Molecular Breeding for Fruit Quality
Challenges and Opportunities
Why fruit quality?

Flavor is generally accepted to be poor. Improved flavor can impact eating habits and therefore, health.

Fruits and vegetables are the major source of micronutrients in the diet. Up to 50% of the world’s population suffers from micronutrient malnutrition (WHO).

Quality fruits and vegetables present significant opportunities for profit and diversification.
The concept of “quality” involves manipulation of secondary metabolite pathways. The major issues are:

Defining the targets – which compounds are really important?
   Nearly 60% of the compounds in a tomato fruit are unidentified

Developing a reliable assay system that minimizes environmental influences

Defining the targeted metabolic pathways

Defining the genes controlling those pathways

Successful metabolic engineering

The cost of accurate phenotyping greatly exceeds cost of genotyping
THE CHEMICAL CONSTITUENTS OF TOMATO FLAVOR

**Sugars:** glucose, fructose  
**Acids:** citrate, malate, ascorbate  
**Volatile:** derived from carotenoids, lipids, amino acids
<table>
<thead>
<tr>
<th>Volatile</th>
<th>[ppb]</th>
<th>Precursor</th>
<th>Odor</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-3-hexenal</td>
<td>12,000</td>
<td>lipid</td>
<td>tomato/green</td>
</tr>
<tr>
<td>β-ionone</td>
<td>4</td>
<td>carotenoid</td>
<td>fruity/floral</td>
</tr>
<tr>
<td>hexanal</td>
<td>3,100</td>
<td>lipid</td>
<td>green/grassy</td>
</tr>
<tr>
<td>β-damascenone</td>
<td>1</td>
<td>carotenoid</td>
<td>fruity</td>
</tr>
<tr>
<td>1-penten-3-one</td>
<td>520</td>
<td>lipid</td>
<td>fruity floral/green</td>
</tr>
<tr>
<td>2+3-methylbutanal</td>
<td>27</td>
<td>Ile/Leu</td>
<td>musty</td>
</tr>
<tr>
<td>trans-2-hexenal</td>
<td>270</td>
<td>lipid</td>
<td>green/grassy</td>
</tr>
<tr>
<td>2-isobutylthiazole</td>
<td>36</td>
<td>Ile?</td>
<td>tomato vine</td>
</tr>
<tr>
<td>1-nitro-2-phenylethane</td>
<td>17</td>
<td>Phe</td>
<td>musty, earthy</td>
</tr>
<tr>
<td>trans-2-heptenal</td>
<td>60</td>
<td>lipid</td>
<td>green/grassy</td>
</tr>
<tr>
<td>phenylacetaldehyde</td>
<td>15</td>
<td>Phe</td>
<td>floral/alcohol</td>
</tr>
<tr>
<td>6-methyl-5-hepten-2-one</td>
<td>130</td>
<td>carotenoid</td>
<td>fruity, floral</td>
</tr>
<tr>
<td>cis-3-hexenol</td>
<td>150</td>
<td>lipid</td>
<td>green/grassy</td>
</tr>
<tr>
<td>2-phenylethanol</td>
<td>1,900</td>
<td>Phe</td>
<td>nutty, floral</td>
</tr>
<tr>
<td>3-methylbutanol</td>
<td>380</td>
<td>Leu</td>
<td>earthy, musty</td>
</tr>
<tr>
<td>methyl salicylate</td>
<td>48</td>
<td>chorismate</td>
<td>wintergreen</td>
</tr>
</tbody>
</table>
The next steps: How do we convert this information into valuable materials?

Sequences of genes and SNPs are far and away the limiting step at present.

For non-transgenic approaches to quality improvement, there is very little genetic diversity in breeding materials.

For transgenic approaches, knowledge of pathway regulation will severely restrict progress.
Some examples

- erythrose-4-phosphate + PEP
- chorismate
- phenylalanine
- phenylethylamine
- methyl salicylate
- phenylacetaldehyde
- 1-nitro-2-phenethane
- 2-phenylethanol
- E-cinnamic acid
- p-coumaric acid
- ferulic acid
- flavonoids
- methyl benzoate
- eugenol
- methyl salicylate
- phenylacetaldehyde
- 1-nitro-2-phenethane
- methyl benzoate
- eugenol
Engineering the first step in a pathway

erthrose-4-phosphate + PEP

chorismate

phenylalanine

E-cinnamic acid

p-coumaric acid

ferulic acid

flavonoids

phenylethylamine

methyl salicylate

phenylacetaldehyde

1-nitro-2-phenethane

methyl benzoate

2-phenylethanol

eugenol
Engineering the last step in a pathway

erthrose-4-phosphate + PEP
chorismate
phenylalanine
E-cinnamic acid
p-coumaric acid
ferulic acid

flavonoids

salicylate
phenylethylamine

phenylacetaldheyde
1-nitro-2-phenethane
methyl benzoate

methyl salicylate

2-phenylethanol

eugenol
Engineering transcriptional control of the entire shikimate pathway

- erythrose-4-phosphate + PEP
- chorismate
- phenylalanine
- E-cinnamic acid
- p-coumaric acid
- ferulic acid
- methyl salicylate
- phenylethylamine
- phenylacetaldehyde
- 1-nitro-2-phenethane
- methyl benzoate
- 2-phenylethanol
- flavonoids
- eugenol
Discussion points – what’s limiting?

Separating genetics from environment. Reliable, high-throughput assays of complex phenotypes.

Sequences and alleles of genetic variants. You can’t select what’s not there.

Basic understanding of pathways and their regulation. How do we break through an iterative process?
A biochemical database for diverse germplasm

*L. esculentum*  
*L. pennellii*  
*L. hirsutum*