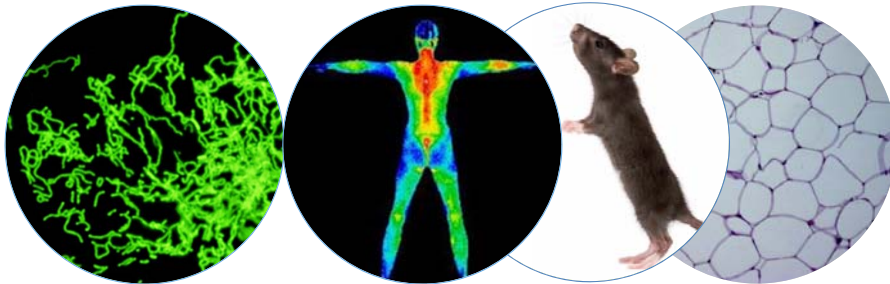


## Hands-on working with MetaCore: pros and cons working with complex datasets

Dr. Evert van Schothorst  
Human and Animal Physiology  
Wageningen University, NL



## Pathway analysis using microarray datasets

### Microarray Pipeline

Design and perform experiment

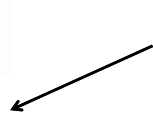
Process and normalise data

Statistical analysis

Differentially expressed genes

**Biological interpretation**

Pathway Analysis:  
MetaCore



## Login via browser: portal.genego.com

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## Start Screen overview

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ProbeName T-test group 1 vs2 FC

A_55_P2116272	0.000001	12.9
A_51_P112223	0.002868	1.66
A_55_P2002745	0.004809	-1.93
A_51_P339344	0.008842	1.22
A_55_P1967478	0.009344	-1.76
A_66_P112773	0.009767	1.31
A_55_P2025883	0.0101747	1.23
A_51_P386899	0.0232158	1.63
A_55_P2092030	0.0351611	1.61
A_30_P01023496	0.0434288	-1.16

Tab delimited text file to upload

Step 3

Species

Choose species

Activate/Deactivate

Activate dataset

Name	Type
My Data	
NETWORKS LISTS	
WORKFLOWS	
EXPERIMENTS	
NuGO-Copenhagen	
ANOVA_CRvsC	GX
MetaCore_4groups_Mario_FDR_all_Ratio HFD (Tg/Wt)	GX
HAP-Guests	
HAP	

The screenshot displays the MetaCore software interface. The main window shows a list of pathways with columns for Name, Type, and Size. A search bar is visible at the top right, and a red arrow points to the 'AND/OR' button within it. The interface includes various toolbars and a sidebar with navigation options.

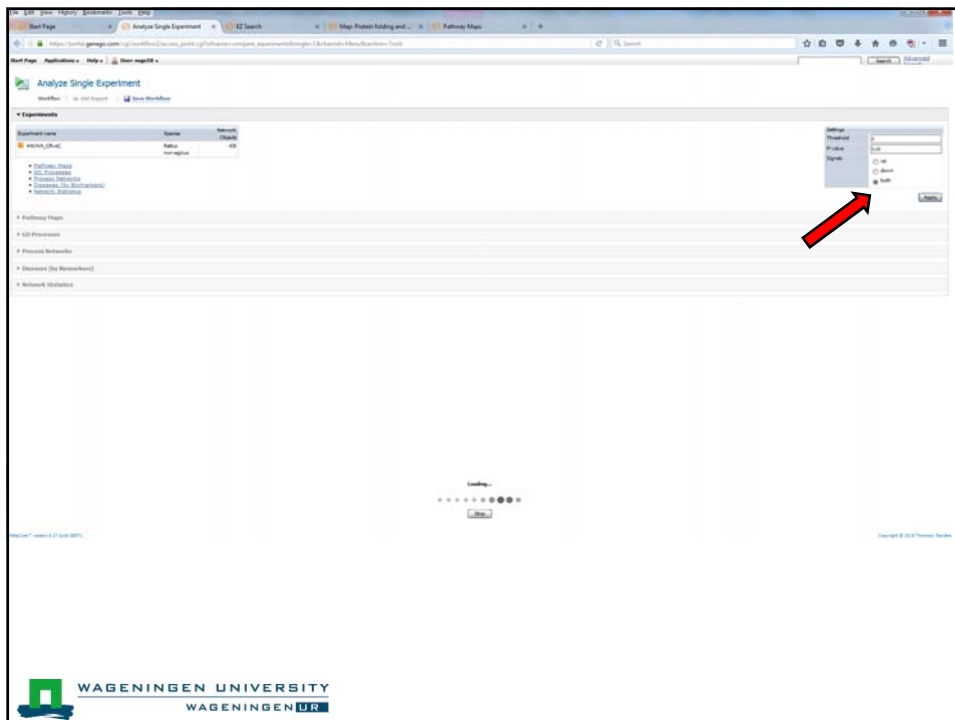
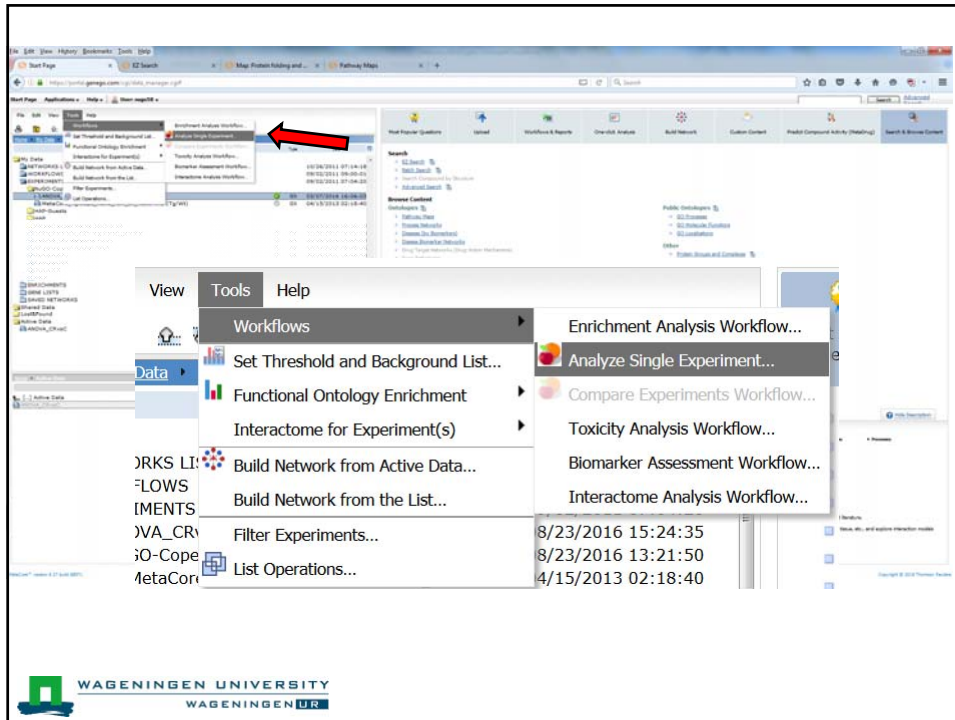
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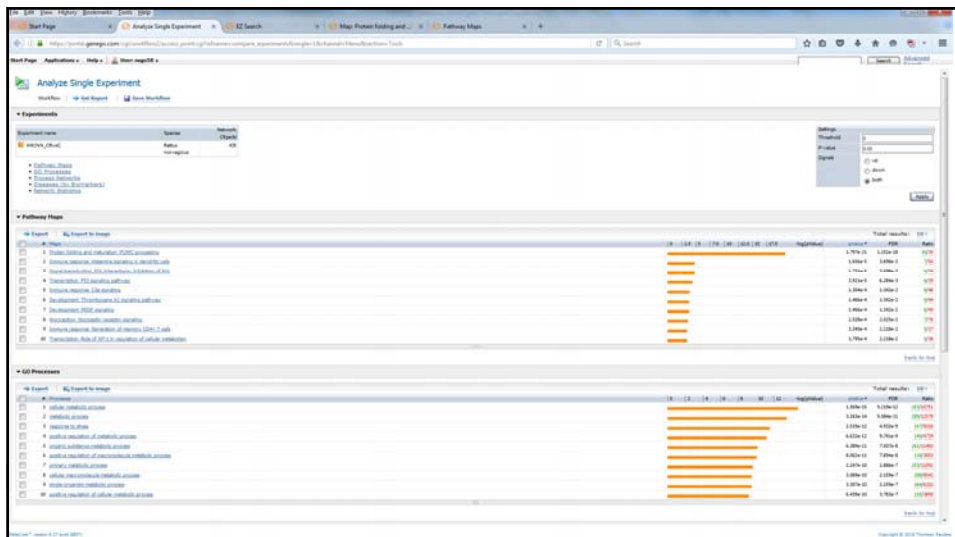
## Focus of analysis in MetaCore

For simplicity, I focus here on:

- **Pathway Analysis**
- **Using a two group comparison**

However, MetaCore can do many more sophisticated analyses, including multiple study groups





Result: **TOP10** Pathways, GO processes, Process networks, Diseases (by biomarkers), and network statistics  
So now a side step: what if you are only interested in pathways??



The screenshot shows the software interface with a menu open. The 'Tools' menu is expanded, and the 'Pathway Maps...' option is highlighted with a red arrow. Other options in the menu include 'Map Folders...', 'Process Networks...', 'Diseases (by Biomarkers)...', 'Disease Biomarker Networks...', 'Metabolic Networks (Endogenous)...', 'GO Processes...', 'GO Molecular Functions...', 'GO Localizations...', and 'Saved Custom Networks...'. The background shows the main software interface with various panels and data.



## List of Pathway maps

The screenshot shows the Pathway Maps software interface. At the top, there are tabs for 'Experiments' and 'Network Objects'. Below, a table lists various pathway maps with their enrichment profiles. A red arrow points to the top entry:

#	Maps	0	2.5	5	7.5	10	12.5	15	17.5	20	-log(pValue)	pValue	FDR
1	Protein folding and maturation, POMC processing	[Bar chart showing high enrichment]										1.797e-21	1.152e-18
2	Immune response, Histamine signaling in dendritic cells	[Bar chart showing moderate enrichment]										1.656e-5	3.698e-3
3	Signal transduction, Erk Interactions, Inhibition of Erk	[Bar chart showing moderate enrichment]										1.731e-5	3.698e-3
4	Transcription, p53 signaling pathway	[Bar chart showing moderate enrichment]										3.921e-5	6.284e-3

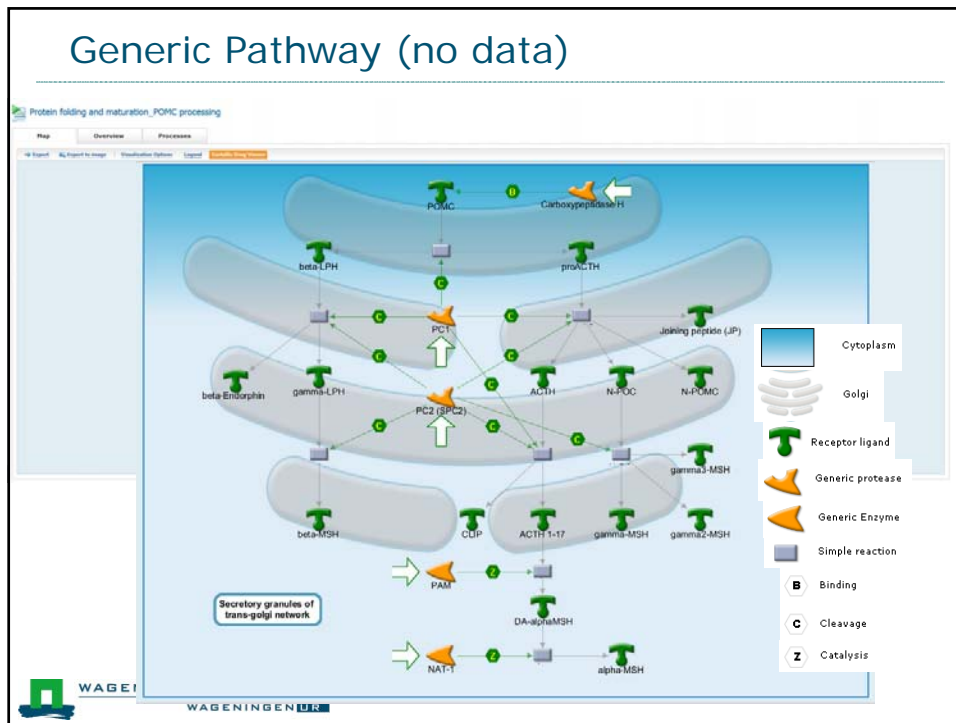
At the bottom of the screenshot, the Wageningen University logo is visible.

The legend is organized into several columns:

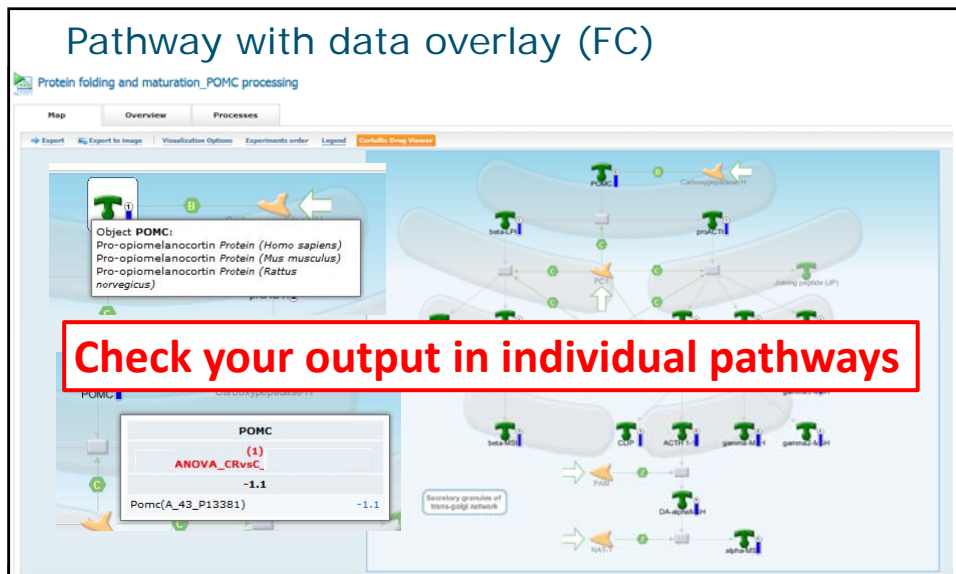
- Enzymes:**
  - Generic Enzyme (orange arrow)
  - KINASE:**
    - Generic kinase (orange arrow)
    - Protein kinase (orange arrow)
    - Lipid kinase (red arrow)
  - PHOSPHATASE:**
    - Generic phosphatase (yellow arrow)
    - Protein phosphatase (orange arrow)
    - Lipid phosphatase (red arrow)
  - PHOSPHOLIPASE:**
    - Generic phospholipase (orange arrow)
  - PROTEASE:**
    - Generic protease (orange arrow)
    - Metalloprotease (red arrow)
  - GTPase:**
    - G-alpha (green arrow)
    - RAS - superfamily (blue arrow)
- G protein Adaptor/regulators:**
  - G beta/gamma (green arrow)
  - Regulators (GDI, GAP, GEF) (green arrow)
  - Heterotrimeric G-protein (green arrow)
- Channels/Transporters:**
  - Generic channel (purple arrow)
  - Ligand-gated ion channel (purple arrow)
  - Voltage-gated ion channel (purple arrow)
  - Transporter (purple arrow)
- Blocks:**
  - Simple reaction (grey square)
  - Metabolic pathway (blue square)
  - Expanded network (dark blue square)
  - Global cell process (black square)
- Arrows:**
  - Generic arrow (green arrow)
  - Regulated arrow (green arrow with red dot)
  - Inhibited arrow (green arrow with red T-bar)
  - Activated arrow (green arrow with red dot)
- Generic classes:**
  - Protein (blue circle)
  - Transfactor (red star)
  - Molecule (purple circle)
  - Anchoring phospholipid (blue circle)
  - Cell membrane glycoprotein (red Y-shape)
  - Inorganic ion (green circle)
  - Receptor ligand (green T-shape)
- Adaptors/regulators:**
  - Generic binding protein (blue circle)
  - Adaptor (blue circle)
- Receptors:**
  - Generic receptor (blue Y-shape)
  - GPCR (blue Y-shape)
  - Receptors with enzyme activity (blue Y-shape)
  - Nuclear receptor (blue Y-shape)
- Localization:**
  - Mitochondria (orange oval)
  - EPR (orange oval)
  - Golgi (orange oval)
  - Nucleus (blue circle)
  - Lysosome (green circle)
  - Peroxisome (orange circle)
  - Unspecified (grey circle)
  - Cytoplasm (blue circle)
  - Extracellular (grey circle)
- Mechanisms:**
  - B** Binding
  - C** Cleavage
  - CM** Covalent modifications
  - Cn** Competition
  - +P** Phosphorylation
  - P** Dephosphorylation
  - T** Transformation
  - Tn** Transport
  - Z** Catalysis
  - TR** Transcription regulation
  - IE** Influence on expression
  - ?** Unspecified interactions
  - CR** Class relation
  - CS** Complex subunit
  - Group of similar objects (grey star)
- Comments:**
  - Note (light blue circle)
  - Normal process (purple circle)
  - Pathological process (red circle)



## Generic Pathway (no data)



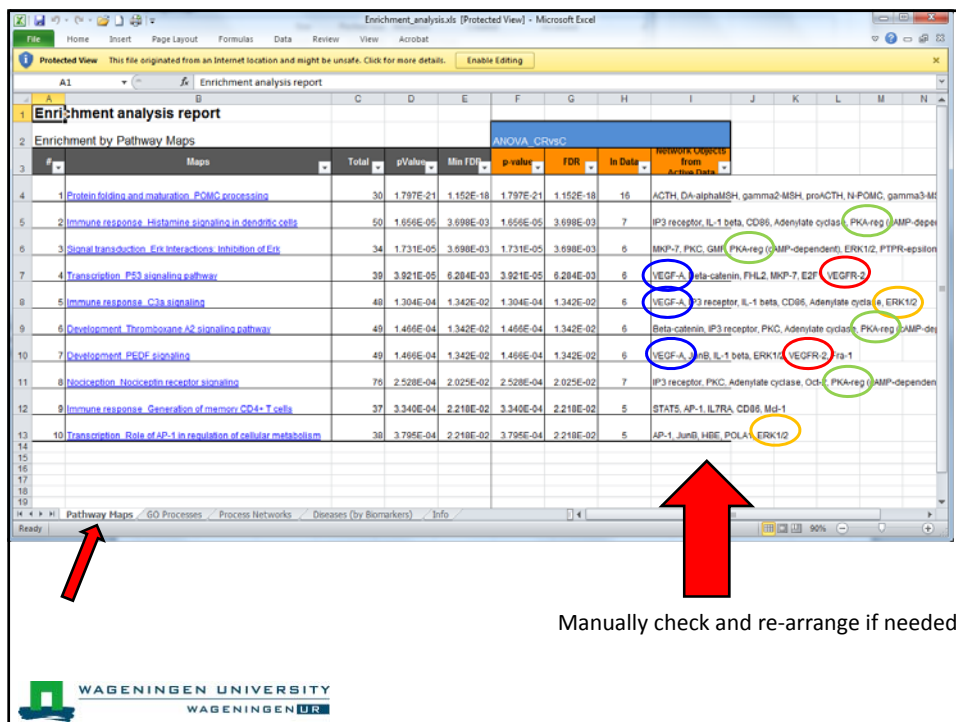
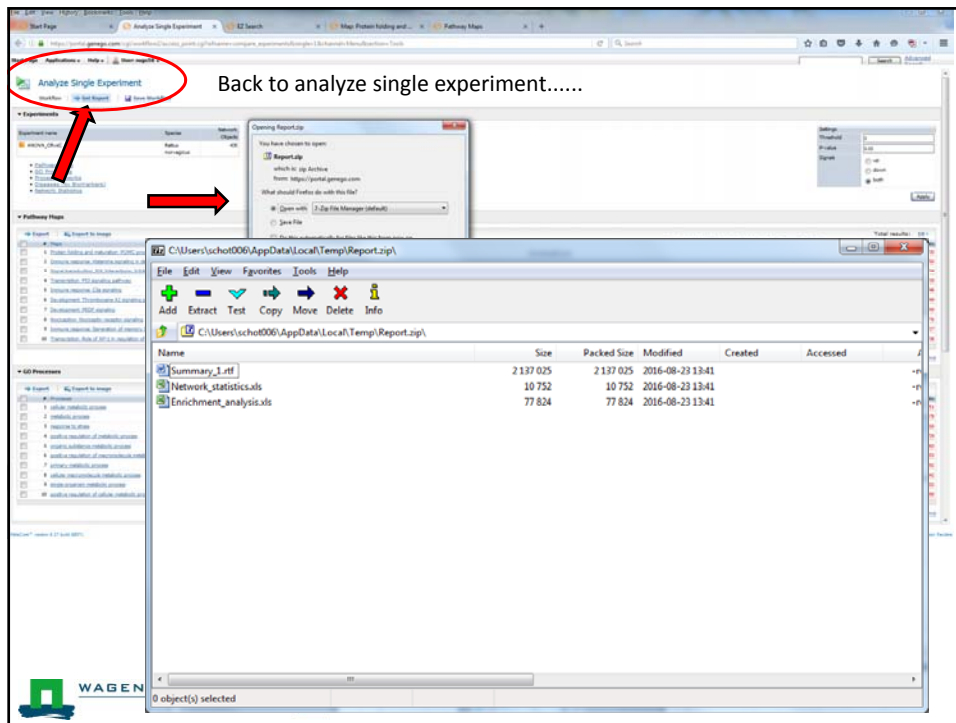
## Pathway with data overlay (FC)



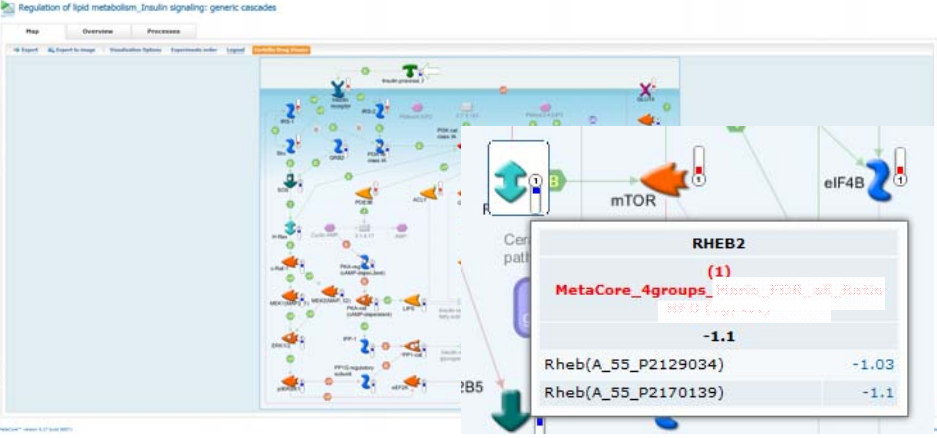
Thermometers indicate level of Fold Change (FC): red up, blue down regulated.

**All 16 proteins** in this pathway/scheme are derived from a single transcript (*Pomc*), so all show the same FC and the same significance






## Thermometers




RHEB2	
(1)	
MetaCore_4groups_Homo_FIG6_w8_Rhebl	
369-02_E_0101-0001	
-1.1	
Rheb(A_55_P2129034)	-1.03
Rheb(A_55_P2170139)	-1.1


Thermometers indicate level of Fold Change (FC): red up, blue down. Moreover, if multiple probes recognize same transcript, strongest FC is shown.


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## Check with a biological point-of-view



**Check biological meaning of regulation:** i.e. eEF2K phosphorylates and inhibits eEF2  
 eEF2K itself is down regulated, and thus decreased inhibition of eEF2, and its upregulation seems reasonable.


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## Take home message

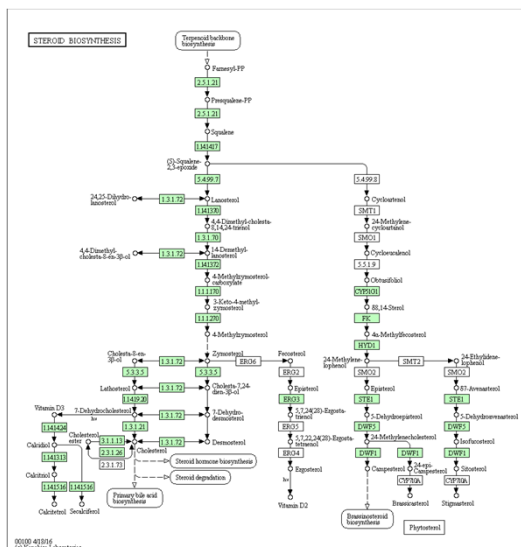
- Unless you have a very clear outcome of the study (minority of all studies), you spend most time on subsequent integration of the pathways/transcripts/.. being regulated.

See i.e. study of Mario Ost.

- MetaCore helps enormously to analyse in an unbiased way, a huge number of transcripts in pathways/networks/processes



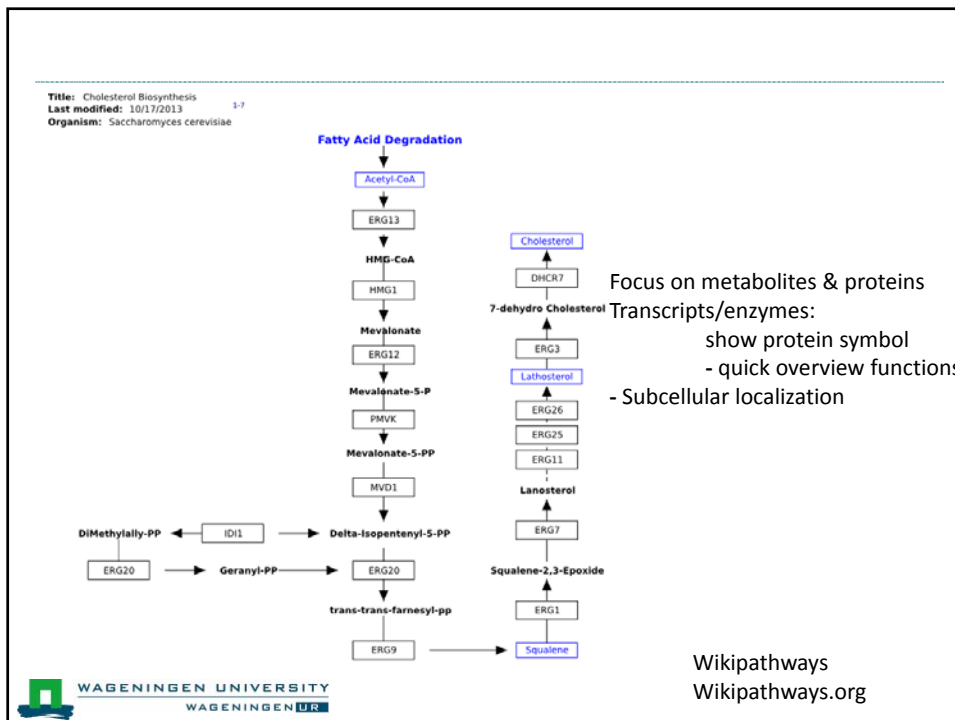
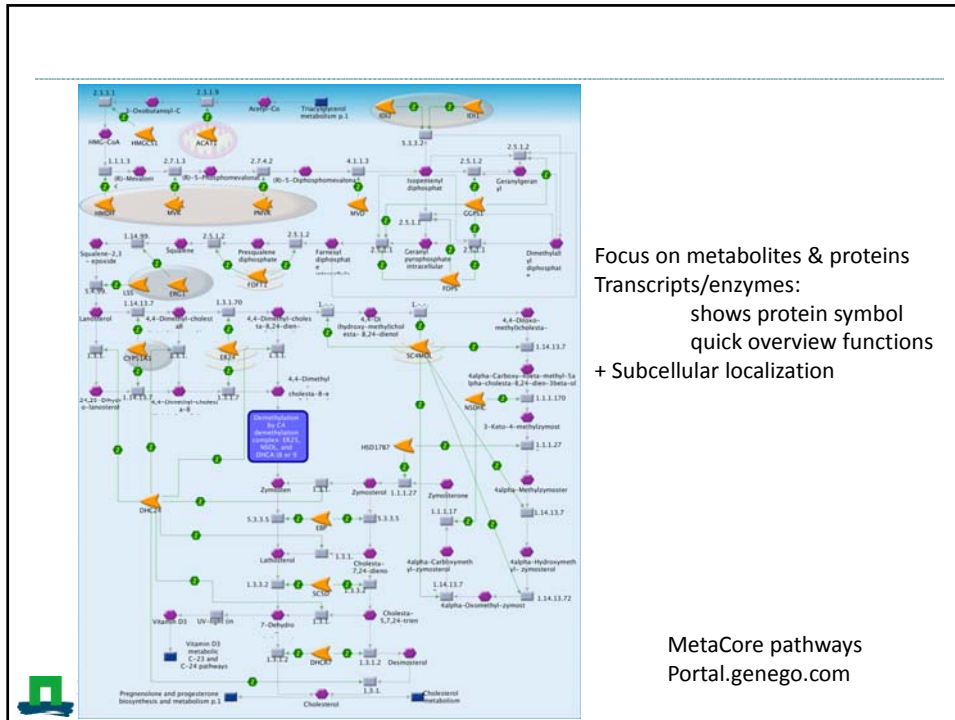
## Cholesterol biosynthesis: how to show and what?



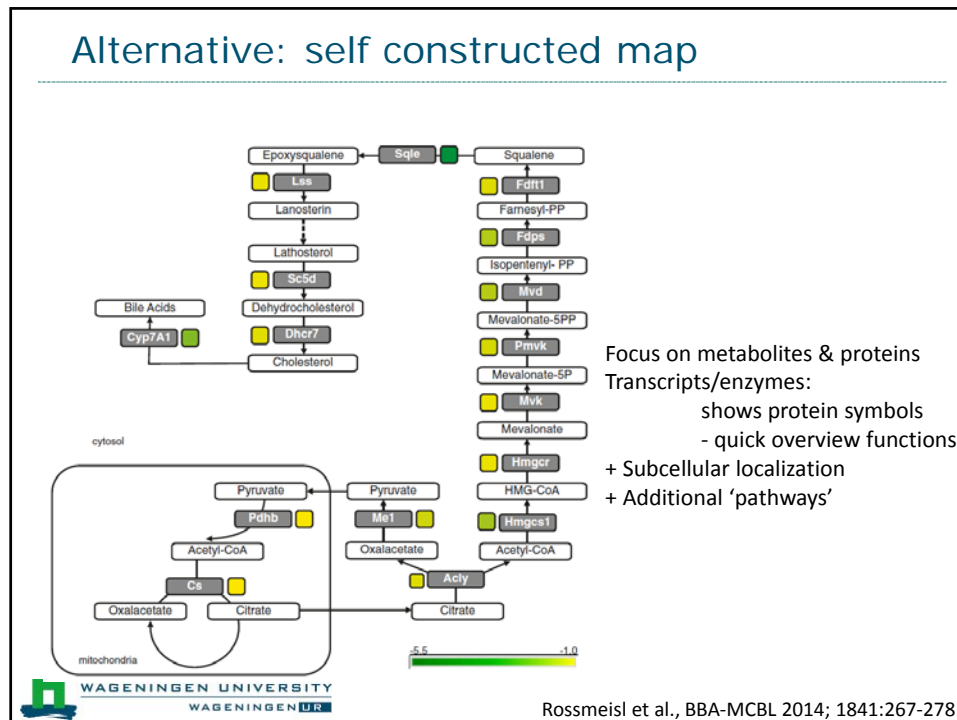
Focus on metabolites  
Transcripts/enzymes:  
shows EC classification  
- subcellular localization



KEGG pathways (<http://www.genome.jp/kegg/kegg2.html>)



## Alternative: self constructed map



## So now it is up to you!

- Get a MetaCore account by email to:  
[nugo@wur.nl](mailto:nugo@wur.nl) subject 'Metacore access' and  
 cc email to your NuGO-delegate!
- Import your data.....
- And start analyzing!!
- **It is that easy**
- I hope we offered you enough insights how MetaCore can help you  
 to achieve your biological research goals



## Acknowledgements

### **HAP, Wageningen University**

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(University Balearic islands, Palma de Mallorca, Spain)

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