AT4G10260	3.916714621	3.962576351
AT4G10320	n/a	n/a
AT4G11030	3.343350088	4.325395405
AT4G11290	4.16659696	4.226622076
AT4G11600	9.516770144	10.85288263
AT4G11640	4.421974172	4.462960695
AT4G11820	8.612033363	9.071645147
AT4G11840	4.262189846	4.173389883
AT4G11850	6.237391443	6.237029396
AT4G12440	4.329285651	4.416694696
AT4G13180	5.877290451	9.752439632
AT4G13250	8.385317125	8.678188363
AT4G13290	2.856634669	2.940479577
AT4G13310	2.856634669	2.940479577
AT4G13430	10.92906817	11.02449611
AT4G13610	4.200529002	4.032293905
AT4G13660	8.97722374	9.023227956
AT4G13720	9.470641748	9.556327715
AT4G13780	9.750745023	9.688219097

AT4G13890	10.50356568	10.68330368
AT4G13930	10.50356568	10.68330368
AT4G14070	6.220941614	6.375905879
AT4G14090	4.168862267	4.106203865
AT4G14140	6.854237484	7.398849161
AT4G14210	7.642445034	8.003315405
AT4G14680	6.178972896	6.60556125
AT4G14910	7.909212028	8.155436463
AT4G14930	8.549497379	8.754226258
AT4G14940	4.209953117	4.196823158
AT4G15340	3.931667062	5.372103896
AT4G15350	3.220262723	3.169394893
AT4G15360	3.210633562	3.14452046
AT4G15440	3.636162763	3.744047936
AT4G15530	4.712306291	5.304131126
AT4G16155	9.303366694	9.369964222
AT4G16210	8.722096676	8.713418041
AT4G16270	5.070526307	4.931714747
AT4G16570	7.640484681	7.460308906
AT4G16700	7.708146985	7.432960008
AT4G16710	7.83522763	7.88855101
AT4G16730	3.342634255	3.422030024
AT4G16760	8.189763679	9.620912935
AT4G16800	7.131009031	7.287164496
AT4G17090	5.527094879	5.602179361
AT4G17260	8.068408502	7.850171044
AT4G17690	3.271172685	3.41780477
AT4G17830	9.577842599	9.417207282
AT4G18230	7.342629477	7.272798008
AT4G18360	8.452311617	9.917731176
AT4G18440	8.434329379	8.88924465
AT4G19710	8.808855301	8.763563035
AT4G20960	6.900894083	7.003380548
AT4G20990	3.805736185	3.892137436
AT4G21000	3.805736185	3.892137436
AT4G21470	7.751934538	7.976457819
AT4G21490	5.796407772	6.011568
AT4G21960	4.983879259	6.611917938
AT4G22330	9.09246654	8.942112325
AT4G22570	9.926489497	10.26762755
AT4G22870	3.784122518	3.799652888

AT4G22880	3.784122518	3.799652888
AT4G23850	10.58699663	10.28252266
AT4G23900	4.483066532	4.489930279
AT4G23920	6.014424962	6.314423793
AT4G24040	7.824612821	8.108733628
AT4G24620	9.036337702	9.089036352
AT4G24650	3.562268478	3.669360951
AT4G24830	10.57544318	10.76248676
AT4G25420	4.00503047	3.888410775
AT4G25700	7.868458131	7.872221904
AT4G26010	3.897909715	3.737360371
AT4G26260	3.991441839	4.056752237
AT4G26270	7.406334061	7.492924142
AT4G26300	9.60361456	9.579457945
AT4G26390	4.582017467	4.735436728
AT4G26520	4.664164993	4.736569123
AT4G26530	4.333976314	4.192220097
AT4G26770	4.236975371	3.958799421
AT4G26850	9.230221693	9.888013628
AT4G26910	9.89803502	10.21981267
AT4G26970	10.98533701	11.23863289
AT4G27070	9.506756307	10.08229902
AT4G27550	3.64148332	3.442815053
AT4G28220	6.450202274	6.308506156
AT4G29010	9.131376244	9.327759525
AT4G29130	8.98873714	9.112908507
AT4G29220	7.306178132	7.593082303
AT4G29510	10.5110501	10.40430661
AT4G29840	10.1360755	10.27498871
AT4G29890	6.25470666	6.286882666
AT4G30000	5.853115068	5.713386896
AT4G30170	3.523784689	3.498305433
AT4G30190	11.40540572	12.12090779
AT4G30580	7.807300504	7.684800271
AT4G31050	5.834422129	5.700185386
AT4G31400	5.124368861	5.378216674
AT4G31760	4.226721336	4.115047054
AT4G31780	7.864167332	7.927415161
AT4G31790	9.184794433	9.339578301
AT4G31870	6.342069865	6.873413753
AT4G31940	2.835079113	2.876266601

AT4G31990	9.363621037	9.606317381
AT4G32180	7.519078565	7.439250617
AT4G32360	6.974769548	6.969235069
AT4G32520	10.81308322	10.71209472
AT4G32540	4.316702612	4.022225471
AT4G32770	6.746820133	7.037446014
AT4G32840	7.940473472	7.888022131
AT4G33010	9.132572904	9.78914778
AT4G33030	7.929109325	8.462598005
AT4G33070	7.484858905	7.807540983
AT4G33150	4.623643562	4.845588498
AT4G33240	5.200992583	5.278332175
AT4G33420	3.688630351	3.600070637
AT4G33670	8.341693889	8.501223341
AT4G33680	10.13620141	10.21250477
AT4G34030	7.519466397	7.479531559
AT4G34050	9.146609776	12.01301022
AT4G34200	11.62494573	12.91772572
AT4G34230	10.9656918	11.52321815
AT4G34350	8.632410717	8.8624433
AT4G34640	8.910630857	8.957737504
AT4G34650	4.093828508	3.959719381
AT4G34700	11.08113386	10.96721206
AT4G34710	9.888428418	9.590883327
AT4G34740	8.433545178	8.098653673
AT4G34890	6.944172991	7.535472891
AT4G35090	7.351507052	7.510399245
AT4G35460	10.14384174	10.18511383
AT4G35830	11.43302015	12.01472573
AT4G36220	3.52625574	3.358281251
AT4G36380	4.689041978	4.583913925
AT4G36430	4.246173291	4.122115067
AT4G36810	7.216948838	7.251500859
AT4G37150	5.240896945	5.110715728
AT4G37310	4.783992146	4.7399647
AT4G37320	4.624541212	4.516683047
AT4G37330	7.446459121	7.261980937
AT4G37340	4.284917714	4.3638742
AT4G37360	3.952539762	4.11542183
AT4G37370	7.696413164	8.4497396
AT4G37400	5.158238208	4.565769529

AT4G37410	4.1153011	4.216715338
AT4G37430	4.172445397	4.050412849
AT4G37520	3.637687646	3.42158552
AT4G37530	3.637687646	3.42158552
AT4G37770	6.569418693	5.844313112
AT4G37840	3.603013068	3.394866732
AT4G37930	8.843411829	8.989915954
AT4G38240	7.697052476	7.718821667
AT4G38880	5.362046556	6.05350857
AT4G38970	9.408173786	9.382150006
AT4G39210	4.432579295	4.818404945
AT4G39280	10.31086686	10.24173569
AT4G39370	5.918959075	5.778951373
AT4G39540	7.79454166	8.429689733
AT4G39640	5.679242564	4.787667813
AT4G39660	7.828042241	8.092594329
AT4G39800	5.415761031	6.365448636
AT5G01220	5.70212176	5.809691682
AT5G02080	7.524476951	7.794745473
AT5G02410	6.040318939	5.862487278
AT5G03080	8.979592036	8.923820295
AT5G03650	7.636357609	8.145768193
AT5G03690	6.710833632	7.306880627
AT5G03760	3.334683719	3.42161685
AT5G03770	5.803393561	5.823613499
AT5G03860	4.143336263	3.976278814
AT5G04140	5.918831591	6.388162903
AT5G04180	4.570300969	4.58701187
AT5G04230	4.199046114	3.798868341
AT5G04330	5.155778327	5.219823406
AT5G04590	9.90931697	10.28424044
AT5G04620	5.946301206	5.972659761
AT5G04630	5.064748697	4.79199819
AT5G04660	5.551290287	5.485042899
AT5G04970	3.589971854	3.581347325
AT5G05340	6.159612397	4.789030666
AT5G05590	8.794528366	8.919773389
AT5G05690	6.947502298	5.594062982
AT5G05730	8.726617232	9.51883632
AT5G05860	9.378120171	8.7996973
AT5G05870	5.955023518	6.091059587

AT5G06060	7.705387566	7.799568362
AT5G06580	7.626816663	7.433124396
AT5G06720	10.47815798	9.440862213
AT5G06730	7.517743176	7.422791948
AT5G07200	3.652093301	3.439957658
AT5G07990	5.43961929	5.297701926
AT5G08280	9.281174946	9.069205521
AT5G08300	11.82618054	11.83749262
AT5G08415	8.349682914	8.367851302
AT5G08530	11.08986056	10.98810165
AT5G08570	10.03910172	10.33590251
AT5G08640	3.47947479	3.523855173
AT5G09300	7.127142519	8.310825363
AT5G09640	4.205166192	4.1762639
AT5G09970	3.860543041	4.033927525
AT5G10050	n/a	n/a
AT5G10160	10.06795429	9.969275173
AT5G10170	3.787240402	3.829754109
AT5G10240	10.48282915	10.5416229
AT5G10300	4.432888829	4.739526213
AT5G10480	9.348862996	9.185855866
AT5G10870	8.39961276	8.307455713
AT5G10920	10.35585673	10.60205892
AT5G11160	8.057559171	7.582983572
AT5G11380	7.379149754	7.359234284
AT5G11520	10.75098017	11.50284863
AT5G11720	8.872025783	8.87260141
AT5G11880	11.21484031	11.30495767
AT5G12180	3.050245965	3.204768789
AT5G12200	9.757909205	9.958460583
AT5G13110	10.21146344	10.10470387
AT5G13420	11.85567444	12.05234158
AT5G13520	10.30827635	10.2876465
AT5G13630	10.3719064	10.48221117
AT5G13690	8.081614505	8.368815357
AT5G13710	10.58353626	10.62414258
AT5G13930	4.64111837	4.569114988
AT5G14130	3.648296796	3.557032229
AT5G14220	7.715351794	7.855543322
AT5G14590	10.027955	10.21131556
AT5G14740	4.444162187	4.246809155



AT5G14760	8.366853615	7.790295658
AT5G14780	10.11131832	11.81723378
AT5G14800	9.927185691	9.822011638
AT5G14850	7.138741808	7.189340805
AT5G14950	8.256202186	8.13356673
AT5G15140	3.398127817	3.134451223
AT5G15180	3.05568179	3.102347316
AT5G15490	9.587543371	9.353670686
AT5G15770	8.306624486	8.309259411
AT5G15950	8.179059986	8.535539039
AT5G16230	4.048291778	4.228527789
AT5G16240	5.213059208	5.033001148
AT5G16440	9.704921393	9.936733259
AT5G16710	10.46435078	10.61082811
AT5G16870	5.779176149	5.155863947
AT5G17050	8.100869665	8.918347798
AT5G17230	8.920111739	9.216707861
AT5G17310	9.521986118	9.534367241
AT5G17330	11.34006112	11.0319422
AT5G17770	10.46801445	10.78083324
AT5G17820	4.709450933	4.417508744
AT5G17990	7.508518037	8.836589269
AT5G18070	7.487069081	7.262226459
AT5G18200	7.289464069	7.317513173
AT5G18280	8.002887706	7.511461698
AT5G18800	11.23051753	11.29582774
AT5G18930	3.742629727	3.64546168
AT5G19040	4.246936703	4.114671408
AT5G19220	3.533883831	3.502614107
AT5G19550	12.16776467	12.27558847
AT5G19730	4.818050079	4.771087376
AT5G19880	4.67197854	4.670519297
AT5G19890	3.798210381	3.81235991
AT5G20410	4.726214671	4.995959887
AT5G20830	10.78988445	10.73581126
AT5G20980	8.820031291	8.599463886
AT5G22130	n/a	n/a
AT5G22300	5.082120668	7.930430804
AT5G22410	3.20787466	3.293181071
AT5G23010	4.075605447	3.920079331
AT5G23020	3.158453437	3.039402248

AT5G23070	8.842981467	8.97026005
AT5G23190	3.387221559	3.409189936
AT5G23250	10.09285431	10.15896547
AT5G23670	7.904435014	7.746414435
AT5G24070	3.699597338	3.774463775
AT5G24140	3.406827322	3.515046385
AT5G24400	8.909408301	9.119533957
AT5G24410	5.082194858	4.964906342
AT5G24420	3.848600628	4.145196425
AT5G24950	5.974143534	5.749376706
AT5G24960	5.974143534	5.749376706
AT5G25120	7.649466214	7.677845983
AT5G25130	2.872240138	2.773224605
AT5G25140	2.803639044	2.821891763
AT5G25180	3.661545116	3.723024125
AT5G25370	4.305098711	4.138388265
AT5G25480	7.35211308	7.17816133
AT5G25900	8.831834711	9.052502398
AT5G26030	9.46041823	9.573784373
AT5G26667	n/a	n/a
AT5G26830	11.4012432	11.2626111
AT5G27430	9.250742905	9.042908887
AT5G27450	8.441730981	8.456042117
AT5G27470	11.92457008	11.82518455
AT5G27600	8.658876372	9.1250335
AT5G27730	6.68828125	6.835378423
AT5G28030	3.406042351	3.413887255
AT5G28840	9.281405204	9.223020444
AT5G34780	3.377147889	3.323455519
AT5G35630	9.745450699	9.758257579
AT5G35790	6.721982459	6.6203419
AT5G36150	3.961869534	3.661780485
AT5G36220	5.25657357	9.410303298
AT5G36880	9.449035695	9.61469539
AT5G37510	10.58013118	10.54251381
AT5G37600	9.609450079	9.603700811
AT5G37830	8.420160901	8.62456145
AT5G37850	7.016755997	7.453238288
AT5G38410	12.38552704	12.34864195
AT5G38420	12.38552704	12.34864195
AT5G38430	12.38552704	12.34864195

AT5G38450	4.504042684	4.25248981
AT5G38460	7.255340319	7.171062539
AT5G38530	8.340453255	9.627037017
AT5G38830	10.62993056	10.70963684
AT5G38970	3.77448174	3.634982519
AT5G39320	7.656325941	7.086729482
AT5G40150	8.528239361	7.565959335
AT5G40390	8.324419216	7.88521464
AT5G40760	8.589425308	9.476523508
AT5G40850	8.504270644	8.254847555
AT5G41360	7.675892199	7.728321025
AT5G41480	6.044600071	6.366448517
AT5G41670	10.5877582	10.25197752
AT5G42180	3.842615963	3.815009093
AT5G42250	6.462281884	6.092779246
AT5G42260	2.908601117	2.963448108
AT5G42400	7.058785189	7.044874228
AT5G42590	3.866722519	4.215199864
AT5G42600	4.813856637	4.873424631
AT5G42650	5.066128425	7.917359172
AT5G42740	9.977212031	10.14567297
AT5G42800	4.654485488	4.432122243
AT5G43280	8.139582949	8.480182067
AT5G43780	8.173509679	8.590806861
AT5G43860	5.861418023	6.058107726
AT5G43940	11.59210496	11.74994383
AT5G44240	9.590344134	9.579700293
AT5G44640	2.908601117	2.963448108
AT5G46330	4.402866587	4.239900047
AT5G47000	3.578221491	3.524264329
AT5G47840	8.491433432	8.736494349
AT5G47890	10.21365738	10.19711501
AT5G48220	7.561335051	7.425122153
AT5G48230	8.962465426	9.070541738
AT5G48300	9.283642542	9.689518874
AT5G48840	7.377615911	7.512338178
AT5G48880	5.097779985	5.10615295
AT5G49160	9.933090542	9.596361184
AT5G49180	3.453008408	3.559307368
AT5G49190	5.483375335	5.23734045
AT5G49460	8.395235241	8.562347631

AT5G49570	7.541318321	7.113835422
AT5G49650	7.873989745	7.976023474
AT5G49810	8.833484533	8.856997041
AT5G50210	7.700833664	8.039350241
AT5G50370	9.349580014	10.08306888
AT5G50375	9.147622167	8.877990049
AT5G50950	10.72314153	10.91080248
AT5G51280	8.619369327	8.58716398
AT5G51810	3.572423182	3.534900844
AT5G51830	8.481148063	9.599857579
AT5G51890	4.661657509	4.608480555
AT5G52100	5.140146196	4.968687749
AT5G52320	3.820765562	3.542325026
AT5G52520	9.344849754	9.085105106
AT5G52560	9.060652748	8.762816246
AT5G52570	7.047812344	7.270404769
AT5G52840	10.58175063	10.43312308
AT5G52920	9.971722429	10.10640926
AT5G53460	10.01388133	11.68916106
AT5G53970	6.992434167	7.184116818
AT5G54160	9.764534056	10.94348522
AT5G54810	9.506756307	10.08229902
AT5G55070	10.48184053	10.83707356
AT5G55500	7.305444719	7.175994534
AT5G56090	8.334892192	8.53326516
AT5G56350	10.24781905	10.3007545
AT5G56630	8.179263399	8.23070015
AT5G56640	3.739003834	3.680704611
AT5G56650	5.998914919	6.324047822
AT5G56680	10.60178249	10.57475026
AT5G56760	9.092318192	9.506881813
AT5G57030	7.532346826	7.592007581
AT5G57220	6.050937182	5.568796341
AT5G57260	3.697467477	3.697468171
AT5G57300	7.76283362	7.801617941
AT5G57350	7.255685127	7.87246797
AT5G57590	7.663772353	8.000275216
AT5G57655	7.939162788	7.631115227
AT5G57850	6.762205503	6.87785862
AT5G57890	8.2440986	9.243245788
AT5G58240	8.772494974	8.823075558

AT5G58390	3.790168954	6.953631194
AT5G58400	4.180131297	4.160669333
AT5G58770	3.536165698	3.722881343
AT5G58860	4.120048302	4.066209632
AT5G59750	6.984074997	7.094138238
AT5G60600	7.858465374	8.210901616
AT5G61140	8.540929189	8.498473687
AT5G61410	9.439008665	9.610038279
AT5G61580	6.930037727	7.114405488
AT5G62530	8.636072722	8.967532079
AT5G62670	6.950518337	7.03523834
AT5G62790	8.387945979	8.541350475
AT5G63290	6.092229571	6.328260195
AT5G63310	9.607184884	9.493186946
AT5G63570	8.613213315	8.941270474
AT5G63590	5.14937117	5.303640388
AT5G63680	9.585773044	9.684701081
AT5G63840	10.30908136	10.25473562
AT5G63890	9.222533102	9.455796166
AT5G63910	7.583720011	7.286982102
AT5G63980	8.316325692	8.960908903
AT5G64100	5.05682032	5.083309885
AT5G64110	5.310751512	4.916198064
AT5G64120	8.181738878	7.71144503
AT5G64370	7.661635072	7.840157865
AT5G64860	7.250025845	7.307961836
AT5G65010	9.155500926	9.413260384
AT5G65110	8.131963267	8.57832046
AT5G65720	10.02561062	9.933571955
AT5G65740	n/a	n/a
AT5G65750	8.298841376	8.590598605
AT5G65800	3.319399085	3.270934438
AT5G66120	9.229032272	9.8370192
AT5G66190	8.692829563	8.994225532
AT5G66220	4.208128827	4.073453039
AT5G66280	5.023804641	4.741014163
AT5G66390	3.356424625	3.193890623
AT5G66680	11.23196599	10.91215066
AT5G67030	7.66406463	7.917525591
AT5G67400	3.984100786	4.095495394
AT5G67520	5.600690238	5.707886382

AT5G67590	10.05681986	10.35804973
AT5G67630	8.948104786	8.902883101
NP_174083	n/a	n/a
NP_188430	n/a	n/a
NP_192901	n/a	n/a
NP_194697	n/a	n/a
NP_200227	n/a	n/a
NP_563822	n/a	n/a
NP_565650	n/a	n/a
AT2G44160	10.45995286	10.67263346
AT1G06290	10.33396862	10.25641075
AT1G31180	8.083627336	8.182029957
AT1G62640	4.091637039	4.07828681
AT2G28850	10.09980178	10.72279456
AT2G44160	8.083627336	8.182029957

Table 6 – List of genes included in *A. thaliana* model and relative expression values (from (Pauwels et al. 2008)). Values of “n/a” indicate that the gene was not in the expression dataset for two possible reasons, one being that the gene was not included on the ATH1 chip, and the other being that the probe corresponding to the gene was discarded from the analysis because of technical issues with the microarray.

Reaction name in model	MJ - upper bounds	MJ - lower bounds	MJ+ upper bounds	MJ+ lower bounds
'R00009 x'	357.54	0.00	387.22	0.00
'alpha alpha trehalose glucohydrolase'	265.72	0.00	311.72	0.00
'chitobiose N acetylglucosaminohydrolase'	284.21	0.00	297.94	0.00
'cellobiose glucohydrolase'	463.13	0.00	468.13	0.00
'maltose glucohydrolase'	375.55	0.00	394.22	0.00
'R00028 p'	375.55	0.00	394.22	0.00
'6 7 Dimethyl 8 1 D ribityl lumazine 6 7 dimethyl 8 1 D '	356.12	0.00	371.68	0.00
'ATP phosphohydrolase'	452.65	-452.65	491.29	-491.29
'R00093 p'	362.09	0.00	449.36	0.00
'NAD phosphohydrolase'	296.46	0.00	319.77	0.00
'ADP ATP adenylyltransferase'	296.46	0.00	319.77	0.00
'ATP AMP phosphotransferase'	327.80	0.00	387.62	0.00
'ATP dephospho CoA 3 phosphotransferase'	288.25	0.00	297.21	0.00
'ATP pyridoxal 5 phosphotransferase'	262.24	0.00	286.52	0.00
'ATP L methionine S adenylyltransferase'	500.00	0.00	491.59	0.00
'S adenosyl L methionine carboxy lyase'	420.18	0.00	435.50	0.00
'S adenosyl L methionine methylthioadenosine lyase'	241.34	0.00	224.67	0.00
'AMP aminohydrolase'	296.46	0.00	319.77	0.00
'P1 P4 bis 5 adenosyl tetraphosphate adenylohydrolase'	202.23	0.00	223.88	0.00
'P1 P3 bis 5 adenosyl triphosphate adenylohydrolase'	313.49	0.00	339.18	0.00
'S Adenosyl L homocysteine hydrolase'	362.26	0.00	397.76	0.00
'R00206 p'	165.54	0.00	203.90	0.00
'2 Hydroxyethylenedicarboxylate carboxy lyase pyruvate forming '	297.37	0.00	320.00	0.00
'pyruvate carboxy lyase acetaldehyde forming '	247.45	-247.45	300.14	-300.14
'malonyl CoA carboxy lyase acetyl CoA forming '	296.46	0.00	319.77	0.00
'acetyl adenylate CoA acetyltransferase'	339.70	-339.70	369.61	-369.61
'R00236 m'	339.70	-339.70	369.61	-369.61

'Acetyl CoA acetyl CoA C acetyltransferase'	306.82	-306.82	348.69	-348.69
'R00238 m'	306.82	-306.82	348.69	-348.69
'R00238 p'	306.82	-306.82	348.69	-348.69
'R00238 x'	376.70	-376.70	415.70	-415.70
'5 oxo L proline amidohydrolase ATP hydrolysing '	286.79	0.00	331.55	0.00
'Deamido NAD L glutamine amido ligase AMP forming '	283.48	0.00	312.64	0.00
'L Alanine 2 oxoglutarate aminotransferase'	273.03	-273.03	293.96	-293.96
'R00258 p'	273.03	-273.03	293.96	-293.96
'Acetyl CoA L glutamate N acetyltransferase'	330.82	0.00	351.10	0.00
'L glutamate 1 carboxy lyase 4 aminobutanoate forming '	371.95	0.00	424.09	0.00
'Isocitrate NADP oxidoreductase decarboxylating '	389.44	-389.44	439.61	-439.61
'R00267 m'	389.44	-389.44	439.61	-439.61
'glutathione hydrogen peroxide oxidoreductase'	239.84	-239.84	237.97	-237.97
'UDP glucose NAD 6 oxidoreductase'	427.10	0.00	424.10	0.00
'UTP alpha D glucose 1 phosphate uridylyltransferase'	341.97	0.00	366.52	0.00
'UDP glucose 4 epimerase'	336.27	-336.27	305.49	-305.49
'UDP glucose 4 6 hydro lyase'	323.86	-323.86	345.83	-345.83
'protoheme ferro lyase protoporphyrin forming '	371.59	0.00	368.04	0.00
'R00316 m'	339.70	0.00	369.61	0.00
'GDP phosphohydrolase'	300.12	0.00	288.76	0.00
'ATP GDP phosphotransferase'	355.38	-355.38	364.94	-364.94
'ATP GMP phosphotransferase'	291.15	0.00	305.24	0.00
'R00351 m'	296.46	0.00	319.77	0.00
'R00351 x'	296.46	0.00	319.77	0.00
'acetyl CoA oxaloacetate C acetyltransferase'	309.20	0.00	329.16	0.00
'L Aspartate 2 oxoglutarate aminotransferase'	429.31	-429.31	471.90	-471.90
'R00355 p'	385.51	-385.51	442.20	-442.20
'L Alanine glyoxylate aminotransferase'	285.96	-285.96	311.10	-311.10
'R00405 m'	369.41	-369.41	390.54	-390.54
'R00408 m'	296.46	-296.46	319.77	-319.77



'GTP 7 8 8 9 dihydrolase diphosphate forming '	335.30	0.00	319.75	0.00
'GTP 8 9 hydrolase'	302.30	0.00	313.95	0.00
'ATP GTP 3 diphosphotransferase'	296.46	0.00	319.77	0.00
'meso 2 6 diaminoheptanedioate carboxy lyase L lysine forming '	410.64	0.00	434.59	0.00
'R00475 x'	278.83	0.00	381.26	0.00
'ATP L aspartate 4 phosphotransferase'	325.07	-325.07	336.89	-336.89
'L aspartate oxygen oxidoreductase'	312.58	0.00	299.48	0.00
'L aspartate 1 carboxy lyase beta alanine forming '	296.46	0.00	319.77	0.00
'UTP alpha D galactose 1 phosphate uridylyltransferase'	261.02	-261.02	281.30	-281.30
'R00519 x'	334.67	-334.67	454.28	-454.28
'ATP sulfate adenylyltransferase'	287.60	0.00	330.25	0.00
'Sulfite oxygen oxidoreductase'	296.46	0.00	319.77	0.00
'Aldehyde NAD oxidoreductase'	322.07	-322.07	348.88	-348.88
'Nitrile aminohydrolase'	296.46	0.00	319.77	0.00
'Riboflavin 5 phosphate phosphohydrolase acid optimum '	183.40	0.00	157.26	0.00
'ATP riboflavin 5 phosphotransferase'	284.16	0.00	306.63	0.00
'L Arginine amidinohydrolase'	256.85	-256.85	294.26	-294.26
'L arginine carboxy lyase agmatine forming '	372.58	0.00	368.93	0.00
'ATP CDP phosphotransferase'	355.38	-355.38	364.94	-364.94
'R00575 m'	402.74	0.00	420.55	0.00
'acetyl CoA L serine O acetyltransferase'	328.17	0.00	365.47	0.00
'methanol hydrogen peroxide oxidoreductase'	407.10	0.00	436.83	0.00
'sarcosine oxygen oxidoreductase demethylating '	142.17	0.00	169.04	0.00
'Thiamin diphosphate phosphohydrolase'	300.12	0.00	288.76	0.00
'ATP thiamine diphosphate phosphotransferase'	296.46	0.00	319.77	0.00
'ATP thiamine diphosphotransferase'	296.46	0.00	319.77	0.00
'R00621 m'	388.47	0.00	409.53	0.00
'primary alcohol NAD oxidoreductase'	296.46	-296.46	319.77	-319.77
'Aldehyde NAD oxidoreductase'	327.24	0.00	394.12	0.00
'2 oxo acid carboxy lyase aldehyde forming '	247.45	-247.45	300.14	-300.14

'L Galactono 1 4 lactone ferricytochrome c oxidoreductase'	331.13	0.00	347.32	0.00
'S Adenosyl L methionine L methionine S methyltransferase'	318.96	-318.96	340.48	-340.48
'S Adenosyl L methionine L homocysteine S methyltransferase'	141.38	0.00	148.84	0.00
'2 phospho D glycerate hydro lyase phosphoenolpyruvate forming '	458.53	-458.53	495.40	-495.40
'Uridine triphosphate pyrophosphohydrolase'	339.82	0.00	367.37	0.00
'N2 Acetyl L ornithine amidohydrolase'	344.92	0.00	362.02	0.00
'L Phenylalanine 2 oxoglutarate aminotransferase'	429.31	-429.31	471.90	-471.90
'L phenylalanine ammonia lyase trans cinnamate forming '	246.58	-246.58	368.59	-368.59
'L phenylalanine oxygen oxidoreductase decarboxylating '	407.10	0.00	436.83	0.00
'Farnesyl diphosphate farnesyl diphosphate farnesyltransferase'	326.54	0.00	344.36	0.00
' S Lactate NAD oxidoreductase'	298.42	-298.42	301.78	-301.78
'3 Oxopropanoate NAD oxidoreductase decarboxylating '	296.46	0.00	319.77	0.00
'R00709 m'	296.46	-296.46	319.77	-319.77
'Acetaldehyde NADP oxidoreductase'	296.46	-296.46	319.77	-319.77
'L Tyrosine 2 oxoglutarate aminotransferase'	296.46	-296.46	319.77	-319.77
'L tyrosine carboxy lyase tyramine forming '	296.46	0.00	319.77	0.00
'L tyrosine ammonia lyase trans p hydroxycinnamate forming '	296.46	0.00	319.77	0.00
'R00742 p'	296.46	0.00	319.77	0.00
'4 hydroxy 2 oxopentanoate pyruvate lyase acetaldehyde forming '	296.46	-296.46	319.77	-319.77
'Ethanol NAD oxidoreductase'	296.46	-296.46	319.77	-319.77
'R00762 p'	277.38	0.00	294.93	0.00
'beta D fructose 2 6 bisphosphate 2 phosphohydrolase'	333.17	-333.17	347.21	-347.21
'D glucose 6 phosphate aldose ketose isomerase'	323.89	-323.89	349.40	-349.40
' S ureidoglycolate urea lyase glyoxylate forming '	296.46	0.00	319.77	0.00
'Ammonia ferredoxin oxidoreductase'	454.78	0.00	418.71	0.00

'R00790 p'	454.78	0.00	418.71	0.00
'UDP glucose D fructose 2 alpha D glucosyltransferase'	376.79	-376.79	412.71	-412.71
'D Sorbitol 6 phosphate NADP 1 oxidoreductase'	374.16	-374.16	404.46	-404.46
'Hydrogen sulfide ferredoxin oxidoreductase'	361.08	0.00	395.35	0.00
'ATP D fructose 6 phosphotransferase'	288.03	0.00	312.46	0.00
'D Glucitol NAD 2 oxidoreductase'	296.46	-296.46	319.77	-319.77
'GDP D mannose 1 phosphate guanylyltransferase'	403.85	-403.85	429.84	-429.84
'GDP mannose 4 6 hydro lyase'	358.20	0.00	363.14	0.00
'L Cysteine 2 oxoglutarate aminotransferase'	227.59	-227.59	254.98	-254.98
'3 aminopropanal NAD oxidoreductase'	327.24	0.00	394.12	0.00
'N Carbamoyl beta alanine amidohydrolase'	282.67	-282.67	301.39	-301.39
'Propionyladenylate CoA propionyltransferase'	296.46	-296.46	319.77	-319.77
'S Methylmalonate semialdehyde NAD oxidoreductase'	285.97	0.00	299.77	0.00
'5 6 7 8 Tetrahydrofolate NADP oxidoreductase'	298.77	-298.77	315.33	-315.33
'Tetrahydrofolate L glutamate gamma ligase ADP forming '	233.13	-233.13	244.74	-244.74
'Formate tetrahydrofolate ligase ADP forming '	350.96	-350.96	405.71	-405.71
'5 10 Methylene tetrahydrofolate glycine hydroxymethyltransferase'	386.74	-386.74	410.69	-410.69
'R00945 m'	398.47	-398.47	411.80	-411.80
'5 Methyltetrahydrofolate L homocysteine S methyltransferase'	296.46	0.00	319.77	0.00
'ATP alpha D glucose 1 phosphate adenylyltransferase'	130.74	-130.74	134.65	-134.65
'R00948 p'	128.39	-128.39	130.83	-130.83
'alpha D Glucose 1 phosphate 1 6 phosphomutase'	296.46	-296.46	319.77	-319.77
'R00959 p'	296.46	-296.46	319.77	-319.77
'5 6 Dihydrouracil NADP oxidoreductase'	296.46	-296.46	319.77	-319.77
'2R 3S 3 methylmalate NAD oxidoreductase'	381.64	-381.64	394.28	-394.28

'L threonine ammonia lyase 2 oxobutanoate forming '	380.23	0.00	408.71	0.00
'2 Hydroxybutyrate NAD oxidoreductase'	298.42	0.00	301.78	0.00
'Dolichyl diphosphate phosphohydrolase'	330.15	0.00	343.05	0.00
'UDPglucose dolichyl phosphate beta D glucosyltransferase'	343.24	-343.24	354.29	-354.29
'GDPmannose dolichyl phosphate O beta D mannosyltransferase'	328.03	0.00	332.55	0.00
'R01015 p'	410.98	-410.98	451.12	-451.12
'ATP choline phosphotransferase'	296.46	-296.46	319.77	-319.77
'sn Glycero 3 phosphocholine glycerophosphohydrolase'	296.46	0.00	319.77	0.00
'ATP D ribose 5 phosphate diphosphotransferase'	332.31	-332.31	339.19	-339.19
'ADP ribose ribophosphohydrolase'	143.76	0.00	152.35	0.00
'D ribose 5 phosphate aldose ketose isomerase'	300.96	-300.96	322.17	-322.17
'R01056 p'	300.96	-300.96	322.17	-322.17
'D glyceraldehyde 3 phosphate NAD oxidoreductase phosphorylating '	350.82	-350.82	379.81	-379.81
'1 5 phospho D ribosyl ATP diphosphate'	278.43	0.00	282.99	0.00
'5 phosphoribosylamine diphosphate phospho alpha D ribosyltransferase'	448.53	0.00	467.10	0.00
'N 5 Phospho D ribosyl anthranilate pyrophosphate'	263.41	-263.41	339.70	-339.70
'dethiobiotin sulfur sulfurtransferase'	369.18	0.00	430.55	0.00
'R01082 m'	385.64	-385.64	419.44	-419.44
'N6 1 2 dicarboxyethyl AMP AMP lyase fumarate forming '	311.87	-311.87	341.72	-341.72
'2 Nomega L arginino succinate arginine lyase fumarate forming '	385.28	-385.28	407.57	-407.57
'L Leucine 2 oxoglutarate aminotransferase'	296.46	-296.46	319.77	-319.77
'ATP D galactose 1 phosphotransferase'	325.90	-325.90	346.76	-346.76
'Lactose galactohydrolase'	237.13	-237.13	251.93	-251.93
'melibiose galactohydrolase'	375.55	0.00	394.22	0.00
'Raffinose galactohydrolase'	350.47	0.00	386.82	0.00
'Galactosylglycerol galactohydrolase'	350.47	-350.47	386.82	-386.82

'Isopentenyl diphosphate delta3 delta2 isomerase'	355.46	-355.46	381.99	-381.99
'IMP 1 2 hydrolase decyclizing '	420.11	-420.11	437.86	-437.86
'IMP NAD oxidoreductase'	363.34	-363.34	388.56	-388.56
'ATP inosine 5 phosphotransferase'	296.46	-296.46	319.77	-319.77
'IMP diphosphate phospho D ribosyltransferase'	281.39	-281.39	282.31	-282.31
'IMP L aspartate ligase GDP forming '	416.25	-416.25	433.61	-433.61
'ATP dADP phosphotransferase'	355.38	-355.38	364.94	-364.94
'dATP pyruvate 2 O phosphotransferase'	115.32	-115.32	118.72	-118.72
'Putrescine oxygen oxidoreductase deaminating '	296.46	-296.46	319.77	-319.77
'S adenosyl L methionine L histidine N methyltransferase'	401.42	0.00	421.63	0.00
'L Histidinal NAD oxidoreductase'	296.46	0.00	319.77	0.00
'L histidine carboxy lyase histamine forming '	404.96	0.00	414.83	0.00
'R01177 x'	376.70	-376.70	415.70	-415.70
'myo Inositol oxygen oxidoreductase'	234.97	0.00	245.46	0.00
'1D myo Inositol 4 phosphate phosphohydrolase'	364.07	0.00	394.85	0.00
'1D myo Inositol 3 phosphate phosphohydrolase'	364.07	0.00	394.85	0.00
'R01197 m'	296.46	0.00	319.77	0.00
'2 3 Dihydroxy 3 methylbutanoate hydro lyase'	415.61	0.00	431.01	0.00
'L Valine 2 oxoglutarate aminotransferase'	296.46	-296.46	319.77	-319.77
'5 10 methylenetetrahydrofolate NADP oxidoreductase'	352.97	-352.97	387.14	-387.14
'5 methyltetrahydrofolate NADP oxidoreductase'	364.42	0.00	412.21	0.00
'5 10 Methylene tetrahydrofolate 3 methyl 2 oxobutanoate'	296.46	0.00	319.77	0.00
'P1 P4 bis 5 guanosyl tetraphosphate guanylylhydrolase'	202.23	0.00	223.88	0.00
'Thioglucoside glucohydrolase'	348.18	-348.18	362.80	-362.80
'L Proline NADP 5 oxidoreductase'	349.15	-349.15	377.58	-377.58
'L Proline 2 oxoglutarate oxygen oxidoreductase 4 hydroxylating '	319.02	0.00	338.37	0.00

'glutathione L amino acid 5 glutamyltransferase'	226.63	0.00	184.05	0.00
'R01279 x'	263.32	0.00	293.14	0.00
'R01280 x'	337.10	-337.10	349.71	-349.71
'Palmitoyl CoA L serine C palmitoyltransferase decarboxylating '	294.77	0.00	304.15	0.00
'Acyl CoA 1 acyl sn glycerol 3 phosphocholine O acyltransferase'	298.95	-298.95	309.90	-309.90
'R01324 m'	442.88	-442.88	483.08	-483.08
'ATP D mannose 6 phosphotransferase'	296.46	0.00	319.77	0.00
'Epimelibiose galactohydrolase'	350.47	-350.47	386.82	-386.82
'R01334 p'	296.46	0.00	319.77	0.00
'ATP propanoate adenyltransferase'	296.46	-296.46	319.77	-319.77
'S 3 hydroxy 3 methylglutaryl CoA acetoacetate lyase'	304.30	-304.30	317.77	-317.77
'4 fumarylacetoacetate fumarylhydrolase'	311.26	-311.26	359.80	-359.80
'Phenylpyruvate oxygen oxidoreductase hydroxylating decarboxylating '	312.99	0.00	352.65	0.00
'phenylpyruvate carboxy lyase phenylacetaldehyde forming '	297.37	-297.37	320.00	-320.00
'UDP D glucuronate carboxy lyase UDP D xylose forming '	296.46	0.00	319.77	0.00
'R01388 x'	296.46	-296.46	319.77	-319.77
'ATP carbamate phosphotransferase'	296.46	0.00	319.77	0.00
'R01398 m'	373.00	-373.00	412.04	-412.04
'R01398 p'	373.00	-373.00	412.04	-412.04
'Cyanohydrin aldehyde lyase cyanide forming '	200.43	0.00	196.85	0.00
'5 6 Dihydrothymine NADP oxidoreductase'	296.46	-296.46	319.77	-319.77
'D xylose aldose ketose isomerase'	277.35	-277.35	293.36	-293.36
'Cholesterol NAD delta7 oxidoreductase'	401.36	0.00	413.04	0.00
'Cholesterol NADP delta7 oxidoreductase'	401.36	0.00	413.04	0.00
'cholesterol NADP Delta24 oxidoreductase'	399.89	0.00	413.88	0.00
'ATP ethanolamine O phosphotransferase'	296.46	0.00	319.77	0.00
'sn Glycerol 3 phosphoethanolamine glycerophosphohydrolase'	296.46	0.00	319.77	0.00

'ATP ceramide 1 phosphotransferase'	296.46	0.00	319.77	0.00
'ATP 3 phospho D glycerate 1 phosphotransferase'	375.78	-375.78	398.92	-398.92
'3 Phospho D glycerate NAD 2 oxidoreductase'	417.08	-417.08	496.59	-496.59
'R01518 p'	295.58	-295.58	326.10	-326.10
'ATP D ribulose 5 phosphate 1 phosphotransferase'	315.62	0.00	337.13	0.00
'R01523 p'	315.62	0.00	337.13	0.00
'R01528 p'	289.08	0.00	301.51	0.00
'D Ribulose 5 phosphate 3 epimerase'	290.40	-290.40	318.86	-318.86
'R01529 p'	350.82	-350.82	369.43	-369.43
'ATP dAMP phosphotransferase'	296.46	-296.46	319.77	-319.77
'D Glucose 1 epimerase'	125.64	-125.64	120.50	-120.50
'4 Hydroxycinnamyl aldehyde NADP oxidoreductase'	347.62	0.00	375.12	0.00
'4 Coumarate CoA ligase AMP forming	296.86	0.00	328.39	0.00
'APS ATP adenylyltransferase'	296.46	0.00	319.77	0.00
'acetyl CoA [acyl carrier protein] S acetyltransferase'	246.03	-246.03	254.82	-254.82
'R01624 p'	292.57	-292.57	314.54	-314.54
'Malonyl CoA [acyl carrier protein] S malonyltransferase'	296.46	-296.46	319.77	-319.77
'R01626 p'	296.46	-296.46	319.77	-319.77
'3 Dehydroshikimate hydro lyase'	344.53	-344.53	373.94	-373.94
'ATP D xylulose 5 phosphotransferase'	287.80	-287.80	306.62	-306.62
'Sedoheptulose 7 phosphate D glyceraldehyde 3 phosphate'	425.82	-425.82	454.92	-454.92
'R01641 p'	324.83	-324.83	398.05	-398.05
'4 hydroxy 2 oxo heptandioate succinate semialdehyde lyase'	284.00	0.00	364.09	0.00
'4 hydroxy 2 oxo heptandioate succinate semialdehyde lyase'	296.46	0.00	319.77	0.00
'Dimethylallyl diphosphate isopentenyl diphosphate'	277.26	0.00	278.77	0.00
'dCMP aminohydrolase'	234.97	0.00	245.46	0.00
'2 Deoxycytidine 5 monophosphate phosphohydrolase'	234.97	0.00	245.46	0.00
'ATP dCMP phosphotransferase'	234.97	0.00	245.46	0.00
'ATP deoxycytidine 5 phosphotransferase'	296.46	0.00	319.77	0.00

'Lactose galactohydrolase'	296.46	0.00	319.77	0.00
'3 Sulfo L alanine carboxy lyase taurine forming '	296.46	0.00	319.77	0.00
' 5 L glutamyl peptide taurine 5 glutamyltransferase'	226.63	0.00	184.05	0.00
'Dihydrolipoamide NAD oxidoreductase'	397.70	-397.70	429.91	-429.91
'3 methyl 2 oxobutanoate [dihydrolipoyllysine residue']	118.60	0.00	127.76	0.00
'4 methyl 2 oxopentanoate [dihydrolipoyllysine residue']	118.60	0.00	127.76	0.00
'Oleoyl [acyl carrier protein] hydrolase'	296.46	-296.46	319.77	-319.77
'R01706 p'	296.46	-296.46	319.77	-319.77
'5 O 1 Carboxyvinyl 3 phosphoshikimate phosphate lyase'	360.46	0.00	425.19	0.00
'Chorismate pyruvatemutase'	295.43	-295.43	319.36	-319.36
'chorismate L glutamine aminotransferase'	215.64	0.00	225.71	0.00
'chorismate hydroxymutase'	123.80	-123.80	129.94	-129.94
'Nicotinate D ribonucleotide diphosphate phosphoribosyltransferase'	321.45	-321.45	341.53	-341.53
'Prephenate NAD oxidoreductase decarboxylating '	296.46	0.00	319.77	0.00
' R S Lactoylglutathione hydrolase'	296.46	0.00	319.77	0.00
'ATP D Gluconate 6 phosphotransferase'	276.77	0.00	309.15	0.00
'D Glyceraldehyde NAD oxidoreductase'	327.24	-327.24	394.12	-394.12
'Mandelonitrile benzaldehyde lyase cyanide forming '	166.32	-166.32	177.64	-177.64
'ATP L homoserine O phosphotransferase'	325.64	0.00	353.93	0.00
'Succinyl CoA L homoserine O succinyltransferase'	296.46	0.00	319.77	0.00
'CTP phosphatidate cytidyltransferase'	151.82	0.00	152.19	0.00
'D Mannose 6 phosphate 1 6 phosphomutase'	389.55	-389.55	421.62	-421.62
'D mannose 6 phosphate aldose ketose isomerase'	163.62	-163.62	177.79	-177.79
'Phosphoenolpyruvate D erythrose 4 phosphate'	363.61	0.00	423.23	0.00



'sedoheptulose 7 phosphate D glyceraldehyde 3 phosphate'	296.46	-296.46	319.77	-319.77
'R01827 p'	322.64	-322.64	355.16	-355.16
'R01829 p'	471.55	0.00	500.00	0.00
'beta D Fructose 6 phosphate D glyceraldehyde 3 phosphate'	425.82	-425.82	454.92	-454.92
'R01830 p'	324.83	-324.83	398.05	-398.05
'dGTP pyruvate 2 O phosphotransferase'	115.32	0.00	118.72	0.00
'inosine phosphate alpha D ribosyltransferase'	296.46	-296.46	319.77	-319.77
' S Dihydroorotate oxygen oxidoreductase'	296.46	-296.46	319.77	-319.77
'Uridine phosphate alpha D ribosyltransferase'	296.46	-296.46	319.77	-319.77
'Cytidine aminohydrolase'	295.39	0.00	305.15	0.00
'ATP pyridoxine 5 phosphotransferase'	262.24	0.00	286.52	0.00
'S adenosylmethioninamine putrescine 3 aminopropyltransferase'	363.76	0.00	416.73	0.00
'2 oxoadipate dehydrogenase complex'	392.88	0.00	416.60	0.00
'2 Oxoadipate lipoamide 2 oxidoreductase decarboxylating and'	305.36	0.00	330.24	0.00
'Caffeic aldehyde NADP oxidoreductase CoA caffeoylating '	347.62	-347.62	375.12	-375.12
'S Adenosyl L methionine caffeoyl CoA 3 O methyltransferase'	179.36	0.00	191.01	0.00
'L Citrulline L aspartate ligase AMP forming '	380.26	-380.26	413.74	-413.74
'ATP D glucosamine 6 phosphotransferase'	296.46	0.00	319.77	0.00
'Deoxyguanosine orthophosphate ribosyltransferase'	296.46	-296.46	319.77	-319.77
'R01975 x'	316.64	-316.64	358.58	-358.58
' S 3 Hydroxybutanoyl CoA NADP oxidoreductase'	266.39	-266.39	284.76	-284.76
'acetyl CoA acetoacetyl CoA C acetyltransferase'	307.60	-307.60	348.74	-348.74
'Geranyl diphosphate isopentenyl diphosphate geranyltrans transferase'	152.66	0.00	152.02	0.00
'Geranyl diphosphate diphosphate lyase cyclizing '	147.86	0.00	153.26	0.00

'geranyldiphosphate diphosphate lyase myrcene forming '	135.30	-135.30	142.17	-142.17
'geranyl diphosphate diphosphate lyase cyclizing '	147.86	-147.86	153.26	-153.26
'geranyl diphosphate diphosphate lyase cyclizing Comphene forming '	147.86	-147.86	153.26	-153.26
'NADPH oxidized thioredoxin oxidoreductase'	369.26	0.00	391.54	0.00
'2 Deoxyadenosine 5 diphosphate oxidized thioredoxin'	323.31	0.00	351.99	0.00
'2 Deoxyuridine 5 diphosphate oxidized thioredoxin'	323.31	0.00	351.99	0.00
'2 Deoxyguanosine 5 diphosphate oxidized thioredoxin'	323.31	0.00	351.99	0.00
'adenosine 3 5 bisphosphate sulfite oxidized thioredoxin'	296.46	0.00	319.77	0.00
'2 Deoxycytidine diphosphate oxidized thioredoxin 2 oxidoreductase'	323.31	0.00	351.99	0.00
'O acetyl L homoserine acetate lyase L homocysteine forming '	386.63	-386.63	400.98	-400.98
'Phosphatidylglycerophosphate phosphohydrolase'	296.46	-296.46	319.77	-319.77
'6 Phospho D glucono 1 5 lactone lactonohydrolase'	256.85	-256.85	221.89	-221.89
'R02035 p'	334.19	-334.19	350.58	-350.58
'S Adenosyl L methionine ethanolamine phosphate N methyltransferase'	296.46	0.00	319.77	0.00
'CTP ethanolamine phosphate cytidyltransferase'	407.06	0.00	417.77	0.00
'Phosphatidylethanolamine phosphatidohydrolase'	147.08	0.00	159.09	0.00
'Phosphatidyl L serine carboxy lyase'	280.46	0.00	285.74	0.00
'CDPEthanolamine 1 2 diacylglycerol ethanolaminephosphotransferase'	380.78	0.00	378.29	0.00
'acetyl CoA D glucosamine 6 phosphate N acetyltransferase'	311.05	-311.05	319.43	-319.43
'trans trans Farnesyl diphosphate isopentenyl diphosphate'	152.66	0.00	152.02	0.00
'geranylgeranyl reductase'	407.74	0.00	440.07	0.00
'Geranylgeranyl diphosphate geranylgeranyl diphosphate'	322.23	-322.23	354.31	-354.31

'ent Copalyl diphosphate lyase'	141.16	0.00	147.44	0.00
'diphosphate beta D fructose 6 phosphate 1 phosphotransferase'	381.60	-381.60	404.28	-404.28
'3 4 Dihydroxy L phenylalanine carboxy lyase'	203.74	0.00	234.75	0.00
'2 Deoxyadenosine 5 monophosphate phosphohydrolase'	234.97	-234.97	245.46	-245.46
'ATP dGMP phosphotransferase'	296.46	-296.46	319.77	-319.77
'dTDP phosphohydrolase'	296.46	0.00	319.77	0.00
'ATP dTDP phosphotransferase'	355.38	-355.38	364.94	-364.94
'ATP dUMP phosphotransferase'	296.46	-296.46	319.77	-319.77
'5 10 Methylene tetrahydrofolate dUMP C methyltransferase'	298.77	-298.77	315.33	-315.33
'xanthine NAD oxidoreductase'	252.46	0.00	289.68	0.00
'Urate oxygen oxidoreductase'	302.05	0.00	327.82	0.00
'Xanthine oxygen oxidoreductase'	252.46	0.00	289.68	0.00
'R02111 p'	345.35	0.00	354.12	0.00
'XMP pyrophosphate phosphoribosyltransferase'	296.46	-296.46	319.77	-319.77
'1H Imidazole 4 ethanamine oxygen oxidoreductase deaminating '	296.46	0.00	319.77	0.00
'S Adenosyl L methionine 3 5 7 3 4 pentahydroxyfavone'	346.90	0.00	420.69	0.00
'UDP glucose flavonol 3 O D glucosyltransferase'	289.10	0.00	342.84	0.00
'Tryptamine oxygen oxidoreductase deaminating '	381.60	0.00	404.28	0.00
'S adenosyl L methionine 2 hexaprenyl 3 methyl 5 hydroxy 6 methoxy '	263.00	0.00	282.22	0.00
'Coniferyl aldehyde NADP oxidoreductase CoA feruloylating '	347.62	0.00	375.12	0.00
'Ferulate CoA ligase AMP forming '	312.54	0.00	480.17	0.00
'L Isoleucine 2 oxoglutarate aminotransferase'	347.19	0.00	375.68	0.00
'sinapoyl aldehyde NADP oxidoreductase CoA sinapoylating '	347.62	0.00	375.12	0.00
'Sinapate CoA ligase AMP forming '	312.54	0.00	480.17	0.00
'7 8 dihydropteroate L glutamate ligase ADP forming '	296.46	0.00	319.77	0.00
'1 2 Diacyl sn glycerol 3 phosphate phosphohydrolase'	296.46	-296.46	319.77	-319.77

'acyl CoA 1 acyl sn glycerol 3 phosphate 2 O acyltransferase'	283.49	0.00	295.42	0.00
'trans cinnamate NADPH oxygen oxidoreductase 4 hydroxylating '	325.23	0.00	431.67	0.00
'trans Cinnamate CoA ligase AMP forming '	312.54	0.00	480.17	0.00
'trans Cinnamate CoA ligase AMP forming '	129.83	-129.83	143.93	-143.93
'5 6 Dihydrouracil amidohydrolase'	352.46	-352.46	382.83	-382.83
'S 4 Amino 5 oxopentanoate 4 5 aminomutase'	361.08	0.00	378.10	0.00
'N2 Acetyl L ornithine 2 oxoglutarate aminotransferase'	365.75	-365.75	393.43	-393.43
'L Aspartate 4 semialdehyde NADP oxidoreductase phosphorylating '	417.65	-417.65	442.73	-442.73
'L Aspartate 4 semialdehyde hydro lyase adding pyruvate and'	429.60	0.00	444.21	0.00
'Cytidine orthophosphate alpha D ribosyltransferase'	296.46	-296.46	319.77	-319.77
'S Aminomethyldihydrolipoylprotein 6S tetrahydrofolate'	360.74	0.00	401.68	0.00
'5 Formyltetrahydrofolate cyclo ligase ADP forming '	283.60	0.00	302.82	0.00
'Nicotinamide ribonucleotide phosphohydrolase'	234.97	0.00	245.46	0.00
'ATP dCDP phosphotransferase'	355.38	-355.38	364.94	-364.94
'dCTP uridine 5 phosphotransferase'	296.46	0.00	319.77	0.00
'ATP dUDP phosphotransferase'	355.38	-355.38	364.94	-364.94
'1S 2R 1 C indol 3 yl glycerol 3 phosphate'	296.46	-296.46	319.77	-319.77
'dCTP cytidine 5 phosphotransferase'	296.46	0.00	319.77	0.00
'S Adenosyl L methionine 3 4 dihydroxy trans cinnamate'	296.46	0.00	319.77	0.00
'UDPglucose sinapate D glucosyltransferase'	159.06	-159.06	158.70	-158.70
'Tyramine oxygen oxidoreductase deaminating flavin containing '	216.36	0.00	251.41	0.00
'Monophenol L dopa oxygen oxidoreductase'	159.01	-159.01	166.10	-166.10
'L Cystine L Cysteine lyase deaminating '	296.46	0.00	319.77	0.00
'Raffinose fructohydrolase'	204.94	0.00	239.38	0.00

'1 alpha D Galactosyl myo inositol sucrose'	296.46	0.00	319.77	0.00
'ATP shikimate 3 phosphotransferase'	263.91	0.00	324.06	0.00
'Allantoate amidohydrolase'	296.46	0.00	319.77	0.00
' S Allantoin amidohydrolase'	201.15	0.00	224.77	0.00
'L Cysteate 2 oxoglutarate aminotransferase'	429.31	-429.31	471.90	-471.90
'flavanone lyase decyclizing '	164.44	0.00	227.32	0.00
'Sphinganine 1 phosphate pammltaldehyde lyase'	290.18	0.00	316.62	0.00
'3 Sulfino L alanine carboxy lyase'	296.46	0.00	319.77	0.00
' R Pantoate beta alanine ligase AMP forming '	277.26	0.00	288.79	0.00
'Deoxycytidine aminohydrolase'	296.46	0.00	319.77	0.00
'ATP pyridoxal 5 phosphotransferase'	262.24	0.00	286.52	0.00
'cinnamaldehyde NADP oxidoreductase CoA cinnamoylating '	347.62	-347.62	375.12	-375.12
'Homogentisate oxygen 1 2 oxidoreductase decyclizing '	296.46	0.00	319.77	0.00
'4 Hydroxyphenylpyruvate oxygen oxidoreductase'	312.99	0.00	352.65	0.00
'aminoacetone oxygen oxidoreductase deaminating '	216.36	0.00	251.41	0.00
' R S Lactoylglutathione methylglyoxal lyase isomerizing '	296.46	-296.46	319.77	-319.77
'Deoxyadenosine aminohydrolase'	223.49	-223.49	239.11	-239.11
'p cumic alcohol NAD oxidoreductase'	356.64	0.00	391.57	0.00
'p Cumic alcohol NADP oxidoreductase'	356.64	0.00	391.57	0.00
'D Fructose 1 phosphate D glyceraldehyde 3 phosphate lyase'	156.00	-156.00	161.16	-161.16
'acetyl CoA enzyme N6 dihydrolipoyl lysine S acetyltransferase'	337.33	-337.33	360.12	-360.12
'Glutaryl CoA dihydrolipoamide S succinyltransferase'	367.91	-367.91	392.87	-392.87
'ATP [protein] L tyrosine O phosphotransferase'	261.74	-261.74	281.43	-281.43
'coniferyl alcohol NADP oxidoreductase'	296.46	-296.46	319.77	-319.77
'UDPglucose coniferyl alcohol 4 beta D glucosyltransferase'	131.35	-131.35	148.24	-148.24

'3 Sulfino L alanine 2 oxoglutarate aminotransferase'	429.31	0.00	471.90	0.00
'2 methylpropanoyl CoA enzyme N6 dihydrolipoyl lysine'	280.46	0.00	283.12	0.00
'2 methylpropanoyl CoA enzyme N6 dihydrolipoyl lysine'	297.37	-297.37	320.00	-320.00
'4 Hydroxymandelonitrile hydroxybenzaldehyde lyase'	200.43	-200.43	196.85	-196.85
'UDPgalactose 1 2 diacylglycerol 3 beta D galactosyltransferase'	284.49	0.00	304.75	0.00
'4 Hydroxyphenylacetate CoA ligase AMP forming '	288.23	-288.23	334.45	-334.45
'5 Hydroxy L tryptophan decarboxy lyase'	203.74	-203.74	234.75	-234.75
'XTP pyrophosphohydrolase'	339.82	0.00	367.37	0.00
'D Fructose 2 6 bisphosphate 2 phosphohydrolase'	333.17	0.00	347.21	0.00
'ATP D fructose 6 phosphate 2 phosphotransferase'	333.17	0.00	347.21	0.00
'N Succinyl LL 2 6 diaminoheptanedioate amidohydrolase'	296.46	0.00	319.77	0.00
'LL 2 6 Diaminoheptanedioate 2 epimerase'	370.34	-370.34	391.51	-391.51
'beta D Glucose 6 phosphate NADP 1 oxoreductase'	300.97	0.00	364.30	0.00
'R02736 p'	376.02	0.00	388.45	0.00
'UDPglucose D glucose 6 phosphate 1 alpha D glucosyltransferase'	390.19	0.00	387.13	0.00
'R02739 p'	296.46	-296.46	319.77	-319.77
'R02740 p'	323.89	-323.89	349.40	-349.40
'Deoxyinosine orthophosphate ribosyltransferase'	296.46	0.00	319.77	0.00
'2 Deoxy D ribose 1 phosphate 1 5 phosphomutase'	296.46	-296.46	319.77	-319.77
'2 Deoxy D ribose 1 phosphate 1 5 phosphomutase'	270.86	-270.86	293.13	-293.13
'2 Deoxy D ribose 1 phosphate 1 5 phosphomutase'	234.97	0.00	245.46	0.00
'Trehalose 6 phosphate phosphohydrolase'	390.19	0.00	387.13	0.00
'P1 P4 Bis 5 nucleosyl tetraphosphate nucleotidohydrolase'	296.46	0.00	319.77	0.00

'Cyanohydrin ketone lyase'	200.43	0.00	196.85	0.00
'nitrile NADPH oxygen oxidoreductase'	159.01	-159.01	166.10	-166.10
'pseudotropine NADP 3 oxidoreductase'	124.68	0.00	120.78	0.00
'Cortisol NAD 11 oxidoreductase'	234.97	0.00	245.46	0.00
'Cortisol NADP 11 oxidoreductase'	234.97	0.00	245.46	0.00
'Cysteine hydrogen sulfide lyase adding HCN '	378.15	-378.15	448.08	-448.08
'S adenosylmethioninamine spermidine 3 aminopropyltransferase'	296.46	-296.46	319.77	-319.77
'Presqualene diphosphate farnesyl diphosphate farnesyltransferase'	326.54	0.00	344.36	0.00
'Flavanone lyase decyclizing '	164.44	-164.44	227.32	-227.32
'serotonin O methyltransferase'	401.42	0.00	421.63	0.00
'L Tyrosine tRNA Tyr ligase AMP forming '	353.09	0.00	366.02	0.00
'Melibiitol galactohydrolase'	350.47	-350.47	386.82	-386.82
'2 Propyn 1 al NAD oxidoreductase'	327.24	0.00	394.12	0.00
' R R Butane 2 3 diol NAD oxidoreductase'	273.25	0.00	285.75	0.00
'phenylacrylic acid decarboxylase'	297.37	0.00	320.00	0.00
'D Glucuronolactone NAD oxidoreductase'	327.24	-327.24	394.12	-394.12
'Sphinganine NADP 3 oxidoreductase'	296.46	0.00	319.77	0.00
'Sphinganine NADP 3 oxidoreductase'	263.32	-263.32	293.14	-293.14
'dTDPglucose 4 epimerase'	336.27	-336.27	317.71	-317.71
'L Histidinol NAD oxidoreductase'	338.83	0.00	363.50	0.00
'L Histidinol phosphate phosphohydrolase'	296.46	0.00	319.77	0.00
'4 Nitrophenyl phosphate phosphohydrolase'	296.46	0.00	319.77	0.00
'R03026 x'	314.59	-314.59	334.96	-334.96
'L Alanine tRNA Ala ligase AMP forming '	385.72	-385.72	402.06	-402.06
'3 Hydroxypropionyl CoA hydro lyase'	316.64	-316.64	358.58	-358.58
'2 Acetolactate pyruvate lyase carboxylating '	408.93	0.00	435.73	0.00
'2 3 Dihydroxy 3 methylbutanoate NADP oxidoreductase isomerizing '	473.53	0.00	488.68	0.00
'5 6 Dihydrothymine amidohydrolase'	352.46	-352.46	382.83	-382.83
'7E 9E 11Z 14Z 5S 6S 5 6 Epoxyicosa 7 9 11 14 tetraenoate'	368.69	0.00	395.48	0.00

'2 Amino 4 hydroxy 6 hydroxymethyl 7 8 dihydropteridine 4 '	296.46	0.00	319.77	0.00
'2 Amino 4 hydroxy 6 hydroxymethyl 7 8 dihydropteridine '	214.01	-214.01	219.64	-219.64
'1 O 4 Hydroxy 3 5 dimethoxycinnamoyl beta D glucose choline'	153.81	0.00	160.55	0.00
'UTP beta L arabinose 1 phosphate uridylyltransferase'	339.87	-339.87	336.86	-336.86
'2 Dehydro 3 deoxy D arabino heptonate 7 phosphate phosphate lyase'	318.66	0.00	378.16	0.00
'3 Dehydroquinone hydro lyase'	458.53	-458.53	495.40	-495.40
'3 Indoleacetonitrile aminohydrolase'	382.20	0.00	480.64	0.00
'UDPglucose indole 3 acetate beta D glucosyltransferase'	122.26	-122.26	120.64	-120.64
'3 Mercaptolactate NAD oxidoreductase'	298.42	0.00	301.78	0.00
'cis 3 4 leucopelargonidin NADP 4 oxidoreductase'	162.24	0.00	170.38	0.00
'O3 Acetyl L serine acetate lyase adding hydrogen sulfide '	378.15	0.00	448.08	0.00
'primary amine oxidase'	329.54	0.00	341.84	0.00
'R03140 p'	234.97	0.00	245.46	0.00
'Hydroxymethylbilane hydro lyase cyclizing '	296.46	0.00	319.77	0.00
' S 2 methylbutanoyl CoA enzyme N6 dihydrolipoyl lysine'	280.46	0.00	283.12	0.00
'S Adenosyl L methionine uroporphyrin III C methyltransferase'	325.49	0.00	317.34	0.00
'Uroporphyrinogen III carboxy lyase'	296.46	0.00	319.77	0.00
' S 2 3 Epoxysqualene mutase cyclizing lanosterol forming '	167.28	0.00	176.73	0.00
' S 2 3 Epoxysqualene mutase cyclizing cycloartenol forming '	343.34	0.00	366.53	0.00
'6 Carboxyhexanoate CoA ligase AMP forming '	317.56	0.00	343.36	0.00
'6 Carboxyhexanoyl CoA L alanine C carboxyhexanoyltransferase'	219.05	0.00	229.60	0.00
'UDPglucose thiohydroximate S beta D glucosyltransferase'	236.52	-236.52	266.46	-266.46
'O Acetyl L homoserine succinate lyase adding cysteine '	296.46	-296.46	319.77	-319.77



'Coproporphyrinogen oxygen oxidoreductase decarboxylating '	305.65	0.00	310.83	0.00
'Protoporphyrinogen IX oxygen oxidoreductase'	284.60	0.00	301.99	0.00
'2 Methyl 4 amino 5 hydroxymethylpyrimidine diphosphate 4 methyl 5 '	280.52	0.00	296.06	0.00
'S Adenosyl L methionine 8 amino 7 oxononanoate aminotransferase'	275.19	0.00	307.55	0.00
'5 Amino 2 oxopentanoate 2 oxoglutarate aminotransferase'	367.71	-367.71	380.69	-380.69
'ATP R 5 phosphomevalonate phosphotransferase'	297.12	0.00	329.61	0.00
'2 Dehydro 3 deoxy D octonate 8 phosphate'	261.98	0.00	295.28	0.00
'O Succinyl L homoserine succinate lyase adding cysteine '	386.63	0.00	400.98	0.00
'UDPglucose flavonol 3 O D glucosyltransferase'	289.10	-289.10	342.84	-342.84
'N [ R 4 Phosphopantothenoyl] L cysteine carboxy lyase'	238.91	0.00	269.68	0.00
'N [ R 4 Phosphopantothenoyl] L cysteine carboxy lyase'	284.00	0.00	364.09	0.00
'trans 4 Hydroxy L proline NAD 5 oxidoreductase'	349.15	0.00	377.58	0.00
'trans 4 Hydroxy L proline NADP 5 oxidoreductase'	349.15	0.00	377.58	0.00
'5alpha cholest 7 en 3beta ol NADH oxygen 5 oxidoreductase'	325.88	-325.88	347.60	-347.60
'1 O Sinapoyl beta D glucose S malate O sinapoyltransferase'	111.43	0.00	125.66	0.00
'1 Phosphatidyl D myo inositol inositolphosphohydrolase'	291.25	-291.25	296.99	-296.99
'4 hydroxyphenylpyruvate carboxy lyase'	296.46	0.00	319.77	0.00
'3 Deoxy D manno octulosonate 8 phosphate 8 phosphohydrolase'	296.46	0.00	319.77	0.00
'CTP 3 deoxy D manno octulosonate cytidyltransferase'	351.19	0.00	350.42	0.00
'5alpha Cholest 7 en 3beta ol delta7 delta8 isomerase'	392.64	-392.64	409.62	-409.62

'5alpha Cholest 7 en 3beta ol delta7 delta8 isomerase'	317.29	-317.29	300.42	-300.42
'beta D Galactosyl 1 4 beta D glucosylceramide galactohydrolase'	234.97	0.00	245.46	0.00
'ATP 1 Phosphatidyl 1D myo inositol 4 phosphotransferase'	280.15	0.00	297.02	0.00
'ATP 1 phosphatidyl 1D myo inositol 3 phosphotransferase'	272.63	0.00	281.08	0.00
'S Adenosyl L methionine 3 4 dihydroxy trans cinnamate'	296.46	0.00	319.77	0.00
'S Adenosyl L methionine 3 4 dihydroxy trans cinnamate'	297.37	-297.37	320.00	-320.00
'D myo Inositol 1 4 bisphosphate 1 phosphohydrolase'	297.30	0.00	344.48	0.00
'8 [ 1R 2R 3 oxo 2 { Z pent 2 enyl}cyclopentyl]octanoate NADP '	266.30	-266.30	448.42	-448.42
'8 [ 1R 2R 3 oxo 2 { Z pent 2 enyl}cyclopentyl]octanoate NADP '	292.41	0.00	307.67	0.00
'R03425 m'	369.27	0.00	398.03	0.00
'D myo Inositol 1 3 4 trisphosphate 1 phosphohydrolase'	297.30	0.00	344.48	0.00
'N Acetyl L glutamate 5 semialdehyde NADP 5 oxidoreductase'	332.73	-332.73	347.85	-347.85
'D erythro 1 Imidazol 4 yl glycerol 3 phosphate hydro lyase'	287.36	0.00	313.51	0.00
'5 amino 6 5 phosphoribitylamino uracil NADP 1 oxidoreductase'	269.44	0.00	275.31	0.00
'2 5 Diamino 6 hydroxy 4 5 phosphoribosylamino pyrimidine'	269.44	0.00	275.31	0.00
'Phosphoenolpyruvate 3 phosphoshikimate'	395.81	-395.81	464.82	-464.82
'ATP 1 phosphatidyl 1D myo inositol 4 phosphate 5 phosphotransferase'	310.17	0.00	293.38	0.00
'ATP 2 amino 4 hydroxy 6 hydroxymethyl 7 8 dihydropteridine'	214.01	-214.01	219.64	-219.64
'2 Amino 4 hydroxy 6 D erythro 1 2 3 trihydroxypropyl 7 8 '	241.43	0.00	258.58	0.00
'1 2 Carboxyphenylamino 1 deoxy D ribulose 5 phosphate'	279.20	0.00	285.44	0.00
'N 5 Phospho beta D ribosyl anthranilate ketol isomerase'	313.60	0.00	342.90	0.00
'RX glutathione R transferase'	413.70	0.00	488.11	0.00

'L cysteine hydrogen sulfide lyase adding HCN '	378.15	-378.15	448.08	-448.08
'D Glucoside glucohydrolase'	228.29	0.00	234.57	0.00
'ATP dIDP phosphotransferase'	355.38	-355.38	364.94	-364.94
'2 Deoxyinosine 5 triphosphate pyrophosphohydrolase'	339.82	0.00	367.37	0.00
'2 Deoxyinosine 5 triphosphate pyrophosphohydrolase'	341.95	0.00	362.77	0.00
'alpha Aminopropionitrile aminohydrolase'	382.20	0.00	480.64	0.00
'Cyanate C N lyase'	337.16	-337.16	363.09	-363.09
'Hydrogen sulfide ferredoxin oxidoreductase'	361.08	-361.08	395.35	-395.35
'UDPglucose coniferyl alcohol 4 beta D glucosyltransferase'	131.35	-131.35	148.24	-148.24
'Galactosylglycerol galactohydrolase'	234.97	0.00	245.46	0.00
'Stachyose fructohydrolase'	187.30	0.00	189.96	0.00
'leucocyanidin NADP 4 oxidoreductase'	162.24	0.00	170.38	0.00
'L Arginine tRNA Arg ligase AMP forming '	333.31	-333.31	353.03	-353.03
'L Aspartate tRNAAsp ligase AMP forming '	296.46	0.00	319.77	0.00
'L Asparagine tRNA Asn ligase AMP forming '	389.97	0.00	406.52	0.00
'L Cysteine tRNA Cys ligase AMP forming '	179.06	0.00	181.66	0.00
'Glycine tRNA Gly ligase AMP forming '	347.96	0.00	365.21	0.00
'L Histidine tRNA His ligase AMP forming '	336.83	0.00	348.67	0.00
'L Isoleucine tRNA Ile ligase AMP forming '	234.97	0.00	245.46	0.00
'L Phenylalanine tRNA Ala ligase AMP forming '	399.02	0.00	414.45	0.00
'L Proline tRNA Pro ligase AMP forming '	338.91	0.00	349.25	0.00
'L Serine tRNA Ser ligase AMP forming '	440.92	0.00	454.59	0.00
'L Threonine tRNA Thr ligase AMP forming '	422.47	0.00	432.96	0.00
'L Tryptophan tRNA Trp ligase AMP forming '	320.42	0.00	338.02	0.00

'L Valine tRNAVal ligase AMP forming '	388.72	0.00	407.91	0.00
'L dopachrome oxygen oxidoreductase'	296.46	0.00	319.77	0.00
' 3Z phytochromobilin ferredoxin oxidoreductase'	209.33	0.00	223.15	0.00
'lanosterol delta24 reductase'	399.89	-399.89	413.88	-413.88
'3 alpha S Strictosidine tryptamine lyase'	322.23	0.00	396.17	0.00
'Cycloeucalenol lyase cyclopropane decyclizing '	331.69	-331.69	341.29	-341.29
'R03778 x'	318.77	-318.77	358.58	-358.58
'dihydrolipoylprotein NAD oxidoreductase'	418.28	0.00	449.62	0.00
'dihydrolipoylprotein NAD oxidoreductase'	350.00	0.00	409.68	0.00
' 2S Flavan 4 ol NADP 4 oxidoreductase'	162.24	-162.24	170.38	-170.38
'GDPmannose glucomannan 1 4 beta D mannosyltransferase'	126.31	-126.31	131.54	-131.54
'Chlorophyllide a NADP 7 8 oxidoreductase'	296.46	0.00	319.77	0.00
'Corticosterone NADP 11 oxidoreductase'	234.97	0.00	245.46	0.00
'R03858 x'	376.70	-376.70	415.70	-415.70
' 5 Glutamyl peptide amino acid 5 glutamyltransferase'	226.63	0.00	184.05	0.00
' S Methylmalonate semialdehyde NAD oxidoreductase'	327.24	0.00	394.12	0.00
'Magnesium protoporphyrin IX chelatase'	367.34	0.00	402.96	0.00
'UDP L rhamnose flavonol 3 O D glucoside L rhamnosyltransferase'	199.69	-199.69	237.30	-237.30
'Sinapyl alcohol NADP oxidoreductase'	394.71	0.00	442.98	0.00
'ATP D fructose 6 phosphotransferase'	362.65	0.00	411.73	0.00
'Sucrose 6 phosphate fructohydrolase'	187.30	0.00	189.96	0.00
' S Allantoin racemase'	296.46	-296.46	319.77	-319.77
'10 Formyltetrahydrofolate L methionyl tRNA N formyltransferase'	187.47	0.00	199.24	0.00
'10 Formyltetrahydrofolate L methionyl tRNA N formyltransferase'	401.42	0.00	421.63	0.00
'10 Formyltetrahydrofolate L methionyl tRNA N formyltransferase'	401.42	0.00	421.63	0.00

'10 Formyltetrahydrofolate L methionyl tRNA N formyltransferase'	296.46	0.00	319.77	0.00
'2 Isopropylmalate hydro lyase'	447.65	-447.65	469.55	-469.55
'5 Glutamyl peptide amino acid 5 glutamyltransferase'	226.63	0.00	184.05	0.00
'R03991 x'	296.46	-296.46	319.77	-319.77
'3 Isopropylmalate hydro lyase'	394.84	-394.84	423.81	-423.81
'UDPglucose coniferyl alcohol 4 beta D glucosyltransferase'	131.35	-131.35	148.24	-148.24
'Digalactosylceramide galactohydrolase'	350.47	0.00	386.82	0.00
'primary amine oxidase'	216.36	0.00	251.41	0.00
'Phosphoribosyl ATP pyrophosphohydrolase'	349.29	0.00	374.52	0.00
'1 5 phospho D ribosyl AMP 1 6 hydrolase'	349.29	0.00	374.52	0.00
'3 phosphonopyruvate carboxy lyase'	296.46	0.00	319.77	0.00
'Imidazole acetaldehyde NAD oxidoreductase'	327.24	0.00	394.12	0.00
'3 Indoleacetaldoxime hydro lyase'	167.49	-167.49	184.92	-184.92
'Thioglucoside glucohydrolase'	212.82	0.00	248.74	0.00
'3 Methylbutanoyl CoA acceptor 2 3 oxidoreductase'	301.98	0.00	327.74	0.00
'3 methylbutanoyl CoA enzyme N6 dihydrolipoyl lysine'	280.46	0.00	283.12	0.00
'S aminomethyl dihydrolipoyl protein 6S tetrahydrofolate'	360.74	-360.74	401.68	-401.68
'S aminomethyl dihydrolipoyl protein 6S tetrahydrofolate'	344.53	-344.53	373.94	-373.94
'S aminomethyl dihydrolipoyl protein 6S tetrahydrofolate'	296.88	0.00	326.00	0.00
'3 Hydroxyisopentyl CoA hydro lyase'	316.64	-316.64	358.58	-358.58
'3 Methylcrotonoyl CoA carbon dioxide ligase ADP forming '	268.58	0.00	287.53	0.00
'5 Phospho D ribosylamine glycine ligase ADP forming '	300.54	0.00	324.93	0.00
'R04170 x'	296.46	-296.46	319.77	-319.77
'S 3 Hydroxydodecanoyl CoA hydro lyase'	297.37	0.00	320.00	0.00
'3 Phosphoserine 2 oxoglutarate aminotransferase'	397.15	-397.15	431.21	-431.21

'2 3 4 5 Tetrahydrodipicolinate NAD oxidoreductase'	191.23	0.00	191.01	0.00
'2 3 4 5 Tetrahydrodipicolinate NADP oxidoreductase'	191.23	0.00	191.01	0.00
'2S 3S 3 hydroxy 2 methylbutanoyl CoA NAD oxidoreductase'	333.08	-333.08	382.60	-382.60
'2S 3S 3 Hydroxy 2 methylbutanoyl CoA hydro liase'	316.64	0.00	358.58	0.00
'2 Formamido N1 5 phosphoribosyl acetamide cyclo ligase'	296.46	0.00	319.77	0.00
'1 5 phospho D ribosyl 5 amino 4 imidazolecarboxylate carboxy lyase'	376.04	-376.04	391.17	-391.17
'1 5 phospho D ribosyl 5 amino 4 imidazolecarboxylate carboxy lyase'	297.37	0.00	320.00	0.00
'S 3 Hydroxyisobutyryl CoA hydro lyase'	333.08	0.00	382.60	0.00
'S adenosyl L methionine magnesium protoporphyrin IX'	296.46	0.00	319.77	0.00
'Aminoacyl tRNA aminoacylhydrolase'	230.74	-230.74	198.20	-198.20
'Aminoacyl tRNA aminoacylhydrolase'	314.18	0.00	376.57	0.00
'CTP ethanolamine phosphate cytidylyltransferase'	407.06	0.00	417.77	0.00
'CTP ethanolamine phosphate cytidylyltransferase'	350.00	0.00	409.68	0.00
'Peptidylproline cis trans isomerase'	336.62	-336.62	354.16	-354.16
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	140.06	0.00	146.07	0.00
'4 2 Aminoethyl 1 2 benzenediol oxygen'	216.36	0.00	251.41	0.00
'trans 2 3 didehydroacyl CoA NADP 4 oxidoreductase'	361.19	-361.19	410.12	-410.12
'Acyl [acyl carrier protein] malonyl [acyl carrier protein]'	296.46	0.00	319.77	0.00
'R04355 p'	292.57	0.00	314.54	0.00
'Acyl [acyl carrier protein] malonyl [acyl carrier protein]'	234.97	0.00	245.46	0.00
'Acetyl CoA 1 alkyl sn glycerol 3 phosphate 2 O acetyltransferase'	283.49	0.00	295.42	0.00
'Succinyl CoA 2 3 4 5 tetrahydropyridine 2 6 dicarboxylate'	296.46	0.00	319.77	0.00
'Succinyl CoA 2 3 4 5 tetrahydropyridine 2 6 dicarboxylate'	403.55	0.00	400.32	0.00

'1 5 Phosphoribosyl 5 amino 4 imidazolecarboxamide pyrophosphate'	419.51	-419.51	436.39	-436.39
'biotin carboxyl carrier protein carbon dioxide ligase ADP forming '	153.59	-153.59	181.09	-181.09
'Acetyl CoA carbon dioxide ligase ADP forming '	153.59	-153.59	181.09	-181.09
'5 Methyltetrahydropteroyltri L glutamate L homocysteine'	310.96	0.00	330.58	0.00
'3 Isopropylmalate NAD oxidoreductase'	389.84	-389.84	419.19	-419.19
'S Adenosyl L methionine zymosterol C methyltransferase'	400.21	-400.21	408.42	-408.42
'3R 3 Hydroxybutanoyl [acyl carrier protein] hydro lyase'	296.46	-296.46	319.77	-319.77
'R04428 p'	296.46	-296.46	319.77	-319.77
'Butyryl [acyl carrier protein] malonyl CoA'	296.46	-296.46	319.77	-319.77
'R04429 p'	296.46	-296.46	319.77	-319.77
' R 2 3 Dihydroxy 3 methylbutanoate NADP oxidoreductase'	473.53	-473.53	488.68	-488.68
' R 2 3 Dihydroxy 3 methylbutanoate hydro lyase'	415.61	0.00	431.01	0.00
'ATP 4 methyl 5 2 hydroxyethyl thiazole 2 phosphotransferase'	289.84	0.00	301.64	0.00
'ATP 4 methyl 5 2 hydroxyethyl thiazole 2 phosphotransferase'	433.21	0.00	459.11	0.00
'Digalactosyl diacylglycerol galactohydrolase'	350.47	0.00	386.82	0.00
'N Succinyl L 2 6 diaminoheptanedioate 2 oxoglutarate'	296.46	0.00	319.77	0.00
'3alpha 7alpha Dihydroxy 5beta cholestan 26 al NAD oxidoreductase'	327.24	0.00	394.12	0.00
'ATP 4 amino 2 methyl 5 phosphomethylpyrimidine phosphotransferase'	280.52	0.00	296.06	0.00
'Phosphatidylinositol 3 4 5 trisphosphate 3 phosphohydrolase'	312.20	0.00	319.52	0.00
'Phosphatidylinositol 3 4 5 trisphosphate 3 phosphohydrolase'	297.37	-297.37	320.00	-320.00
' 3R 3 Hydroxybutanoyl [acyl carrier protein] NADP oxidoreductase'	296.46	-296.46	319.77	-319.77
'R04533 p'	296.46	-296.46	319.77	-319.77

' 3R 3 Hydroxydecanoyl [acyl carrier protein] NADP oxidoreductase'	296.46	-296.46	319.77	-319.77
'R04534 p'	296.46	-296.46	319.77	-319.77
' 3R 3 Hydroxybutanoyl [acyl carrier protein] hydro lyase'	296.46	-296.46	319.77	-319.77
'R04535 p'	296.46	-296.46	319.77	-319.77
' 3R 3 Hydroxyoctanoyl [acyl carrier protein] NADP oxidoreductase'	296.46	-296.46	319.77	-319.77
'R04536 p'	296.46	-296.46	319.77	-319.77
' 3R 3 Hydroxypalmitoyl [acyl carrier protein] NADP oxidoreductase'	296.46	-296.46	319.77	-319.77
'R04543 p'	296.46	-296.46	319.77	-319.77
' 3R 3 Hydroxypalmitoyl [acyl carrier protein] hydro lyase'	296.46	-296.46	319.77	-319.77
'R04544 p'	296.46	-296.46	319.77	-319.77
'Propanoyl CoA acetyl CoA C acyltransferase'	318.77	0.00	358.58	0.00
'Propanoyl CoA acetyl CoA C acyltransferase'	263.32	0.00	293.14	0.00
'1 5 Phosphoribosyl 5 amino 4 N succinocarboxamide imidazole'	311.87	-311.87	341.72	-341.72
'10 Formyltetrahydrofolate 5 phosphoribosyl 5 amino 4 '	420.11	0.00	437.86	0.00
' 3R 3 Hydroxytetradecanoyl [acyl carrier protein] NADP '	296.46	-296.46	319.77	-319.77
'R04566 p'	296.46	-296.46	319.77	-319.77
' R 3 Hydroxytetradecanoly [acyl carrier protein] UDP N acetyl '	296.46	0.00	319.77	0.00
' 3R 3 Hydroxypalmitoyl [acyl carrier protein] hydro lyase'	296.46	-296.46	319.77	-319.77
'R04568 p'	296.46	-296.46	319.77	-319.77
'1 5 Phosphoribosyl 5 amino 4 carboxyimidazole L aspartate ligase'	357.64	-357.64	362.64	-362.64
'1 5 Phosphoribosyl 5 amino 4 carboxyimidazole L aspartate ligase'	263.32	0.00	293.14	0.00
'1 5 Phosphoribosyl 5 amino 4 carboxyimidazole L aspartate ligase'	369.89	0.00	393.90	0.00
'1 5 Phosphoribosyl 5 amino 4 carboxyimidazole L aspartate ligase'	369.89	0.00	393.90	0.00
'N 5 Phospho D ribosylformimino 5 amino 1 '	335.42	0.00	344.49	0.00



'N 5 Phospho D ribosylformimino 5 amino 1 '	217.52	0.00	223.87	0.00
'3 Ureidoisobutyrate amidohydrolase'	282.67	0.00	301.39	0.00
'4alpha Methylcholesta 8 en 3beta ol delta7 delta8 isomerase'	392.64	0.00	409.62	0.00
' S 2 Acetolactate pyruvate lyase carboxylating '	408.93	0.00	435.73	0.00
' S 2 Aceto 2 hydroxybutanoate pyruvate lyase carboxylating '	408.93	0.00	435.73	0.00
' S 2 Aceto 2 hydroxybutanoate pyruvate lyase carboxylating '	296.46	0.00	319.77	0.00
'S adenosyl L methionine 3 hexaprenyl 4 5 dihydroxylate'	263.00	0.00	282.22	0.00
'Dodecanoyl [acyl carrier protein] malonyl CoA'	296.46	-296.46	319.77	-319.77
'R04724 p'	296.46	-296.46	319.77	-319.77
'dodecanoyl [acyl carrier protein] malonyl [acyl carrier protein]'	246.03	0.00	254.82	0.00
'R04726 p'	292.57	0.00	314.54	0.00
'dodecanoyl [acyl carrier protein] malonyl [acyl carrier protein]'	297.37	0.00	320.00	0.00
'R04737 x'	316.64	-316.64	358.58	-358.58
'R04738 x'	296.46	-296.46	319.77	-319.77
'R04739 x'	316.64	-316.64	358.58	-358.58
'R04740 x'	296.46	-296.46	319.77	-319.77
'R04741 x'	316.64	-316.64	358.58	-358.58
'R04742 x'	318.77	-318.77	358.58	-358.58
'R04743 x'	316.64	-316.64	358.58	-358.58
'R04744 x'	296.46	-296.46	319.77	-319.77
'R04745 x'	316.64	-316.64	358.58	-358.58
'R04746 x'	296.46	-296.46	319.77	-319.77
'R04747 x'	318.77	-318.77	358.58	-358.58
'R04748 x'	316.64	-316.64	358.58	-358.58
'R04749 x'	296.46	-296.46	319.77	-319.77
'11beta Hydroxyandrost 4 ene 3 17 dione NADP 11 oxidoreductase'	234.97	0.00	245.46	0.00
'11beta Hydroxyandrost 4 ene 3 17 dione NADP 11 oxidoreductase'	401.42	0.00	421.63	0.00
'ATP L selenomethione S adenosyltransferase'	500.00	0.00	491.59	0.00
'S Adenosyl L methionine L methionine S methyltransferase'	318.96	0.00	340.48	0.00

'Selenomethionine tRNAMet ligase AMP forming '	354.78	0.00	372.44	0.00
'Selenomethionine tRNAMet ligase AMP forming '	203.29	0.00	206.75	0.00
'ATP D fructose 6 phosphate 1 phosphotransferase'	286.88	0.00	316.41	0.00
'R04779 p'	346.58	0.00	351.04	0.00
'beta D Fructose 1 6 bisphosphate 1 phosphohydrolase'	378.74	0.00	399.44	0.00
'R04780 p'	277.38	0.00	294.93	0.00
'3 Ketolactose galactohydrolase'	234.97	-234.97	245.46	-245.46
'3 Ketolactose galactohydrolase'	297.37	-297.37	320.00	-320.00
'3 Ketolactose galactohydrolase'	350.00	0.00	409.68	0.00
'delta8 24 Cholestadien 3beta ol delta7 delta8 isomerase'	392.64	0.00	409.62	0.00
'delta8 24 Cholestadien 3beta ol delta7 delta8 isomerase'	411.22	0.00	451.70	0.00
'3alpha 7alpha 24 trihydroxy 5beta cholestanoyl CoA NAD '	333.08	-333.08	382.60	-382.60
'S Adenosyl L methionine DNA cytosine 5 methyltransferase'	388.58	0.00	400.35	0.00
'O3 Acetyl L serine acetate lyase adding hydrogen sulfide '	296.46	0.00	319.77	0.00
'S Adenosyl L methionine cytochrome c L lysine N6 methyltransferase'	254.40	-254.40	270.82	-270.82
'S Adenosyl L methionine cytochrome c L lysine N6 methyltransferase'	336.36	0.00	347.63	0.00
'3 4 Dihydroxyphenylethyleneglycol NAD oxidoreductase'	411.22	-411.22	451.70	-451.70
'3 4 Dihydroxyphenylethyleneglycol NAD oxidoreductase'	297.37	0.00	320.00	0.00
'UDP L rhamnose flavonol 3 O D glucoside L rhamnosyltransferase'	199.69	-199.69	237.30	-237.30
'5 Hydroxyindoleacetaldehyde NAD oxidoreductase'	327.24	0.00	394.12	0.00
'5 Hydroxyindoleacetaldehyde NAD oxidoreductase'	407.74	0.00	440.07	0.00
'CMP 2 aminoethylphosphonate 1 2 diacylglycerol'	380.78	0.00	378.29	0.00
'ATP adenylylsulfate 3 phosphotransferase'	218.39	0.00	219.42	0.00
'ATP sulfate adenylyltransferase'	287.60	-287.60	330.25	-330.25

' 5 L glutamyl peptide Se Methylselenocysteine 5 glutamyltransferase'	226.63	0.00	184.05	0.00
'Se Adenosylselenohomocysteine hydrolase'	362.26	0.00	397.76	0.00
'Selenocystathionine L homocysteine lyase deaminating '	334.35	0.00	357.69	0.00
'O Phosphorylhomoserine succinate lyase adding cysteine '	386.63	0.00	400.98	0.00
'O Acetylhomoserine succinate lyase adding cysteine '	386.63	0.00	400.98	0.00
'O Succinyl L homoserine succinate lyase adding cysteine '	386.63	0.00	400.98	0.00
'Cyanoglycoside glucohydrolase'	463.13	0.00	468.13	0.00
'Cyanoglycoside glucohydrolase'	402.29	0.00	449.37	0.00
' 3R 3 Hydroxyhexanoyl [acyl carrier protein] NADP oxidoreductase'	296.46	-296.46	319.77	-319.77
'R04953 p'	296.46	-296.46	319.77	-319.77
' 3R 3 Hydroxybutanoyl [acyl carrier protein] hydro lyase'	296.46	-296.46	319.77	-319.77
'R04954 p'	296.46	-296.46	319.77	-319.77
'Hexanoyl [acyl carrier protein] malonyl CoA'	296.46	-296.46	319.77	-319.77
'R04955 p'	296.46	-296.46	319.77	-319.77
'hexanoyl [acyl carrier protein] malonyl [acyl carrier protein]'	246.03	0.00	254.82	0.00
'R04957 p'	292.57	0.00	314.54	0.00
'Octanoyl [acyl carrier protein] malonyl CoA'	296.46	-296.46	319.77	-319.77
'R04958 p'	296.46	-296.46	319.77	-319.77
'Octanoyl [acyl carrier protein] malonyl [acyl carrier protein]'	246.03	0.00	254.82	0.00
'R04960 p'	292.57	0.00	314.54	0.00
'Decanoyl [acyl carrier protein] malonyl CoA'	296.46	-296.46	319.77	-319.77
'R04961 p'	296.46	-296.46	319.77	-319.77
'Decanoyl [acyl carrier protein] malonyl [acyl carrier protein]'	246.03	0.00	254.82	0.00
'R04963 p'	292.57	0.00	314.54	0.00
' 3R 3 Hydroxydodecanoyl [acyl carrier protein] NADP oxidoreductase'	296.46	-296.46	319.77	-319.77
'R04964 p'	296.46	-296.46	319.77	-319.77

' 3R 3 Hydroxybutanoyl [acyl carrier protein] hydro lyase'	296.46	-296.46	319.77	-319.77
'R04965 p'	296.46	-296.46	319.77	-319.77
'Tetradecanoyl [acyl carrier protein] malonyl CoA'	296.46	-296.46	319.77	-319.77
'R04966 p'	296.46	-296.46	319.77	-319.77
'Tetradecanoyl [acyl carrier protein] malonyl [acyl carrier protein]'	246.03	0.00	254.82	0.00
'R04968 p'	292.57	0.00	314.54	0.00
'Hexadecanoyl [acyl carrier protein] malonyl CoA'	296.46	-296.46	319.77	-319.77
'R04969 p'	296.46	-296.46	319.77	-319.77
'Uroporphyrinogen I carboxy lyase'	296.46	0.00	319.77	0.00
'Uroporphyrinogen I carboxy lyase'	329.71	0.00	458.33	0.00
'Uroporphyrinogen I carboxy lyase'	401.42	0.00	421.63	0.00
'Uroporphyrinogen I carboxy lyase'	159.01	-159.01	166.10	-166.10
'3 octaprenyl 4 hydroxybenzoate carboxy lyase'	297.37	-297.37	320.00	-320.00
'3 octaprenyl 4 hydroxybenzoate carboxy lyase'	265.28	-265.28	272.52	-272.52
'3 octaprenyl 4 hydroxybenzoate carboxy lyase'	159.01	-159.01	166.10	-166.10
'UDP L rhamnose flavonol 3 O D glucoside L rhamnosyltransferase'	401.42	0.00	421.63	0.00
'UDP L rhamnose flavonol 3 O D glucoside L rhamnosyltransferase'	401.42	0.00	421.63	0.00
'beta D Glucosyl 2 coumarinate glucohydrolase'	463.13	0.00	468.13	0.00
'Undecaprenyl diphospho N acetylmuramoyl N acetylglucosamine L '	422.74	0.00	495.17	0.00
'UDP N acetyl D glucosamine undecaprenyl diphospho N acetylmuramoyl '	237.61	0.00	254.65	0.00
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	140.06	0.00	146.07	0.00
'leucodelphinidin NADP 4 oxidoreductase'	162.24	0.00	170.38	0.00
'formamidopyrimidine nucleoside triphosphate amidohydrolase'	302.30	0.00	313.95	0.00
'2 5 Diaminopyrimidine nucleoside triphosphate mutase'	302.30	0.00	313.95	0.00

'3 Hydroxy 2 methylpropanoate NAD oxidoreductase'	316.64	-316.64	358.58	-358.58
' S 2 Aceto 2 hydroxybutanoate NADP oxidoreductase isomerizing '	296.46	-296.46	319.77	-319.77
' R 2 3 Dihydroxy 3 methylpentanoate hydro lyase'	415.61	0.00	431.01	0.00
' S 2 Acetolactate methylmutase'	296.46	-296.46	319.77	-319.77
' S 2 Acetolactate methylmutase'	217.52	0.00	223.87	0.00
'O Phospho 4 hydroxy L threonine 2 oxoglutarate aminotransferase'	397.15	-397.15	431.21	-431.21
'O Phospho 4 hydroxy L threonine phospho lyase adding water '	370.92	0.00	395.00	0.00
'ent Copalyl diphosphate diphosphate lyase'	229.04	0.00	227.56	0.00
'3 Butyn 1 al NAD oxidoreductase'	327.24	0.00	394.12	0.00
'3 Butyn 1 al NAD oxidoreductase'	344.53	-344.53	373.94	-373.94
'S Adenosyl L methionine [3 phospho D glycerate carboxy lyase'	218.63	-218.63	231.69	-231.69
'ATP 1D myo inositol 1 3 4 5 6 pentakisphosphate 2 phosphotransferase'	296.46	0.00	319.77	0.00
'2 5 dichloro 2 5 cyclohexadiene 1 4 diol NAD oxidoreductase'	280.08	-280.08	357.58	-357.58
'2 5 dichloro 2 5 cyclohexadiene 1 4 diol NAD oxidoreductase'	159.01	-159.01	166.10	-166.10
'2 5 dichloro 2 5 cyclohexadiene 1 4 diol NAD oxidoreductase'	350.00	0.00	409.68	0.00
'4 hydroxybutyryl CoA dehydratase'	344.53	-344.53	373.94	-373.94
'4 hydroxybutyryl CoA dehydratase'	365.40	-365.40	394.15	-394.15
'4 hydroxybutyryl CoA dehydratase'	307.15	0.00	337.17	0.00
'4 hydroxybutyryl CoA dehydratase'	297.37	0.00	320.00	0.00
'4 hydroxybutyryl CoA dehydratase'	407.74	0.00	440.07	0.00
'4 hydroxybutyryl CoA dehydratase'	407.74	0.00	440.07	0.00
'4 hydroxybutyryl CoA dehydratase'	280.08	-280.08	357.58	-357.58
'4 hydroxybutyryl CoA dehydratase'	159.01	-159.01	166.10	-166.10
'Benzoyl acetate CoA ligase'	288.23	-288.23	334.45	-334.45
'Benzoyl acetate CoA ligase'	159.01	-159.01	166.10	-166.10
'Benzoyl acetate CoA ligase'	159.01	-159.01	166.10	-166.10
'Benzoyl acetate CoA ligase'	292.41	0.00	307.67	0.00
'Benzoyl acetate CoA ligase'	350.00	0.00	409.68	0.00
'Benzoyl acetate CoA ligase'	318.77	0.00	358.58	0.00
'Benzoyl acetate CoA ligase'	395.18	-395.18	434.35	-434.35

'Benzoyl acetate CoA ligase'	350.47	0.00	386.82	0.00
'4 amino 4 deoxychorismate pyruvate lyase'	237.88	0.00	264.40	0.00
'Farnesyl diphosphate Isopentenyl diphosphate farnesyl transferase'	324.91	-324.91	344.67	-344.67
'L Aspartate tRNA Asp ligase AMP forming '	296.46	0.00	319.77	0.00
'L Aspartate tRNA Asp ligase AMP forming '	203.29	0.00	206.75	0.00
'Nitrile aminohydrolase'	382.20	0.00	480.64	0.00
'Nitrile aminohydrolase'	329.59	0.00	331.84	0.00
'Nitrile aminohydrolase'	316.64	0.00	358.58	0.00
'Nitrile aminohydrolase'	212.61	-212.61	241.86	-241.86
'all trans octaprenyl diphosphate 4 hydroxybenzoate'	212.61	-212.61	241.86	-241.86
'all trans octaprenyl diphosphate 4 hydroxybenzoate'	433.21	0.00	459.11	0.00
'chlorophyll chlorophyllidohydrolase'	207.37	0.00	232.89	0.00
'Phenylphosphate synthase'	296.46	0.00	319.77	0.00
'CTP 2 C Methyl D erythritol 4 phosphate cytidyltransferase'	314.65	-314.65	334.30	-334.30
'ATP 4 Cytidine 5 diphospho 2 C methyl D erythritol'	296.36	0.00	318.81	0.00
'1 Deoxy D xylulose 5 phosphate pyruvate lyase carboxylating '	264.93	0.00	282.91	0.00
'2 Phospho 4 cytidine 5 diphospho 2 C methyl D erythritol'	350.59	-350.59	371.17	-371.17
'4 4 dimethyl 5a cholesta 8 24 dien 3b ol NADP D14 oxidoreductase'	319.10	-319.10	316.44	-316.44
'4 4 dimethyl 5a cholesta 8 24 dien 3b ol NADP D14 oxidoreductase'	287.49	-287.49	296.05	-296.05
'4 4 dimethyl 5a cholesta 8 24 dien 3b ol NADP D14 oxidoreductase'	284.00	0.00	364.09	0.00
'UDP N acetyl D glucosamine N acetyl alpha D muramyl oyl L Ala gamma '	237.61	-237.61	254.65	-254.65
'1 Deoxy D xylulose 5 phosphate isomeroreductase'	294.33	0.00	328.35	0.00
'GDP L fucose NADP 4 oxidoreductase 3 5 epimerizing '	324.60	0.00	331.47	0.00
'lanosterol D24 reductase'	399.89	-399.89	413.88	-413.88
'S adenosyl L methionine jasmonate O methyltransferase'	121.88	-121.88	126.83	-126.83

'geranyl diphosphate diphosphate lyase cyclizing pinene forming '	129.81	0.00	131.55	0.00
'geranyl diphosphate diphosphate lyase cyclizing pinene forming '	129.81	0.00	131.55	0.00
'UDP glucose hydroquinone O b D glucosyltransferase'	123.53	-123.53	125.52	-125.52
'UDP 6 sulfo 6 deoxyglucose sulfohydrolase'	279.74	0.00	325.32	0.00
'polyneuridine aldehyde hydrolase decarboxylating '	311.50	0.00	375.53	0.00
'polyneuridine aldehyde hydrolase decarboxylating '	203.29	-203.29	206.75	-206.75
'R05875 p'	296.46	-296.46	319.77	-319.77
'isopentenyl diphosphate NAD P oxidoreductase'	325.44	0.00	340.69	0.00
'UDP N acetyl D glucosamine dolichyl phosphate N acetyl D glucosamine'	301.02	0.00	316.40	0.00
'squalene synthase'	326.54	0.00	344.36	0.00
'magnesium protoporphyrin IX 13 monomethyl ester NADPH oxygen'	337.85	-337.85	380.74	-380.74
'magnesium protoporphyrin IX 13 monomethyl ester NADPH oxygen'	337.85	-337.85	380.74	-380.74
'magnesium protoporphyrin IX 13 monomethyl ester NADPH oxygen'	337.85	-337.85	380.74	-380.74
'chlorophyllide a phytyl diphosphate phytyltransferase'	348.92	0.00	362.21	0.00
'chlorophyllide a NADP 7 8 oxidoreductase'	296.46	0.00	319.77	0.00
'ent kaur 16 ene NADPH oxygen oxidoreductase hydroxylating '	305.49	0.00	348.00	0.00
'ent Kaur 16 ene NADPH oxygen oxidoreductase hydroxylating '	305.49	0.00	348.00	0.00
'ent Kaur 16 ene NADPH oxygen oxidoreductase hydroxylating '	305.49	0.00	348.00	0.00
'ent Kaur 16 en 19 oate NADPH oxygen oxidoreductase hydroxylating '	214.58	0.00	214.19	0.00
'ent Kaur 16 en 19 oate NADPH oxygen oxidoreductase hydroxylating '	214.58	0.00	214.19	0.00
'Aldehyde NAD oxidoreductase'	327.24	0.00	394.12	0.00
'perillic acid CoA ligase ADP forming '	288.23	-288.23	334.45	-334.45
'perillic acid CoA ligase AMP forming '	288.23	-288.23	334.45	-334.45

'2 hydroxy 4 isopropenylcyclohexane 1 carboxyl CoA hydro lyase'	344.53	-344.53	373.94	-373.94
'alpha Pinene monooxygenase'	350.00	0.00	409.68	0.00
'Pinocarveol dehydrogenase'	304.86	0.00	337.17	0.00
'cis 2 Methyl 5 isopropylhexa 2 5 dienoyl CoA hydro lyase'	333.08	0.00	382.60	0.00
'trans 2 Methyl 5 isopropylhexa 2 5 dienoyl CoA hydro lyase'	333.08	0.00	382.60	0.00
'3 Hydroxy 2 6 dimethyl 5 methylene heptanoyl CoA dehydrogenase'	304.86	0.00	337.17	0.00
'2 6 Dimethyl 5 methylene 3 oxo heptanoyl CoA C acetyltransferase'	203.29	0.00	206.75	0.00
'3 Isopropylbut 3 enoyl CoA thioesterase'	329.59	0.00	331.84	0.00
'3 Isopropylbut 3 enoyl CoA thioesterase'	147.86	-147.86	153.26	-153.26
'3 Isopropylbut 3 enoyl CoA thioesterase'	147.86	-147.86	153.26	-153.26
'dTDPglucose 4 6 hydro lyase'	350.99	-350.99	364.94	-364.94
'sphingosine 1 phosphate palmitaldehyde lyase'	290.18	0.00	316.62	0.00
'N acylsphingosine amidohydrolase'	296.46	0.00	319.77	0.00
'3 sn phosphatidate phosphohydrolase'	225.52	-225.52	239.50	-239.50
'3 sn phosphatidate phosphohydrolase'	225.52	-225.52	239.50	-239.50
'UDP glucose anthocyanidin 3 O D glucosyltransferase'	296.46	0.00	319.77	0.00
'UDP glucose anthocyanidin 3 O D glucosyltransferase'	296.46	0.00	319.77	0.00
'UDP glucose anthocyanidin 3 O D glucosyltransferase'	296.46	0.00	319.77	0.00
'flavonoid NADPH oxygen oxidoreductase 3 hydroxylating '	193.28	0.00	203.66	0.00
'dihydroflavonol 2 oxoglutarate oxygen oxidoreductase'	131.74	0.00	135.47	0.00
'flavan 3 ol NADP oxidoreductase'	176.56	0.00	178.39	0.00
'flavan 3 ol NADP oxidoreductase'	176.56	0.00	178.39	0.00
'flavan 3 ol NADP oxidoreductase'	176.56	0.00	178.39	0.00
'flavanone lyase decyclizing '	164.44	-164.44	227.32	-227.32
'cinnamaldehyde NADP oxidoreductase CoA cinnamoylating '	347.62	-347.62	375.12	-375.12
'5 Hydroxyisourate amidohydrolase'	296.46	0.00	319.77	0.00
'5 Hydroxyisourate amidohydrolase'	296.46	0.00	319.77	0.00



'UDPglucose flavonol 3 O D glucosyltransferase'	289.10	0.00	342.84	0.00
'acetyl CoA tropine O acetyltransferase'	203.29	0.00	206.75	0.00
'acetyl CoA pseudotropine O acetyltransferase'	203.29	0.00	206.75	0.00
'primary amine oxidase'	216.36	0.00	251.41	0.00
'4 carboxymethylenebut 2 en 4 olide lactonohydrolase'	395.18	-395.18	434.35	-434.35
'shikimate NAD 3 oxidoreductase'	296.46	-296.46	319.77	-319.77
'shikimate NAD 3 oxidoreductase'	159.01	-159.01	166.10	-166.10
'shikimate NAD 3 oxidoreductase'	304.86	0.00	337.17	0.00
'shikimate NAD 3 oxidoreductase'	304.86	0.00	337.17	0.00
'shikimate NAD 3 oxidoreductase'	433.21	0.00	459.11	0.00
'shikimate NAD 3 oxidoreductase'	401.42	0.00	421.63	0.00
'UDP sulfoquinovose diacylglycerol alpha D sulfoquinovosyltransferase'	403.55	0.00	400.32	0.00
'S Adenosyl L methionine methylethanolamine phosphate'	296.46	0.00	319.77	0.00
'S Adenosyl L methionine phosphodimethylethanolamine'	296.46	0.00	319.77	0.00
'coproporphyrinogen III S adenosyl L methionine'	230.49	0.00	243.27	0.00
'coproporphyrinogen III S adenosyl L methionine'	263.32	0.00	293.14	0.00
'coproporphyrinogen III S adenosyl L methionine'	344.53	-344.53	373.94	-373.94
'coproporphyrinogen III S adenosyl L methionine'	304.86	0.00	329.19	0.00
'coproporphyrinogen III S adenosyl L methionine'	203.29	0.00	206.75	0.00
'coproporphyrinogen III S adenosyl L methionine'	344.53	-344.53	373.94	-373.94
'coproporphyrinogen III S adenosyl L methionine'	159.01	-159.01	166.10	-166.10
'alcohol NAD oxidoreductase'	411.22	0.00	451.70	0.00
'alcohol NAD oxidoreductase'	307.15	0.00	337.17	0.00
'alcohol NAD oxidoreductase'	344.53	-344.53	373.94	-373.94
'alcohol NAD oxidoreductase'	297.37	0.00	320.00	0.00
'alcohol NAD oxidoreductase'	159.01	-159.01	166.10	-166.10
'alcohol NAD oxidoreductase'	411.22	0.00	451.70	0.00
'alcohol NAD oxidoreductase'	341.95	0.00	362.77	0.00

' S 3 hydroxyacyl CoA NAD oxidoreductase'	333.08	-333.08	382.60	-382.60
' 3S 3 hydroxyacyl CoA hydro lyase'	314.59	0.00	334.96	0.00
' 3S 3 hydroxyacyl CoA hydro lyase'	263.32	-263.32	293.14	-293.14
' 3S 3 hydroxyacyl CoA hydro lyase'	288.23	-288.23	334.45	-334.45
'zeaxanthin NADH oxygen oxidoreductase'	267.95	-267.95	304.37	-304.37
'9 cis Neoxanthin 9 cis epoxy-carotenoid dioxygenase'	128.74	-128.74	128.75	-128.75
'9 cis Violaxanthin 9 cis epoxy-carotenoid dioxygenase'	128.74	-128.74	128.75	-128.75
'abscisic aldehyde oxygen oxidoreductase'	250.31	-250.31	287.97	-287.97
'abscisic aldehyde oxygen oxidoreductase'	297.37	-297.37	320.00	-320.00
' 1R hydroxy 2R glutathionyl 1 2 dihydronaphthalene'	413.70	0.00	488.11	0.00
' 1R glutathionyl 2R hydroxy 1 2 dihydronaphthalene'	413.70	0.00	488.11	0.00
' 1S hydroxy 2S glutathionyl 1 2 dihydronaphthalene'	413.70	0.00	488.11	0.00
'1 nitro 7 hydroxy 8 glutathionyl 7 8 dihydronaphthalene'	413.70	0.00	488.11	0.00
'1 nitro 7 glutathionyl 8 hydroxy 7 8 dihydronaphthalene'	413.70	0.00	488.11	0.00
'1 nitro 5 hydroxy 6 glutathionyl 5 6 dihydronaphthalene'	413.70	0.00	488.11	0.00
'1 nitro 5 glutathionyl 6 hydroxy 5 6 dihydronaphthalene'	413.70	0.00	488.11	0.00
'Glutathione 5 HPETE oxidoreductase'	359.14	0.00	417.21	0.00
'Glutathione 15 HPETE oxidoreductase'	359.14	0.00	417.21	0.00
'3 4 dihydro 3 hydroxy 4 S glutathionyl bromobenzene'	413.70	0.00	488.11	0.00
'2 3 dihydro 2 S glutathionyl 3 hydroxy bromobenzene'	413.70	0.00	488.11	0.00
'4 5 dihydro 4 hydroxy 5 S glutathionyl benzo[a]pyrene'	413.70	0.00	488.11	0.00
'7 8 dihydro 7 hydroxy 8 S glutathionyl benzo[a]pyrene hydrolase'	413.70	0.00	488.11	0.00
'S 2 2 dichloro 1 hydroxy ethyl glutathione'	413.70	0.00	488.11	0.00

'1 1 dichloroethylene epoxide glutathione'	413.70	0.00	488.11	0.00
'chloroacetyl chloride glutathione S chloroacetyltransferase'	413.70	0.00	488.11	0.00
'2 S glutathionyl acetyl chloride glutathione'	413.70	0.00	488.11	0.00
'trichloroethene glutathione S 1 2 dichlorovinyl transferase'	413.70	0.00	488.11	0.00
'Trichloroethanol NAD oxidoreductase'	411.22	0.00	451.70	0.00
'1 2 dibromoethane glutathione ethylenetransferase'	413.70	0.00	488.11	0.00
'2 bromoacetaldehyde glutathione S formylmethyl transferase'	413.70	0.00	488.11	0.00
'antheraxanthin ascorbate oxidoreductase'	221.49	-221.49	203.77	-203.77
'zeaxanthin NAD P H oxygen oxidoreductase'	286.44	-286.44	313.79	-313.79
'di trans poly cis decaprenyl diphosphate isopentenyl diphosphate'	126.07	-126.07	134.61	-134.61
'prephytoene diphosphate geranylgeranyl diphosphate'	322.23	-322.23	354.31	-354.31
'D ribulose 5 phosphate formate lyase L 3 4 dihydroxybutan 2 one'	335.30	0.00	319.75	0.00
'flavan 3 ol NADP oxidoreductase'	176.56	-176.56	178.39	-178.39
'S prenyl L cysteine oxygen oxidoreductase'	266.77	-266.77	280.13	-280.13
'S prenyl L cysteine oxygen oxidoreductase'	296.46	0.00	319.77	0.00
'S prenyl L cysteine oxygen oxidoreductase'	296.46	0.00	319.77	0.00
'S prenyl L cysteine oxygen oxidoreductase'	171.03	-171.03	180.58	-180.58
'S prenyl L cysteine oxygen oxidoreductase'	380.78	-380.78	378.29	-378.29
'S prenyl L cysteine oxygen oxidoreductase'	147.08	0.00	159.09	0.00
'S prenyl L cysteine oxygen oxidoreductase'	296.46	0.00	319.77	0.00
'choline reduced ferredoxin oxygen oxidoreductase'	225.35	0.00	241.68	0.00
'choline reduced ferredoxin oxygen oxidoreductase'	324.91	0.00	344.67	0.00

'choline reduced ferredoxin oxygen oxidoreductase'	306.61	0.00	328.04	0.00
'choline reduced ferredoxin oxygen oxidoreductase'	160.60	0.00	170.10	0.00
'Campest 4 en 3 one NADPH steroid 5alpha reductase'	263.32	0.00	293.14	0.00
'6 Oxocampestanol NADPH brassinosteroid C22alpha hydroxylase'	260.76	0.00	168.01	0.00
'p coumaroyl CoA caffeoyl CoA 3 hydroxylase'	329.71	0.00	458.33	0.00
'4 coumaryl alcohol NADP oxidoreductase'	394.71	0.00	442.98	0.00
'4 coumaryl alcohol NADP oxidoreductase'	329.71	0.00	458.33	0.00
'sinapoyl aldehyde NAD oxidoreductase'	183.99	0.00	216.71	0.00
'sinapoyl aldehyde NADP oxidoreductase'	183.99	0.00	216.71	0.00
'Campesterol NADPH brassinosteroid C22alpha hydroxylase'	260.76	0.00	168.01	0.00
'22alpha Hydroxy campest 4 en 3 one NADPH steroid 5alpha reductase'	263.32	0.00	293.14	0.00
'6 Deoxocathasterone brassinosteroid C23alpha hydroxylase'	319.10	0.00	215.05	0.00
'6 Deoxoteasterone brassinosteroid C3 oxidoreductase'	171.99	-171.99	196.06	-196.06
'6 Deoxocastasterone brassinosteroid C6 oxidase'	136.32	0.00	140.66	0.00
'6 Deoxocastasterone brassinosteroid C6 oxidase'	136.32	0.00	140.66	0.00
'Campest 4 en 3 one NADPH brassinosteroid C22alpha hydroxylase'	260.76	0.00	168.01	0.00
'5alpha Campestan 3 one NADPH brassinosteroid C22alpha hydroxylase'	260.76	0.00	168.01	0.00
'Campesterol NADPH brassinosteroid C22alpha hydroxylase'	260.76	0.00	168.01	0.00
'6 Deoxytyphasterol brassinosteroid C6 oxidase'	136.32	0.00	140.66	0.00
'L cysteine [ThiI] sulfurtransferase'	367.19	0.00	381.87	0.00
'L cysteine [ThiI] sulfurtransferase'	182.60	-182.60	191.12	-191.12
'L cysteine [ThiI] sulfurtransferase'	212.61	-212.61	241.86	-241.86
'L cysteine [ThiI] sulfurtransferase'	400.21	0.00	408.42	0.00

'L cysteine [ThiI] sulfurtransferase'	319.10	0.00	316.44	0.00
'L cysteine [ThiI] sulfurtransferase'	392.64	0.00	409.62	0.00
'L cysteine [ThiI] sulfurtransferase'	325.88	0.00	347.60	0.00
'L cysteine [ThiI] sulfurtransferase'	401.36	0.00	413.04	0.00
'L cysteine [ThiI] sulfurtransferase'	399.89	0.00	413.88	0.00
'L cysteine [ThiI] sulfurtransferase'	364.42	0.00	412.21	0.00
'L cysteine [ThiI] sulfurtransferase'	325.88	0.00	347.60	0.00
'L cysteine [ThiI] sulfurtransferase'	401.36	0.00	413.04	0.00
'L cysteine [ThiI] sulfurtransferase'	399.89	0.00	413.88	0.00
'L cysteine [ThiI] sulfurtransferase'	399.89	0.00	413.88	0.00
'L cysteine [ThiI] sulfurtransferase'	399.89	0.00	413.88	0.00
'phytyl diphosphate homogentisate phytyltransferase decarboxylating '	259.59	0.00	298.46	0.00
'phytyl diphosphate homogentisate phytyltransferase decarboxylating '	265.28	-265.28	272.52	-272.52
'phytyl diphosphate homogentisate phytyltransferase decarboxylating '	254.69	0.00	270.54	0.00
'phytyl diphosphate homogentisate phytyltransferase decarboxylating '	254.69	0.00	270.54	0.00
'S adenosyl L methionine delta tocopherol 5 O methyltransferase'	264.51	0.00	302.23	0.00
'S adenosyl L methionine delta tocopherol 5 O methyltransferase'	399.89	0.00	413.88	0.00
'S adenosyl L methionine delta tocopherol 5 O methyltransferase'	286.22	0.00	302.63	0.00
'S adenosyl L methionine delta tocopherol 5 O methyltransferase'	296.71	0.00	303.46	0.00
'S adenosyl L methionine delta tocopherol 5 O methyltransferase'	286.22	0.00	302.63	0.00
'S adenosyl L methionine delta tocopherol 5 O methyltransferase'	286.22	0.00	302.63	0.00
'S adenosyl L methionine delta tocopherol 5 O methyltransferase'	286.22	0.00	302.63	0.00
'S adenosyl L methionine delta tocopherol 5 O methyltransferase'	286.22	0.00	302.63	0.00
'S adenosyl L methionine delta tocopherol 5 O methyltransferase'	286.22	0.00	302.63	0.00
'S adenosyl L methionine delta tocopherol 5 O methyltransferase'	286.22	0.00	302.63	0.00
'S adenosyl L methionine delta tocopherol 5 O methyltransferase'	286.22	0.00	302.63	0.00
'S adenosyl L methionine delta tocopherol 5 O methyltransferase'	286.22	0.00	302.63	0.00

'S adenosyl L methionine delta tocopherol 5 O methyltransferase'	286.22	0.00	302.63	0.00
'S adenosyl L methionine delta tocopherol 5 O methyltransferase'	118.60	0.00	127.76	0.00
'S adenosyl L methionine delta tocopherol 5 O methyltransferase'	118.60	0.00	127.76	0.00
'S adenosyl L methionine delta tocopherol 5 O methyltransferase'	118.60	0.00	127.76	0.00
'S adenosyl L methionine delta tocopherol 5 O methyltransferase'	118.60	0.00	127.76	0.00
'LL 2 6 diaminoheptanedioate 2 oxoglutarate aminotransferase'	371.25	-371.25	392.59	-392.59
'R07618 m'	418.28	-418.28	449.62	-449.62
'enzyme N6 dihydrolipoyl lysine NAD oxidoreductase'	370.48	-370.48	390.43	-390.43
'enzyme N6 dihydrolipoyl lysine NAD oxidoreductase'	340.07	-340.07	354.56	-354.56
'enzyme N6 dihydrolipoyl lysine NAD oxidoreductase'	340.07	-340.07	354.56	-354.56
'enzyme N6 dihydrolipoyl lysine NAD oxidoreductase'	364.07	0.00	394.85	0.00
'enzyme N6 dihydrolipoyl lysine NAD oxidoreductase'	294.91	0.00	326.81	0.00
'enzyme N6 dihydrolipoyl lysine NAD oxidoreductase'	162.78	-162.78	160.00	-160.00
'enzyme N6 dihydrolipoyl lysine NAD oxidoreductase'	321.55	0.00	380.12	0.00
'enzyme N6 dihydrolipoyl lysine NAD oxidoreductase'	350.00	0.00	409.68	0.00
'enzyme N6 dihydrolipoyl lysine NAD oxidoreductase'	307.15	0.00	329.19	0.00
'enzyme N6 dihydrolipoyl lysine NAD oxidoreductase'	350.00	0.00	409.68	0.00
'aniline dioxygenase'	350.00	0.00	409.68	0.00
'nitrobenzene 1 2 dioxygenase'	350.00	0.00	409.68	0.00
'nitrobenzene 1 2 dioxygenase'	479.92	-479.92	496.17	-496.17
'nitrobenzene 1 2 dioxygenase'	224.41	0.00	227.29	0.00
'nitrobenzene 1 2 dioxygenase'	338.22	0.00	353.13	0.00
'nitrobenzene 1 2 dioxygenase'	328.93	0.00	346.60	0.00
'nitrobenzene 1 2 dioxygenase'	321.01	0.00	331.51	0.00
'nitrobenzene 1 2 dioxygenase'	224.41	0.00	227.29	0.00
'nitrobenzene 1 2 dioxygenase'	349.98	0.00	383.24	0.00

'nitrobenzene 1 2 dioxygenase'	328.93	-328.93	346.60	-346.60
'octanoyl [acp] protein N6 octanoyltransferase'	255.74	0.00	270.57	0.00
'protein N6 octanoyl lysine sulfur sulfurtransferase'	354.45	0.00	384.50	0.00
'octanoyl [acp] sulfur sulfurtransferase'	354.45	0.00	384.50	0.00
'lipoyl [acp] protein N6 lipoyltransferase'	255.74	0.00	270.57	0.00
'bromoxynil NADPH oxygen oxidoreductase'	124.30	0.00	135.13	0.00
'bromoxynil NADPH oxygen oxidoreductase'	182.60	-182.60	191.12	-191.12
'bromoxynil NADPH oxygen oxidoreductase'	296.45	0.00	357.58	0.00
'p coumarate 3 hydroxylase'	329.71	0.00	458.33	0.00
'p coumarate 3 hydroxylase'	275.49	0.00	291.86	0.00
'p coumarate 3 hydroxylase'	296.71	0.00	303.46	0.00
'p coumarate 3 hydroxylase'	286.22	0.00	302.63	0.00
'phenylacetonitrile aminohydrolase'	382.20	0.00	480.64	0.00
'phenylacetonitrile aminohydrolase'	280.72	0.00	291.78	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	189.22	0.00	304.36	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	283.18	0.00	314.64	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	189.22	0.00	304.36	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	129.83	0.00	143.93	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	283.18	0.00	314.64	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	129.83	0.00	143.93	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	154.16	0.00	157.85	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	288.23	0.00	334.45	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	385.54	0.00	410.28	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	316.64	0.00	358.58	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	316.64	0.00	358.58	0.00

'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	318.77	0.00	358.58	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	385.54	0.00	410.28	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	316.64	0.00	358.58	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	316.64	0.00	358.58	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	318.77	0.00	358.58	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	381.64	0.00	394.28	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	333.08	0.00	382.60	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	333.08	0.00	382.60	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	318.77	0.00	358.58	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	154.16	0.00	157.85	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	322.23	0.00	354.31	0.00
'9Z 11E 14Z 13S hydroperoxyoctadeca 9 11 14 trienoate'	381.64	0.00	394.28	0.00
'1 methylxanthine oxygen oxidoreductase'	252.46	0.00	289.68	0.00
'1 methylxanthine oxygen oxidoreductase'	385.54	0.00	410.28	0.00
'1 7 dimethylxanthine oxygen oxidoreductase'	252.46	0.00	289.68	0.00
'3 7 dimethylxanthine oxygen oxidoreductase'	252.46	0.00	289.68	0.00
'7 methylxanthine oxygen oxidoreductase'	252.46	0.00	289.68	0.00
'flavanone lyase decyclizing '	164.44	0.00	227.32	0.00
'flavanone lyase decyclizing '	164.44	0.00	227.32	0.00
'5 deoxyleucopelargonidin NADP 4 oxidoreductase'	162.24	0.00	170.38	0.00
'5 deoxyleucocyanidin NADP 4 oxidoreductase'	162.24	0.00	170.38	0.00
'flavonoid NADPH oxygen oxidoreductase 3 hydroxylating '	193.28	0.00	203.66	0.00



'flavonoid NADPH oxygen oxidoreductase 3 hydroxylating '	193.28	0.00	203.66	0.00
'2 Isopentenyl diphosphate ATP delta2 isopentenyltransferase'	209.20	0.00	245.39	0.00
'2 Isopentenyl diphosphate ADP delta2 isopentenyltransferase'	209.20	0.00	245.39	0.00
'2 Isopentenyl diphosphate ADP delta2 isopentenyltransferase'	326.54	0.00	338.28	0.00
'2 Isopentenyl diphosphate ADP delta2 isopentenyltransferase'	186.33	0.00	269.47	0.00
'7 methyl 3 oxo 6 octenoyl CoA acetyl CoA C acyltransferase'	318.77	0.00	358.58	0.00
'7 methyl 3 oxo 6 octenoyl CoA acetyl CoA C acyltransferase'	263.32	-263.32	293.14	-293.14
'3 hydroxy 5 methylhex 4 enoyl CoA hydro lyase'	314.59	0.00	334.96	0.00
'3 methylcrotonyl CoA acetyl CoA C acyltransferase'	318.77	0.00	358.58	0.00
'3 methylcrotonyl CoA acetyl CoA C acyltransferase'	296.45	0.00	357.58	0.00
'5 fluoromuconolactone lactonohydrolase'	395.18	0.00	434.35	0.00
'4 fluoromuconolactone lactonohydrolase'	395.18	0.00	434.35	0.00
'4 fluoromuconolactone lactonohydrolase'	280.08	-280.08	357.58	-357.58
'4 fluoromuconolactone lactonohydrolase'	280.80	-280.80	278.83	-278.83
'4 fluoromuconolactone lactonohydrolase'	329.59	0.00	331.84	0.00
'4 fluoromuconolactone lactonohydrolase'	329.59	0.00	331.84	0.00
'4 fluoromuconolactone lactonohydrolase'	329.59	0.00	331.84	0.00
'L tryptophan NADPH oxygen oxidoreductase N hydroxylating '	159.01	-159.01	166.10	-166.10
'L tryptophan NADPH oxygen oxidoreductase N hydroxylating '	329.59	0.00	331.84	0.00
'L tryptophan NADPH oxygen oxidoreductase N hydroxylating '	329.59	0.00	331.84	0.00
'UDP glucose N hydroxy 2 phenylethanethioamide'	236.52	-236.52	266.46	-266.46

'Stearoyl CoA hydrolase'	247.68	0.00	290.27	0.00
'Icosanoyl CoA hydrolase'	247.68	0.00	290.27	0.00
'Oleoyl CoA hydrolase'	247.68	0.00	290.27	0.00
'Linoleoyl CoA hydrolase'	247.68	0.00	290.27	0.00
'Linoleoyl CoA hydrolase'	247.68	0.00	290.27	0.00
'Linoleoyl CoA hydrolase'	247.68	0.00	290.27	0.00
'Linoleoyl CoA hydrolase'	247.68	0.00	290.27	0.00
'Linoleoyl CoA hydrolase'	247.68	0.00	290.27	0.00
'Linoleoyl CoA hydrolase'	247.68	0.00	290.27	0.00
'Linoleoyl CoA hydrolase'	160.79	0.00	170.58	0.00
'Linoleoyl CoA hydrolase'	160.79	0.00	170.58	0.00
'Linoleoyl CoA hydrolase'	160.79	0.00	170.58	0.00
'Linoleoyl CoA hydrolase'	160.79	0.00	170.58	0.00
'Linoleoyl CoA hydrolase'	160.79	0.00	170.58	0.00
'Linoleoyl CoA hydrolase'	160.79	0.00	170.58	0.00
'Linoleoyl CoA hydrolase'	160.79	0.00	170.58	0.00
'Linoleoyl CoA hydrolase'	160.79	0.00	170.58	0.00
'Linoleoyl CoA hydrolase'	160.79	0.00	170.58	0.00
'N Acetyl D glucosamine 1 phosphate 1 6 phosphomutase'	266.03	-266.03	279.18	-279.18
'N Acetyl D glucosamine 1 phosphate 1 6 phosphomutase'	265.79	-265.79	301.08	-301.08
'N Acetyl D glucosamine 1 phosphate 1 6 phosphomutase'	265.79	-265.79	301.08	-301.08
'L serine tRNA <sup>Sec</sup> ligase AMP forming	440.92	-440.92	454.59	-454.59
'5 deoxy 5 fluorocytidine aminohydrolase'	295.39	0.00	305.15	0.00
'5 fluorouridine monophosphate diphosphate'	234.97	0.00	245.46	0.00
'ATP 5 fluorodeoxyuridine 5 phosphotransferase'	319.22	0.00	344.84	0.00
'6 thioinosine 5 monophosphate diphosphate'	281.39	0.00	282.31	0.00
'6 methylthiopurine 5 monophosphate ribonucleotide diphosphate'	281.39	0.00	282.31	0.00
'6 thioinosine 5 monophosphate NAD oxidoreductase'	363.34	0.00	388.56	0.00
'inosine triphosphate pyrophosphatase'	339.82	0.00	367.37	0.00
'6 thioguanosine monophosphate diphosphate'	281.39	0.00	282.31	0.00

'tamoxifen NADPH oxygen oxidoreductase N oxide forming '	355.56	0.00	403.46	0.00
'tamoxifen NADPH oxygen oxidoreductase N oxide forming '	160.79	0.00	170.58	0.00
'4 glutathionyl cyclophosphamide hydrolase'	413.70	0.00	488.11	0.00
'alcohosphamide NAD oxidoreductase'	411.22	0.00	451.70	0.00
'2 phenyl 1 3 propanediol monocarbamate NAD oxidoreductase'	411.22	0.00	451.70	0.00
'4 hydroxy 5 phenyltetrahydro 1 3 oxazin 2 one NAD oxidoreductase'	411.22	0.00	451.70	0.00
'S adenosylmethioninamine cadaverine 3 aminopropyltransferase'	363.76	0.00	416.73	0.00
'2 deoxyribonucleoside diphosphate trypanothione disulfide'	404.18	0.00	414.53	0.00
'retinol NADP oxidoreductase'	304.86	-304.86	337.17	-337.17
'retinol NADP oxidoreductase'	304.86	-304.86	337.17	-337.17
'S Adenosyl L methionine 2 3 carboxy 3 aminopropyl L histidine'	346.94	-346.94	359.04	-359.04
'2 Oxoglutarate dehydrogenase complex'	305.36	-305.36	330.24	-330.24
'2 Oxoglutarate dehydrogenase complex'	135.66	-135.66	139.83	-139.83
'parathion oxidoreductase'	182.60	-182.60	191.12	-191.12
'tryptamine NADPH oxygen oxidoreductase N hydroxylating '	159.01	-159.01	166.10	-166.10
'N hydroxyl tryptamine oxidoreductase'	212.61	-212.61	241.86	-241.86
'N hydroxyl tryptamine oxidoreductase'	212.61	-212.61	241.86	-241.86
'UDP glucose p hydroxyphenylacetothiohydroximate'	236.52	-236.52	266.46	-266.46
'UDP glucose 4 methylthiobutylthiohydroximate'	236.52	-236.52	266.46	-266.46
'UDP glucose thiohydroximate S beta D glucosyltransferase'	236.52	-236.52	266.46	-266.46
'N linked glycopeptide N acetyl beta D glucosaminyl L asparagine'	273.72	-273.72	273.47	-273.47
'putrescine pyruvate aminotransferase'	355.71	-355.71	365.11	-365.11
'ditrans dicis pentaprenyl diphosphate isopentenyl diphosphate'	126.07	-126.07	134.61	-134.61
'ditrans tricis hexaprenyl diphosphate isopentenyl diphosphate'	126.07	-126.07	134.61	-134.61

'ditrans tetrakis heptaprenyl diphosphate isopentenyl diphosphate'	126.07	-126.07	134.61	-134.61
'ditrans pentakis octaprenyl diphosphate isopentenyl diphosphate'	126.07	-126.07	134.61	-134.61
'ditrans hexakis nonaprenyl diphosphate isopentenyl diphosphate'	126.07	-126.07	134.61	-134.61
'3 4 hydroxyphenyl lactate hydro lyase 4 coumarate forming '	344.53	-344.53	373.94	-373.94
'all trans nonaprenyl diphosphate homogentisate nonaprenyltransferase'	136.40	-136.40	143.12	-143.12
'trans cinnamate NADPH oxygen oxidoreductase 4 hydroxylating '	325.23	-325.23	431.67	-431.67
'UDP glucose ferulate D glucosyltransferase'	159.06	-159.06	158.70	-158.70
'ATP 1D 1 guanidino 3 amino 1 3 dideoxy scyllo inositol'	117.65	-117.65	123.20	-123.20
'UDP N acetyl D glucosamine ribostamycin'	234.97	-234.97	245.46	-245.46
'7 hydroxychlorophyllide a NAD oxidoreductase'	335.78	-335.78	333.61	-333.61
'pheophorbide a NADPH oxygen oxidoreductase biladiene forming '	278.05	-278.05	308.75	-308.75
'pheophorbide a hydrolase'	265.28	-265.28	272.52	-272.52
'pheophorbide a hydrolase'	155.84	-155.84	154.62	-154.62
'carbonate hydro lyase carbon dioxide forming ;'	351.65	0.00	284.21	0.00
'R00132 m'	351.65	0.00	284.21	0.00
'R00132 p'	351.65	0.00	284.21	0.00
'carbonate hydro lyase carbon dioxide forming ;'	403.55	0.00	400.32	0.00
'R01068 p'	438.80	0.00	477.26	0.00
'beta D fructose 1 6 bisphosphate D glyceraldehyde 3 phosphate lyase'	156.00	-156.00	161.16	-161.16
'R01070 p'	438.80	-438.80	477.26	-477.26
'R01175 x'	385.54	0.00	410.28	0.00
'R02112 p'	219.74	0.00	215.36	0.00
'R02570 m'	367.91	-367.91	392.87	-392.87
'R03316 m'	305.36	0.00	330.24	0.00
'R03651 m'	296.46	0.00	319.77	0.00
'L Glutamine tRNA Gln ligase AMP forming '	359.32	0.00	369.15	0.00

'L Lysine tRNALys ligase AMP forming '	452.99	0.00	474.81	0.00
'L Methionine tRNAMet ligase AMP forming '	354.78	0.00	372.44	0.00
'R03777 x'	385.54	0.00	410.28	0.00
'R03857 x'	385.54	0.00	410.28	0.00
'R03990 x'	385.54	0.00	410.28	0.00
'2 Amino 4 hydroxy 6 erythro 1 2 3 trihydroxypropyl '	302.30	0.00	313.95	0.00
'R04751 x'	385.54	0.00	410.28	0.00
'R04754 x'	385.54	0.00	410.28	0.00
'1 4 alpha D Glucan 1 4 alpha D glucan 4 alpha D glycosyltransferase'	249.56	0.00	280.94	0.00
'R05196 p'	249.56	0.00	280.94	0.00
'1 4 alpha D Glucan 1 4 alpha D glucan 4 alpha D glycosyltransferase'	293.23	0.00	303.25	0.00
'1 4 alpha D Glucan 1 4 alpha D glucan 4 alpha D glycosyltransferase'	289.33	0.00	304.58	0.00
'1 4 alpha D Glucan 1 4 alpha D glucan 4 alpha D glycosyltransferase'	304.32	-304.32	319.07	-319.07
'diphosphate phosphohydrolase;'	389.05	0.00	401.39	0.00
'pyruvate pyruvate acetaldehydetransferase decarboxylating ;'	408.93	0.00	435.73	0.00
'R00024 p'	234.97	0.00	245.46	0.00
'5 aminolevulinate hydro lyase adding 5 aminolevulinate and'	382.66	0.00	395.68	0.00
'6 hydroxynicotinate hydrogen donor oxygen oxidoreductase'	292.41	0.00	307.67	0.00
'porphobilinogen 4 [2 carboxyethyl] 3 [carboxymethyl]pyrrol 2 '	350.96	0.00	348.64	0.00
'ATP diphosphate lyase cyclizing; 3 5 cyclic AMP forming '	319.92	0.00	336.11	0.00
'NADH ferricytochrome b5 oxidoreductase'	376.15	0.00	414.44	0.00
'Uridine triphosphate AMP phosphotransferase;'	296.46	0.00	319.77	0.00
'R00209 m'	418.28	0.00	449.62	0.00
'R00209 p'	390.47	0.00	415.66	0.00
'L Glutamate 5 semialdehyde NAD oxidoreductase'	306.80	-306.80	344.73	-344.73

'L Glutamate ammonia ligase ADP forming '	422.74	-422.74	495.17	-495.17
'R00253 m'	350.47	-350.47	375.13	-375.13
'phosphate oxaloacetate carboxy lyase adding'	368.24	-368.24	392.81	-392.81
'Acyl CoA acceptor 2 3 oxidoreductase'	301.98	-301.98	327.74	-327.74
'R00415 c'	289.84	-289.84	302.84	-302.84
'GTP diphosphohydrolase diphosphate forming ;'	339.82	0.00	367.37	0.00
'GTP diphosphate lyase cyclizing; 3 5 cyclic GMP forming '	319.92	0.00	336.11	0.00
'R00472 x'	143.73	-143.73	152.86	-152.86
'3 phosphoadenylyl sulfate sulfohydrolase;'	296.46	0.00	319.77	0.00
'serine racemase;'	157.61	-157.61	171.57	-171.57
'ascorbate acceptor oxidoreductase'	350.00	-350.00	409.68	-409.68
'L arogenate hydro lyase decarboxylating; L phenylalanine forming '	296.46	0.00	319.77	0.00
'L glutamine D fructose 6 phosphate isomerase deaminating ;'	240.00	-240.00	283.10	-283.10
'L Cysteine hydrogen sulfide lyase deaminating; pyruvate forming '	296.46	0.00	319.77	0.00
'nitrite NAD oxidoreductase;'	296.46	0.00	319.77	0.00
'Succinyl CoA acetyl CoA C acyltransferase;'	296.46	0.00	319.77	0.00
'R00840 c'	277.35	-277.35	320.14	-320.14
'sn Glycerol 3 phosphate NAD 2 oxidoreductase'	296.46	-296.46	319.77	-319.77
'acyl CoA sn glycerol 3 phosphate 1 O acyltransferase'	296.46	0.00	319.77	0.00
'L Cysteine 2 oxoglutarate aminotransferase'	429.31	0.00	471.90	0.00
'propanoyl CoA NADP 2 oxidoreductase;'	407.74	-407.74	440.07	-440.07
'acetyl CoA propanoyl CoA 2 C acetyltransferase;'	318.77	0.00	358.58	0.00
'chorismate pyruvate lyase amino accepting; anthranilate forming '	300.19	0.00	308.44	0.00
'O Succinyl L homoserine succinate lyase deaminating;'	386.63	-386.63	400.98	-400.98

'L cystathionine cysteine lyase deaminating; 2 oxobutanoate forming '	296.46	0.00	319.77	0.00
'Glycerone phosphate phosphohydrolase'	183.40	-183.40	157.26	-157.26
'uracil hydro lyase adding D ribose'	296.46	-296.46	319.77	-319.77
'ATP R 5 diphosphomevalonate carboxy lyase adding ATP;'	344.84	0.00	361.41	0.00
'acetyl CoA 3 methyl 2 oxobutanoate C acetyltransferase'	321.22	0.00	369.68	0.00
'glycine synthase;'	360.74	-360.74	401.68	-401.68
'R01221 m'	360.74	-360.74	401.68	-401.68
'R01222 c'	364.42	-364.42	410.28	-410.28
'Xanthosine 5 phosphate L glutamine amido ligase AMP forming '	409.42	0.00	420.52	0.00
'L cystathionine L homocysteine lyase deaminating; pyruvate forming '	334.35	0.00	357.69	0.00
'O acetyl L homoserine hydrogen sulfide'	296.46	0.00	319.77	0.00
'O4 succinyl L homoserine hydrogen sulfide'	433.21	-433.21	459.11	-459.11
'L serine hydro lyase adding homocysteine; L cystathionine forming '	296.46	0.00	319.77	0.00
'L serine hydro lyase adding homocysteine; L cystathionine forming '	292.41	0.00	307.67	0.00
'prephenate hydro lyase decarboxylating; phenylpyruvate forming '	296.46	-296.46	319.77	-319.77
'O phospho L homoserine phosphate lyase adding'	370.92	0.00	395.00	0.00
'R01679 c'	234.97	0.00	245.46	0.00
'CDP diacylglycerol sn glycerol 3 phosphate 3 phosphatidyltransferase'	359.18	0.00	369.95	0.00
'gamma Amino gamma cyanobutanoate aminohydrolase'	382.20	0.00	480.64	0.00
'Caffeate CoA ligase AMP forming ;'	312.54	0.00	480.17	0.00
'Phosphatidylglycerol cholinephosphohydrolase'	291.25	-291.25	296.99	-296.99
'R02086 c'	266.03	-266.03	279.18	-279.18
'R02110 p'	260.63	0.00	313.14	0.00
'NADH ubiquinone oxidoreductase'	314.40	-314.40	332.90	-332.90
'R02198 c'	296.46	0.00	319.77	0.00

'N6 L 1 3 Dicarboxypropyl L lysine NAD oxidoreductase;'	167.04	-167.04	186.28	-186.28
'NTP pyruvate O2 phosphotransferase'	238.16	0.00	189.76	0.00
'Aromatase'	296.46	0.00	319.77	0.00
'Pectin pectylhydrolase'	174.07	0.00	134.70	0.00
'Ubiquitin C terminal thiolester hydrolase'	208.06	-208.06	222.16	-222.16
'R02435 c'	270.09	0.00	289.37	0.00
'flavonoid NADPH oxygen oxidoreductase 3 hydroxylating ;'	296.46	0.00	319.77	0.00
'deoxyuridine orthophosphate 2 deoxy D ribosyltransferase;'	296.46	-296.46	319.77	-319.77
'Glutaryl CoA acceptor 2 3 oxidoreductase decarboxylating '	296.46	0.00	319.77	0.00
'Glutaryl CoA acceptor 2 3 oxidoreductase decarboxylating '	297.37	0.00	320.00	0.00
'4 aminobutanal NAD 1 oxidoreductase;'	322.07	-322.07	348.88	-348.88
' 5Z 13E 15S 9alpha 11alpha 15 trihydroxyprosta 5 13 '	296.46	0.00	319.77	0.00
'Donor hydrogen peroxide oxidoreductase'	407.10	0.00	436.83	0.00
'2 methylpropanoyl CoA acceptor 2 3 oxidoreductase'	296.46	0.00	319.77	0.00
'2 methylpropanoyl CoA acceptor 2 3 oxidoreductase'	357.54	0.00	387.22	0.00
'3 Hydroxyanthranilate O methyltransferase'	401.42	0.00	421.63	0.00
'L serine hydro lyase [adding 1 C indol 3 yl glycerol 3 phosphate;'	334.48	-334.48	398.74	-398.74
'R02771 c'	350.99	0.00	364.94	0.00
'1 4 1 3;1 4 beta D Glucan 4 glucanohydrolase'	296.46	0.00	319.77	0.00
' Gibberellin 1 2 oxoglutarate oxygen oxidoreductase'	149.30	0.00	154.46	0.00
'Aromatase'	296.46	0.00	319.77	0.00
' S 2 methylbutanoyl CoA acceptor 2 3 oxidoreductase'	296.46	0.00	319.77	0.00
'11beta Hydroxysteroid NADP 11 oxidoreductase'	234.97	-234.97	245.46	-245.46
'L ribulose 5 phosphate 3 epimerase;'	298.59	-298.59	324.04	-324.04



'4 Trimethylammoniobutanal NAD 1 oxidoreductase;'	296.46	0.00	319.77	0.00
'L Glutamate 5 semialdehyde NADP 5 oxidoreductase phosphorylating '	347.81	-347.81	352.74	-352.74
'L Glutamate 5 semialdehyde NADP 5 oxidoreductase phosphorylating '	307.77	0.00	321.40	0.00
'ATP protamine O phosphotransferase'	143.16	-143.16	162.99	-162.99
'flavanone 2 oxoglutarate oxygen oxidoreductase 3 hydroxylating '	141.76	0.00	164.55	0.00
'3alpha 7alpha 12alpha trihydroxy 5beta cholanoyl CoA propanoyl CoA'	318.77	0.00	358.58	0.00
' Gibberellin 44 2 oxoglutarate oxygen oxidoreductase'	152.49	0.00	149.48	0.00
' gibberellin 44 2 oxoglutarate oxygen oxidoreductase'	152.49	0.00	149.48	0.00
'R03847 c'	234.97	0.00	245.46	0.00
'Ubiquitin protein lysine N ligase AMP forming '	234.97	-234.97	245.46	-245.46
' 5 Glutamyl peptide amino acid 5 glutamyltransferase'	226.63	0.00	184.05	0.00
'Donor hydrogen peroxide oxidoreductase'	407.10	0.00	436.83	0.00
'gamma Glutamyl beta aminopropionitrile amidohydrolase'	226.63	0.00	184.05	0.00
'Donor hydrogen peroxide oxidoreductase'	407.10	0.00	436.83	0.00
'R04008 c'	297.37	-297.37	320.00	-320.00
'1 Alkyl 2 acyl sn glycerol 3 phosphate phosphohydrolase'	296.46	0.00	319.77	0.00
'1 Alkyl 2 acyl sn glycerol 3 phosphate phosphohydrolase'	203.29	0.00	206.75	0.00
'R04254 m'	203.29	0.00	206.75	0.00
'R04254 p'	203.29	0.00	206.75	0.00
'R04254 x'	203.29	0.00	206.75	0.00
'glycerone phosphate iminosuccinate alkyltransferase cyclizing '	276.70	0.00	309.05	0.00
'Propanoyl CoA acceptor 2 3 oxidoreductase'	301.98	-301.98	327.74	-327.74
'5 Phosphoribosylformylglycinamide L glutamine amido ligase'	370.95	0.00	392.19	0.00
' 3R 3 Hydroxybutanoyl [acyl carrier protein] hydro lyase;'	296.46	-296.46	319.77	-319.77

'R04537 p'	296.46	-296.46	319.77	-319.77
' 3R 3 Hydroxybutanoyl [acyl carrier protein] hydro lyase;'	296.46	0.00	319.77	0.00
'R04757 c'	234.97	0.00	245.46	0.00
' 3R 3 Hydroxybutanoyl [acyl carrier protein] hydro lyase;'	292.41	0.00	307.67	0.00
' 3R 3 Hydroxybutanoyl [acyl carrier protein] hydro lyase;'	292.41	-292.41	307.67	-307.67
'zeta Carotene hydrogen donor oxygen oxidoreductase'	249.30	0.00	279.91	0.00
'Neurosporene hydrogen donor oxygen oxidoreductase'	249.30	0.00	279.91	0.00
'Propanoyl CoA acetyl CoA C acyltransferase;'	318.77	0.00	358.58	0.00
' 2S flavan 4 ol NADP 4 oxidoreductase;'	296.46	0.00	319.77	0.00
' 2S flavan 4 ol NADP 4 oxidoreductase;'	401.42	0.00	421.63	0.00
'R05027 c'	237.61	0.00	254.65	0.00
'R05028 c'	422.74	0.00	495.17	0.00
'R05033 c'	422.74	0.00	495.17	0.00
'S Adenosyl L methionine kaempferol 3 O methyltransferase;'	346.90	0.00	420.69	0.00
'flavanone 2 oxoglutarate oxygen oxidoreductase 3 hydroxylating '	141.76	0.00	164.55	0.00
'N4 Acetylaminobutanal NAD oxidoreductase'	327.24	0.00	394.12	0.00
'L erythro 4 Hydroxyglutamate 2 oxoglutarate aminotransferase'	296.46	0.00	319.77	0.00
' R 2 3 Dihydroxy 3 methylpentanoate NADP oxidoreductase'	473.53	-473.53	488.68	-488.68
' gibberellin 44 2 oxoglutarate oxygen oxidoreductase'	152.49	0.00	149.48	0.00
'10 Formyltetrahydrofolyl L glutamate L glutamate gamma ligase'	233.13	-233.13	244.74	-244.74
'R05201 c'	401.42	0.00	421.63	0.00
'Geranylgeranyl diphosphate Isopentenyl diphosphate geranylgeranyl'	433.21	-433.21	459.11	-459.11
'Glutaryl CoA acceptor 2 3 oxidoreductase'	296.46	0.00	319.77	0.00

'Glutaryl CoA acceptor 2 3 oxidoreductase'	307.15	0.00	337.17	0.00
'Glutaryl CoA acceptor 2 3 oxidoreductase'	401.42	0.00	421.63	0.00
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	140.06	0.00	146.07	0.00
'R05883 c'	283.52	0.00	315.65	0.00
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	271.52	0.00	284.97	0.00
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	403.55	0.00	400.32	0.00
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	403.55	0.00	400.32	0.00
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	350.47	-350.47	386.82	-386.82
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	284.21	-284.21	297.94	-297.94
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	304.32	-304.32	319.07	-319.07
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	279.48	0.00	290.95	0.00
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	415.98	0.00	419.49	0.00
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	129.90	0.00	134.01	0.00
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	233.99	-233.99	235.64	-235.64
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	294.41	-294.41	296.73	-296.73
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	301.54	0.00	312.67	0.00
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	284.21	0.00	297.94	0.00
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	234.97	0.00	245.46	0.00
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	187.70	-187.70	198.10	-198.10
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	262.14	-262.14	275.86	-275.86
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	304.32	-304.32	319.07	-319.07

'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	403.55	-403.55	400.32	-400.32
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	304.32	-304.32	319.07	-319.07
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	304.32	-304.32	319.07	-319.07
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	304.32	-304.32	319.07	-319.07
'1 alpha D galactosyl myo inositol raffinose galactosyltransferase'	280.33	-280.33	303.13	-303.13
'1 alpha D galactosyl myo inositol raffinose galactosyltransferase'	304.32	-304.32	319.07	-319.07
'1 alpha D galactosyl myo inositol raffinose galactosyltransferase'	375.55	-375.55	394.22	-394.22
'GDP mannose glycolipid 1 2 alpha D mannosyltransferase'	296.46	0.00	319.77	0.00
'GDP mannose glycolipid 1 2 alpha D mannosyltransferase'	304.32	-304.32	319.07	-319.07
'Galactoside alpha 1 2 L fucosyltransferase'	304.32	0.00	319.07	0.00
'Galactoside alpha 1 2 L fucosyltransferase'	403.55	0.00	400.32	0.00
'Galactoside alpha 1 2 L fucosyltransferase'	403.55	0.00	400.32	0.00
'Galactoside alpha 1 2 L fucosyltransferase'	403.55	0.00	400.32	0.00
'Galactoside alpha 1 2 L fucosyltransferase'	403.55	0.00	400.32	0.00
'Galactoside alpha 1 2 L fucosyltransferase'	403.55	0.00	400.32	0.00
'Galactoside alpha 1 2 L fucosyltransferase'	403.55	0.00	400.32	0.00
'ent Kaur 16 en 19 oate NADPH oxygen oxidoreductase hydroxylating '	214.58	0.00	214.19	0.00
'ent Kaur 16 en 19 oate NADPH oxygen oxidoreductase hydroxylating '	214.58	0.00	214.19	0.00
' gibberellin 44 2 oxoglutarate oxygen oxidoreductase'	152.49	0.00	149.48	0.00
' gibberellin 44 2 oxoglutarate oxygen oxidoreductase'	152.49	0.00	149.48	0.00
' gibberellin 44 2 oxoglutarate oxygen oxidoreductase'	152.49	0.00	149.48	0.00

'gibberellin 1 2 oxoglutarate oxygen oxidoreductase'	125.02	-125.02	136.12	-136.12
'CDPethanolamine 1 alkyl 2 acylglycerol'	380.78	0.00	378.29	0.00
'CDPethanolamine 1 alkyl 2 acylglycerol'	304.86	0.00	329.19	0.00
'alpha Pinene dehydrogenase'	350.00	0.00	409.68	0.00
'Myrtenol dehydrogenase'	304.86	0.00	329.19	0.00
'Myrtenol dehydrogenase'	350.00	0.00	409.68	0.00
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	140.06	0.00	146.07	0.00
'2S flavan 4 ol NADP 4 oxidoreductase;'	296.46	0.00	319.77	0.00
'R06860 c'	200.32	0.00	208.06	0.00
'Acyl CoA sn glycerol 3 phosphate 2 O acyltransferase'	203.29	0.00	206.75	0.00
'flavanone lyase decyclizing '	164.44	-164.44	227.32	-227.32
'n alkanal NADP 2 oxidoreductase'	253.04	-253.04	270.38	-270.38
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	140.06	-140.06	146.07	-146.07
'6 Deoxo cathasterone brassinosteroid C23alpha hydroxylase'	319.10	0.00	215.05	0.00
'donor hydrogen peroxide oxidoreductase'	407.10	0.00	436.83	0.00
'donor hydrogen peroxide oxidoreductase'	234.97	0.00	245.46	0.00
'donor hydrogen peroxide oxidoreductase'	284.21	0.00	297.94	0.00
'donor hydrogen peroxide oxidoreductase'	284.21	0.00	297.94	0.00
'acetyl CoA heparan alpha D glucosaminide N acetyltransferase'	253.87	-253.87	262.77	-262.77
'acetyl CoA heparan alpha D glucosaminide N acetyltransferase'	288.18	0.00	321.72	0.00
'flavanone 2 oxoglutarate oxygen oxidoreductase 3 hydroxylating '	141.76	0.00	164.55	0.00
'flavanone 2 oxoglutarate oxygen oxidoreductase 3 hydroxylating '	141.76	0.00	164.55	0.00
'flavanone 2 oxoglutarate oxygen oxidoreductase 3 hydroxylating '	141.76	0.00	164.55	0.00
'flavanone 2 oxoglutarate oxygen oxidoreductase 3 hydroxylating '	156.29	0.00	163.48	0.00

'flavanone 2 oxoglutarate oxygen oxidoreductase 3 hydroxylating '	156.29	0.00	163.48	0.00
'flavanone 2 oxoglutarate oxygen oxidoreductase 3 hydroxylating '	156.29	0.00	163.48	0.00
'dihydroflavonol 2 oxoglutarate oxygen oxidoreductase'	191.57	0.00	203.88	0.00
'dihydroflavonol 2 oxoglutarate oxygen oxidoreductase'	183.02	0.00	202.52	0.00
'2 succinyl 5 enolpyruvyl 6 hydroxy 3 cyclohexene 1 carboxylate'	200.32	0.00	208.06	0.00
'5 6 dihydro 5 fluorouracil amidohydrolase'	352.46	0.00	382.83	0.00
'alpha fluoro beta ureidopropionic acid amidohydrolase'	282.67	0.00	301.39	0.00
'6 thioxanthine 5 monophosphate L glutamine amido ligase'	409.42	0.00	420.52	0.00
'2 deoxyribonucleoside diphosphate tryparedoxin disulfide'	404.18	0.00	414.53	0.00
'L methionine 2 oxo acid aminotransferase;'	355.71	-355.71	365.11	-365.11
'trans trans farnesyl diphosphate diphosphate lyase [cyclizing '	147.86	-147.86	153.26	-153.26
' 2E 6E farnesyl diphosphate diphosphate lyase'	147.86	-147.86	153.26	-153.26
' 2E 6E farnesyl diphosphate diphosphate lyase'	147.86	-147.86	153.26	-153.26
'trans trans cis geranylgeranyl diphosphate isopentenyl diphosphate'	126.07	-126.07	134.61	-134.61
'RN01 c'	296.46	-296.46	319.77	-319.77
'RN03 c'	296.46	-296.46	319.77	-319.77
'RN03 p'	296.46	-296.46	319.77	-319.77
'RN04 m'	296.46	0.00	319.77	0.00
'RN05 m'	296.46	0.00	319.77	0.00
'RN06 c'	296.46	-296.46	319.77	-319.77
'R00095 tmx'	296.46	-296.46	319.77	-319.77
'R00644 tmx'	296.46	0.00	319.77	0.00
'R00021 p'	340.34	0.00	382.90	0.00
'S adenosyl L methionine S adenosyl L methionine'	150.85	-150.85	162.93	-162.93
'Urea amidohydrolase'	223.96	0.00	237.30	0.00
'R01195 p'	373.23	-373.23	384.27	-384.27

'Malonyl CoA 4 Coumaroyl CoA malonyltransferase cyclizing '	206.57	0.00	209.27	0.00
' R Mevalonate NADP oxidoreductase CoA acylating '	352.97	0.00	372.46	0.00
'ATP R mevalonate 5 phosphotransferase'	304.98	0.00	325.07	0.00
' Gibberellin 20 2 oxoglutarate oxygen oxidoreductase'	218.07	-218.07	224.03	-224.03
'7 8 Diaminononanoate carbon dioxide cyclo ligase'	296.46	0.00	319.77	0.00
'Nicotinate nucleotide pyrophosphate phosphoribosyltransferase'	366.11	0.00	408.58	0.00
' gibberellin 1 2 oxoglutarate oxygen oxidoreductase'	149.30	0.00	154.46	0.00
' gibberellin 1 2 oxoglutarate oxygen oxidoreductase'	296.46	-296.46	319.77	-319.77
'butyryl [acyl carrier protein] malonyl [acyl carrier protein]'	246.03	0.00	254.82	0.00
'R04952 p'	292.57	0.00	314.54	0.00
'leucocyanidin 2 oxoglutarate oxygen oxidoreductase'	140.06	0.00	146.07	0.00
'L Glutamate tRNA Glu ligase AMP forming '	433.21	-433.21	459.11	-459.11
'Lanosterol NADPH oxygen oxidoreductase 14 methyl cleaving '	338.40	-338.40	367.99	-367.99
' gibberellin 20 2 oxoglutarate oxygen oxidoreductase'	199.65	0.00	212.60	0.00
' gibberellin 1 2 oxoglutarate oxygen oxidoreductase'	149.30	0.00	154.46	0.00
' gibberellin 1 2 oxoglutarate oxygen oxidoreductase'	149.30	0.00	154.46	0.00
'di trans poly cis Decaprenyl diphosphate isopentenyl diphosphate'	136.40	0.00	143.12	0.00
'malonyl CoA 4 coumaroyl CoA malonyltransferase cyclizing reducing '	407.74	0.00	440.07	0.00
' gibberellin 44 2 oxoglutarate oxygen oxidoreductase'	152.49	-152.49	149.48	-149.48
'flavanone 2 oxoglutarate oxygen oxidoreductase 3 hydroxylating '	141.76	-141.76	164.55	-164.55
'flavan 3 ol NAD oxidoreductase'	176.56	-176.56	178.39	-178.39
'dihydroflavonol 2 oxoglutarate oxygen oxidoreductase'	131.74	-131.74	135.47	-135.47

'dihydroflavonol 2 oxoglutarate oxygen oxidoreductase'	340.86	0.00	350.97	0.00
'dihydroflavonol 2 oxoglutarate oxygen oxidoreductase'	171.03	-171.03	180.58	-180.58
'dihydroflavonol 2 oxoglutarate oxygen oxidoreductase'	302.05	0.00	327.82	0.00
'malonyl CoA cinnamoyl CoA malonyltransferase cyclizing '	206.57	0.00	209.27	0.00
'malonyl CoA caffeoyl CoA malonyltransferase cyclizing '	206.57	0.00	209.27	0.00
'malonyl CoA feruloyl CoA malonyltransferase cyclizing '	206.57	0.00	209.27	0.00
'malonyl CoA feruloyl CoA malonyltransferase cyclizing '	280.08	-280.08	357.58	-357.58
'6 mercaptopurin oxygen oxidoreductase'	252.46	0.00	289.68	0.00
'6 mercaptopurin oxygen oxidoreductase'	182.60	-182.60	191.12	-191.12
'Glucose drain'	296.46	0.00	319.77	0.00
'Serine drain'	296.46	0.00	319.77	0.00
'Threonine drain'	296.46	0.00	319.77	0.00
'Methionine drain'	296.46	0.00	319.77	0.00
'Cysteine drain'	296.46	0.00	319.77	0.00
'Valine drain'	296.46	0.00	319.77	0.00
'Leucine drain'	296.46	0.00	319.77	0.00
'Isoleucine drain'	296.46	0.00	319.77	0.00
'Glutamine drain'	296.46	0.00	319.77	0.00
'Arginine drain'	296.46	0.00	319.77	0.00
'Proline drain'	296.46	0.00	319.77	0.00
'Sucrose drain'	296.46	0.00	319.77	0.00
'Lysine drain'	296.46	0.00	319.77	0.00
'Histidine drain'	296.46	0.00	319.77	0.00
'Phenylalanine drain'	296.46	0.00	319.77	0.00
'Tyrosine drain'	296.46	0.00	319.77	0.00
'Tryptophan drain'	296.46	0.00	319.77	0.00
'Asparagine drain'	296.46	0.00	319.77	0.00
'4 Coumaryl alcohol drain'	296.46	0.00	319.77	0.00
'Coniferyl alcohol drain'	296.46	0.00	319.77	0.00
'Sinapyl alcohol drain'	296.46	0.00	319.77	0.00
'Fructose drain'	296.46	0.00	319.77	0.00
'Cellulose drain'	296.46	0.00	319.77	0.00
'FA biomass drain'	296.46	0.00	319.77	0.00



'dATP drain'	296.46	0.00	319.77	0.00
'dGTP drain'	296.46	0.00	319.77	0.00
'dCTP drain'	296.46	0.00	319.77	0.00
'dTTP drain'	296.46	0.00	319.77	0.00
'ATP drain'	296.46	0.00	319.77	0.00
'GTP drain'	296.46	0.00	319.77	0.00
'CTP drain'	296.46	0.00	319.77	0.00
'UTP drain'	296.46	0.00	319.77	0.00
'Starch drain'	296.46	0.00	319.77	0.00
'Xylose drain Hemicellulose '	296.46	0.00	319.77	0.00
'Glutamate drain'	296.46	0.00	319.77	0.00
'Alanine drain'	296.46	0.00	319.77	0.00
'Aspartate drain'	296.46	0.00	319.77	0.00
'Glycine drain'	296.46	0.00	319.77	0.00
'Biomass synthesis Leaf '	296.46	0.00	319.77	0.00
'Pyruvate transporter'	296.46	-296.46	319.77	-319.77
'Serine transporter'	296.46	-296.46	319.77	-319.77
'ATP transporter'	296.46	-296.46	319.77	-319.77
'ADP transporter'	296.46	-296.46	319.77	-319.77
'AMP transporter'	296.46	-296.46	319.77	-319.77
'CoA'	296.46	-296.46	319.77	-319.77
'CO2 transporter'	296.46	-296.46	319.77	-319.77
'Acetate transporter'	296.46	-296.46	319.77	-319.77
'Phosphate transporter/Dicarboxylate translocator'	296.46	-296.46	319.77	-319.77
'Malate transporter/Dicarboxylate translocator'	296.46	-296.46	319.77	-319.77
'H2O transporter'	296.46	-296.46	319.77	-319.77
'Proton transporter'	296.46	-296.46	319.77	-319.77
'Glutamine transporter'	296.46	-296.46	319.77	-319.77
'ThPP transporter'	296.46	-296.46	319.77	-319.77
'Oxygen transport'	296.46	-296.46	319.77	-319.77
'NH3 transport'	296.46	-296.46	319.77	-319.77
'Succinate transporter'	296.46	-296.46	319.77	-319.77
'Phosphate transporter/Dicarboxylate translocator'	296.46	-296.46	319.77	-319.77
'Isocitrate transporter'	296.46	-296.46	319.77	-319.77
'L Ornithine transporter'	296.46	-296.46	319.77	-319.77
'L Citrulline transporter'	296.46	-296.46	319.77	-319.77
'Citrate transporter'	296.46	-296.46	319.77	-319.77
'Aspartate transporter'	296.46	-296.46	319.77	-319.77

'Glutamate transporter'	296.46	-296.46	319.77	-319.77
'Oxaloacetate transporter'	296.46	-296.46	319.77	-319.77
'alpha ketoglutarate transporters'	296.46	-296.46	319.77	-319.77
'Glycine transporter'	296.46	-296.46	319.77	-319.77
'Glucose translocator'	296.46	-296.46	319.77	-319.77
'Phosphate transporter/Dicarboxylate translocator'	296.46	-296.46	319.77	-319.77
'Malate transporter'	296.46	-296.46	319.77	-319.77
'Oxaloacetate transporter'	296.46	-296.46	319.77	-319.77
'Sulfate transporter'	296.46	-296.46	319.77	-319.77
'NH3 transport'	296.46	-296.46	319.77	-319.77
'Nitrite transporter'	296.46	-296.46	319.77	-319.77
'Maltose transporter'	296.46	-296.46	319.77	-319.77
'Glycolate transporter'	296.46	-296.46	319.77	-319.77
'alpha ketoglutarate translocator'	296.46	-296.46	319.77	-319.77
'H2O transport'	296.46	-296.46	319.77	-319.77
'CO2 fixation'	296.46	-296.46	319.77	-319.77
'ATP transporter'	296.46	-296.46	319.77	-319.77
'ADP transporter'	296.46	-296.46	319.77	-319.77
'Proton transporter'	296.46	-296.46	319.77	-319.77
'Glutamate transporter'	296.46	-296.46	319.77	-319.77
'Glycerate transport'	296.46	-296.46	319.77	-319.77
'Oxigen transport'	296.46	-296.46	319.77	-319.77
'Phosphate transporter/Dicarboxylate translocator'	296.46	-296.46	319.77	-319.77
'Glutamine transporter'	296.46	-296.46	319.77	-319.77
'L Ornithine transporter'	296.46	-296.46	319.77	-319.77
' Pyruvate transporter'	296.46	-296.46	319.77	-319.77
' L Citrulline transporter'	296.46	-296.46	319.77	-319.77
'Fatty accid in plastid'	296.46	-296.46	319.77	-319.77
' phosphoenolpyruvate transporter'	296.46	-296.46	319.77	-319.77
'2 phosphoglycerate transporter'	296.46	-296.46	319.77	-319.77
'alpha D Glucose 6 phosphate transporter'	296.46	-296.46	319.77	-319.77
'Triose phosphate translocator G3P '	296.46	-296.46	319.77	-319.77
'Triose phosphate trasnlocator glyceroneP '	296.46	-296.46	319.77	-319.77
'Triose phosphate translocator 3 phosphoglycerate '	296.46	-296.46	319.77	-319.77
'Sulfate transporter'	296.46	-296.46	319.77	-319.77
'Malate transporter'	296.46	-296.46	319.77	-319.77

'Nitrate transporter'	296.46	-296.46	319.77	-319.77
'Sucrose transporter'	296.46	-296.46	319.77	-319.77
'Citrate transporter'	296.46	-296.46	319.77	-319.77
'Glycine transport'	296.46	-296.46	319.77	-319.77
'Phosphate transporter/Dicarboxylate translocator'	296.46	-296.46	319.77	-319.77
'H2O transporter'	296.46	-296.46	319.77	-319.77
'Oxygen transport'	296.46	-296.46	319.77	-319.77
'Glycerate transport'	296.46	-296.46	319.77	-319.77
'Citrate transporter'	296.46	-296.46	319.77	-319.77
'Succinate transporter'	296.46	-296.46	319.77	-319.77
'Isocitrate transporter'	296.46	-296.46	319.77	-319.77
'Oxaloacetate transporter'	296.46	-296.46	319.77	-319.77
'Proton transporter'	296.46	-296.46	319.77	-319.77
'CoA'	296.46	-296.46	319.77	-319.77
'Serine transporter'	296.46	-296.46	319.77	-319.77
'Glycolate transporter'	296.46	-296.46	319.77	-319.77
'alpha ketoglutarate translocator'	296.46	-296.46	319.77	-319.77
'Glutamate translocator'	296.46	-296.46	319.77	-319.77
'Malate transporter/Dicarboxylate translocator'	296.46	-296.46	319.77	-319.77
'Fatty acid transporter for beta oxidation '	296.46	-296.46	319.77	-319.77
'ATP transporter'	296.46	-296.46	319.77	-319.77
'AMP transporter'	296.46	-296.46	319.77	-319.77
'V0001'	296.46	0.00	319.77	0.00
'V0002'	296.46	0.00	319.77	0.00
'V0003'	296.46	0.00	319.77	0.00
'V0004'	296.46	0.00	319.77	0.00
'V0005'	296.46	0.00	319.77	0.00
'V0006'	296.46	0.00	319.77	0.00
'V0007'	296.46	0.00	319.77	0.00
'V0008'	296.46	0.00	319.77	0.00
'Biomass exchange'	296.46	-100.00	319.77	-100.00
' CO2 exchange'	296.46	-100.00	319.77	-100.00
' Hydrogen sulfide exchange'	296.46	-100.00	319.77	-100.00
' Sulfate exchange'	296.46	-100.00	319.77	-100.00
' alpha D Glucose exchange'	296.46	-100.00	319.77	-100.00
' beta D Fructose exchange'	296.46	-100.00	319.77	-100.00
' Maltose exchange'	296.46	-100.00	319.77	-100.00
'hvi exchange'	296.46	-100.00	319.77	-100.00

'hvo exchange'	296.46	-100.00	319.77	-100.00
' Sucrose exchange'	296.46	-100.00	319.77	-100.00
' Orthophosphate exchange'	296.46	-100.00	319.77	-100.00
' H2O exchange'	296.46	-100.00	319.77	-100.00
' Oxygen exchange'	296.46	-100.00	319.77	-100.00
' Nitrate exchange'	296.46	-100.00	319.77	-100.00
' NH3 exchange'	296.46	-100.00	319.77	-100.00
'Light rxn 1'	296.46	0.00	319.77	0.00
'serine ammonia lyase pyruvate forming '	234.97	0.00	245.46	0.00
'L asparagine hydro lyase 3 cyanoalanine forming '	296.46	0.00	319.77	0.00
'L asparagine hydro lyase 3 cyanoalanine forming '	296.46	0.00	319.77	0.00
'L asparagine hydro lyase 3 cyanoalanine forming '	296.46	0.00	319.77	0.00
'L asparagine hydro lyase 3 cyanoalanine forming '	296.46	0.00	319.77	0.00
'Carbon monoxide oxygen oxidoreductase'	296.46	0.00	319.77	0.00
'Carbon monoxide oxygen oxidoreductase'	296.46	0.00	319.77	0.00
' R 4 Phosphopantothenate L cysteine ligase'	287.34	0.00	299.65	0.00
'L aspartate ammonia ligase AMP forming '	394.29	0.00	405.25	0.00
'S adenosyl L methionine 3 4 dihydroxy trans cinnamate'	234.97	0.00	245.46	0.00
'L threonine acetaldehyde lyase glycine forming '	234.97	0.00	245.46	0.00
'ATP pantothenate 4 phosphotransferase'	280.41	0.00	285.98	0.00
'ATP pantothenate 4 phosphotransferase'	280.41	0.00	285.98	0.00
' R Pantoate NADP 2 oxidoreductase'	234.97	0.00	245.46	0.00
' R Pantoate NADP 2 oxidoreductase'	296.46	0.00	319.77	0.00
'cellobiose phosphate alpha D glucosyltransferase'	234.97	0.00	245.46	0.00
'N R Pantothenoyl L cysteine carboxy lyase'	234.97	0.00	245.46	0.00
'acetyl CoA 4 hydroxybutanoate CoA transferase'	296.46	0.00	319.77	0.00

'Dephospho CoA nucleotidohydrolase'	234.97	0.00	245.46	0.00
'pyruvate 2 oxobutanoate acetaldehydetransferase decarboxylating '	347.49	0.00	334.46	0.00
'pyruvate 2 oxobutanoate acetaldehydetransferase decarboxylating '	296.46	0.00	319.77	0.00
'ATP pantothenate 4 phosphotransferase'	280.41	0.00	285.98	0.00
'caffeic aldehyde 3 4 dihydroxy trans cinnamate 3 O methyltransferase'	234.97	0.00	245.46	0.00
'caffeic aldehyde 3 4 dihydroxy trans cinnamate 3 O methyltransferase'	296.46	0.00	319.77	0.00

Table 7 – Table of upper and lower bounds for MJ- and MJ+ conditions in E-Flux simulations.

## APPENDIX B

### CRYOSECTIONING AND IMMUNOFLUORESCENCE

#### PROTOCOLS

##### Paraffin Embedding

Fixation and paraffin embedding

1. Prepare 37% (w/v) formaldehyde fresh from powder by dissolving in heated water and adding a few drops of NaOH, alternatively 37% formaldehyde that has been stored frozen can be diluted to 4% immediately prior to use
2. Make fixative solution of 4% (w/v) formaldehyde and 0.1% (v/v) glutaraldehyde
3. Remove aggregates from media by using filter, place aggregates in scintillation vial and cover with excess of fixative solution
4. Place uncapped vials in vacuum chamber and apply vacuum for 1 minute
5. Cap vials and let sit 24 hours at room temperature
6. Remove fixative and apply a graded ethanol series to dehydrate the sample
  - a. Add 30%, 50%, 75% and neat solutions of histological grade ethanol for 30 minutes each, with 3 applications of neat solution
7. Immerse sample in pure xylene for 2 hours
8. Pipette out solven and add molten paraffin to sample, let sit for 1 hour in incubator at 58  
C
9. Pour off paraffin and fresh paraffin to sample, let sit for 1 hour
10. Transfer aggregates to square sample mold and let solidify to room temperature

Sectioning

1. Using a microtome, section wax blocks with embedded sample to 10  $\mu\text{m}$  slices
2. Float slices in water bath and then place on lysine-coated slides
3. Allow to dry on heated drying block for > 2 hours

#### Dewaxing and Rehydration

1. Place slides in a coplin jar, add 35 ml 100% xylene, incubate for 10 minutes, empty coplin jar, add new 100% xylene and incubate another 10 minutes
2. Add 95% ethanol to slides, incubate 10 minutes
3. Add 75% ethanol diluted in PBS, incubate 5 minutes
4. Add 50% ethanol in PBS, incubate 5 minutes
5. Add PBS, incubate 5 minutes
6. Remove slides from coplin jar and dip in distilled water to remove PBS salts

#### Staining

1. Incubate slides at RT in coplin jar with 0.01% calcufluor (diluted in water) in dark for 4 hours
2. Dip slides in distilled water to rinse
3. Stain with PI (1:100 from stock) lay slides flat in dark for 10 minutes
4. Put 1-3 drops mounting media (10:1 glycerol to PBS mixsture) and coverslip
5. Blot edges with Kim wipe
6. Image with microscope

#### **Cryosectioning with sucrose gradient**

##### Solution and Sample preparation

1. 24 hours before fixation, add stock solution of Fluorescent Brightener 28 (calcufluor) to live cells to final concentration in media of 0.01% and incubate in the dark for 24 hours
2. Make fixative solution (10 ml for each sample vial, this makes 50 ml total)
  - a. 5.4 mls 37% formaldehyde (freshly prepared from powder or frozen stock)
  - b. 20  $\mu$ L 25% glutaraldehyde
  - c. add nanopure water to reach 50 ml
3. Make 2.3 M sucrose, dissolved into water
4. Make 0.1 M Sorensen buffer
5. Make PBS from packet or from recipe

#### Cell Fixation

1. Filter aggregates and place cells in scintillation vial
2. Rinse briefly with PBS to remove media, pipette off PBS
3. Pipette 10 ml of prepared fixative into each sample vial
4. Place open sample vials in vacuum chamber and turn on vacuum for 1 min
5. Remove sample vial from vacuum, cap and Incubate 12-24 hours at 4 C

#### Cryoprotection

1. Pipette out fixation solution from scintillation vials, add 10 ml PBS to cells and let sit for 15 min
2. Prepare iterative dilutions of 2.3 M sucrose solution into 0.1 M Sorensen buffer: 25, 33, 50, 66, 75 and 100%
3. Pipette out PBS and pipette 25% sucrose solution into vial, let sit for one hour
4. Pipette out 25% sucrose solution and pipette in 33% sucrose solution, let sit for one hour, repeat until 100% solution is reached



5. For 100% sucrose solution, let sit in refrigerator overnight
6. Freeze cells within 24 hours

#### Freezing

1. In a square plastic mold, submerge cells in OCT freezing solution
2. Submerge mold in liquid nitrogen for 10 seconds until hardened
3. Cover with aluminum foil and freeze at -80 C until use

#### **Cryosectioning without sucrose gradient**

##### Solution and Sample preparation

1. 24 hours before fixation, add stock solution of Fluorescent Brightener 28 (calcufluor) to live cells to final concentration in media of 0.01% and incubate in the dark for 24 hours
2. Make fixative solution (10 ml for each sample vial, this makes 50 ml total)
  - a. 5.4 mls 37% formaldehyde (freshly prepared from powder or frozen stock)
  - b. 20  $\mu$ L 25% glutaraldehyde
  - c. add nanopure water to reach 50 ml
3. Make 2.3 M sucrose, dissolved into water
4. Make PBS from packet or from recipe

##### Cell Fixation

1. Filter aggregates and place cells in scintillation vial
2. Rinse briefly with PBS to remove media, pipette off PBS
3. Pipette 10 ml of prepared fixative into each sample vial
4. Place open sample vials in vacuum chamber and turn on vacuum for 1 min

5. Remove sample vial from vacuum, cap and Incubate 12-24 hours at 4 C

#### Cryoprotection

1. Pipette out fixation solution from scintillation vials, add 10 ml PBS to cells and let sit for 15 min
2. Pipette out PBS and pipette sucrose solution into vial, let sit overnight.
3. Freeze cells within 24 hours

#### Freezing

1. In a square plastic mold, submerge cells in OCT freezing solution
2. Submerge mold in liquid nitrogen for 10 seconds until hardened
3. Cover with aluminum foil and freeze at -80 C until use

#### **Flash freezing of fresh tissue**

1. Filter aggregates and place cells in scintillation vial
2. Rinse briefly with PBS to remove media, pipette off PBS
3. In a square plastic mold, submerge cells in OCT freezing solution
4. Submerge mold in liquid nitrogen for 10 seconds until hardened
5. Cover with aluminum foil and freeze at -80 C until use

#### **Cryosectioning**

1. Cool cryostat, blade and metal sample chucks to -20 C
2. Remove frozen sample from mold and if desired, cut to desired size

3. Add a thick layer of OCT compound to metal sample chuck and lightly press sample onto the OCT
4. Allow sample to harden inside cryostat for >30 minutes
5. Set cryostat to thick slices (50  $\mu\text{m}$ ), set chuck/sample in place and begin turning crank until blade comes into contact with sample. Make some thick slices to even out top of sample
6. Set cryostat to desired slice thickness (5 to 10  $\mu\text{m}$ ) and begin slicing
  - a. After each slice, take a warm poly-lysine coated slide and hover the slide a few mm above sample – sample will jump up to slide
  - b. Allow each slide to sit at room temperature for >1 hour to dry

### **Immunostaining for paclitaxel**

1. Solution preparation:
  - a. Prepare blocking buffer: 2% nonfat milk powder (w/w%) in PBS.
  - b. Prepare primary antibody solution: 1:100 dilution of anti-taxol mouse antibody in blocking buffer
  - c. Prepare secondary antibody solution: 1:200 dilution of anti-mouse antibody in blocking buffer
2. In a coplin jar, immerse dried slides in blocking buffer and let sit 1 hour
3. Dip slides in PBS briefly to remove blocking buffer
4. In a box lined with damp paper towels, lay slides flat. Blot excess blocking buffer from edge of slide using a Kim wipe.
5. Pipette 1 ml of diluted primary antibody onto each slide. Cut a piece of parafilm to cover the slices on the slide, and lay parafilm gently over the slices (do not stretch)
6. Let slides incubate in the dark for 1.5 hours

7. Remove parafilm from slides, place in coplin jar, and immerse in PBS for 15 minutes on shaker, put slides in fresh PBS and wash for 15 minutes, repeat one more time (3 total washes)
8. Pipette 1 ml of diluted secondary antibody onto each slide. Cut a piece of parafilm to cover the slices on the slide, and lay parafilm gently over the slices
9. Let the slides incubate in the dark for 1.5 hours
10. Remove parafilm and perform 3 PBS washes as in step 7 above
11. Dip slides in distilled water to remove salts
12. If desired, stain nuclei with PI as in paraffin embedding protocol above.
13. To preserve slides, add one drop of aqueous mounting medium and gently place coverslip over slices.

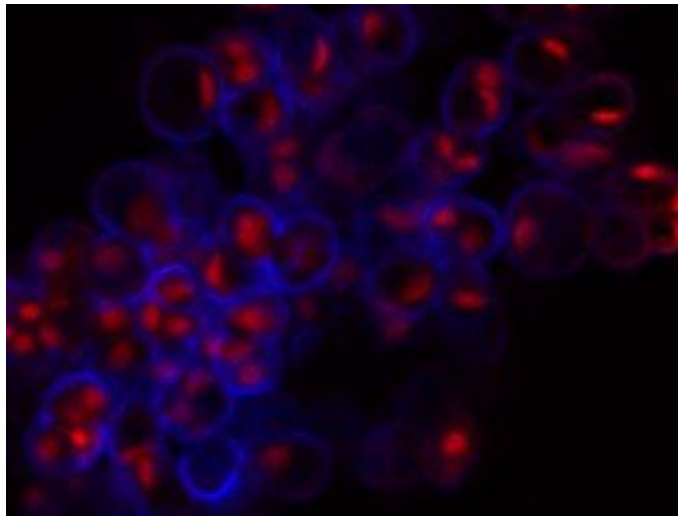


Figure 8 – Example of a cryosectioned *Taxus* aggregate stained with calcofluor (blue) to designate cell wall and propidium iodide (red) to denote the nucleus. This aggregate was sliced into 30  $\mu\text{m}$  slices.

## BIBLIOGRAPHY

- Ajikumar, P.K. et al., 2010. Isoprenoid pathway optimization for Taxol precursor overproduction in *Escherichia coli*. *Science (New York, N.Y.)*, 330(6000), pp.70–4. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3034138&tool=pmcentrez&rendertype=abstract> [Accessed June 10, 2011].
- Baebler, Š. et al., 2005. Establishment of cell suspension cultures of yew (*Taxus* × *media* Rehd.) and assessment of their genomic stability. *In Vitro Cellular & Developmental Biology - Plant*, 41(3), pp.338–343. Available at: <http://www.springerlink.com/index/10.1079/IVP2005642> [Accessed December 10, 2010].
- Becker, S. et al., 2007. Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox. *Nature protocols*, 2(3), pp.727–38. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/17406635> [Accessed July 14, 2012].
- Becker, S. & Palsson, B.O., 2008a. Context-specific metabolic networks are consistent with experiments. *PLoS computational biology*, 4(5), p.e1000082. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2366062&tool=pmcentrez&rendertype=abstract> [Accessed July 13, 2012].
- Becker, S. & Palsson, B.O., 2008b. Three factors underlying incorrect in silico predictions of essential metabolic genes. *BMC systems biology*, 2, p.14. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2248557&tool=pmcentrez&rendertype=abstract> [Accessed July 9, 2013].
- Bordbar, A. & Palsson, B., 2012. Using the reconstructed genome-scale human metabolic network to study physiology and pathology. *Journal of internal medicine*, 271(2), pp.131–41. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/22142339> [Accessed September 7, 2012].
- Borodina, I. & Nielsen, J., 2005. From genomes to in silico cells via metabolic networks. *Current opinion in biotechnology*, 16(3), pp.350–5. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/15961036> [Accessed July 13, 2012].
- Bourgaud, F., 2001. Production of plant secondary metabolites: a historical perspective. *Plant Science*, 161(5), pp.839–851. Available at: <http://linkinghub.elsevier.com/retrieve/pii/S0168945201004903>.
- Bräutigam, A. & Gowik, U., 2010. What can next generation sequencing do for you? Next generation sequencing as a valuable tool in plant research. *Plant biology (Stuttgart, Germany)*, 12(6), pp.831–41. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21040298> [Accessed July 11, 2011].
- Bringi, V. et al., 2007. Enhanced production of Taxol and Taxanes by cell cultures of *Taxus* species.

- Chang, S.S. et al., 1994. Stable genetic transformation of *Arabidopsis thaliana* by *Agrobacterium* inoculation in planta. *The Plant journal : for cell and molecular biology*, 5(November 1993), pp.551–558.
- Chemler, J.A. & Koffas, M.A.G., 2008. Metabolic engineering for plant natural product biosynthesis in microbes. *Current opinion in biotechnology*, 19(6), pp.597–605. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/18992815> [Accessed July 20, 2011].
- Cheng, A.-X. et al., 2007. Plant Terpenoids : Biosynthesis and Ecological Functions. *Journal of Integrative Plant Biology*, 49(2), pp.179–186.
- Christen, A., Gibson, D.M. & Bland, J., 1991. Production of taxol or taxol-like compounds in cell culture.
- Colijn, C. et al., 2009. Interpreting expression data with metabolic flux models: predicting *Mycobacterium tuberculosis* mycolic acid production. *PLoS computational biology*, 5(8), p.e1000489. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2726785&tool=pmcentrez&rendertype=abstract> [Accessed March 20, 2014].
- Conesa, A. et al., 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics (Oxford, England)*, 21(18), pp.3674–6. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/16081474> [Accessed July 16, 2012].
- Cragg, G. et al., 1993. The taxol supply crisis. New NCI policies for handling the large-scale production of novel natural product anticancer and anti-HIV agents. *Journal of natural products*, 56(10), pp.1657–68. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/7903979>.
- Cragg, G. & Snader, K., 1991. Taxol: The Supply Issue. *Cancer Cells*.
- Cragg, G.M. et al., 1995. Pharmaceutical Prospecting and the Potential for Pharmaceutical Crops. Natural Product Drug Discovery and Development at the United States National Cancer Institute. *Annals of the Missouri Botanical Garden*, 82(1), pp.47–53.
- Croteau, R. et al., 2006. Taxol biosynthesis and molecular genetics. *Phytochemistry reviews : proceedings of the Phytochemical Society of Europe*, 5(1), pp.75–97. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2901146&tool=pmcentrez&rendertype=abstract> [Accessed August 2, 2010].
- Dal'Molin, C.G.D.O. et al., 2010. C4GEM, a genome-scale metabolic model to study C4 plant metabolism. *Plant physiology*, 154(4), pp.1871–85. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2996019&tool=pmcentrez&rendertype=abstract> [Accessed August 21, 2012].
- Denis, J.-N. et al., 1988. A Highly Efficient, Practical Approach to Natural Taxol. *J. Am. Chem. Soc.*, 110, pp.5917–5919.

- Dietrich, J. a et al., 2009. A novel semi-biosynthetic route for artemisinin production using engineered substrate-promiscuous P450(BM3). *ACS chemical biology*, 4(4), pp.261–7. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/19271725>.
- Duarte, N.C., Herrgård, M.J. & Palsson, B.Ø., 2004. Reconstruction and validation of *Saccharomyces cerevisiae* iND750, a fully compartmentalized genome-scale metabolic model. *Genome research*, 14(7), pp.1298–309. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=442145&tool=pmcentrez&rendertype=abstract> [Accessed July 19, 2012].
- Edwards, J.S. & Palsson, B.O., 2000. The *Escherichia coli* MG1655 in silico metabolic genotype : Its definition , characteristics , and capabilities. *PNAS*.
- Eibl, R. & Eibl, D., 2007. Design of bioreactors suitable for plant cell and tissue cultures. *Phytochemistry Reviews*, 7(3), pp.593–598. Available at: <http://www.springerlink.com/index/10.1007/s11101-007-9083-z> [Accessed June 30, 2011].
- Evans, B.E. et al., 1988. Methods for Drug Discovery: Development of Potent, Selective, Orally Effective Cholecystokinin Antagonists. *J. Med. Chem.*, 31, pp.2235–2246.
- Expósito, O. et al., 2009. Biotechnological production of taxol and related taxoids: current state and prospects. *Anti-cancer agents in medicinal chemistry*, 9(1), pp.109–21. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/19149486>.
- Farkya, S., Bisaria, V.S. & Srivastava, A.K., 2004. Biotechnological aspects of the production of the anticancer drug podophyllotoxin. *Applied microbiology and biotechnology*, 65(5), pp.504–19. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/15378293> [Accessed November 3, 2011].
- Feist, A.M. & Palsson, B.O., 2010. The biomass objective function. *Current opinion in microbiology*, 13(3), pp.344–349. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/20430689>.
- Firn, R.D. & Jones, C.G., 2003. Natural products: A simple model to explain chemical diversity. *Natural Product Reports*, 20(4), p.382. Available at: <http://xlink.rsc.org/?DOI=b208815k> [Accessed July 21, 2011].
- Fischbach, M.A. & Clardy, J., 2007. One pathway , many products. *Nature Chemical Biology*, 3(7), pp.353–355.
- Van der Fits, L. & Memelink, J., 2000. ORCA3, a Jasmonate-Responsive Transcriptional Regulator of Plant Primary and Secondary Metabolism. *Science*, 289(5477), pp.295–297. Available at: <http://www.sciencemag.org/cgi/doi/10.1126/science.289.5477.295> [Accessed October 12, 2011].
- Fonseca, S. et al., 2009. (+)-7-iso-Jasmonoyl-L-isoleucine is the endogenous bioactive jasmonate. *Nature chemical biology*, 5(5), pp.344–50. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/19349968> [Accessed July 24, 2012].

- Gelvin, S.B., 2003. Improving plant genetic engineering by manipulating the host. *Trends in biotechnology*, 21(3), pp.95–8. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/12628361>.
- Georgiev, M., Ludwig-Muller, J. & Bley, T., 2010. Hairy Root Culture: Copying Nature in New Bioprocesses. In R. Arora, ed. *Medicinal Plant Biotechnology*. Cambridge, MA: CABI, pp. 156–175.
- Georgiev, M., Pavlov, A. & Ilieva, M., 2006. Selection of high rosmarinic acid producing *Lavandula vera* MM cell lines. *Process Biochemistry*, 41(9), pp.2068–2071. Available at: <http://linkinghub.elsevier.com/retrieve/pii/S1359511306001942> [Accessed January 19, 2012].
- Georgiev, M.I., Weber, J. & Maciuk, A., 2009. Bioprocessing of plant cell cultures for mass production of targeted compounds. *Applied microbiology and biotechnology*, 83(5), pp.809–23. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/19488748> [Accessed January 9, 2011].
- De Geyter, N. et al., 2012. Transcriptional machineries in jasmonate-elicited plant secondary metabolism. *Trends in Plant Science*, 17(6), pp.349–359. Available at: <http://linkinghub.elsevier.com/retrieve/pii/S1360138512000532> [Accessed March 29, 2012].
- Gräwert, T. et al., 2011. Biochemistry of the non-mevalonate isoprenoid pathway. *Cellular and molecular life sciences : CMLS*, 68(23), pp.3797–814. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21744068> [Accessed September 4, 2012].
- Gundlach, H. et al., 1992. Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. *Plant biology*, 89(March), pp.2389–2393.
- Hale, V. et al., 2007. Microbially derived artemisinin: a biotechnology solution to the global problem of access to affordable antimalarial drugs. *The American journal of tropical medicine and hygiene*, 77(6 Suppl), pp.198–202. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/18165493>.
- Hampel, D., Mau, C.J.D. & Croteau, R.B., 2009. Taxol biosynthesis: Identification and characterization of two acetyl CoA:taxoid-O-acetyl transferases that divert pathway flux away from Taxol production. *Archives of biochemistry and biophysics*, 487(2), pp.91–7. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2748654&tool=pmcentrez&rendertype=abstract> [Accessed March 3, 2011].
- Hanada, K. et al., 2009. Evolutionary persistence of functional compensation by duplicate genes in Arabidopsis. *Genome biology and evolution*, 1, pp.409–14. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2817435&tool=pmcentrez&rendertype=abstract> [Accessed May 29, 2013].
- Harding, K., 2004. Genetic integrity of cryopreserved plant cells: A review. *Cryoletters*, 25(1), pp.3–22.



- Hartmann, T., 2007. From waste products to ecochemicals: fifty years research of plant secondary metabolism. *Phytochemistry*, 68(22-24), pp.2831–46. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/17980895> [Accessed July 20, 2011].
- Henry, C.S. et al., 2010. High-throughput generation, optimization and analysis of genome-scale metabolic models. *Nature biotechnology*, 28(9), pp.977–82. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/20802497> [Accessed July 16, 2012].
- Holton, R.A. et al., 1994. First Total Synthesis of Taxol. 1. Functionalization of the B Ring. *J. Am. Chem. Soc.*, 116(2), pp.1597–1598.
- Humphreys, J.M. & Chapple, C., 2002. Rewriting the lignin roadmap. *Current opinion in Plant Biology*, 5, pp.224–229.
- Jensen, P.A., Lutz, K.A. & Papin, J.A., 2011. TIGER : Toolbox for integrating genome-scale metabolic models , expression data , and transcriptional regulatory networks. *BMC Systems Biology*, 5(1), p.147. Available at: <http://www.biomedcentral.com/1752-0509/5/147>.
- Kanehisa, M. & Goto, S., 2000. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic acids research*, 28(1), pp.27–30. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=102409&tool=pmcentrez&rendertype=abstract>.
- Kim, Y., Wyslouzil, B.E. & Weathers, P.J., 2002. Secondary metabolism of hairy root cultures in bioreactors. *In Vitro Cellular & Developmental Biology - Plant*, 38(1), pp.1–10. Available at: <http://www.springerlink.com/index/10.1079/IVP2001243> [Accessed June 16, 2011].
- Kingston, D.G.I., 1994. Taxol: The chemistry and structure-activity relationships of a novel anticancer agent. *Trends in biotechnology*, 12(6), pp.222–227.
- Koehn, F.E. & Carter, G.T., 2005. The evolving role of natural products in drug discovery. *Nature reviews. Drug discovery*, 4(3), pp.206–20. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/15729362> [Accessed July 23, 2011].
- Kolewe, M.E., Gaurav, V. & Roberts, S.C., 2008. Pharmaceutically active natural product synthesis and supply via plant cell culture technology. *Molecular pharmaceuticals*, 5(2), pp.243–56. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/18393488>.
- Kolewe, M.E., Henson, M.A. & Roberts, S.C., 2011. Analysis of aggregate size as a process variable affecting paclitaxel accumulation in *Taxus* suspension cultures. *Biotechnology progress*, 27(5), pp.1365–72. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21692199> [Accessed January 18, 2012].
- Kutchan, T.M., 2005. A role for intra- and intercellular translocation in natural product biosynthesis. *Current opinion in plant biology*, 8(3), pp.292–300. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/15860426> [Accessed September 2, 2011].

- Lee, E.-K. et al., 2010. Cultured cambial meristematic cells as a source of plant natural products. *Nature biotechnology*, 28(11), pp.1213–1217. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/20972422> [Accessed October 28, 2010].
- Lee, S.J. et al., 2005. Metabolic Engineering of *Escherichia coli* for Enhanced Production of Succinic Acid , Based on Genome Comparison and In Silico Gene Knockout Simulation Metabolic Engineering of *Escherichia coli* for Enhanced Production of Succinic Acid , Based on Genome Com. *Applied and environmental microbiology*, 71(12), pp.7880–7887.
- Leonard, E. et al., 2008. Strain improvement of recombinant *Escherichia coli* for efficient production of plant flavonoids. *Molecular pharmaceuticals*, 5(2), pp.257–65. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/18333619>.
- Leone, L.M. & Roberts, S.C., 2013. Accessing Anti-Cancer Natural Products by Plant Cell Culture. In F. E. Koehn, ed. *Natural Products and Cancer Drug Discovery*. Springer New York, pp. 193–211.
- Li, L. et al., 2012. Determining degradation and synthesis rates of arabidopsis proteins using the kinetics of progressive <sup>15</sup>N labeling of two-dimensional gel-separated protein spots. *Molecular & cellular proteomics : MCP*, 11(6), p.M111.010025. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3433911&tool=pmcentrez&rendertype=abstract> [Accessed March 21, 2014].
- Lipkus, A.H. et al., 2008. Structural diversity of organic chemistry. A scaffold analysis of the CAS Registry. *The Journal of organic chemistry*, 73(12), pp.4443–51. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/18505297>.
- Lloyd, J. & Meinke, D., 2012. A comprehensive dataset of genes with a loss-of-function mutant phenotype in *Arabidopsis*. *Plant physiology*, 158(3), pp.1115–29. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3291275&tool=pmcentrez&rendertype=abstract> [Accessed September 29, 2013].
- Mintz-Oron, S. et al., 2011. Reconstruction of *Arabidopsis* metabolic network models accounting for subcellular compartmentalization and tissue-specificity. *Proceedings of the National Academy of Sciences*, (7), pp.1–6. Available at: <http://www.pnas.org/cgi/doi/10.1073/pnas.1100358109> [Accessed December 20, 2011].
- Mueller, M.J. et al., 1993. Signaling in the elicitation process is mediated through the octadecanoid pathway leading to jasmonic acid. *Proceedings of the National Academy of Sciences of the United States of America*, 90(16), pp.7490–4. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=47167&tool=pmcentrez&render type=abstract>.
- Mustafa, N.R. et al., 2011. Initiation, growth and cryopreservation of plant cell suspension cultures. *Nature protocols*, 6(6), pp.715–42. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21637194> [Accessed August 1, 2011].
- Nagel, A. et al., 2005. Extension of the Visualization Tool MapMan to Allow Statistical Analysis of Arrays , Display of Corresponding Genes , and Comparison with Known Responses 1. , 138(July), pp.1195–1204.

- Naik, R. et al., 1988. An antiinflammatory cum immunomodulatory piperidinylbenzopyranone from *dysoxylum binectariferum* : isolation, structure and total synthesis. *Tetrahedron*, 44(7), pp.2081–2086.
- Naill, M.C. & Roberts, S.C., 2005a. Culture of isolated single cells from *Taxus* suspensions for the propagation of superior cell populations. *Biotechnology letters*, 27(21), pp.1725–30. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/16247682> [Accessed August 2, 2010].
- Naill, M.C. & Roberts, S.C., 2005b. Flow cytometric identification of Paclitaxel-accumulating subpopulations. *Biotechnology progress*, 21(3), pp.978–83. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/15932283>.
- Nakagawa, A. et al., 2011. A bacterial platform for fermentative production of plant alkaloids. *Nature communications*, 2(May), p.326. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3112539&tool=pmcentrez&rendertype=abstract> [Accessed July 26, 2011].
- Newman, D.J. & Cragg, G.M., 2007. Natural products as sources of new drugs over the last 25 years. *Journal of natural products*, 70(3), pp.461–77. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/17309302>.
- Ni, X. et al., 2011. Enhancement of camptothecin production in *Camptotheca acuminata* hairy roots by overexpressing ORCA3 gene. *Journal of Applied Pharmaceutical Science*, 01(08), pp.85–88.
- Nicolau, K.C. et al., 1994. Total synthesis of taxol. *Nature*, 367, pp.630–634.
- Niklas, J., Schneider, K. & Heinzle, E., 2010. Metabolic flux analysis in eukaryotes. *Current opinion in biotechnology*, 21(1), pp.63–9. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/20163950> [Accessed June 21, 2011].
- Nims, E. et al., 2006. Expression profiling of genes involved in paclitaxel biosynthesis for targeted metabolic engineering. *Metabolic engineering*, 8(5), pp.385–94. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/16793302> [Accessed August 2, 2010].
- O’Grady, J. et al., 2012. Metabolic cartography: experimental quantification of metabolic fluxes from isotopic labelling studies. *Journal of experimental botany*, 63(6), pp.2293–308. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/22371075> [Accessed July 26, 2012].
- De Oliveira Dal’Molin, C.G. et al., 2010. AraGEM, a genome-scale reconstruction of the primary metabolic network in *Arabidopsis*. *Plant physiology*, 152(2), pp.579–89. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2815881&tool=pmcentrez&rendertype=abstract> [Accessed July 28, 2012].
- Orth, J.D., Thiele, I. & Palsson, B.Ø., 2010. What is flux balance analysis? *Nature biotechnology*, 28(3), pp.245–8. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/20212490>.

- Ortholand, J.-Y. & Ganesan, a, 2004. Natural products and combinatorial chemistry: back to the future. *Current opinion in chemical biology*, 8(3), pp.271–80. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/15183325> [Accessed October 11, 2011].
- Patil, R. et al., 2012. Contribution of taxane biosynthetic pathway gene expression to observed variability in paclitaxel accumulation in *Taxus* suspension cultures. *Biotechnology journal*, 7(3), pp.418–27. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/22095859> [Accessed September 16, 2012].
- Patil, R., 2013. *Molecular and Population Level Approaches to Understand Taxus Metabolism in Cell Suspension Cultures*. University of Massachusetts Amherst.
- Patil, R. et al., 2011. Taxane biosynthetic pathway gene expression in *Taxus* suspension cultures with different bulk paclitaxel accumulation patterns & a molecular approach to understand variability in paclitaxel accumulation. *Biotechnology journal*, pp.1–10. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/22095859> [Accessed January 16, 2012].
- Pauwels, L. et al., 2008. Mapping methyl jasmonate-mediated transcriptional reprogramming of metabolism and cell cycle progression in cultured *Arabidopsis* cells. *Proceedings of the National Academy of Sciences of the United States of America*, 105(4), pp.1380–5. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2234147&tool=pmcentrez&rendertype=abstract>.
- Pauwels, L., Inzé, D. & Goossens, A., 2009. Jasmonate-inducible gene: What does it mean? *Trends in plant science*, 14(2), pp.87–91. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/19162528> [Accessed March 10, 2013].
- Piques, M. et al., 2009. Ribosome and transcript copy numbers, polysome occupancy and enzyme dynamics in *Arabidopsis*. *Molecular systems biology*, 5(314), p.314. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2779082&tool=pmcentrez&rendertype=abstract> [Accessed March 21, 2014].
- Poolman, M.G. et al., 2009. A genome-scale metabolic model of *Arabidopsis* and some of its properties. *Plant physiology*, 151(3), pp.1570–81. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2773075&tool=pmcentrez&rendertype=abstract> [Accessed July 20, 2012].
- Poolman, M.G. et al., 2013. Responses to Light Intensity in a Genome-Scale Model of Rice Metabolism. *Plant physiology*, 162(June), pp.1060–1072. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/23640755> [Accessed May 31, 2013].
- Reed, J.L. et al., 2003. An expanded genome-scale model of *Escherichia coli* K-12 (iJR904 GSM/GPR). *Genome biology*, 4(9), p.R54. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=193654&tool=pmcentrez&rendertype=abstract>.
- Ro, D.-K. et al., 2006. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature*, 440(7086), pp.940–3. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/16612385> [Accessed July 6, 2011].

- Roberts, S. & Kolewe, M., 2010. Plant natural products from cultured multipotent cells. *Nature biotechnology*, 28(11), pp.1175–6. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21057485> [Accessed January 7, 2011].
- Roberts, S.C., 2007. Production and engineering of terpenoids in plant cell culture. *Nature chemical biology*, 3(7), pp.387–95. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/17576426>.
- Ruiz-May, E. et al., 2011. Methyl jasmonate induces ATP biosynthesis deficiency and accumulation of proteins related to secondary metabolism in *Catharanthus roseus* (L.) G. hairy roots. *Plant & Cell Physiology*, 52(8), pp.1401–21. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21727181> [Accessed August 20, 2012].
- Saha, R., Suthers, P.F. & Maranas, C.D., 2011. Zea mays i RS1563 : A Comprehensive Genome-Scale Metabolic Reconstruction of Maize Metabolism. *PLoS biology*, 6(7).
- Schäfer, H. & Wink, M., 2009. Medicinally important secondary metabolites in recombinant microorganisms or plants: progress in alkaloid biosynthesis. *Biotechnology journal*, 4(12), pp.1684–703. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/19946877> [Accessed November 29, 2011].
- Schnur, D.M. et al., 2011. *Cheminformatics and Computational Chemical Biology* J. Bajorath, ed., Totowa, NJ: Humana Press. Available at: <http://www.springerlink.com/index/10.1007/978-1-60761-839-3> [Accessed June 17, 2011].
- Schwanhäusser, B. et al., 2011. Global quantification of mammalian gene expression control. *Nature*, 473(7347), pp.337–42. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21593866> [Accessed March 20, 2014].
- Senger, R.S. et al., 2006. Development of a culture sub-population induction model: signaling pathways synergy and taxanes production by *Taxus canadensis*. *Biotechnology progress*, 22(6), pp.1671–82. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/17137317>.
- Solomon, L.D., 2011. *Synthetic Biology: Science, Business, and Policy*, Transaction Publishers.
- Sriram, G., Fulton, D.B. & Shanks, J. V, 2007. Flux quantification in central carbon metabolism of *Catharanthus roseus* hairy roots by <sup>13</sup>C labeling and comprehensive bondomer balancing. *Phytochemistry*, 68(16-18), pp.2243–57. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/17532015> [Accessed September 14, 2012].
- Stitt, M., 2013. Systems-integration of plant metabolism: means, motive and opportunity. *Current opinion in plant biology*, 16(3), pp.381–388. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/23642787> [Accessed May 27, 2013].
- Stitt, M., Sulpice, R. & Keurentjes, J., 2010. Metabolic networks: how to identify key components in the regulation of metabolism and growth. *Plant physiology*, 152(2), pp.428–44. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2815907&tool=pmcentrez&rendertype=abstract> [Accessed July 28, 2011].

- Swiatek, A. et al., 2002. Differential Effect of Jasmonic Acid and Abscisic Acid on Cell Cycle Progression in Tobacco BY-2 Cells 1. *Plant physiology*, 128(January), pp.201–211.
- Verpoorte, R. et al., 1999. Metabolic engineering of plant secondary metabolite pathways for the production of fine chemicals. *Cultures*, (Verpoorte), pp.467–479.
- Weathers, P. et al., 2008. Mist reactors : principles , comparison of various systems , and case studies. *Biotechnology and Bioengineering*, 3(October), pp.29–37.
- Werck-Reichhart, D. & Feyereisen, R., 2000. Protein family review Cytochromes P450 : a success story. *Genome biology*, 1(6), pp.1–9.
- Wilson, R.M. & Danishefsky, S.J., 2006. Small molecule natural products in the discovery of therapeutic agents: the synthesis connection. *The Journal of organic chemistry*, 71(22), pp.8329–51. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/17064003>.
- Wilson, S. & Roberts, S.C., 2011. Recent advances towards development and commercialization of plant cell culture processes for the synthesis of biomolecules. *Plant biotechnology journal*, pp.1–20. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/22059985> [Accessed November 15, 2011].
- Yukimune, Y. et al., 1996. Methyl jasmonate-induced overproduction of paclitaxel and baccatin III in *Taxus* cell suspension cultures. *Nature Biotechnology*, 14.
- Zhao, J., Davis, L.C. & Verpoorte, R., 2005. Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnology advances*, 23(4), pp.283–333. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/15848039> [Accessed July 24, 2011].