

Response gut epithelium to digested berries

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Goal of D3.3

- How can berries be better positioned based on health effects
 - Content
 - Health claims
- Identify potential leads for health effects of berry based on effects towards the gut & immune system
- Review on what is content of berries and what can be potentially used for positioning
- Study effects of berries towards gut epithelium
 - Potential new health benefit

Preparation of review on effects berries on gut & immune system

- What compounds do berries contain
- Human trials (berries and or compounds highly present in berries)
- Animal trials
- In vitro supportive analysis
- Gaps and new strategies
- Ready for 90%



What is in and can be used for positioning

	Raspberry				Strawberry		Blackberry		Blueberry		Black currant	
	Red Raspberry		Black Raspberry									
												
	/ 100 g	ADH/100g ADH/portion	/ 100 g	ADH/100g ADH/portion	/ 100 g	ADH/100g ADH/portion	/ 100 g	ADH/100g ADH/portion	/ 100 g	ADH/100g ADH/portion	/ 100 g	ADH/100g ADH/portion
Vitamins												
Vitamin C	26 mg	33% ** 22% *	26 mg	33% **	58.8 mg	74%						6% ** % **
Vitamin K	7.8 mcg											
Minerals												
Manganase	0.7 mg											13% 9%

Indication of content based on databases.
These are variable due to
genetic x environment x harvest time x postharvest conditions.
Know the variation in your product and know that you are above
threshold when using these content of health claims.

- * Source of; ** Rich in
- Many EFSA health claims on Vitamin C, K and manganese when product is 'source of'
- Vitamine C: 15 autoriseer claims

What is in and can not be used for promotion in EU yet

		Raspberry		Strawberry	Blackberry	Blueberry	Black currant
		Red	Black				
							
		/ 100 g	/ 100 g	/ 100 g	/ 100 g	/ 100 g	/ 100 g
Anthocyanidins	Cyanidin	45.77 mg	669.01 mg	1.68 mg	99.95 mg	8.46 mg	61.30 mg
	Delphinidin	1.32 mg		0.31 mg	0.00 mg	35.43 mg	87.86 mg
	Malvidin	0.13 mg		0.01 mg	0.00 mg	67.59 mg	
	Peonidin	0.12 mg	1.09 mg	0.05 mg	0.21 mg	20.29 mg	0.57 mg
	Petunidin	0.31 mg		0.11 mg	0.00 mg	31.53 mg	3.87 mg
	Pelargonidin	0.98 mg	16.69 mg	24.85 mg	0.45 mg	0.00 mg	1.17 mg
Flavan-3-ols	(-)-Epicatechin	3.52 mg		0.42 mg	4.66 mg	0.62 mg	0.47 mg
	(+)-Catechin	1.31 mg		3.11 mg	37.06 mg	5.29 mg	0.70 mg
Flavonols	Quercetin	1.05 mg	19 mg	1.11 mg	3.58 mg	7.67 mg	4.48 mg
	Myricetin	0.00 mg		0.04 mg	0.67 mg	1.30 mg	6.18 mg
Ellagitannins	Sanguin H-6	81.34 mg		4 mg	63 mg		
	Lambertianin C	35.68 mg		15 mg	90 mg		
Phenolic acids	Total ellagic acids	47 mg	90 mg	42 mg	140 mg		
	p-coumaric acid 4-O glucoside	0.32 mg		0.21 mg	0.27 mg	0.95 mg	0.51 mg
	Chlorogenic acid					30-110 mg	
Stilbenoids	Resveratrol					16.1 mcg	
	Trans-resveratrol			0.35 mg			

Interesting but can not be used for promotion yet.

Berries general:

48 submitted, 0 authorised

Berries specific:

14 submitted, 0 authorised

Goal

- Do berries have effect towards the gut epithelial and what can be learned from the response in relation to potential health effects
- Do all berries have similar effects or do different berries have different effects
- Can it be related to certain phytochemicals present in specific cultivars
- Develop product stably high in bioactivity
- Perform human trial with selected product and specific health biomarker in future
- Support direction of breeding & selection
- Give positive media attention to berries

Overview

Methods

Preparation of digested berry samples

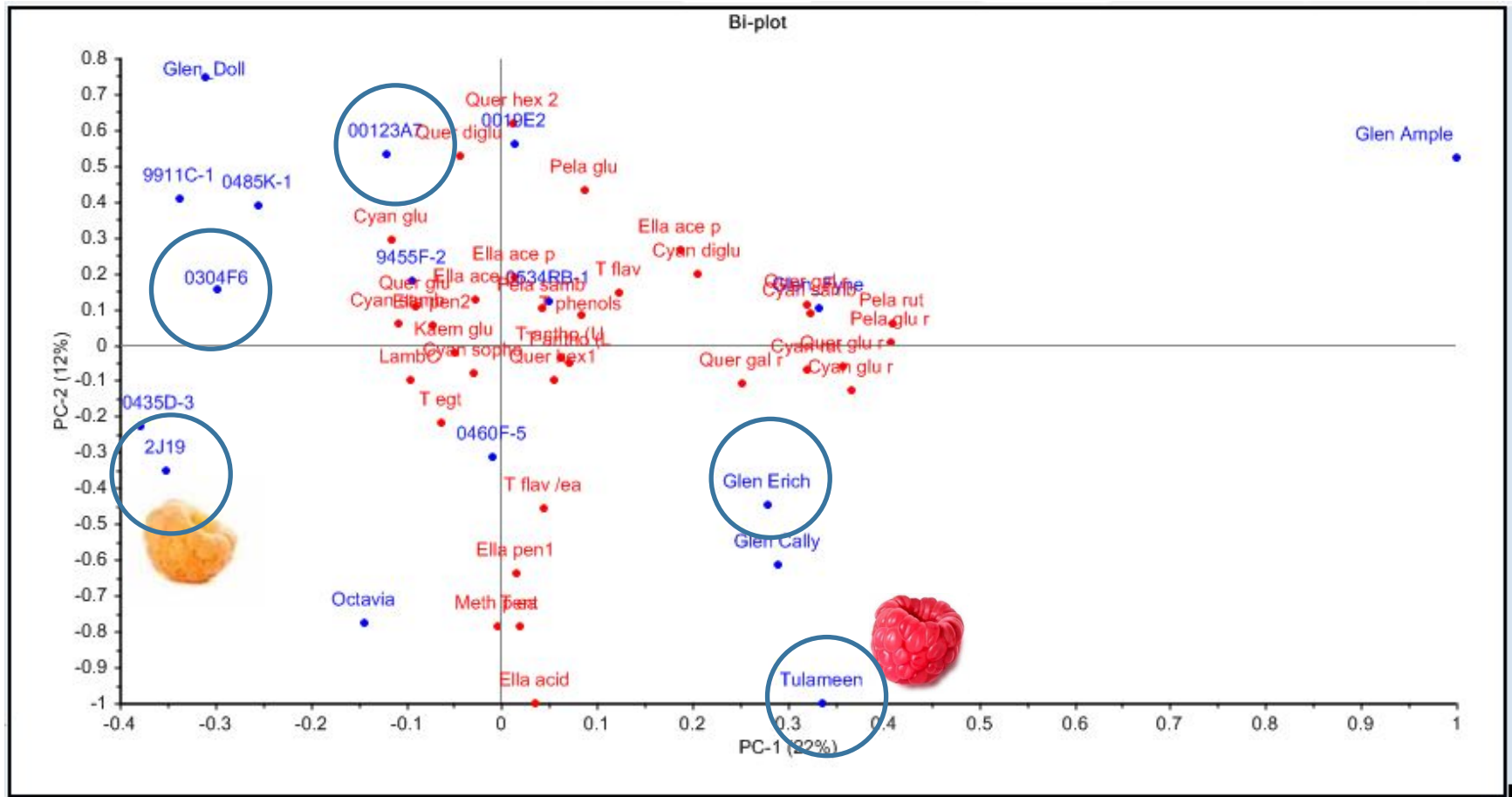
Exposure gut epithelial cells (Caco-2) and subsequent analysis

Gut epithelial very important for barrier, uptake nutrients and signalling to other cells and organs

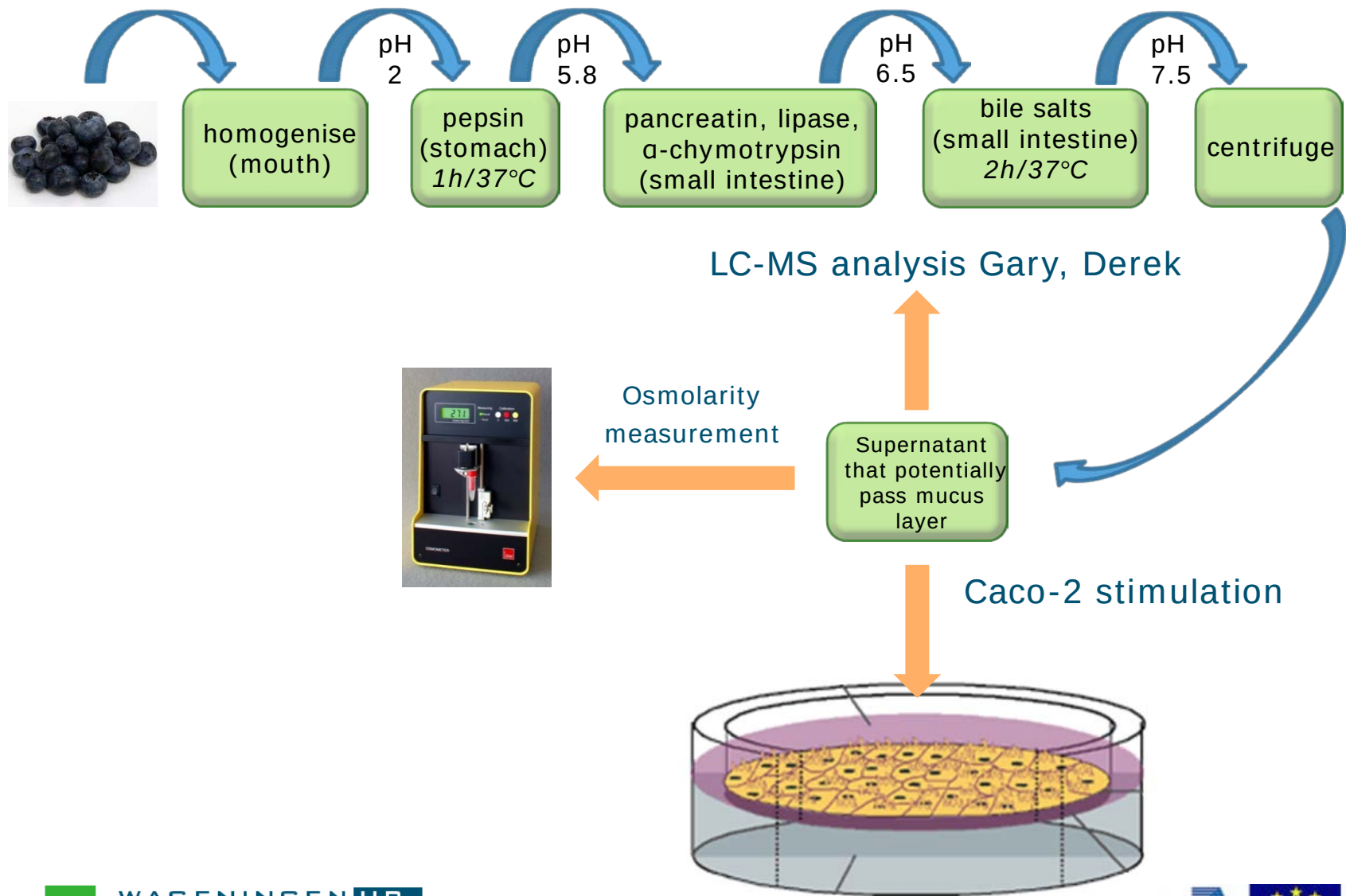
Caco-2 gene expression analysis by full human microarray's

- Top 10 up and down
- Linking osmolarity to genetic responses
- expression induced by all berries
- expression unique for species
- gene expression unique for groups following clustering

Selected most differential cultivars

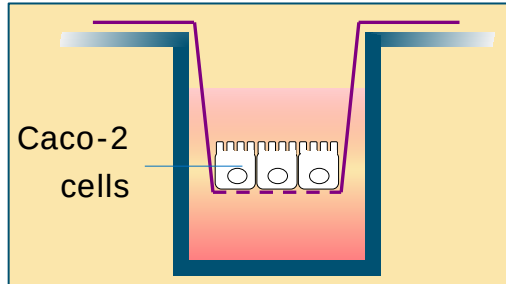


Preparation of digested berry samples



Caco-2 sample exposure and subsequent analysis

Harvesting mRNA from cells



Gene selection criteria

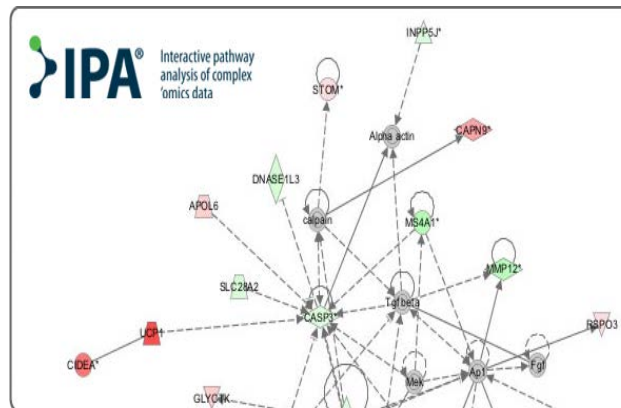
- UPC value >0.5 for at least 1 treatments
- $p < 0.05$ vs digest control for at least 1 treatment

Yielded 8814 genes of interest

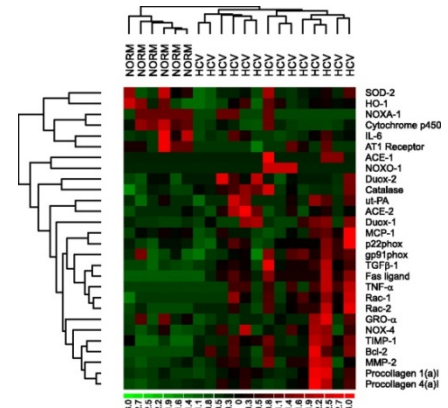
trans-epithelial electrical resistance (TEER)



Pathway analysis

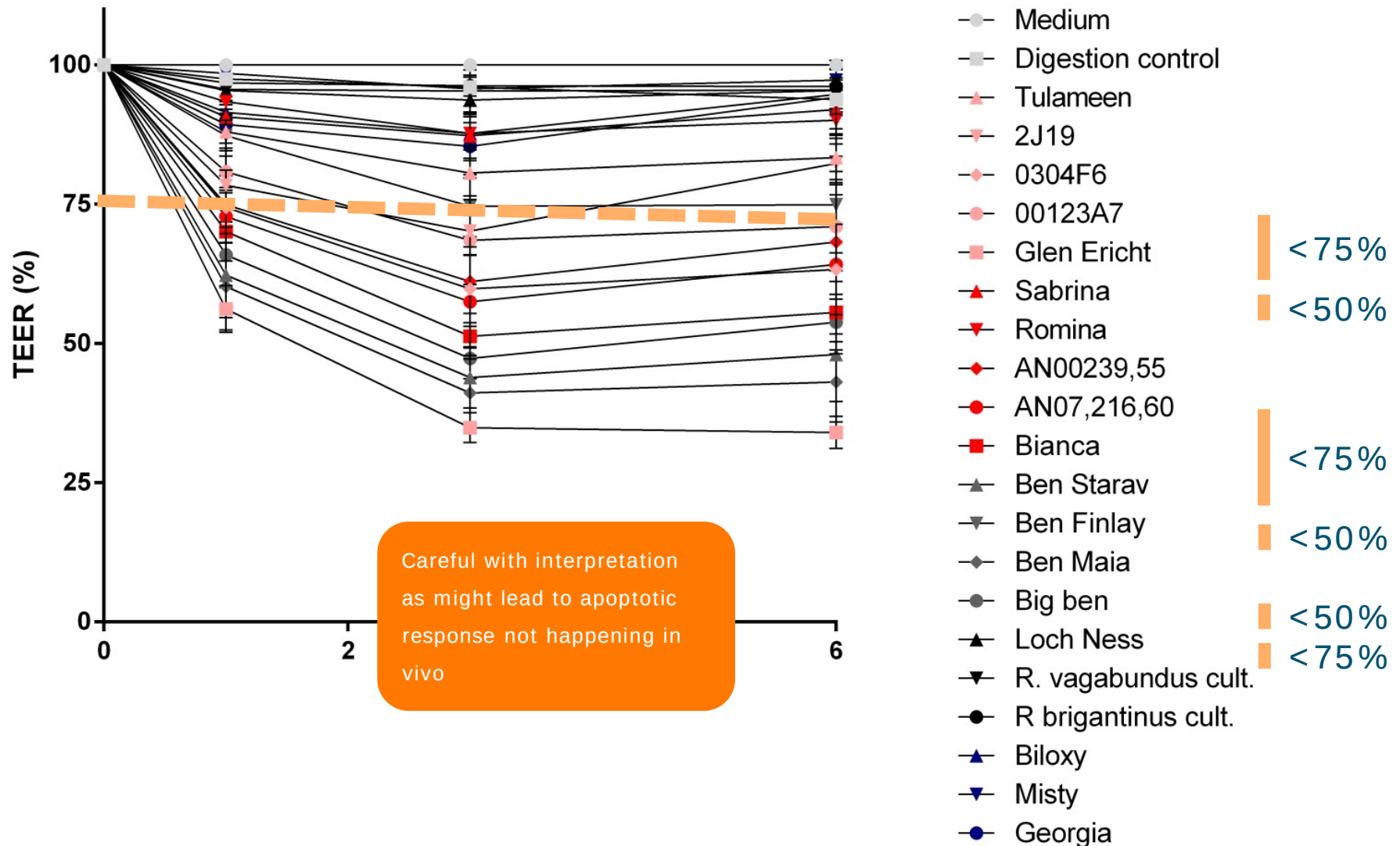


Cluster analysis



Berries affect membrane integrity

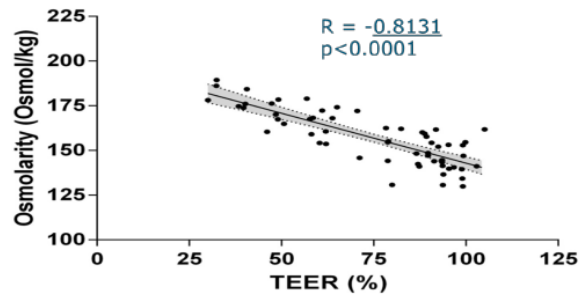
All berries



Some general background

- Cell lines are variable
- Food are mild modulators of epithelial cell responses
- Subtle changes in gene expression
- In vitro digest (remaining of enzyme/bile acids etc.) often higher effect then food compound itself
- Careful statistical/comparative analysis make use of biological interpretation, relevant for tissue function

Pathway analysis of genes clustering with TEER/osmolarity



Genes related to TEER (osmolarity)

Genes regulated by all berries

159 genes

112 genes

**99 genes
Up-regulated**

**153 genes
Down-regulated**

More reliable effects of berries when
only including berries with no/low
effect on TEER

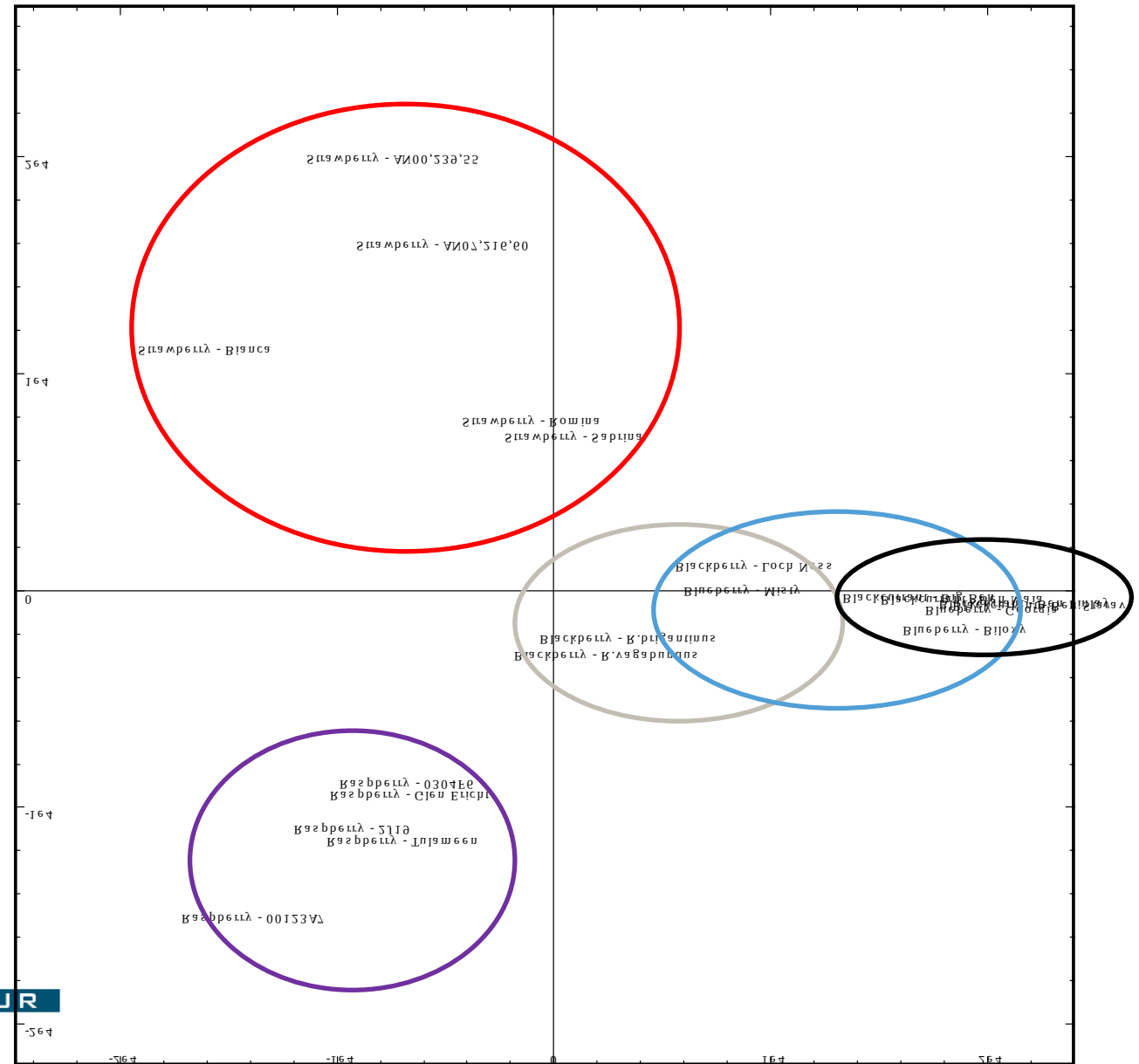
Cell-cell junction

Inflammation
response

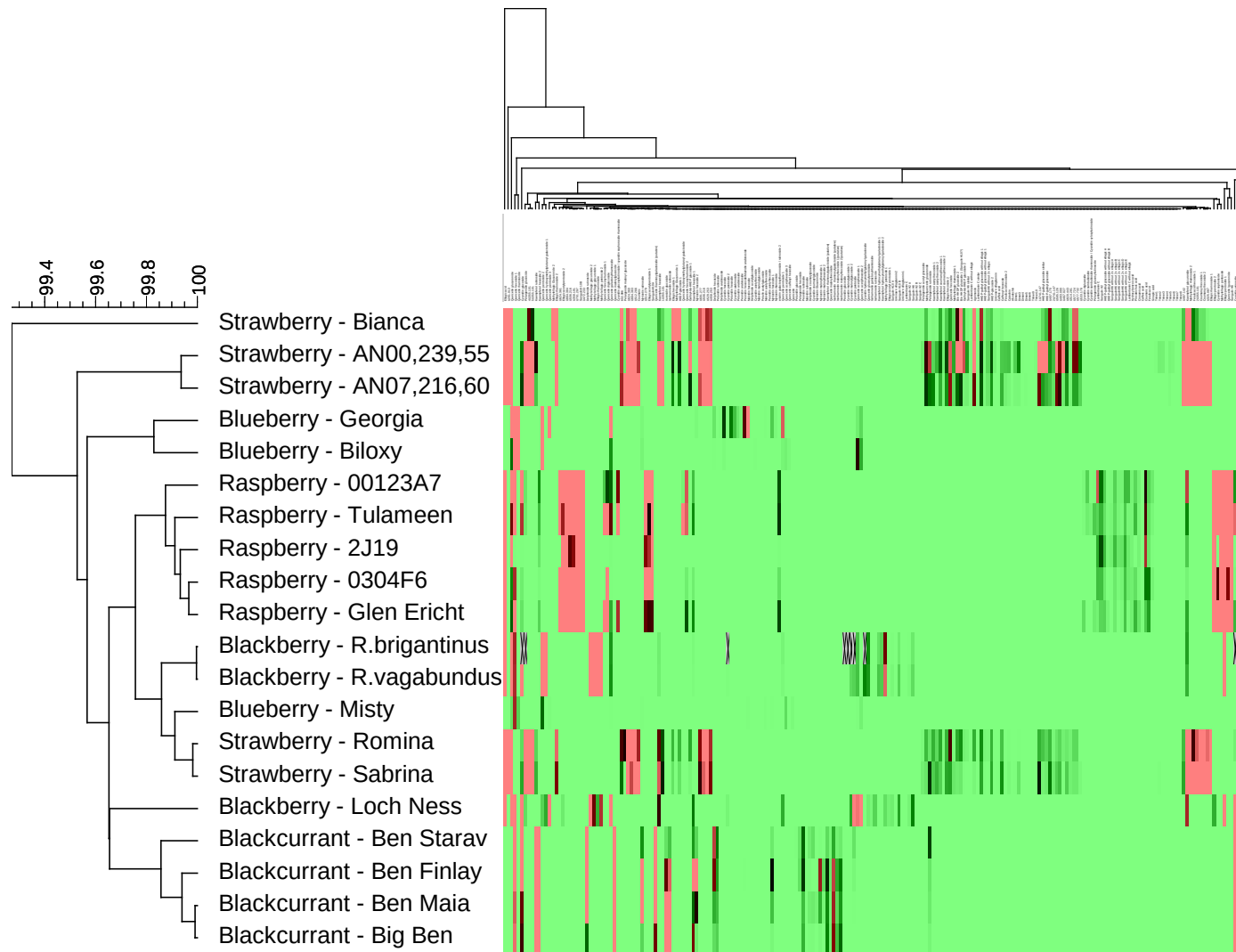
Stress response

Lipid metabolism

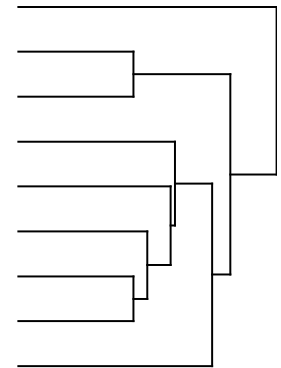
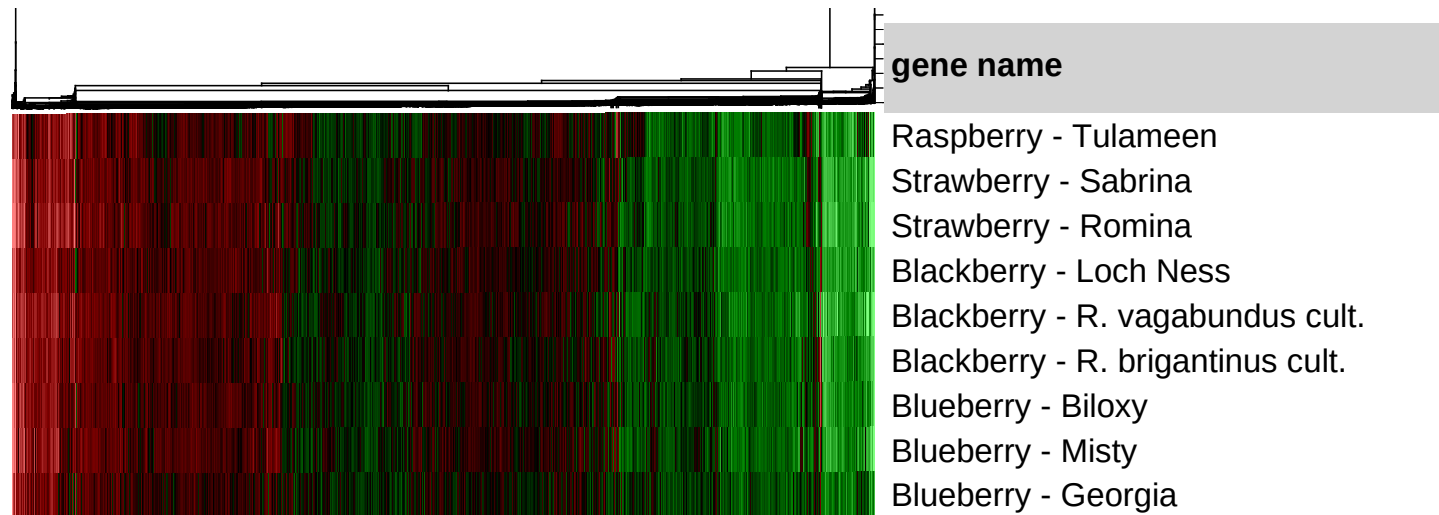
PCA clustering of all berry based on phytochemicals



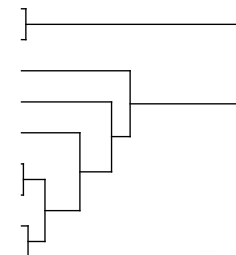
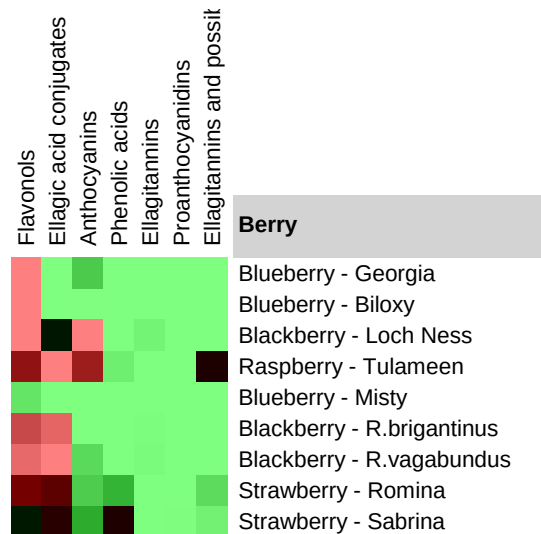
Clustering of all berry contents



Heatmap of Berries with TEER > 75%



Can we relate
epithelial
responses to
berry content?
Currently opinion:
unlikely, but still
ongoing



Still to be done

- Interpretation of the responses in biological relevant pathways in order to build hypothesis
 - IPA pathways
 - Reading and discussing literature
- Can it be related to berry content as measured by Gary
- Write and submit paper
- Finish and submit review article on gut & immune effects berries

Questions?

Thanks for the
collaboration

Hope to collaborate
more in near future

