

# The use of transcriptomics for biomarker development to trace anabolic hormone functions

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# Outline

- Anabolic Steroid Hormones and their use in animal husbandry
- Gene Expression Biomarkers
- Study design
- Results
- Conclusions
- Perspectives

# Anabolic Steroid Hormones in animal husbandry

- Important for establishment of muscle tissue
- Enhance body protein accretion
- Mobilization of fat stores
- Enhanced feed efficiency and reduced costs
- Use and misuse as growth promoters in animal husbandry

# Anabolic Steroid Hormones in animal husbandry

- Use of specific anabolic agents is licenced in Canada, USA, Australia, South Africa...
  - Since 1988: use of anabolic agents is prohibited in the EU (Directive 88/146/EEC)
  - Misuse of anabolics  $\Rightarrow$  permanent control necessary
  - Detection of hormone residues using chromatography in combination with mass spectrometry
  - Problem: Development of new xenobiotics and administration of hormone cocktails
- $\Rightarrow$  New sensitive test systems required

# Gene Expression Biomarker

- Detection of physiological changes caused by treatment with anabolic agents  
⇒ Development of a biomarker pattern based on changes in gene expression
- Activated steroid hormone receptors act as transcription factors  
⇒ direct influence on gene expression

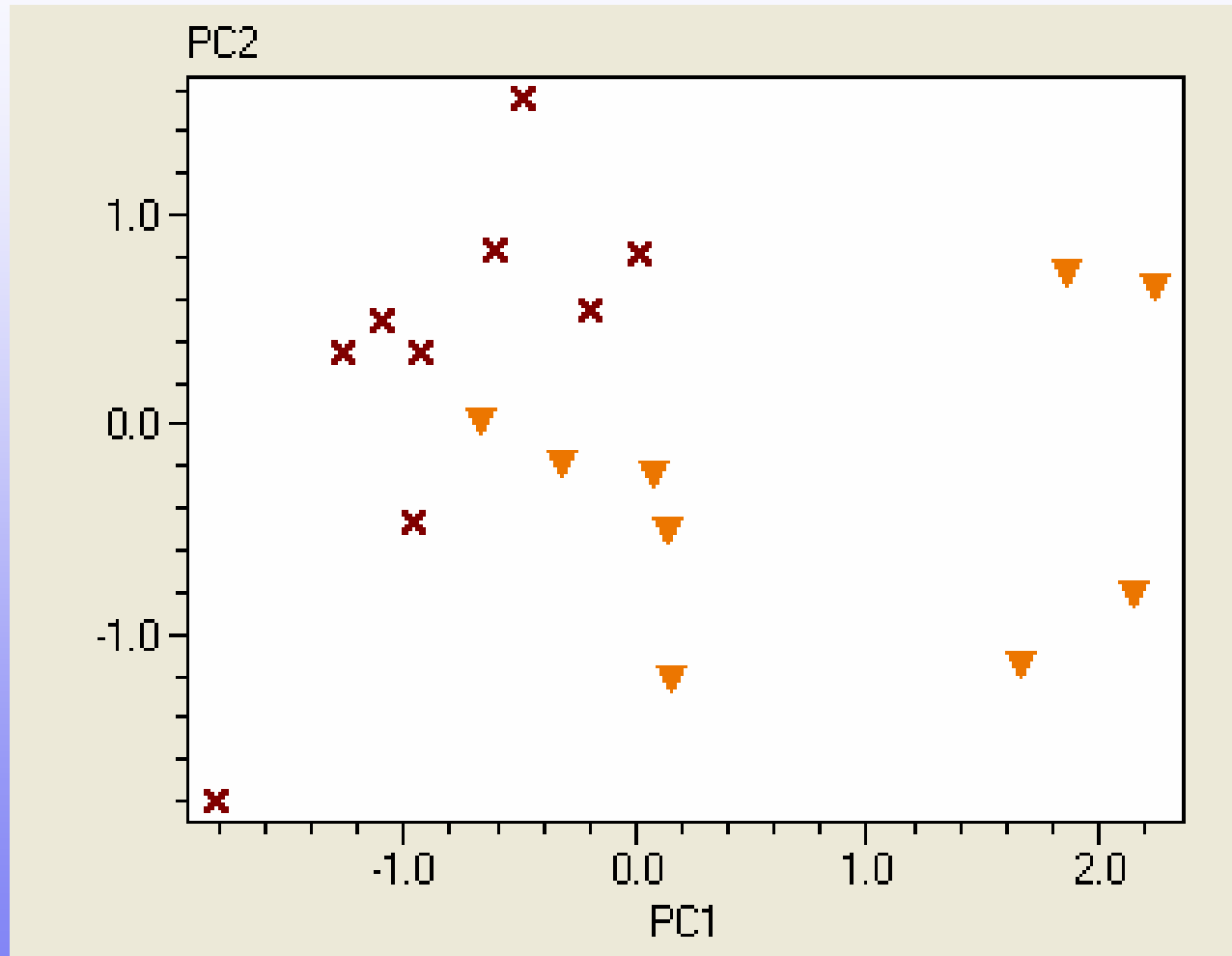
# Study design

- 18 Nguni heifers (9 treated, 9 untreated)
- Treatment with Revalor H by implantation for 42 days
  - 140 mg Trenbolone acetate, 14 mg estradiol
  - Implant is placed under the skin on the posterior aspect of the ear
- Tissues for biomarker screening: liver, blood, vaginal smear (containing vaginal epithelial cells)
- Sampling of blood and vaginal smear at day 0, 2, 16 and 39 of treatment
- Liver samples obtained at slaughter (day 42)

# Procedure

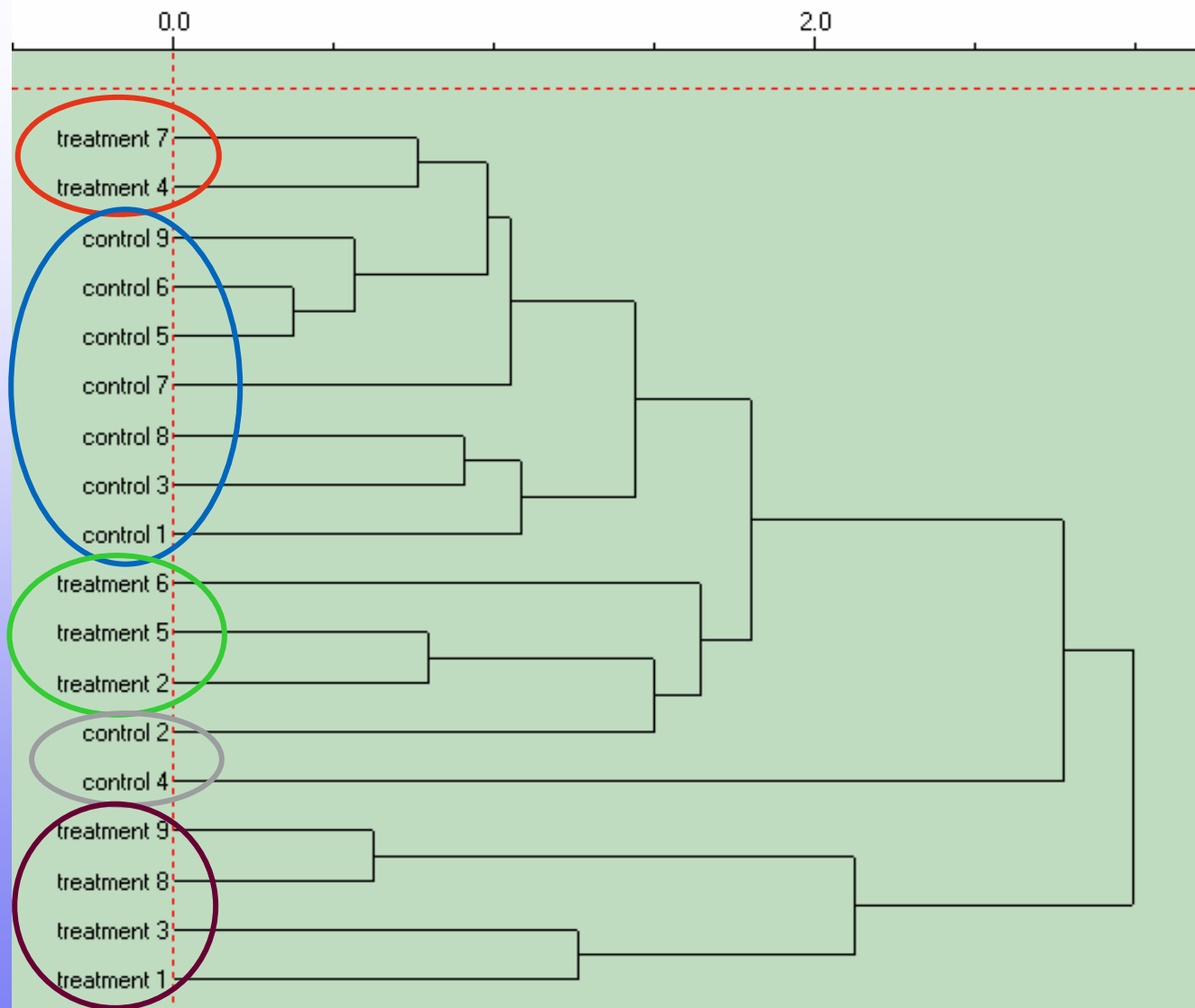
- Selection of target genes by screening the actual literature for steroidal effects on analyzed tissues
- Quantification of gene expression via qRT-PCR
- Normalization with reference genes
- Determination of significant regulations between treatment and control using the t-test ( $p < 0.05$ )
- Principle Components Analysis (PCA) and Hierarchical Cluster Analysis using Genex Pro. Ver. 4.3.8 (MultiD Analyses AB, Gothenburg, Sweden)

# Liver samples: 5 regulated genes



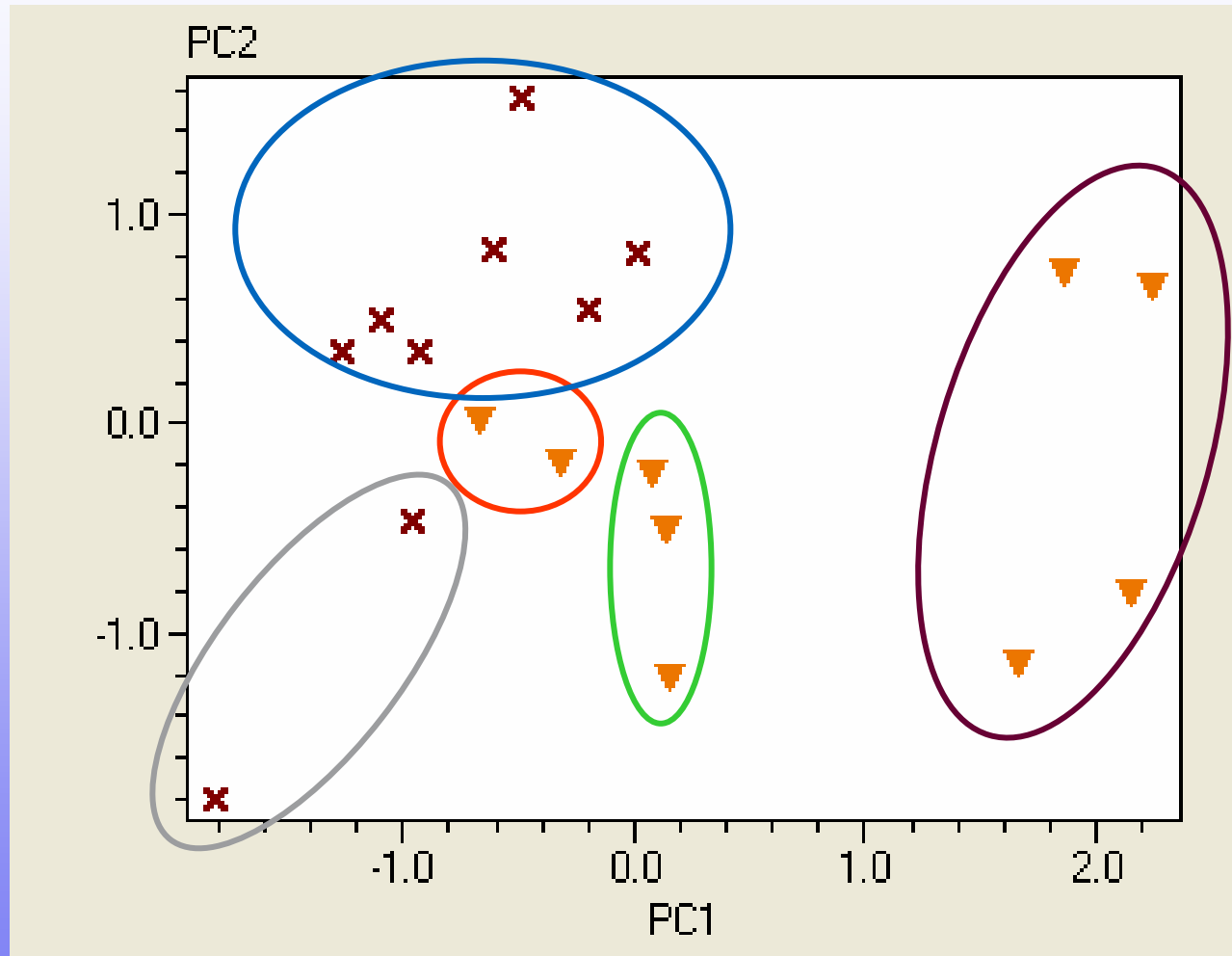
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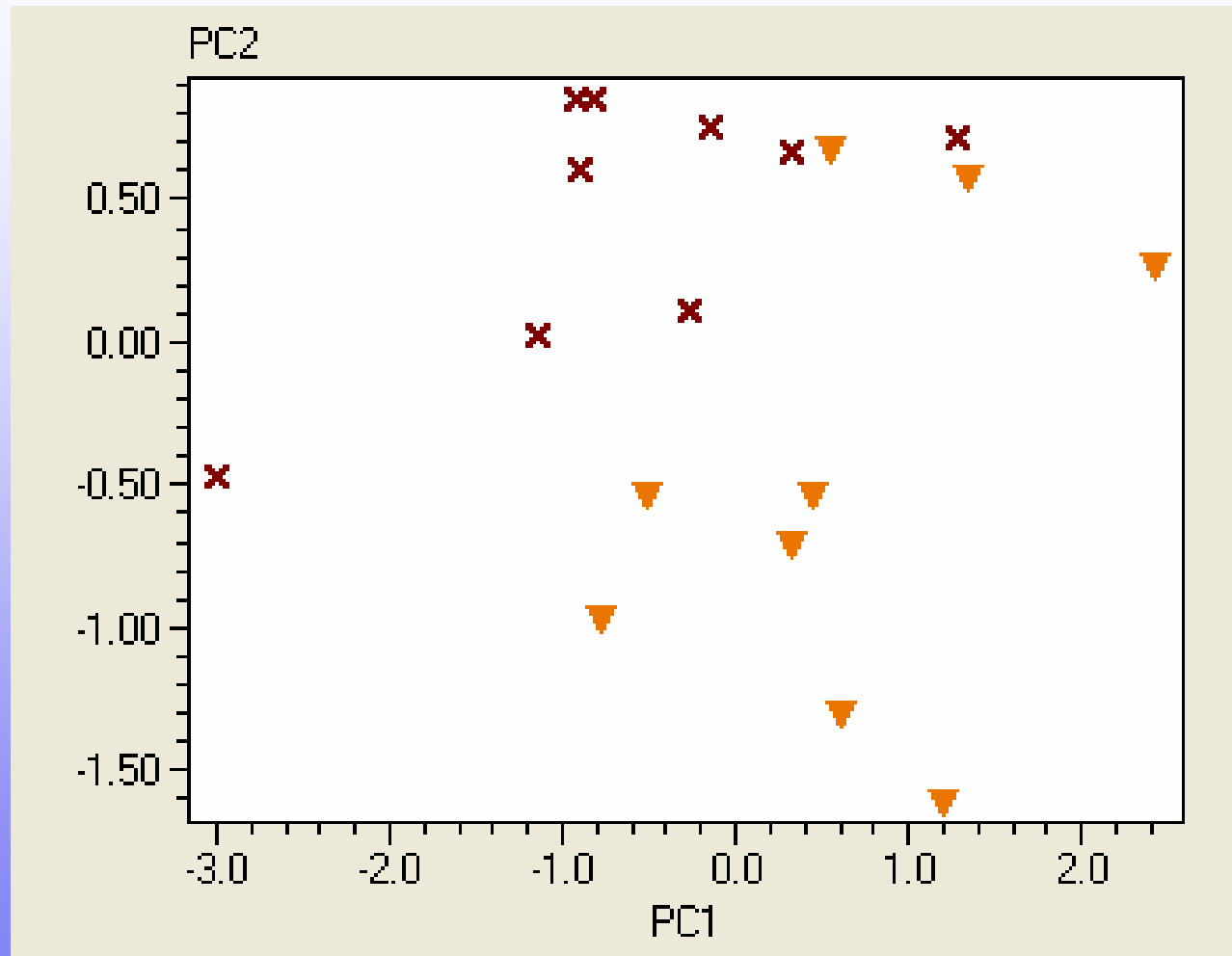
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# Liver samples: 5 regulated genes

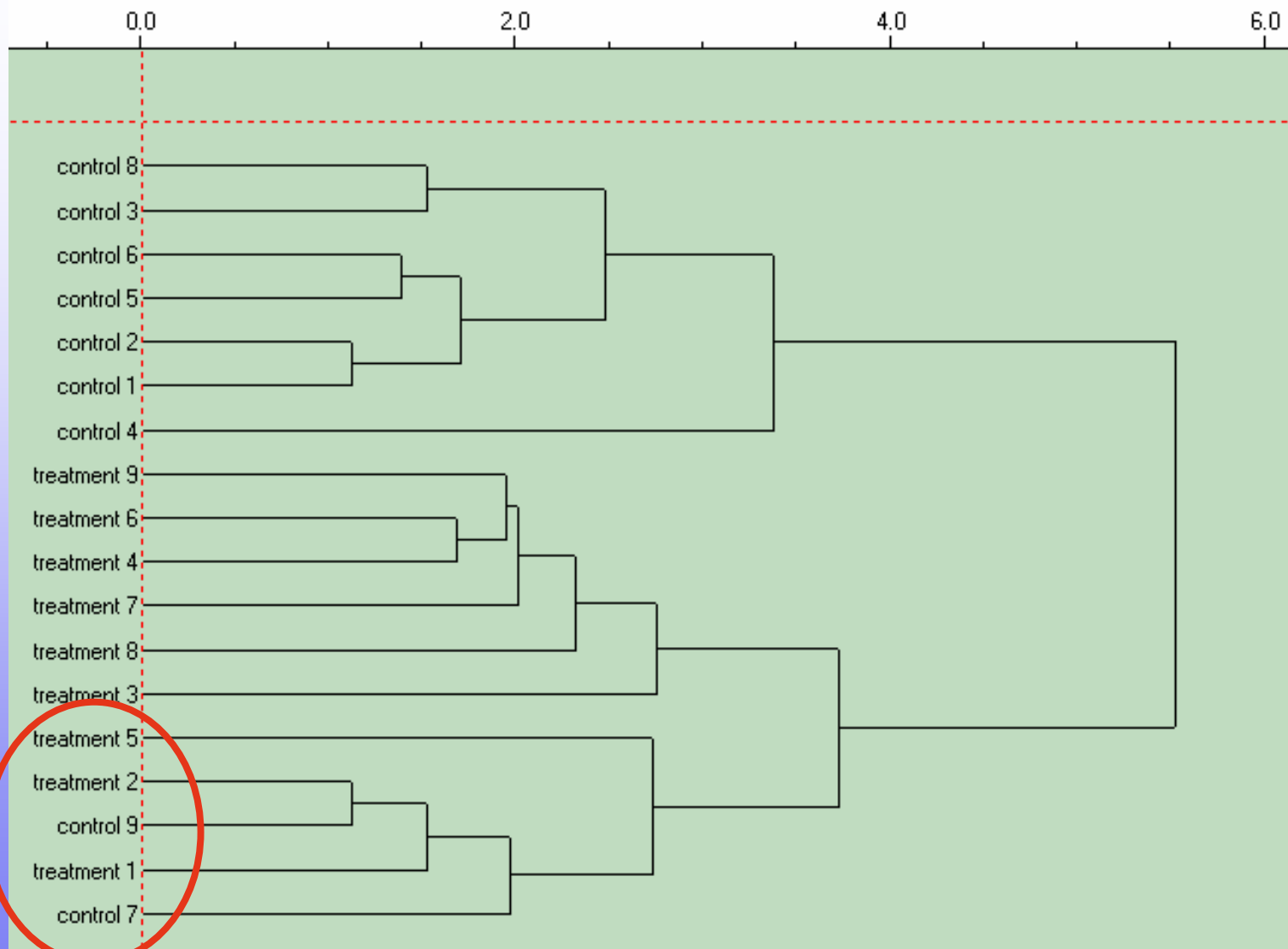


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## Blood: 11 regulated genes

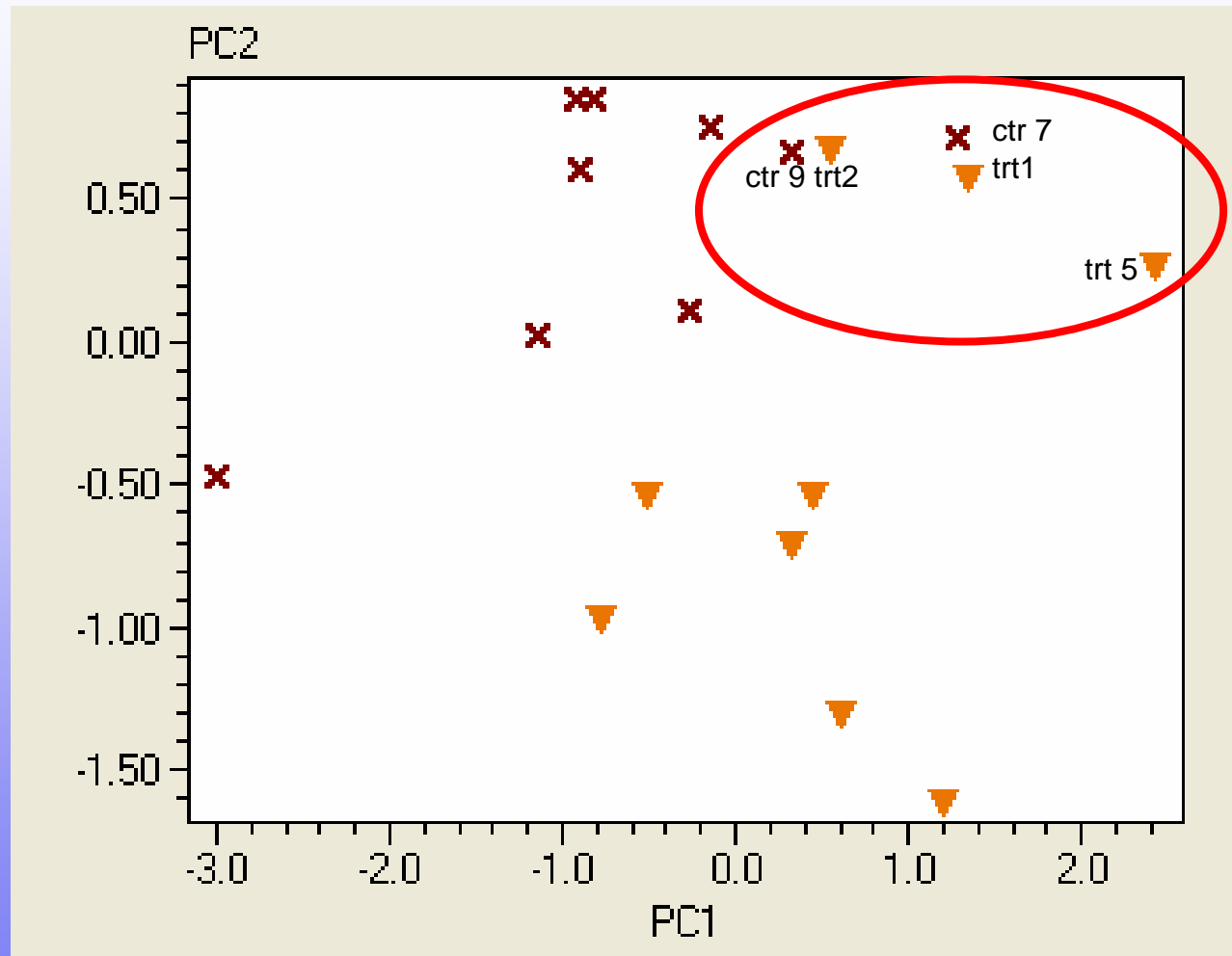


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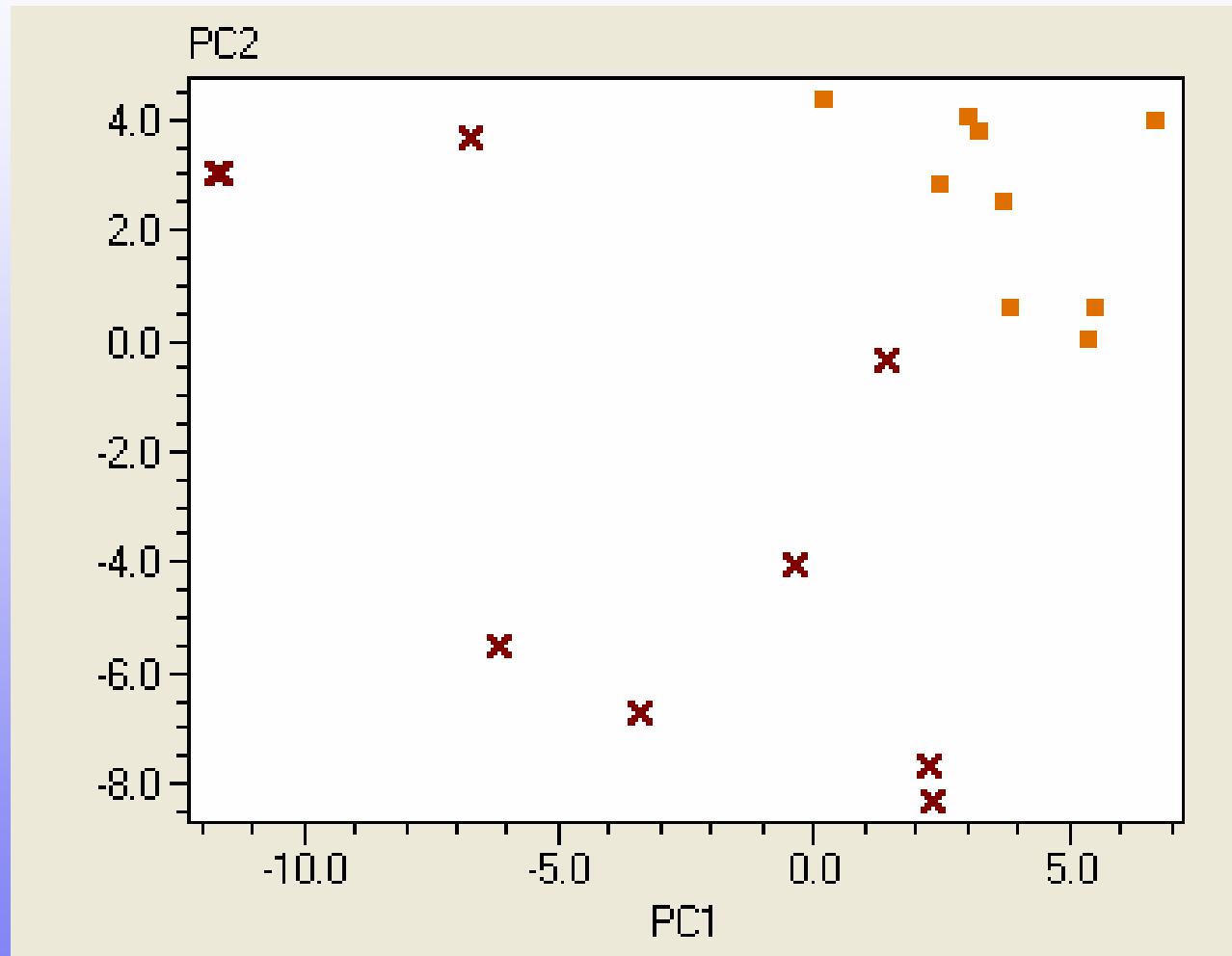
Genex Ver. 4.3.8, MultiD Analyses AB, Gothenburg, Sweden

# Blood: 11 regulated genes

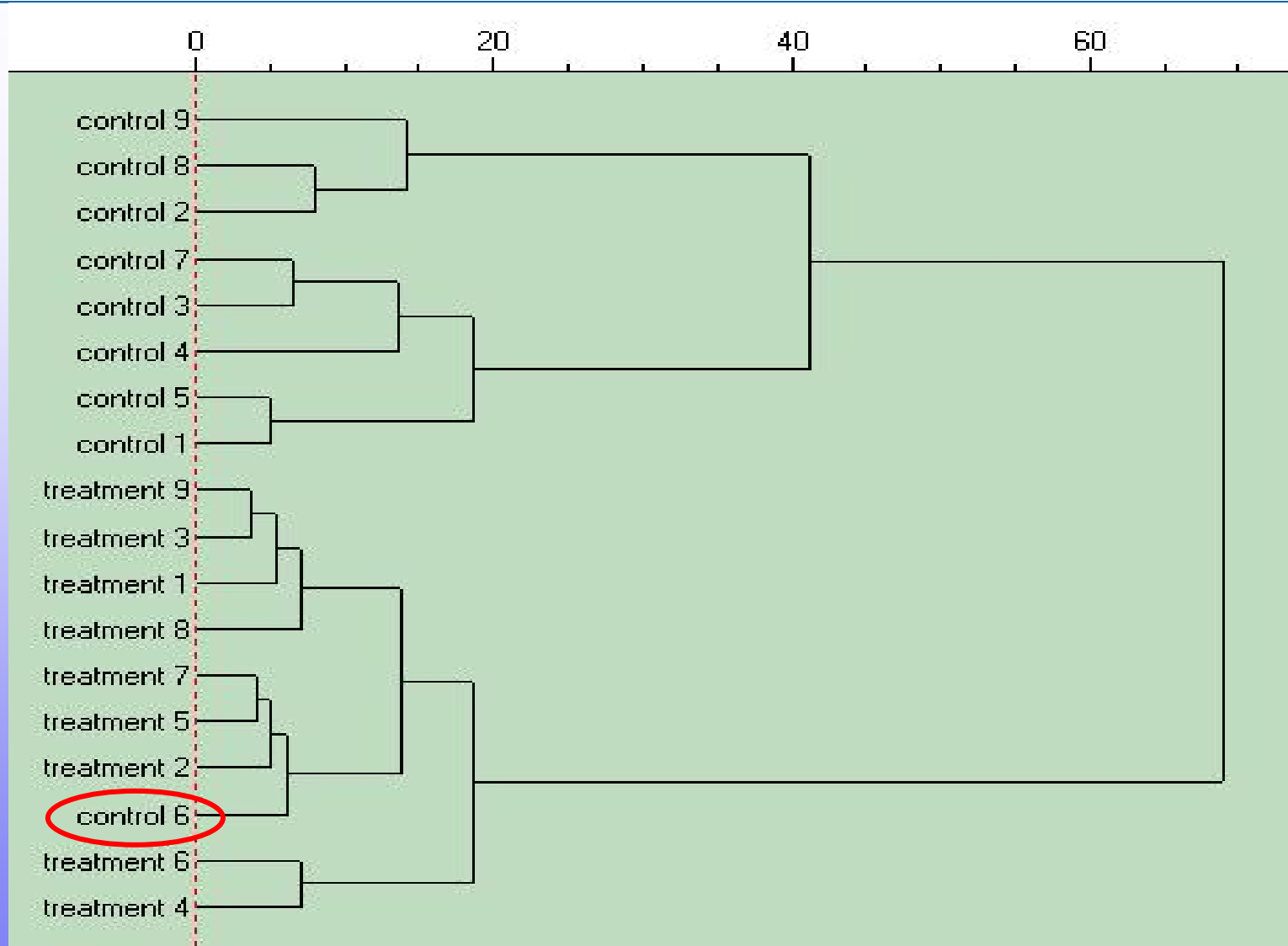


Genex Ver. 4.3.8, MultiD Analyses AB, Gothenburg, Sweden

## Vaginal smear: 27 measured genes

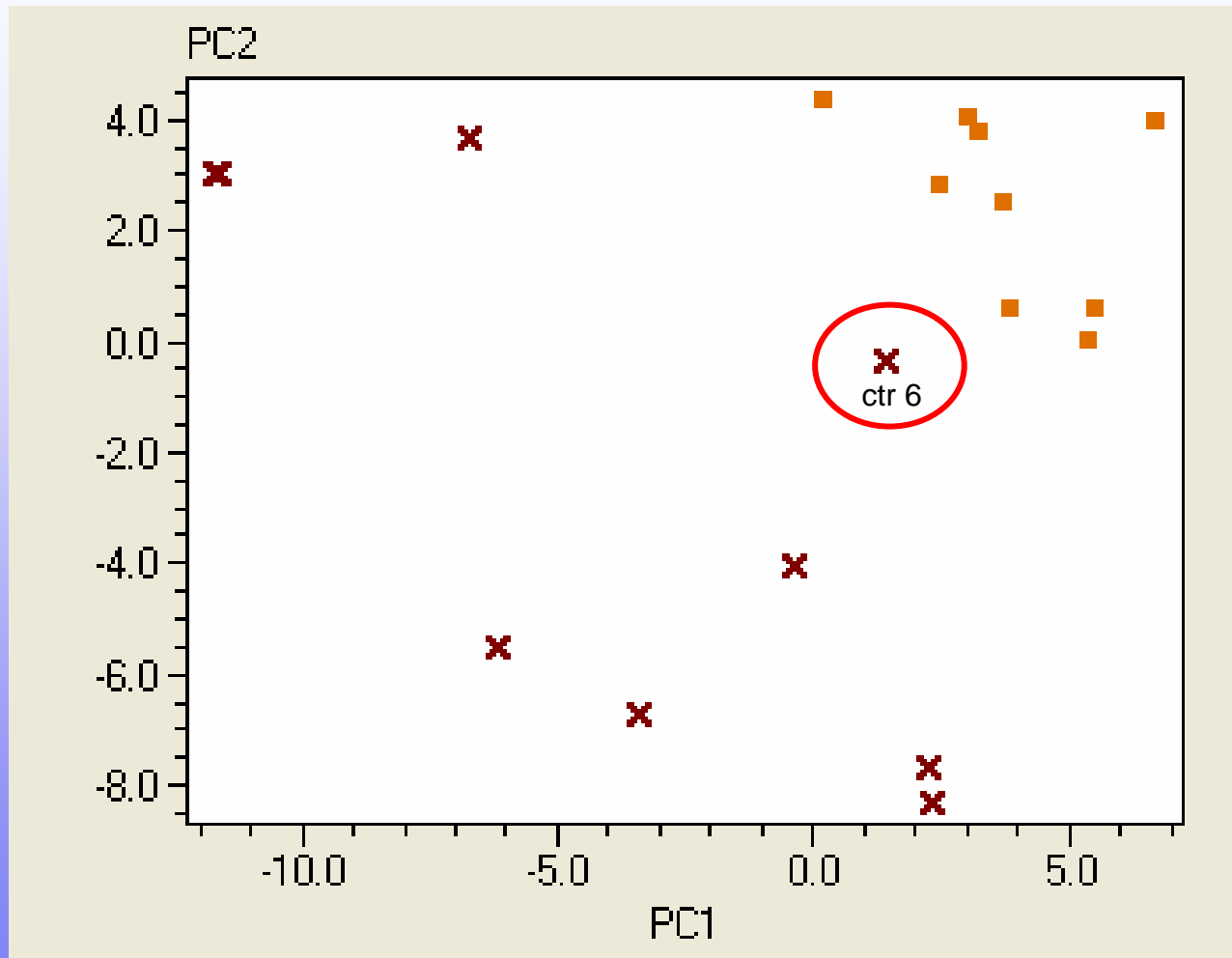


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# Vaginal smear: 27 measured genes



Genex Ver. 4.3.8, MultiD Analyses AB, Gothenburg, Sweden



# Conclusions

- Potential of transcriptomics for the development of a biomarker pattern to screen for the abuse of anabolic agents
- The more regulated genes the better results using PCA or Hierarchical Clustering
- Tissues that are directly influenced by steroid hormones are better for the development of gene expression biomarkers

# Perspectives

- More trials with more animals and other anabolic agents
- Large number of untreated control samples
- Combination of transcriptomics with other „omic“ technologies, like proteomics or metabolomics

**Thank you for your attention!**

