

# **„Comparative analysis of veins and arteries used in coronary artery bypass grafting”**

**PhD Thesis**



**Piotr Zawierucha**

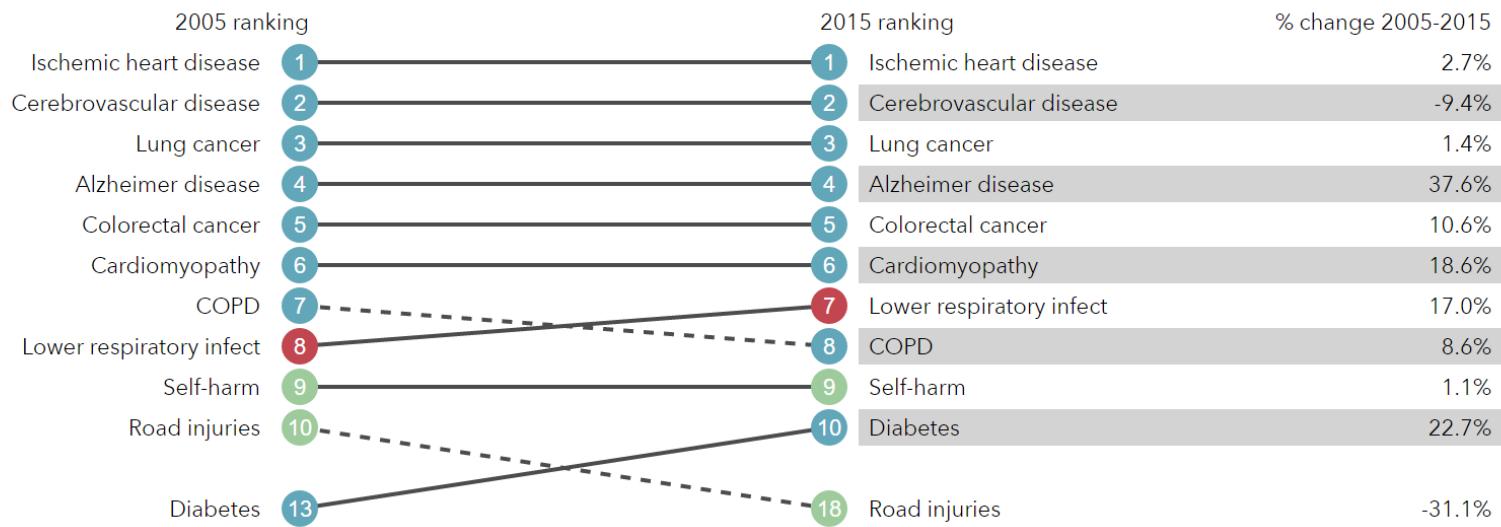
Thesis supervisor: prof. Marcin Ruciński  
Department of Histology and Embriology  
Poznan University of Medical Sciences



# Introduction

# IHME – profile Poland (than)

## What causes the most deaths?

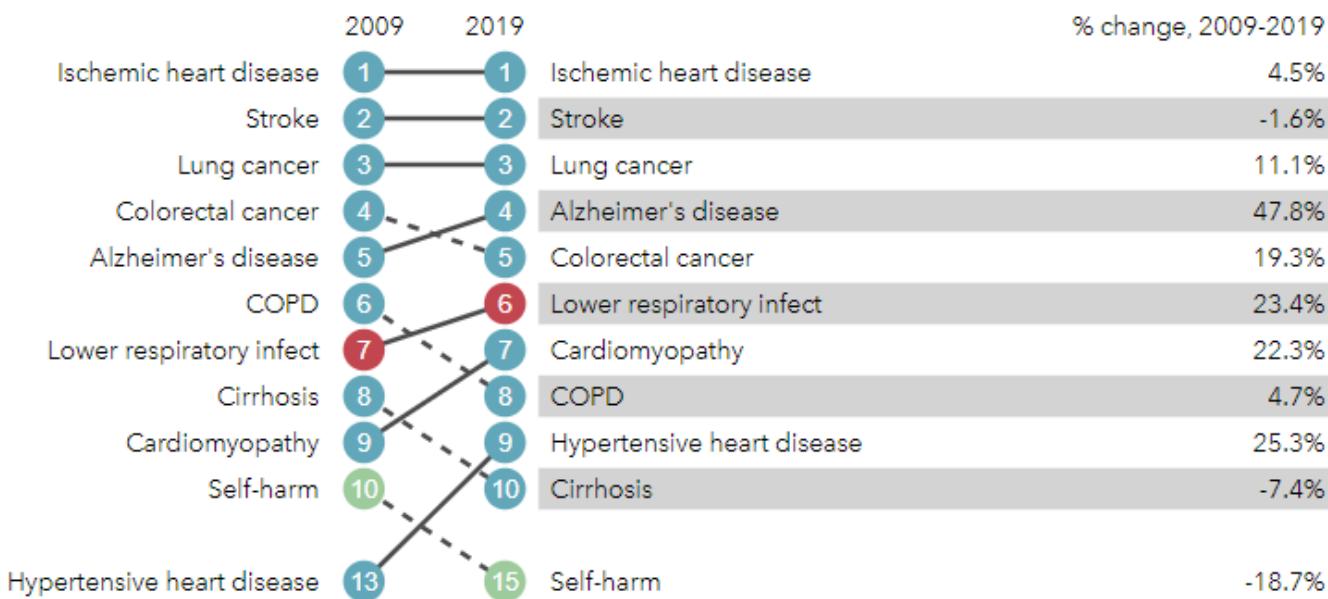


Top 10 causes of death by rate in 2015 and percent change, 2005-2015

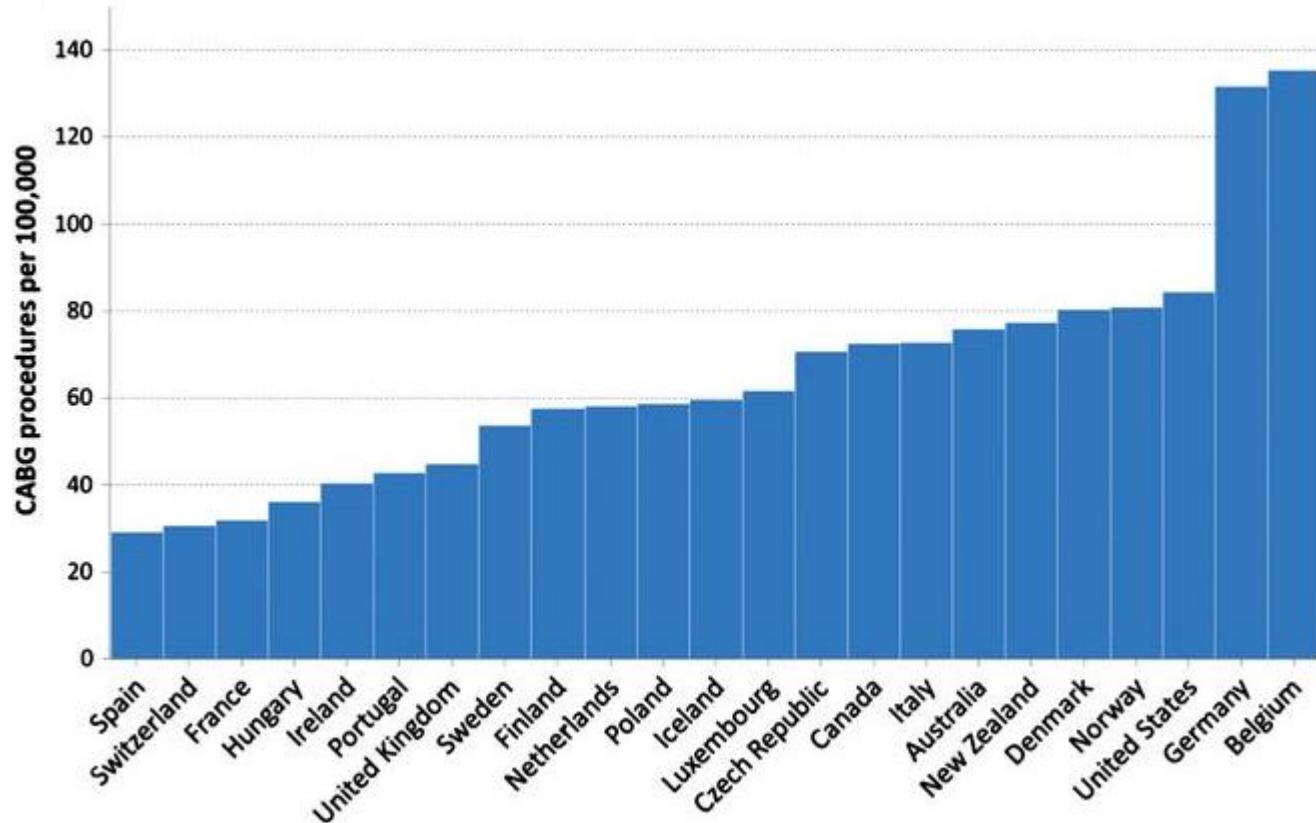
# IHME – profile Poland (now)

## What causes the most deaths?

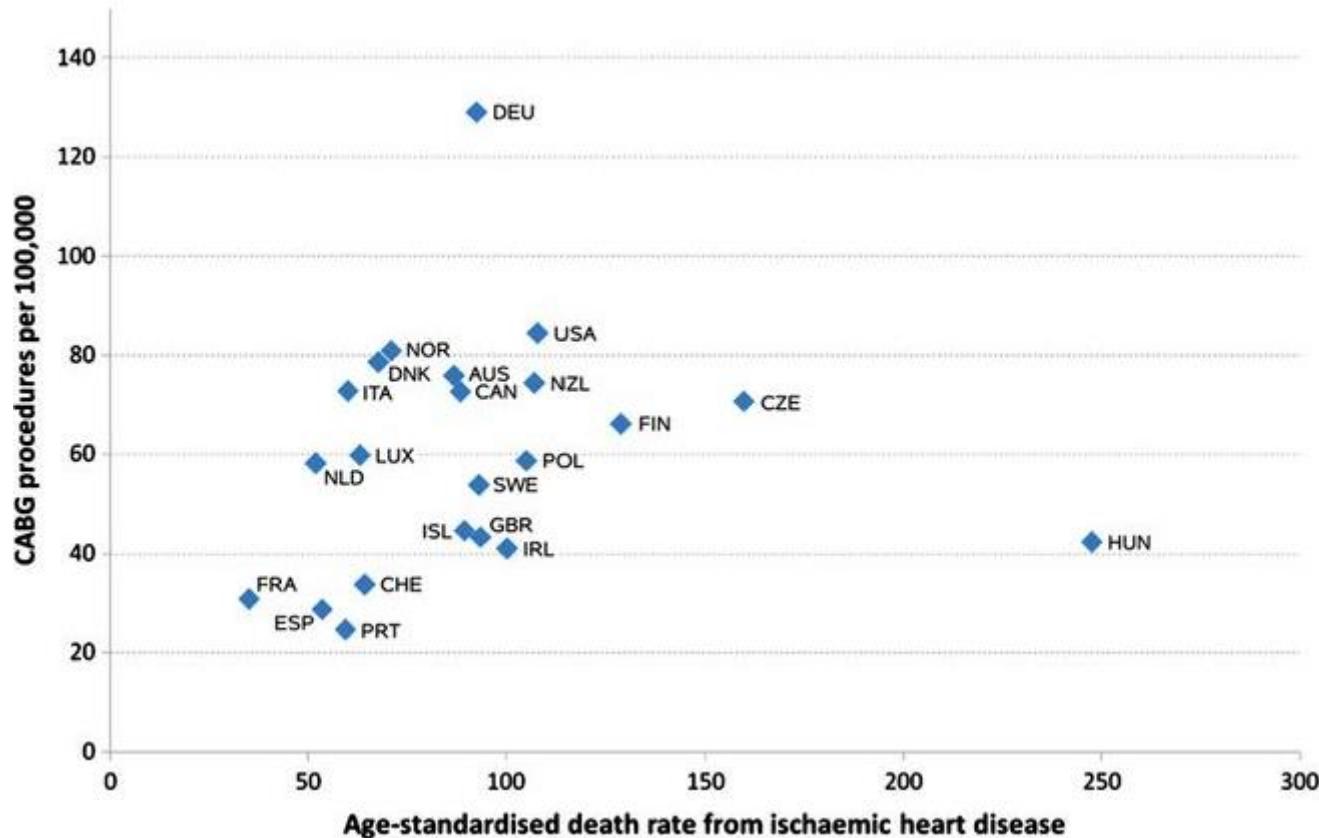
- Communicable, maternal, neonatal, and nutritional diseases
- Non-communicable diseases
- Injuries



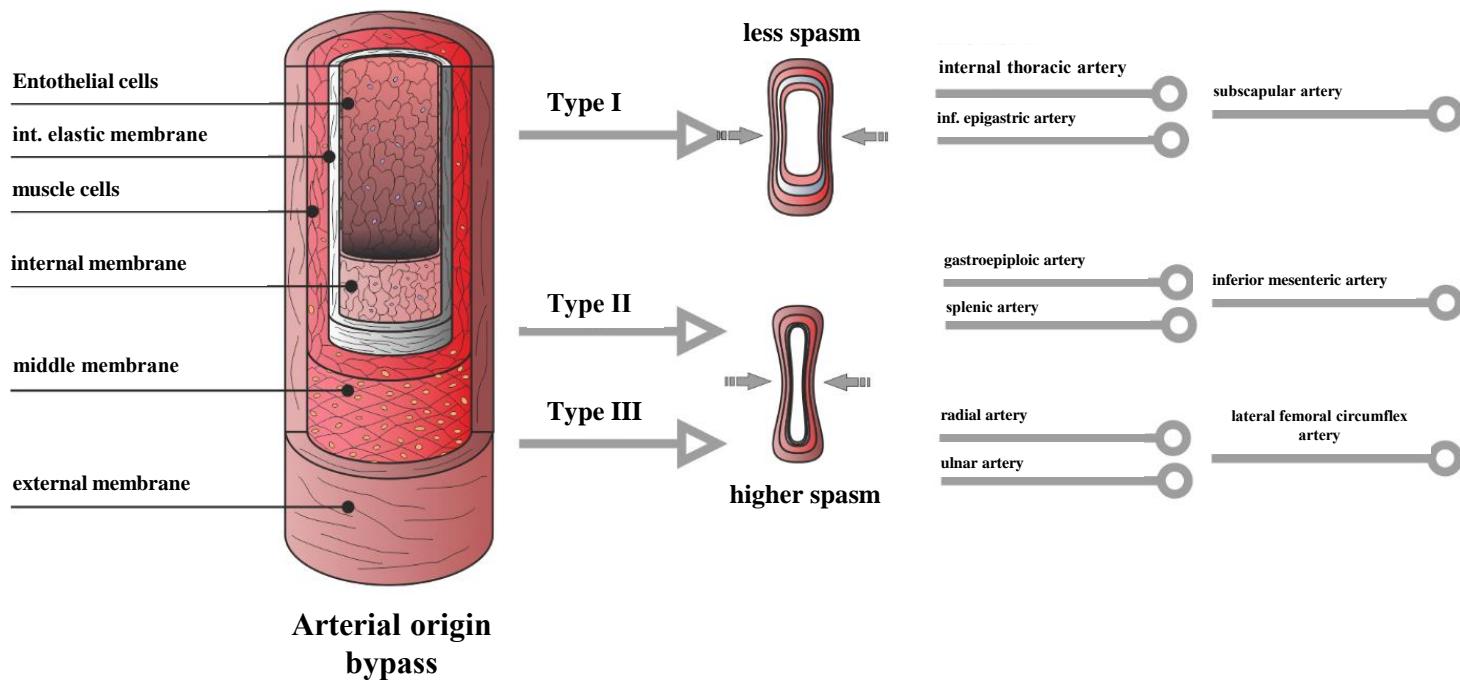
## Scale of the problem



## Scale of the problem

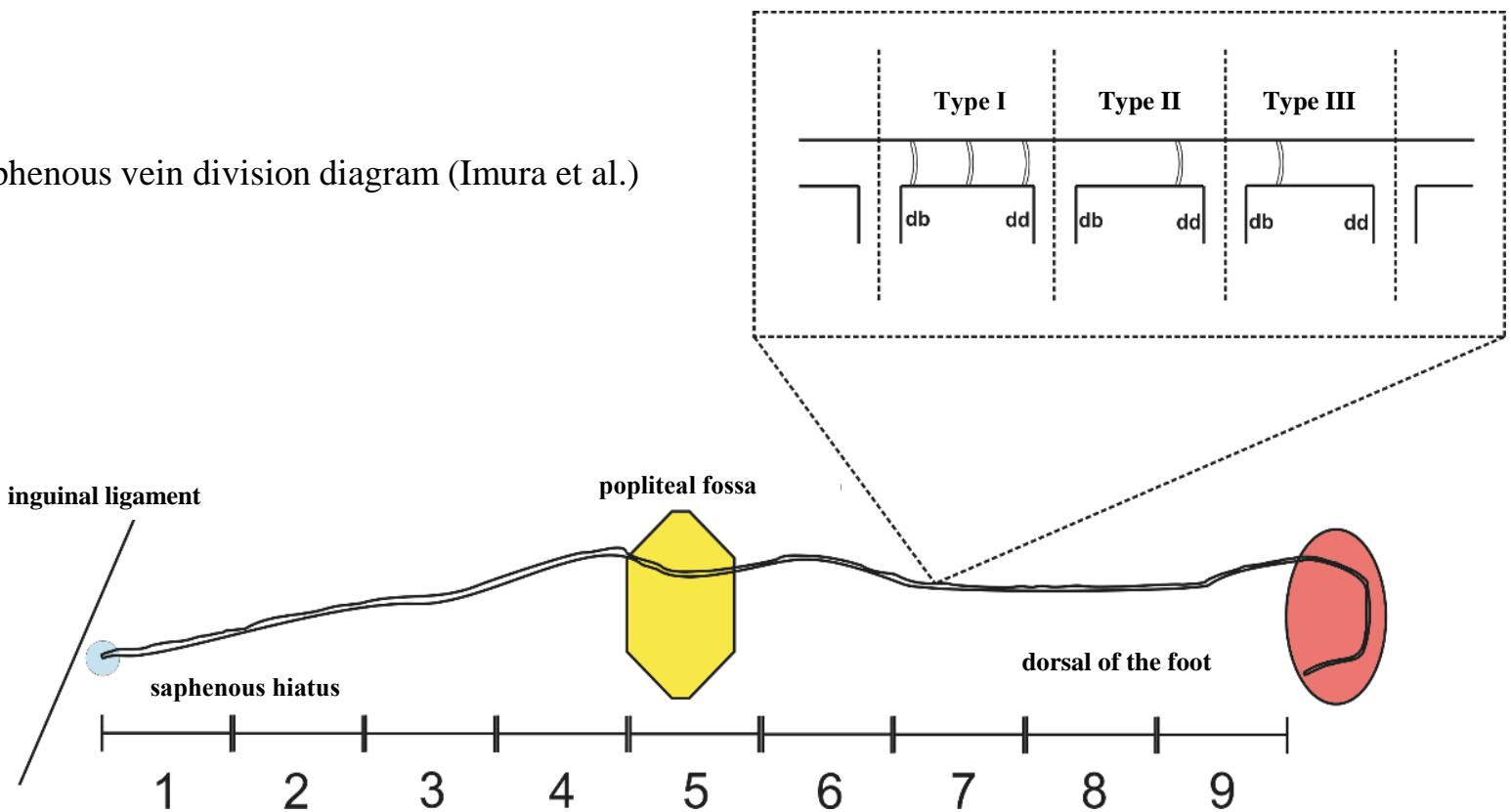


# Blood vessels used in CABG procedure



# Blood vessels used in CABG procedure

Saphenous vein division diagram (Imura et al.)



**CABG  
(clinical)**

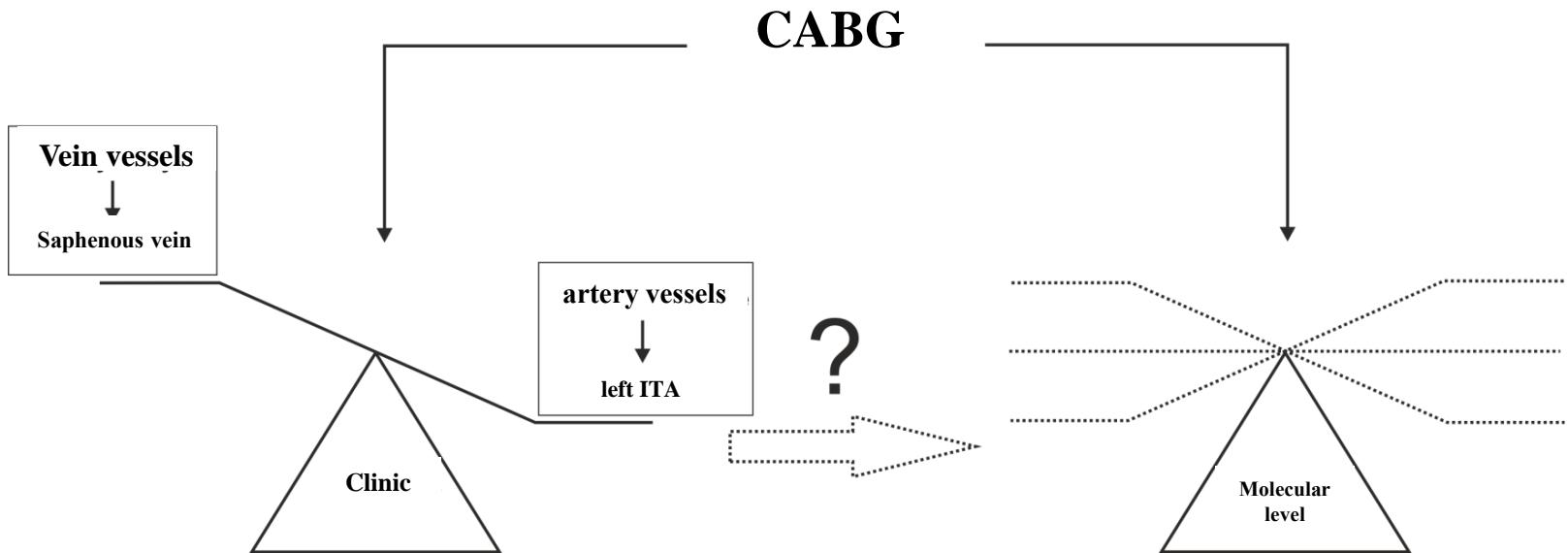
**Saphenous  
vein**

- availability
- preparation
- length
- vascularization
- permeability
- thrombosis, accelerated atherosclerosis

**Left internal  
thoracic artery**

- permeability
- length
- physical parameters
- no SMC migration
- low susceptibility to atherosclerosis
- preparation

# The Question



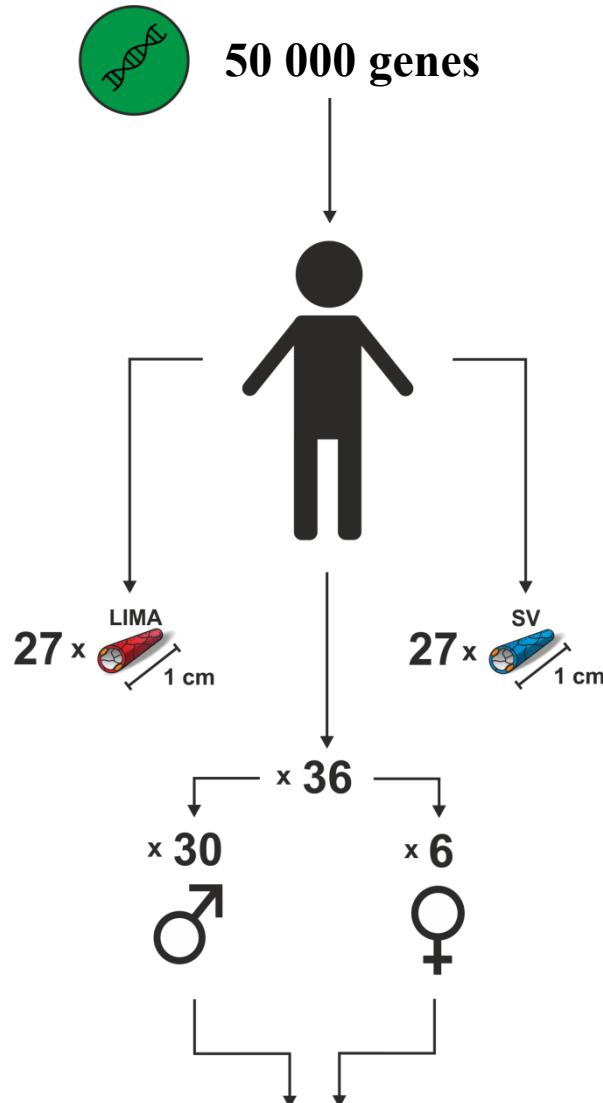
assumptions and purpose of the work

The main goal of the thesis was to answer following question:  
Does blood vessel choose to CABG procedure based on clinical  
outcomes has a confirmation on the molecular level?

The achievement of the main research objective was based on the  
following specific objectives:

1. Evaluation of global gene expression in two study groups representing veins (SV) and arteries (LIMA), respectively
2. Identify specific ontology groups overrepresented in a given vessel type
3. Gene expression validation of ontology group data using QPCR.
4. Comparison of the applied bioinformatics methods for the analysis of the obtained results.

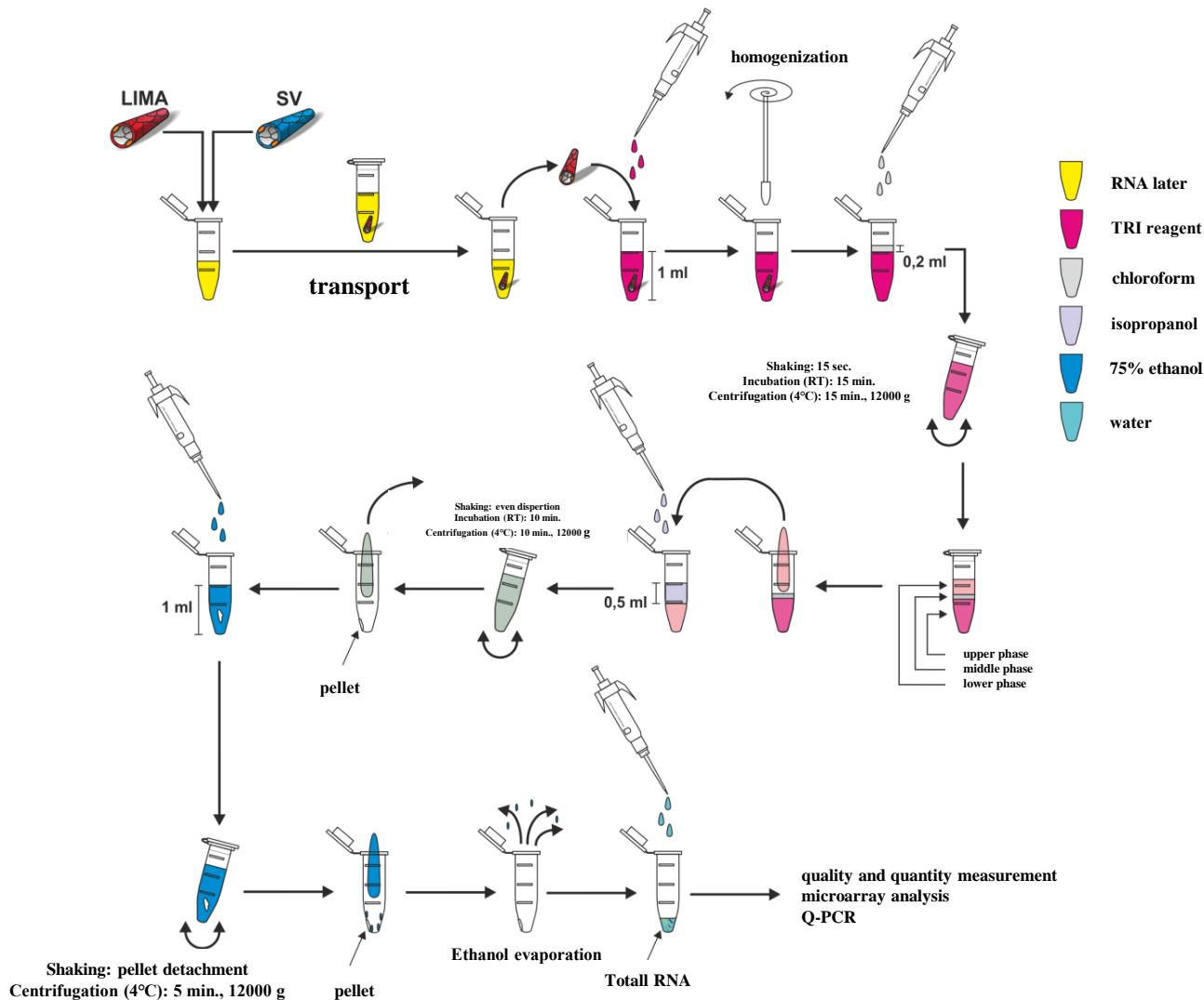
## Materials and Methods



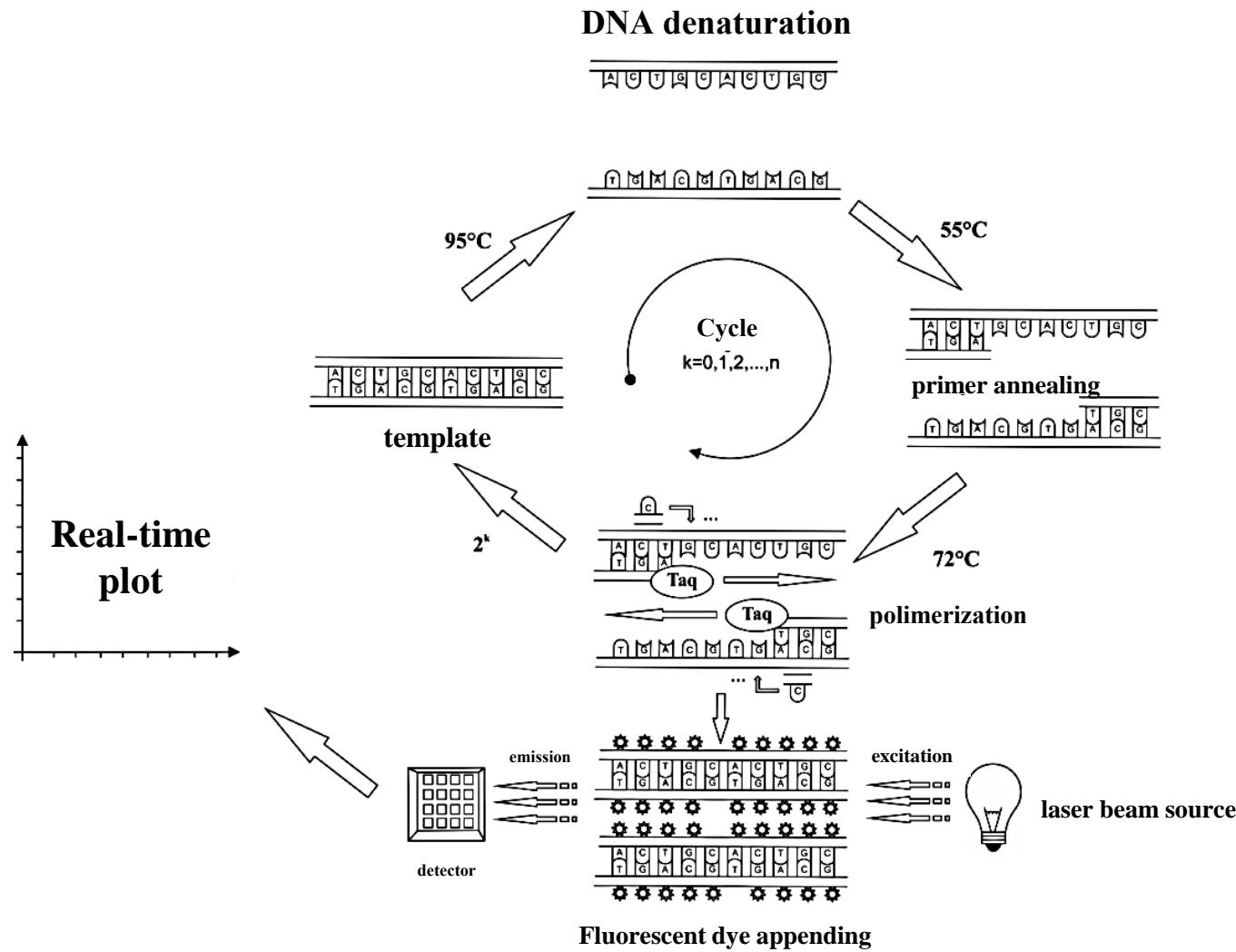
Research material

Cardiac surgery clinic, Department of  
Cardiothoracic surgery, Poznan  
University of Medical Sciences

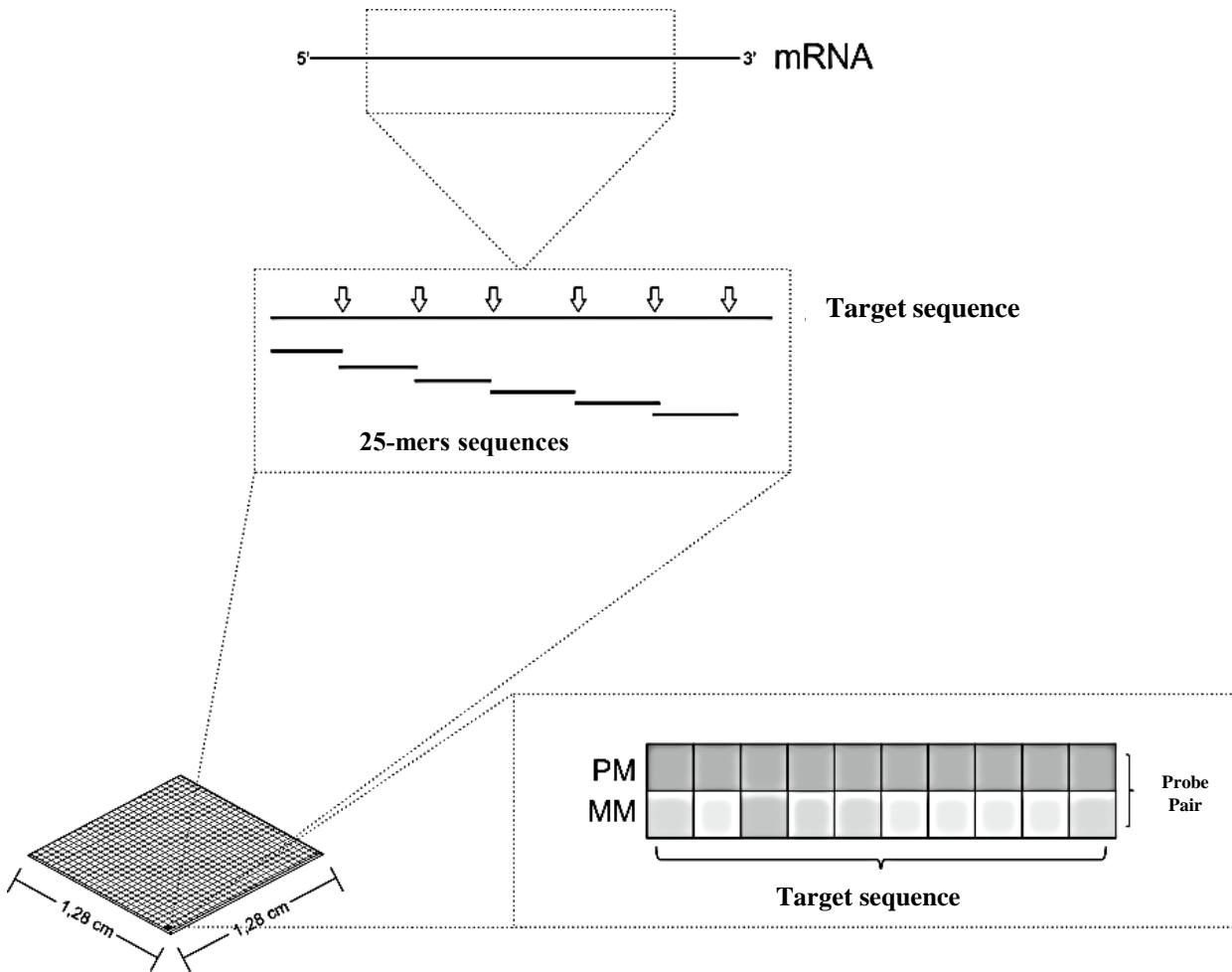
# Methods – isolation of the total RNA



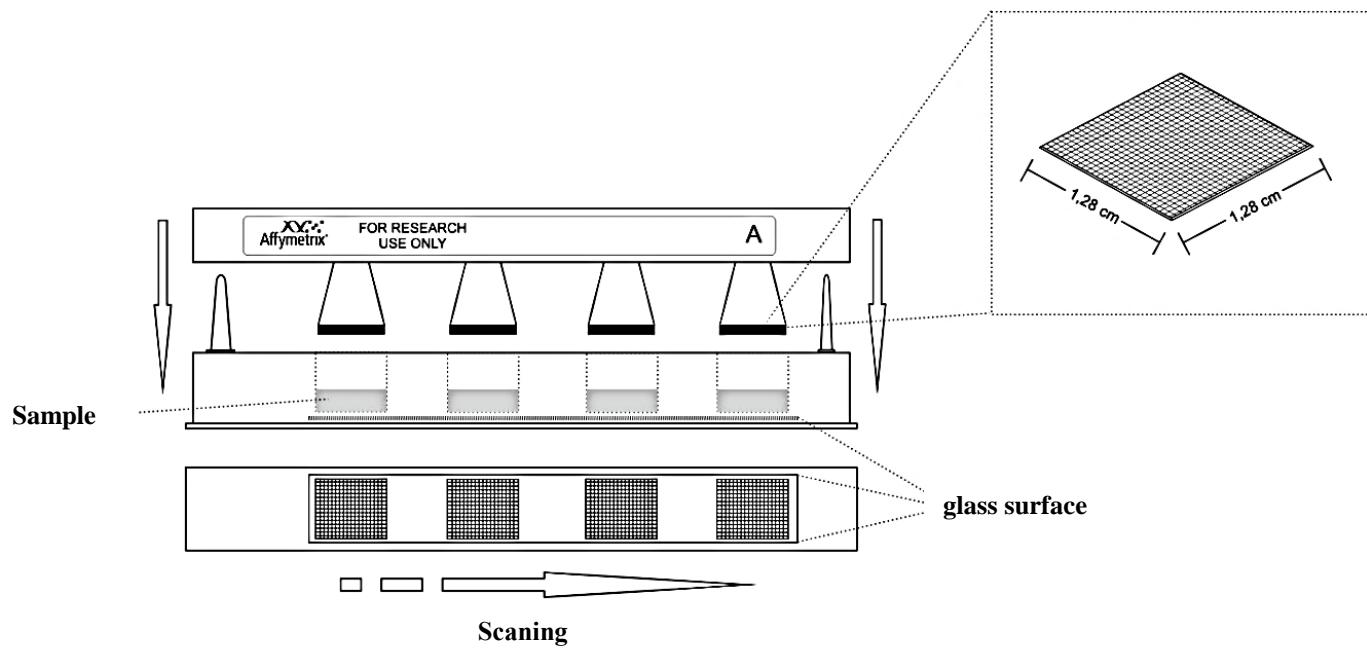
# Methods – Q-PCR



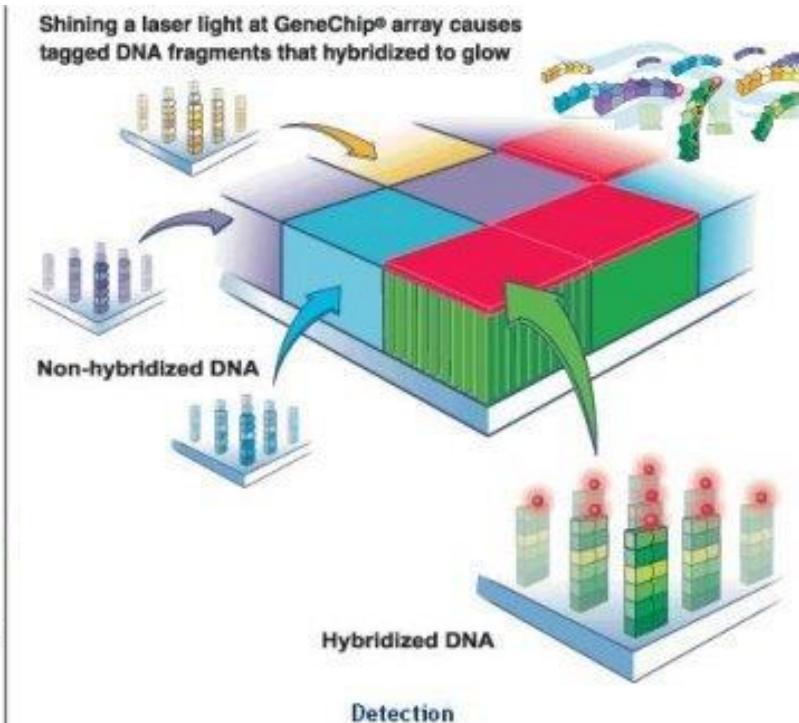
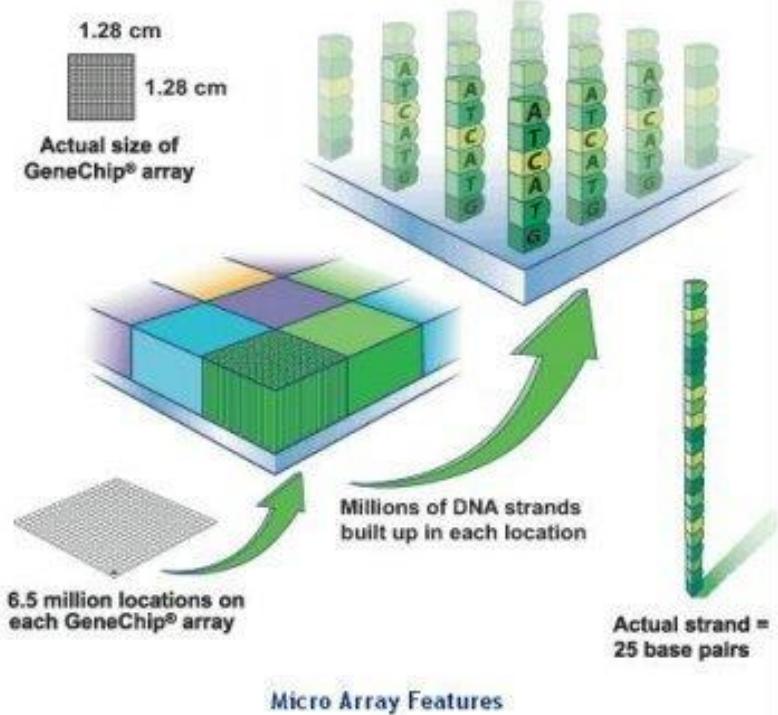
# Methods – microarray (Affymetrix)



## Methods – microarray (Affymetrix)



# Methods – microarray (Affymetrix)



## Methods – bioinformatic analysis



STRING (*Search Tool for the Retrieval of Interacting Genes/Proteins*)

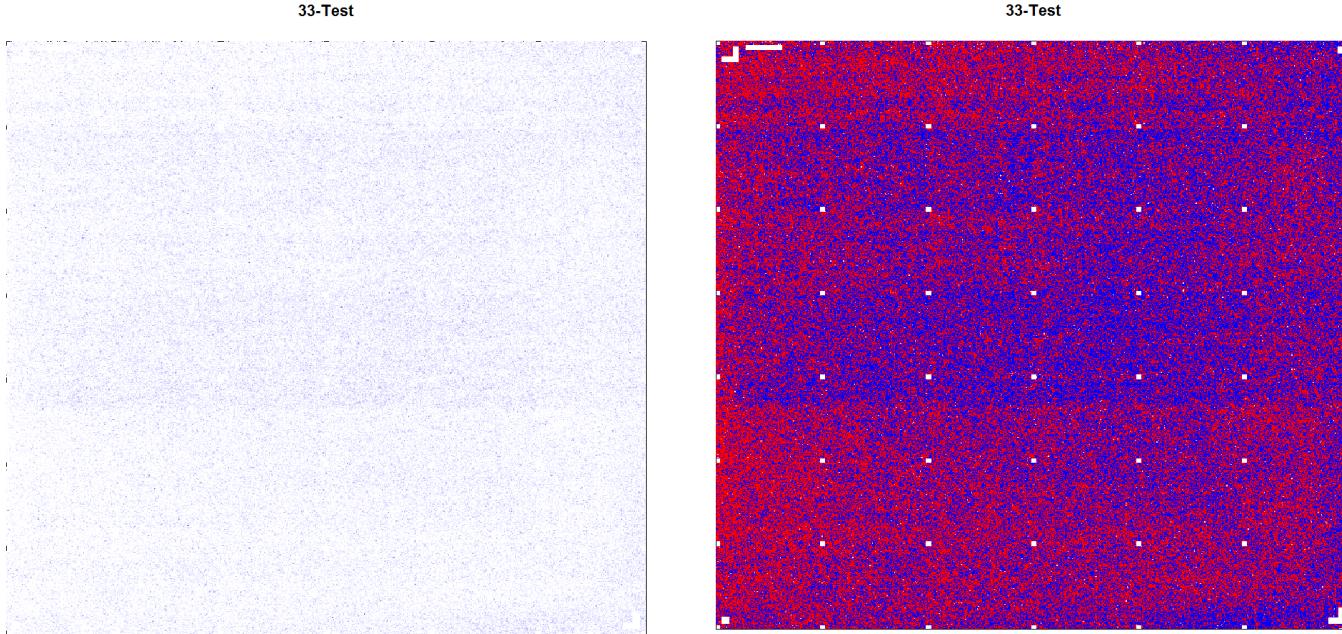


**DAVID Bioinformatics Resources 6.7**  
National Institute of Allergy and Infectious Diseases (NIAID), NIH

Database for Annotation, Visualization and Integrated Discovery (DAVID )

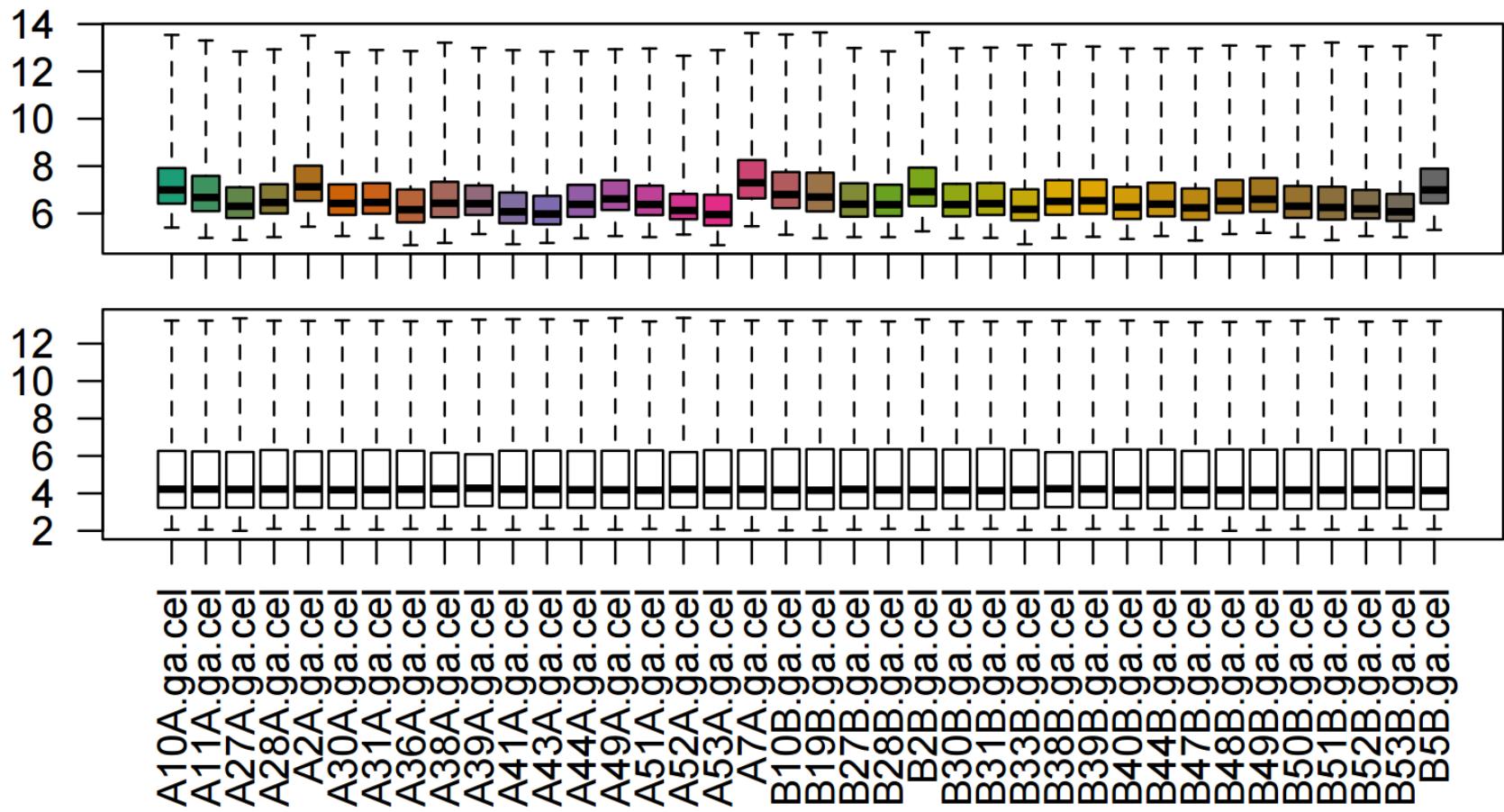
# Results

# Quality Control



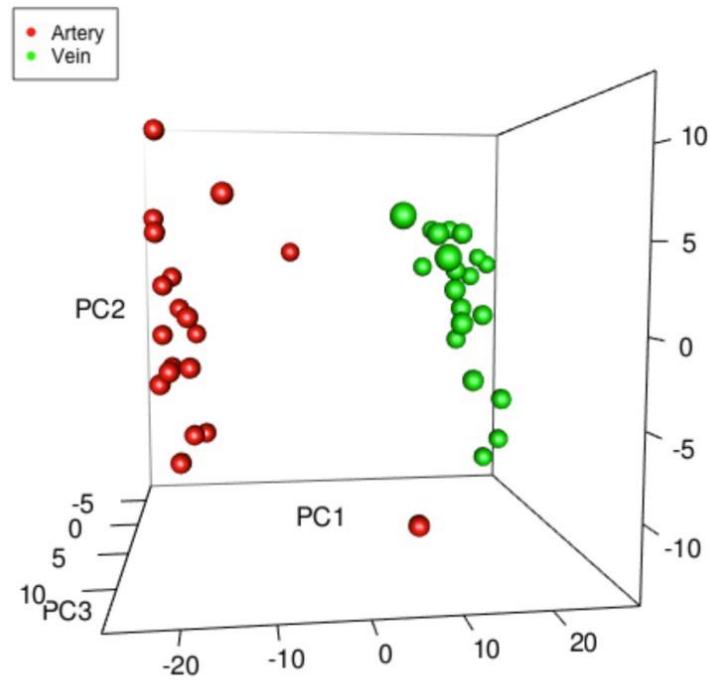
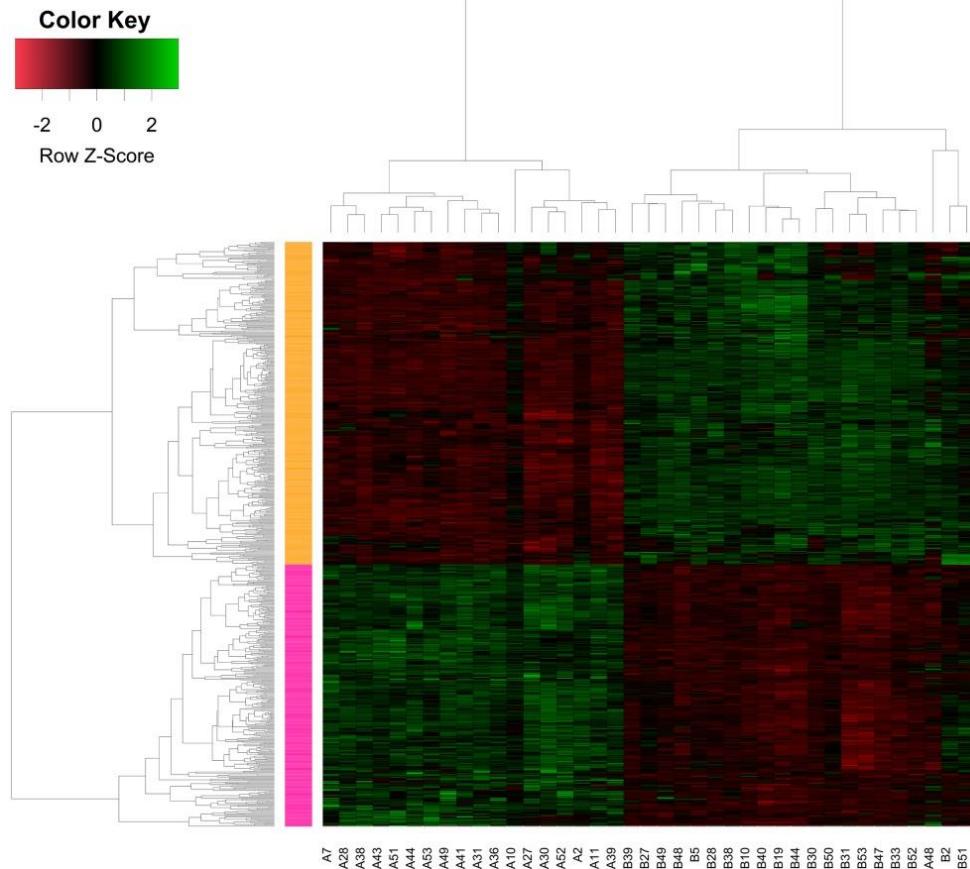
Affymetrix HG-U219 microarray staining with negative. The image shows RNA hybridization derived from the patient's artery number  
33.

# Quality Control

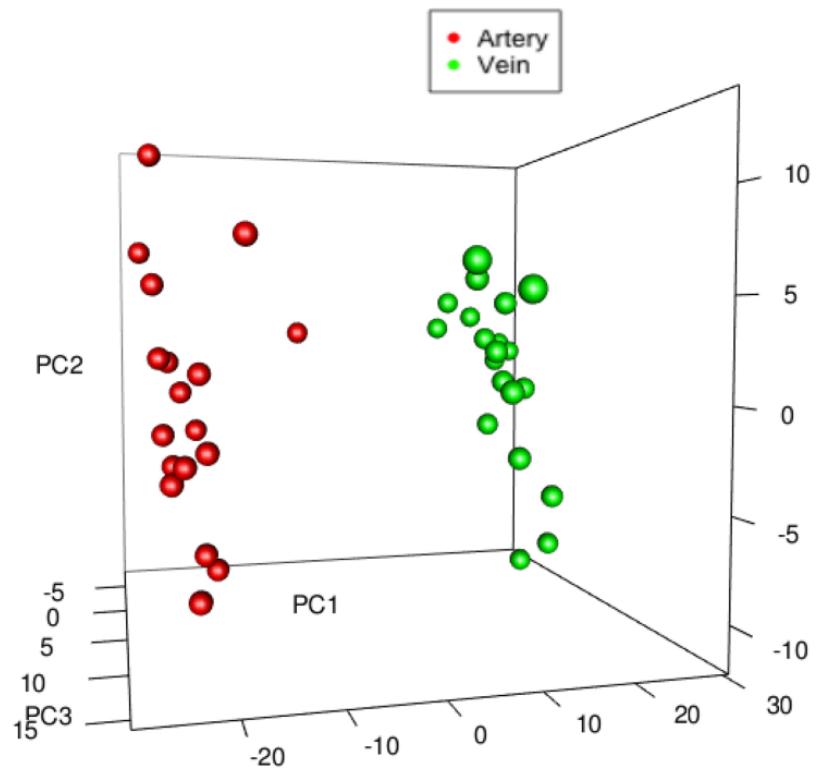
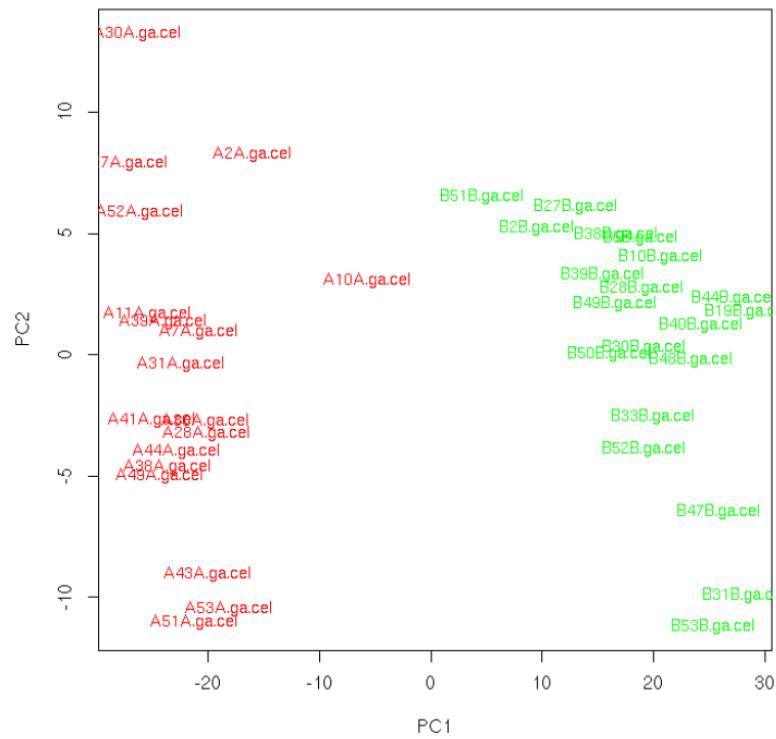


Results of normalization of microarray fluorescence throughout the experiment. Top panel - before normalization, bottom panel - after normalization.

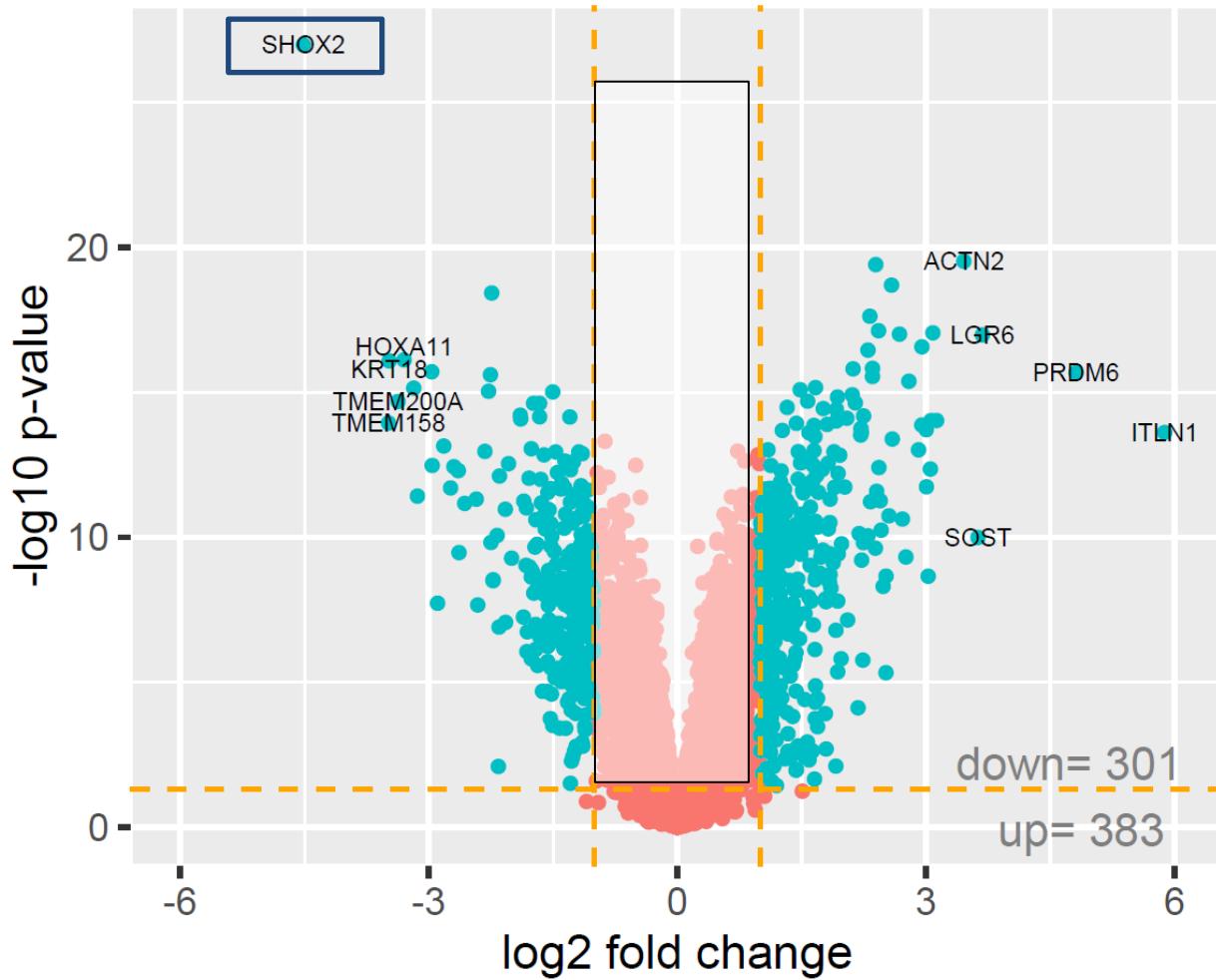
# PCA - heatmap



# PCA

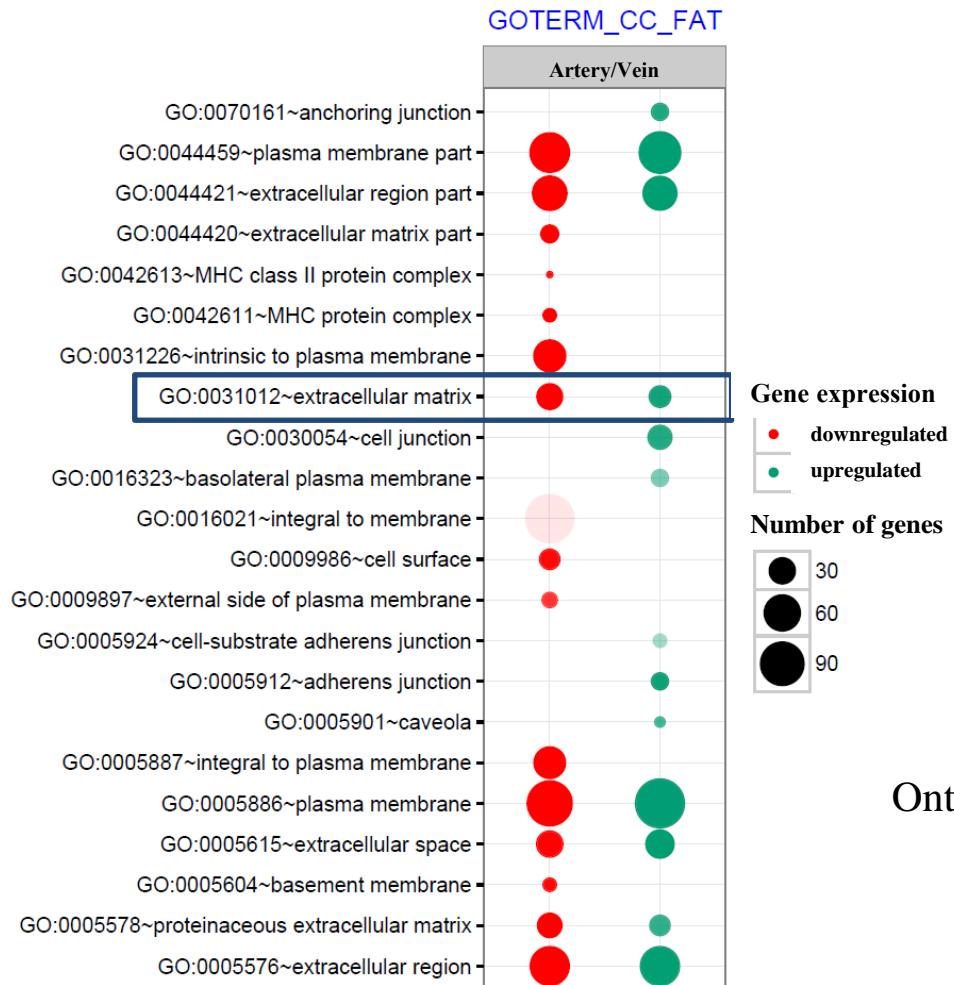


# Volcano plot



Results correlated with  $p\text{-value} < 0.05$  and  $\text{fold change} > 2.0$ .

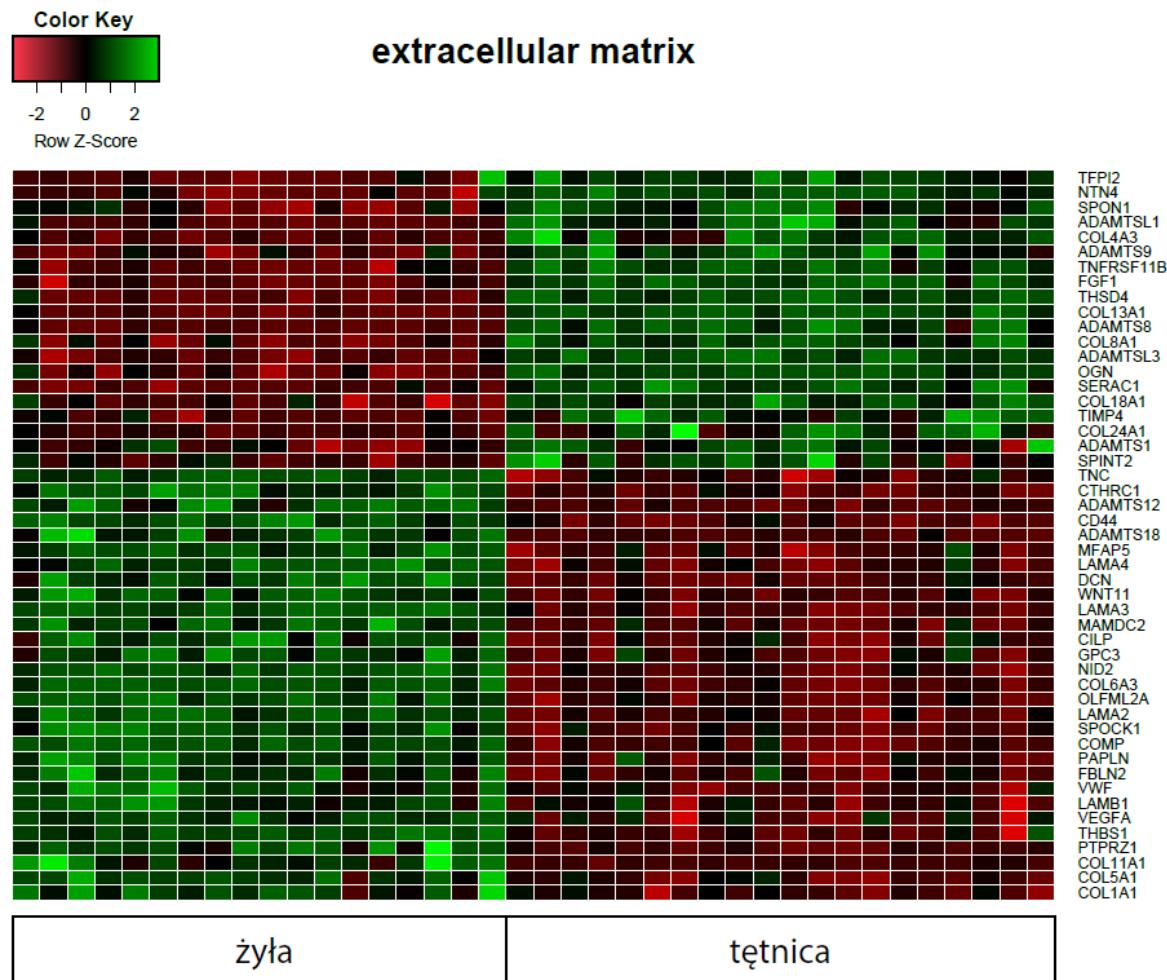
# Gene Ontology



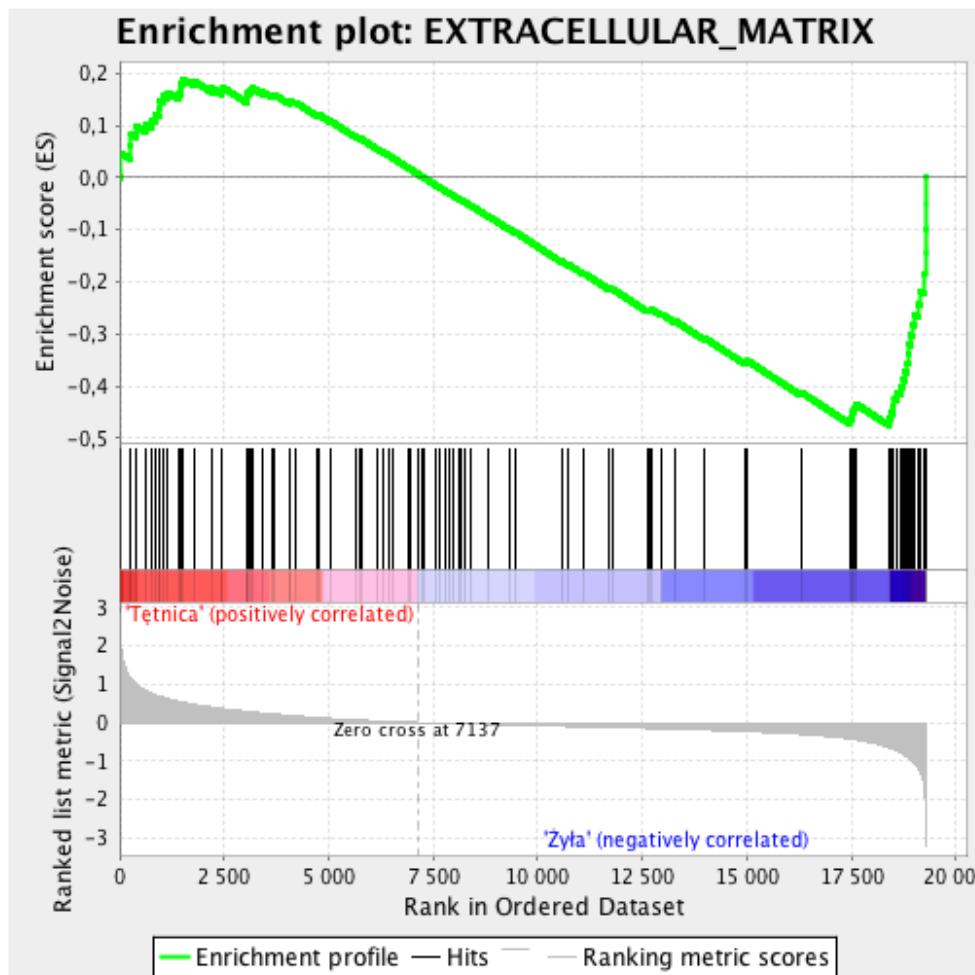
Ontology analysis results for cellular components.

# DAVID

Results of  
microarray analysis  
for extracellular  
matrix genes.

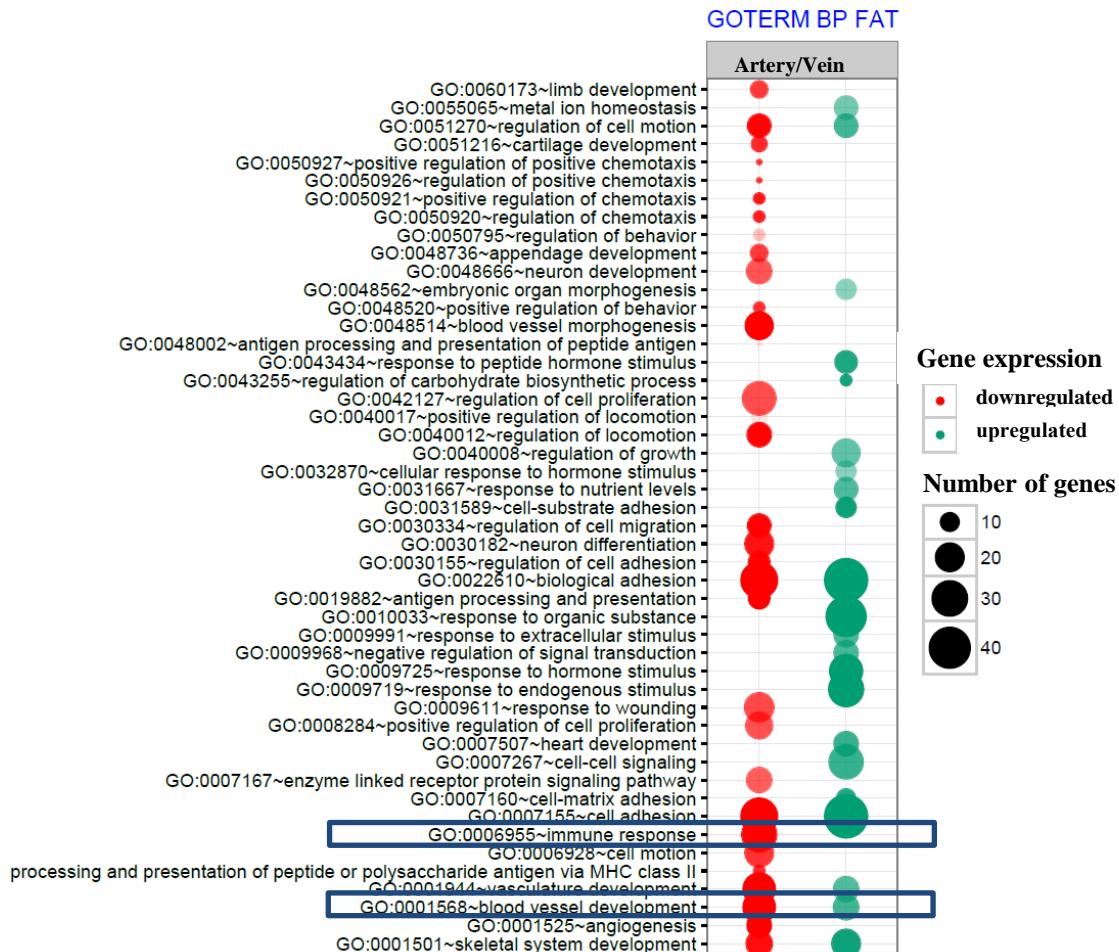


# GEA



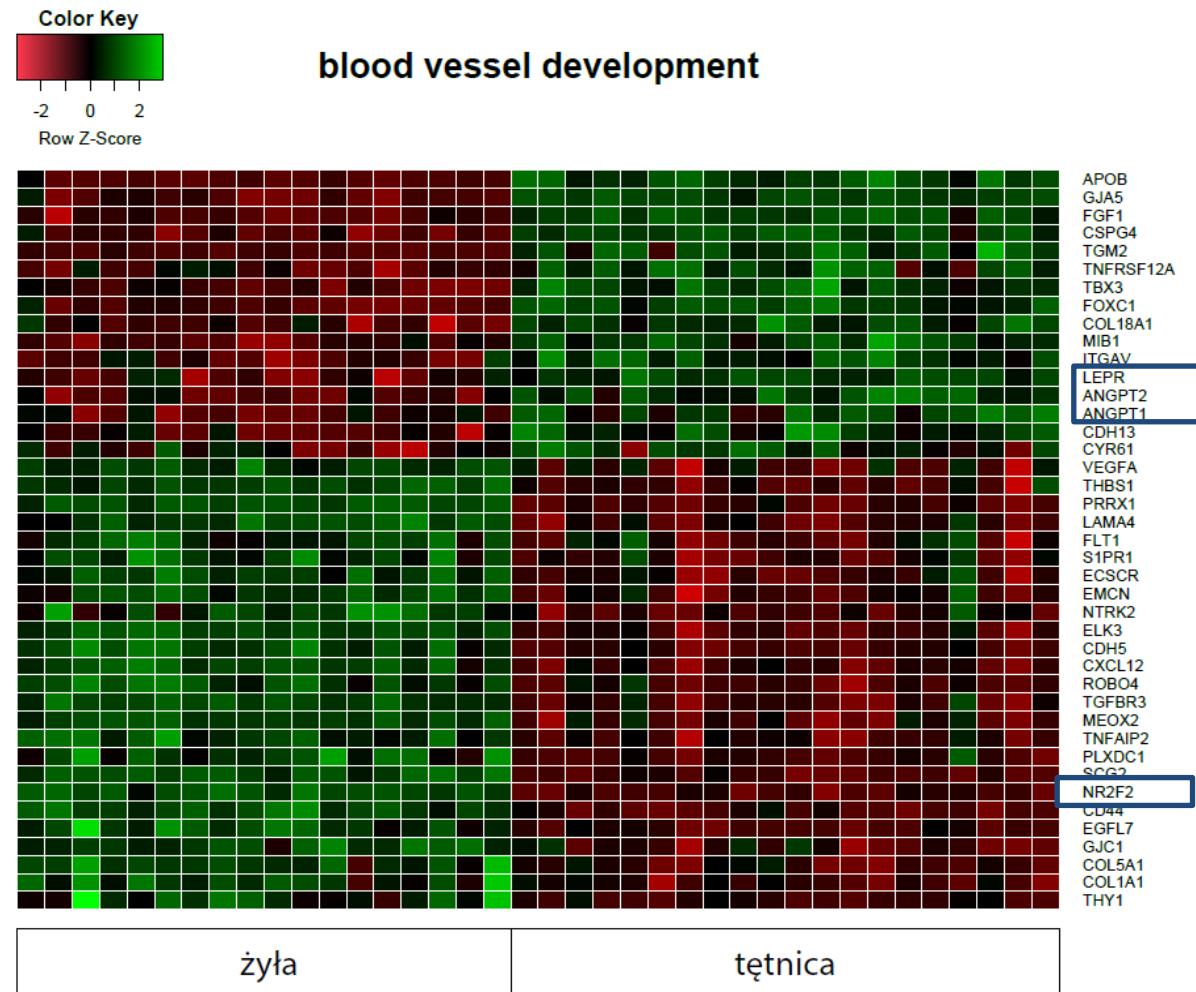
**Fig 1: Enrichment plot: EXTRACELLULAR\_MATRIX**  
*Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List*

# Gene Ontology



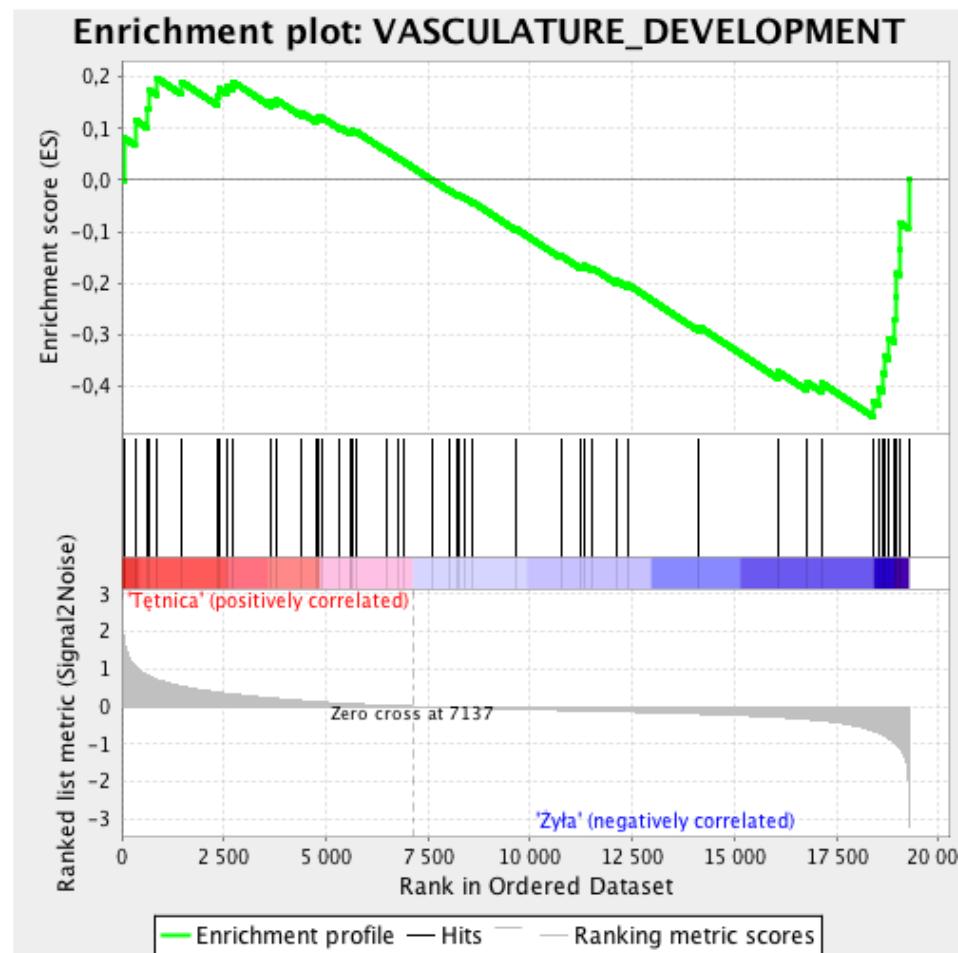
Ontological analysis results for biological processes.

# DAVID



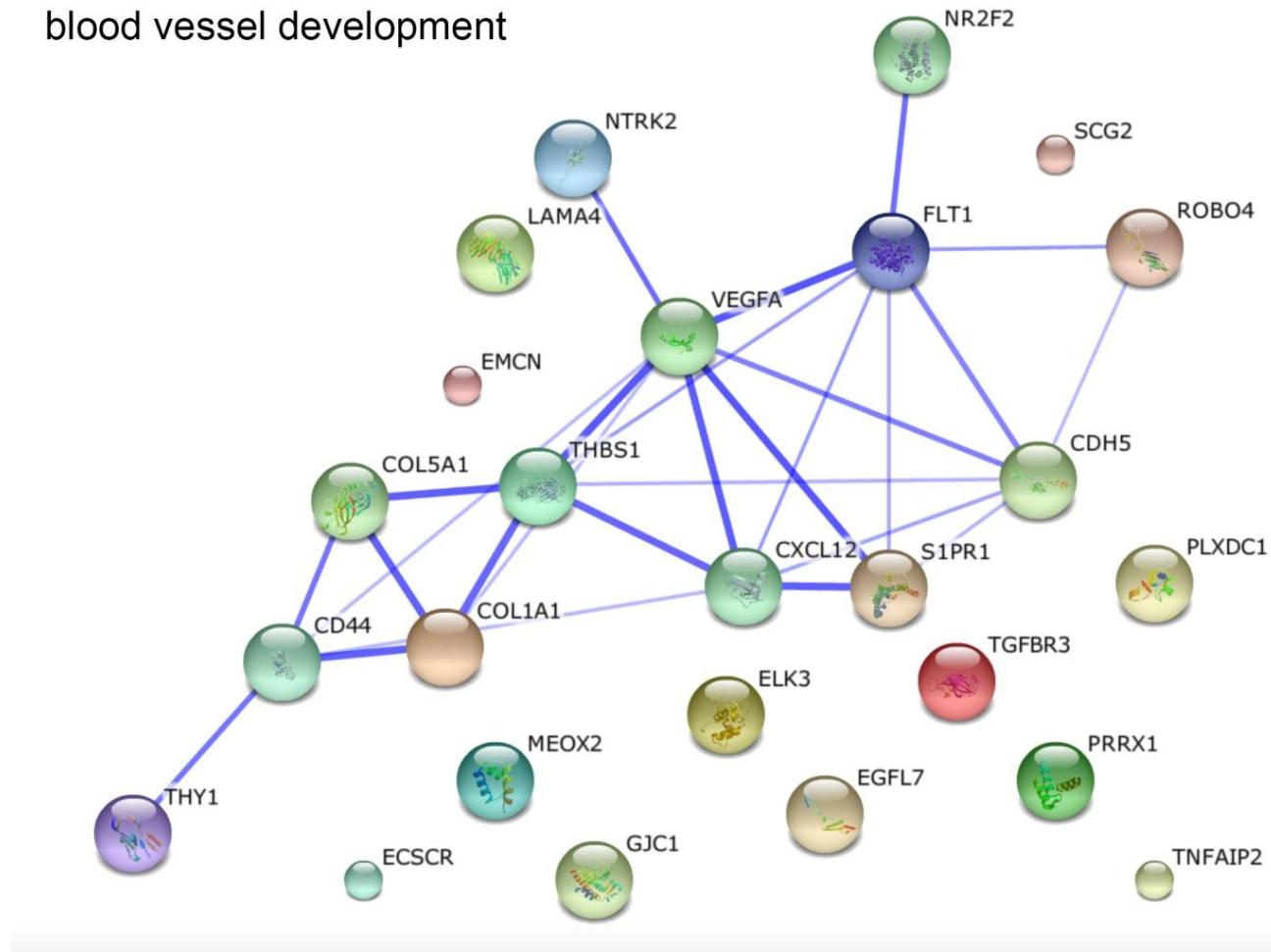
Results of microarray analysis for genes associated with vascular development.

# GEA



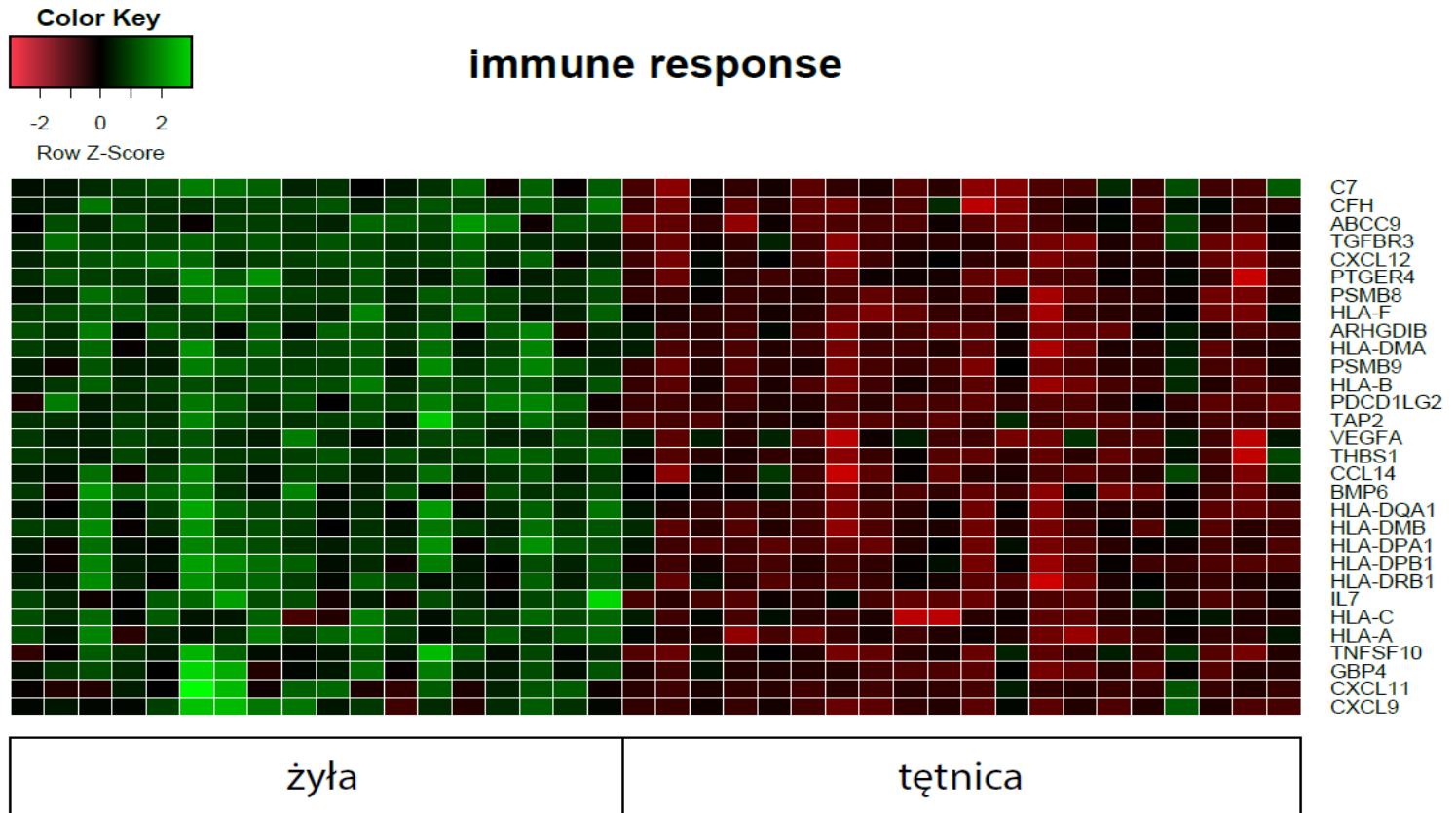
**Fig 1: Enrichment plot: VASCULATURE\_DEVELOPMENT**  
Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List

## blood vessel development



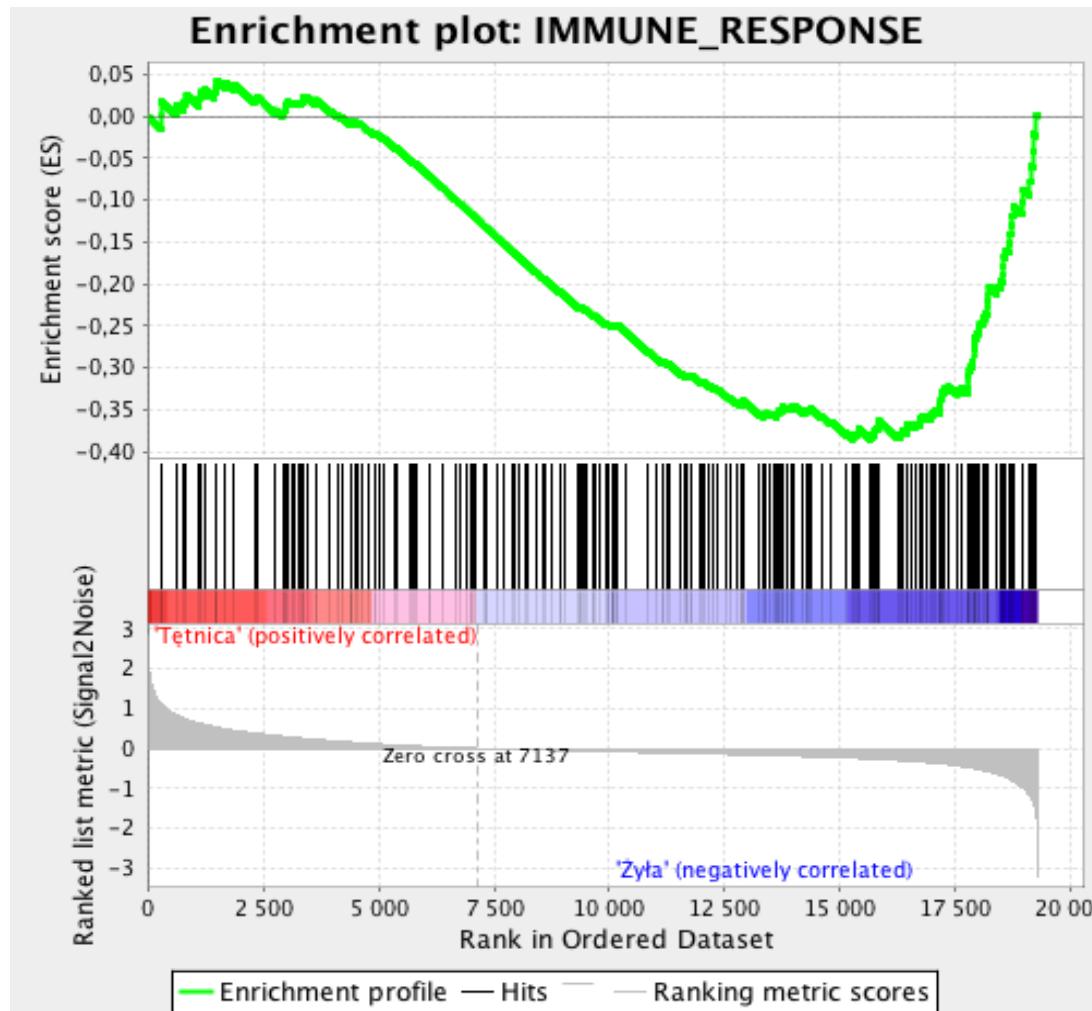
Interactions of differential genes representing a subset of blood vessel development obtained from literature and experimental data of the STRING database. The thickness of the connecting line corresponds to the strength of the interaction.

# DAVID

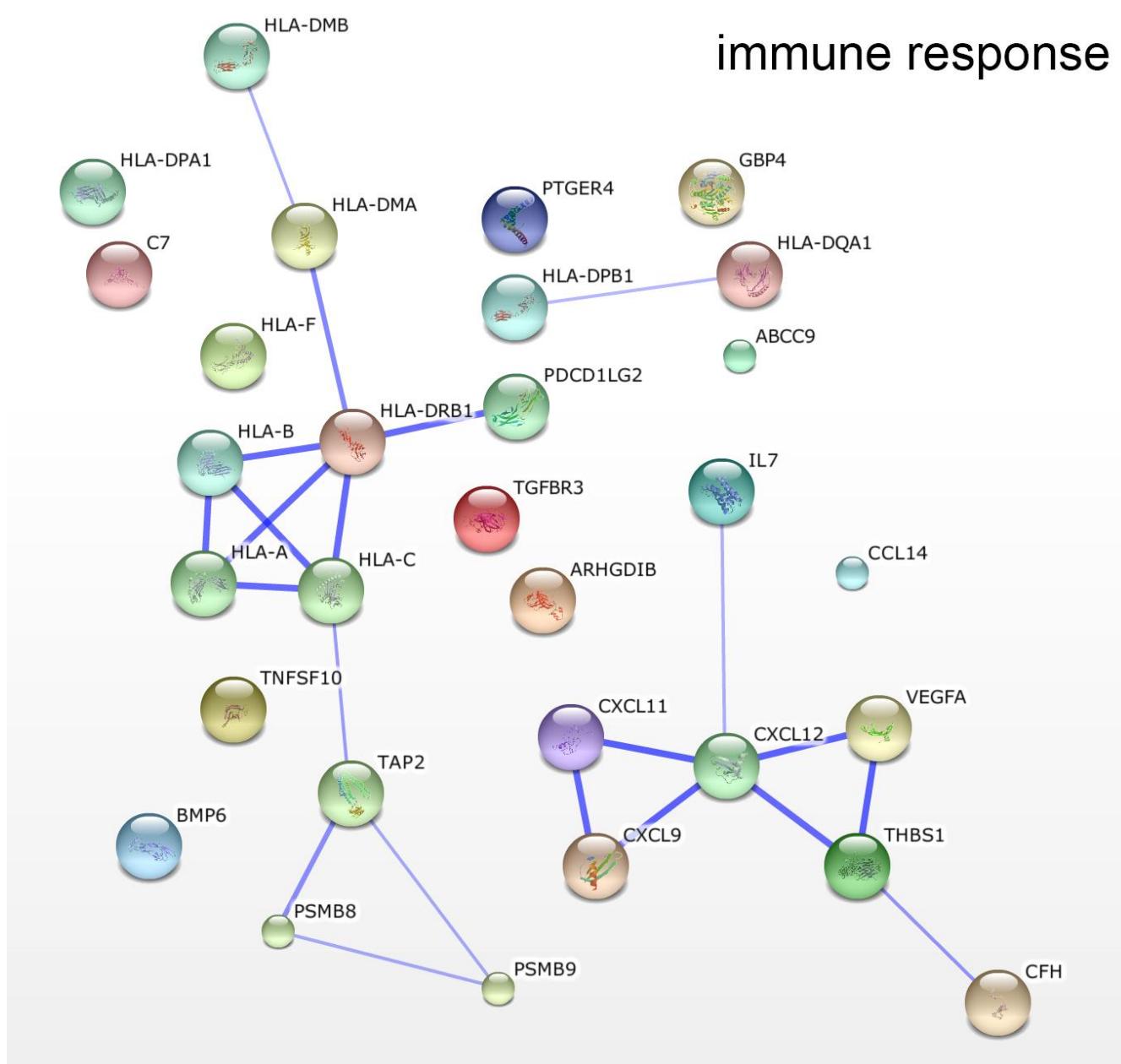


Results of microarray analysis for genes involved in immune response.

# GSEA

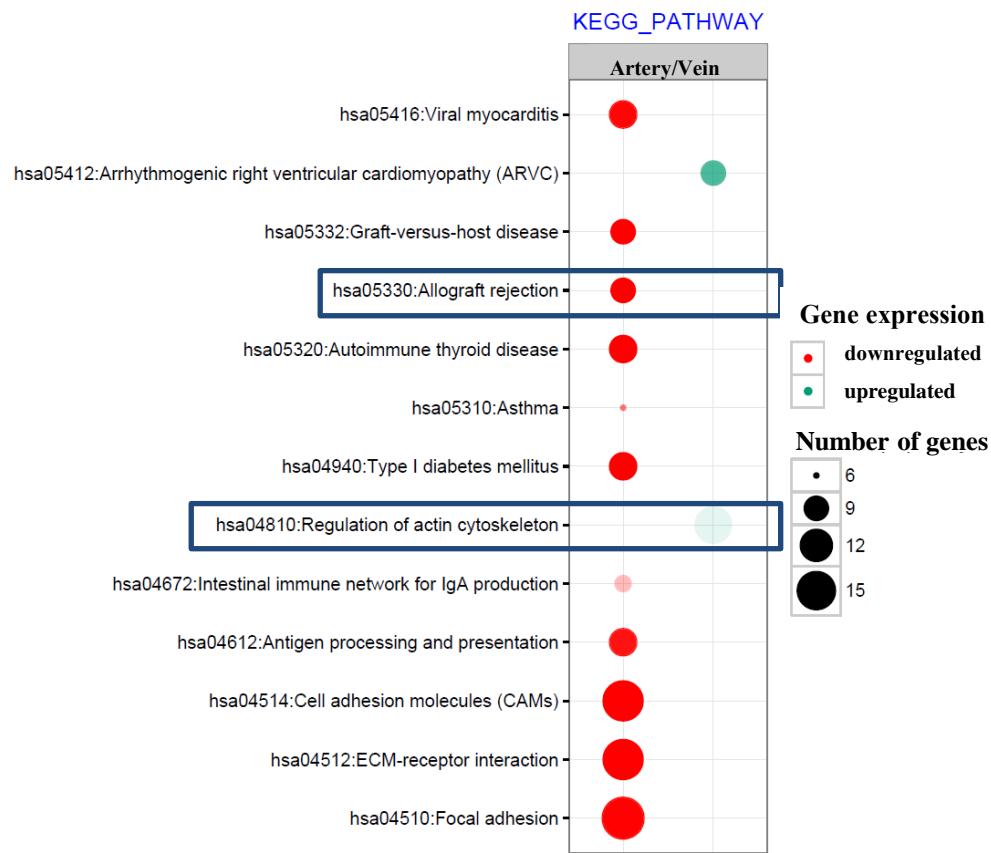


# immune response

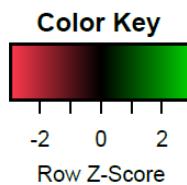


Interactions of differential genes, representing a subset of the immune response (immune response), obtained from literature and experimental data of the STRING database. The thickness of the connecting line corresponds to the strength of the interaction

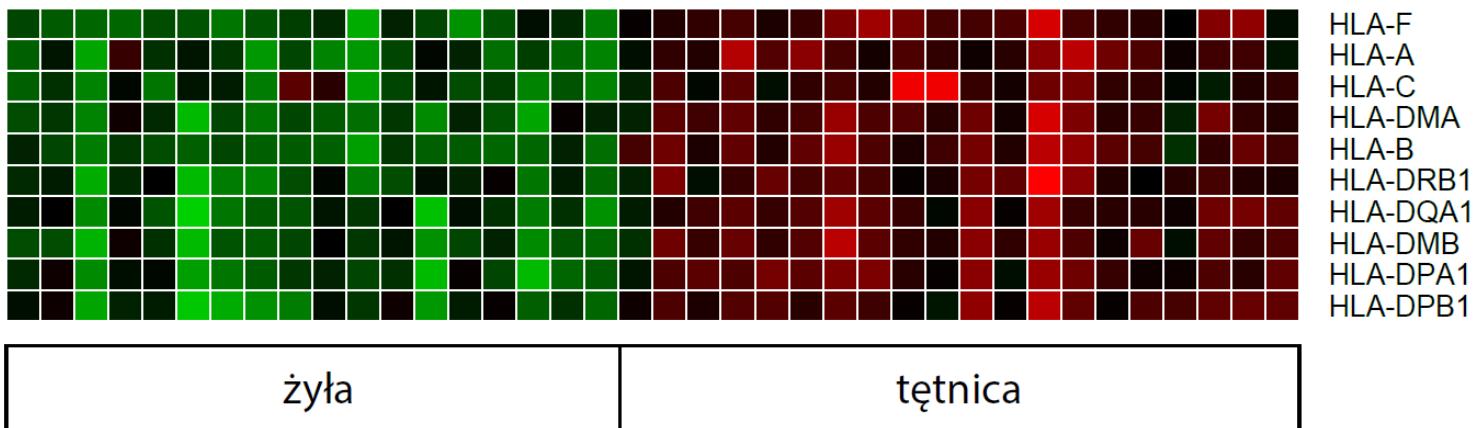
# KEGG



# DAVID

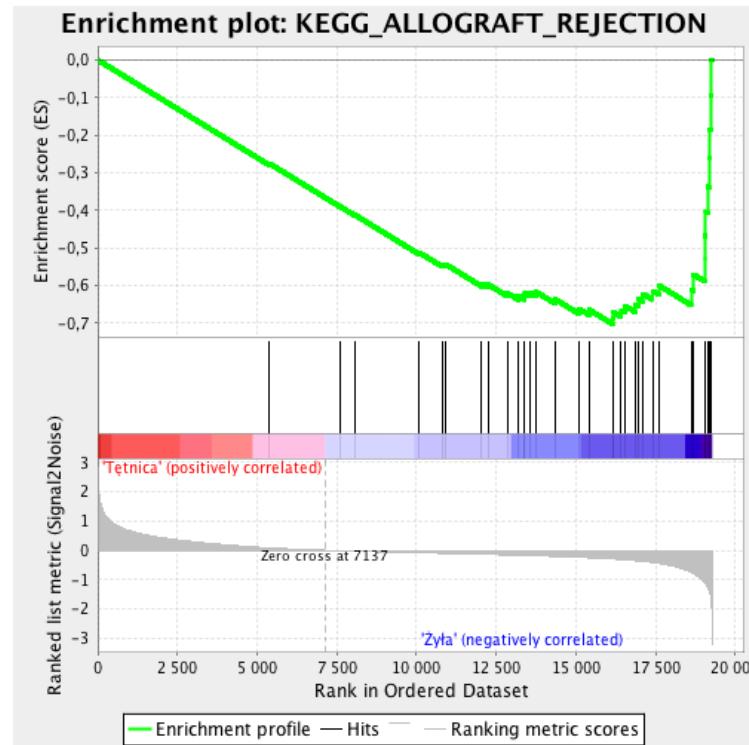


## Allograft rejection



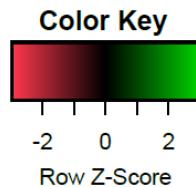
Results of microarray analysis for genes involved in graft rejection.

# GSEA

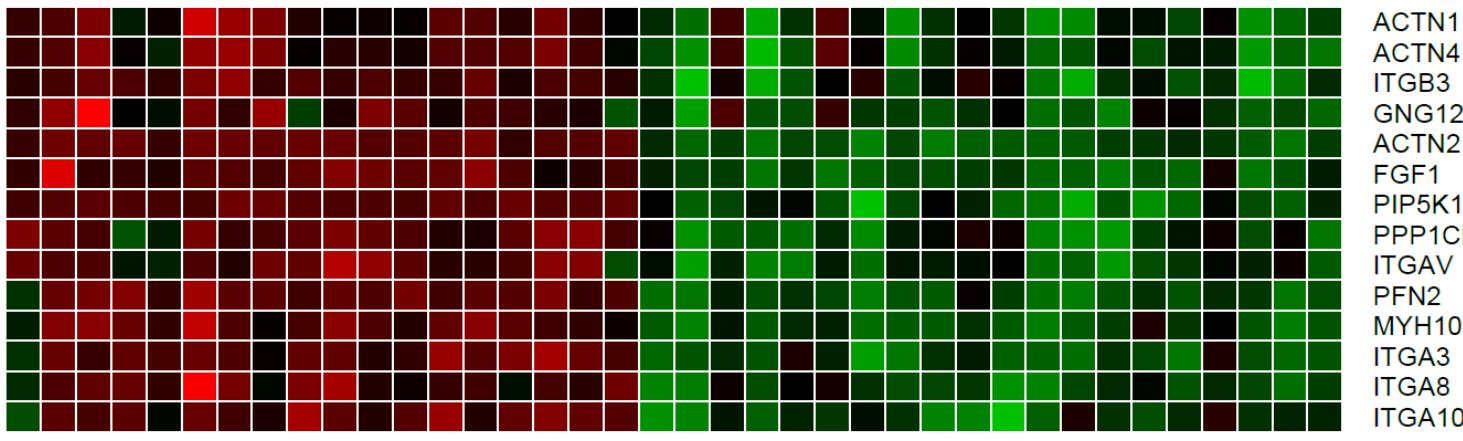


**Fig 1: Enrichment plot: KEGG\_ALLOGRAFT\_REJECTION**  
*Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List*

# DAVID



## Regulation of actin cytoskeleton



żyła

tętnica

Results of microarray analysis for genes regulating cytoskeletal structure.

# GSEA

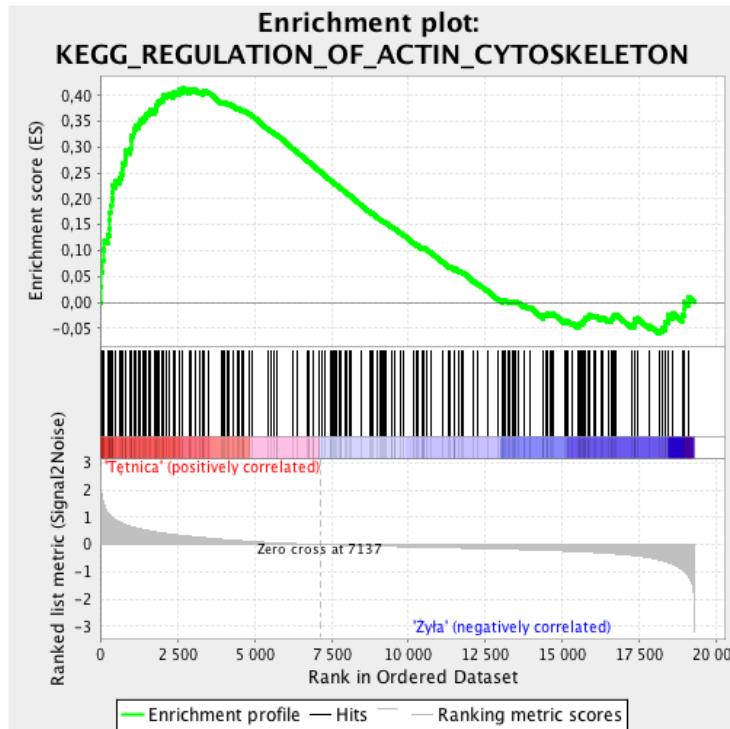
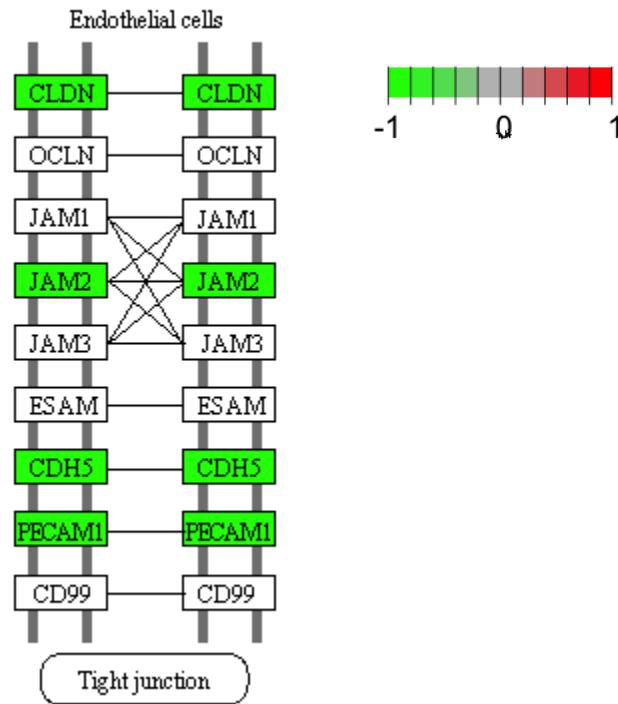


Fig 1: Enrichment plot: KEGG\_REGULATION\_OF\_ACTIN\_CYTOSKELETON  
Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List

# KEGG



Results of KEGG analysis for genes involved in encoding proteins responsible for intercellular connections between endothelial cells.

# GSEA

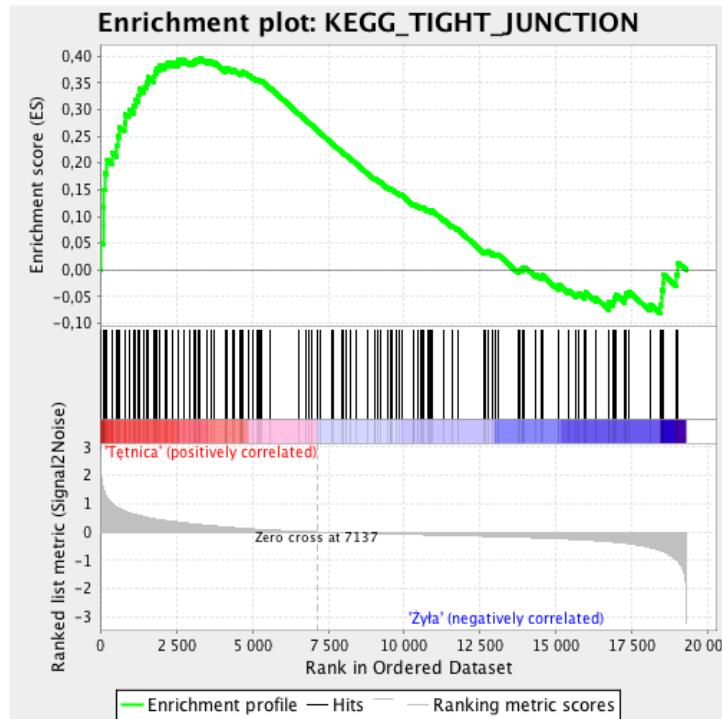
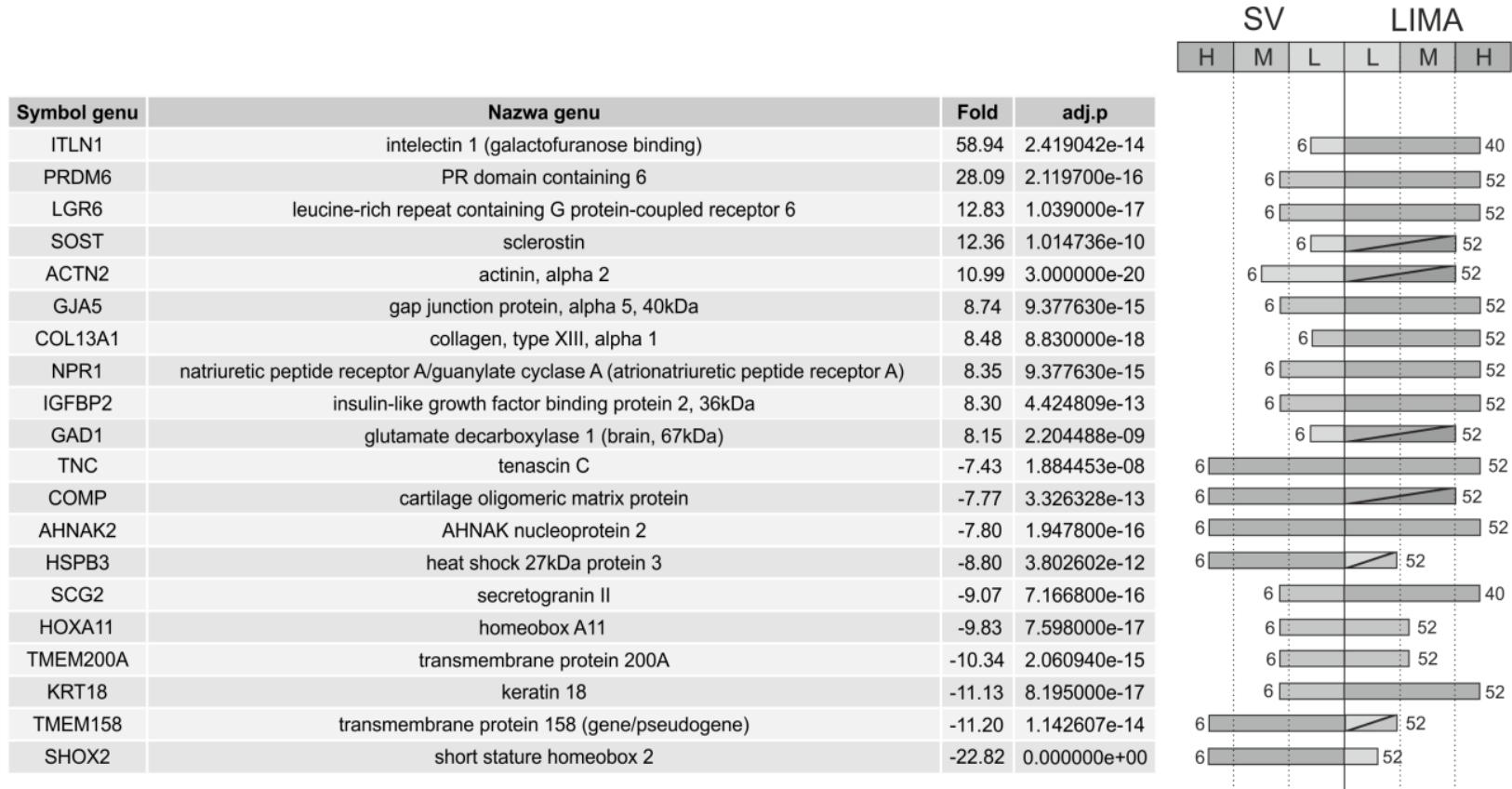


Fig 1: Enrichment plot: KEGG\_TIGHT\_JUNCTION  
Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List



Alteration of LIMA gene expression in relation to SV

# RT-PCR

## Angiogenesis PCR Array

### **Angiogenic Factors:**

Growth Factors and Receptors: ANG, ANGPT1, ANGPT2, ANPEP, TYMP, FGF1, FGF2 (bFGF), FIGF (VEGFD), FLT1, JAG1, KDR, NRP1, NRP2, PGF, VEGFA, VEGFB, VEGFC.

Adhesion Molecules: BAI1, COL4A3, IL8, NRP1, NRP2.

Proteases, inhibitors and Other Matrix Proteins: ANGPTL4, F3, PECAM1, PF4, PROK2, SERPINE1 (PAI-1), SERPINF1.

Other Genes: HIF1A, NOS3, SPHK1.

### **Other Factors Involved in Angiogenesis:**

Cytokines: CCL11 (Eotaxin), CCL2 (MCP-1), CXCL1, CXCL10 (INP10), CXCL5 (ENA78/LIX), CXCL6 (GCP-2), CXCL9 (MIG), EDN1, IFNA1, IFNG, IL1B, IL6, MDK, TNF.

Growth Factors and Receptors: CTGF, EFNA1, EFNB2, EGF, EPHB4, FGFR3, HGF, IGF1, ITGB3, PDGFA, S1PR1, TEK (TIE2), TGFA, TGFB1, TGFB2, TGFBR1.

Adhesion Molecules: CCL11 (Eotaxin), CCL2 (MCP-1), CDH5, COL18A1, CTGF, ENG (EVI-1), ERBB2 (HER2), FN1, ITGAV, ITGB3, S1PR1, THBS1, THBS2.

Proteases, Inhibitors and Other Matrix Proteins: LECT1, LEP, MMP14, MMP2, MMP9, PLAU (uPA), PLG, TIMP1, TIMP2, TIMP3.

Other Genes: AKT1, HPSE, ID1, NOTCH4, PTGS1, TIE1.

# RT-PCR

## Endothelial Cell Biology PCR Array

**Angiogenesis:** ANGPT1, CCL2 (MCP-1), CCL5 (RANTES), CX3CL1, EDN1, EDNRA, ENG (EVI-1), F3, FASLG (TNFSF6), FGF1, FGF2 (bFGF), FLT1, FN1, HIF1A, HMOX1, IL1B, IL6, ITGA5, ITGAV, ITGB1, ITGB3, KDR, KIT (CD117), KLK3, MMP2, MMP9, NOS3 (eNOS), NPPB, NPR1, PF4, PGF, PLAU (uPA), PTGS2 (COX2), SERPINE1 (PAI-1), SPHK1, TEK (TIE2), THBS1, TYMP, VEGFA.

**Vasoconstriction & Vasodilation:** ACE, AGT, AGTR1, ALOX5, APOE, CALCA, CAV1, CX3CL1, EDN1, EDN2, EDNRA, F2R, HMOX1, ICAM1, NOS3 (eNOS), NPPB, NPR1, PTGIS, PTGS2 (COX2), SOD1.

**Inflammatory Response:** ACE, AGT, AGTR1, ALOX5, APOE, CALCA, CCL2 (MCP-1), CCL5 (RANTES), CX3CL1, EDNRA, F2R, F3, FN1, HIF1A, HMOX1, IL1B, IL6, NPPB, PTGS2 (COX2), SELE, SPHK1, TGFB1, THBS1, TNF, VCAM1.

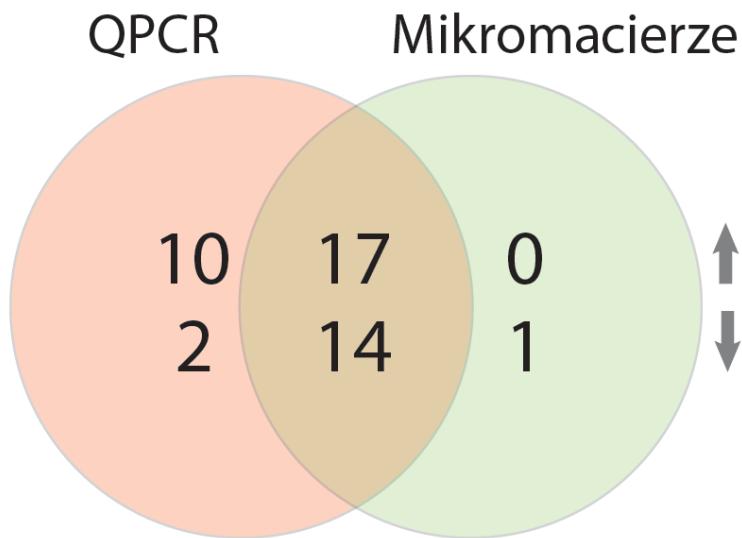
**Apoptosis:** ANXA5, BAX, BCL2, BCL2L1 (BCL-X), CASP1 (ICE), CASP3, CAV1, CCL2 (MCP-1), CCL5 (RANTES), CFLAR (CASPER), CX3CL1, EDN1, EDNRA, FAS (TNFRSF6), FASLG (TNFSF6), FGF2, HIF1A, HMOX1, IL1B, IL3, IL6, IL7, OCLN, PF4, PTK2, SPHK1, TEK (TIE2), THBS1, TNF, TNFSF10 (TRAIL).

**Cell Adhesion:** ADAM17 (CD156B), AGT, BCL2, CALCA, CDH5, COL18A1 (Endostatin), CX3CL1, ENG (EVI-1), FGF1, FN1, ICAM1, IL1B, ITGA5, ITGAV, ITGB1, ITGB3, KDR, PDGFRA, PECAM1, PLAU (uPA), PLG, PTK2, SELE, SELL (LECAM1), SELPLG (P-selectin), SERPINE1 (PAI-1), TGFB1, THBS1, TNF, VCAM1, VEGFA, VWF.

**Coagulation:** ANXA5, CAV1, EDN1, F2R, F3, FN1, MMP1, PECAM1, PF4, PLAT (tPA), PLAU (uPA), PLG, PROCR, PTK2, SELL (LECAM1), SELPLG (P-selectin), SERPINE1 (PAI-1), TEK (TIE2), TFPI, THBD, THBS1, TIMP1, VWF.

**Platelet Activation:** APOE, CX3CL1, F2R, FN1, IL11, IL6, ITGB3, NOS3 (eNOS), PDGFRA, PECAM1, PF4, PLG, SERPINE1 (PAI-1), SOD1, TGFB1, THBD, THBS1, TIMP1, VEGFA, VWF.

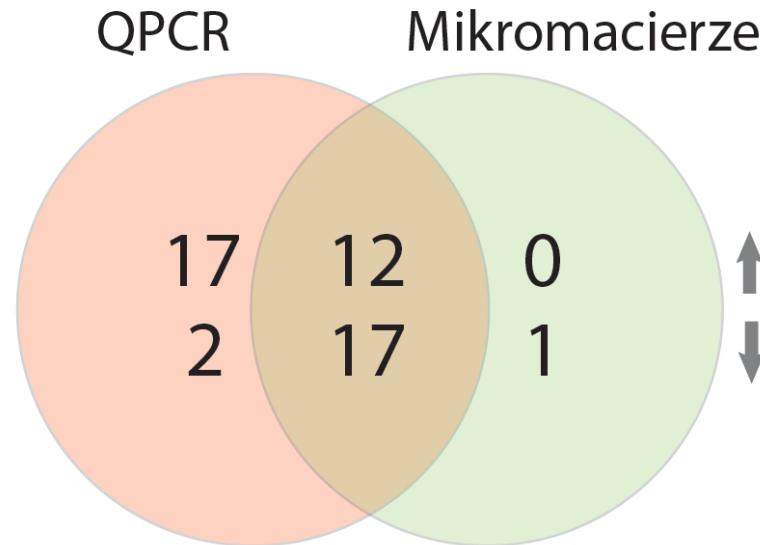
# Q-PCR Angiogenesis



	AKT1	ANGPTL4	CCL2	IL6	NRP1	TGFB1	TGFB2	TGFBR1	TIMP1	TIMP2	CXCL10	CXCL6
QPCR	1.39	1.59	2.9	3	1.29	1.84	1.66	1.5	1.48	1.32	-1.7	-2.4
Mikromacierze	1.05	1.04	1.44	1.62	-1.07	1.09	-1.02	1.02	-1.04	-1.16	-1.69	-1.02

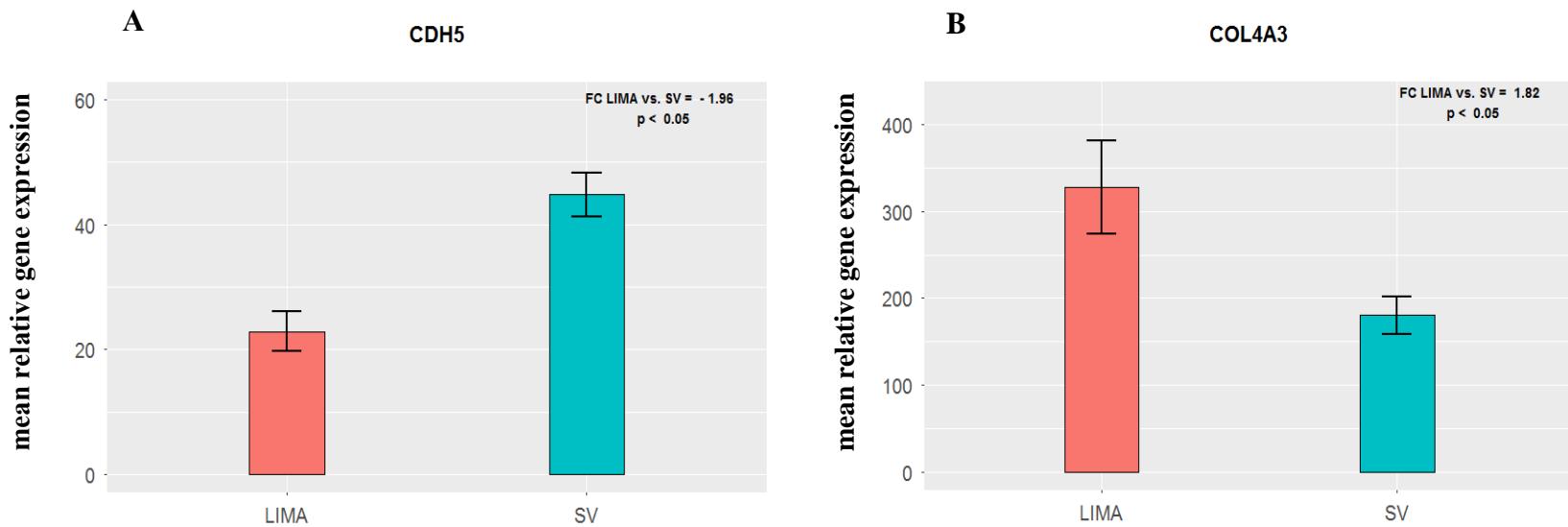
# Q-PCR

## Endothelial Cell biology

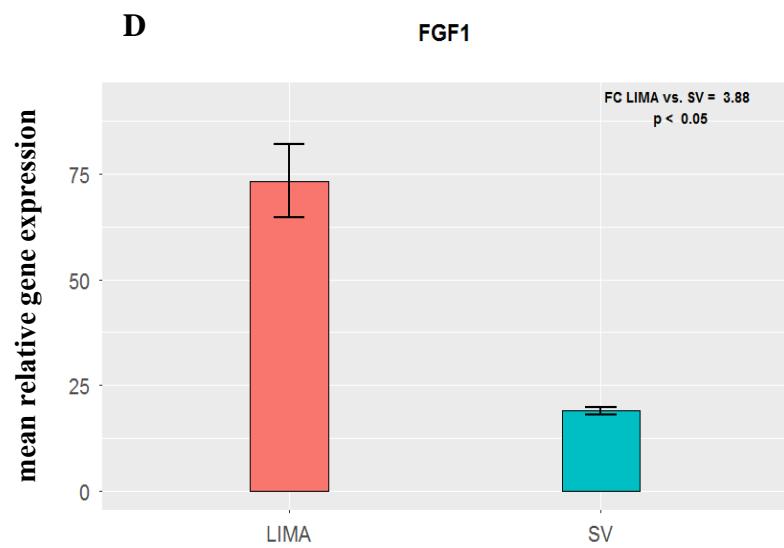
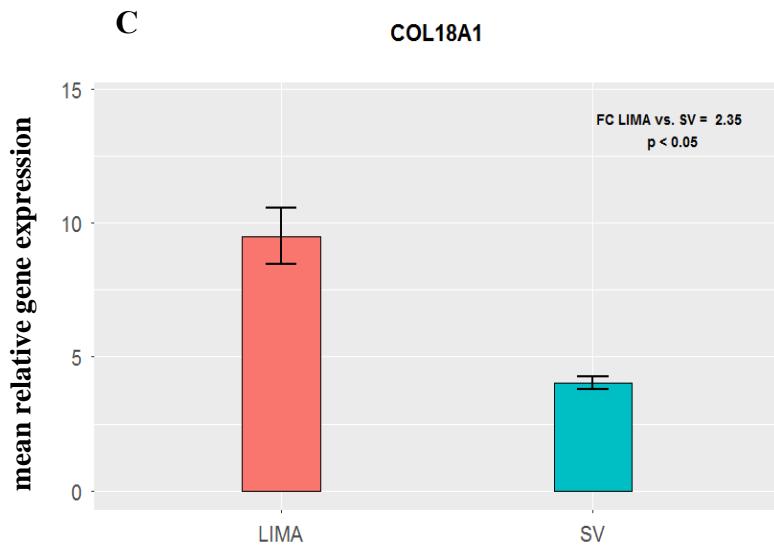


	VCAM1	SERPINE1	TFPI	SOD1	THBD	TIMP1	TNFRSF10C	RHOB	RIPK1	IL6	FN1	CX3CL1	CASP3	CCL2	BCL2L1	ADAM17	BCL2	KIT	PLG	TFPI.1
QPCR	1.61	2.30	1.27	1.28	2.23	1.60	1.70	2.26	1.30	3.70	1.70	1.70	1.30	3.04	1.40	1.18	1.51	-1.58	-2.07	1.27
Mikromacierze	1.17	1.13	-1.30	1.07	1.08	-1.04	1.03	1.07	1.07	1.62	1.07	1.01	1.08	1.44	-1.07	1.05	1.02	-1.02	-1.01	-1.30

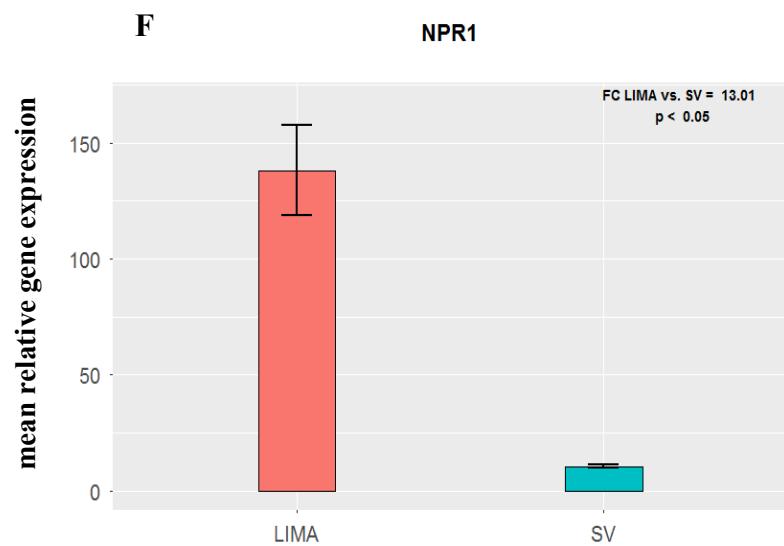
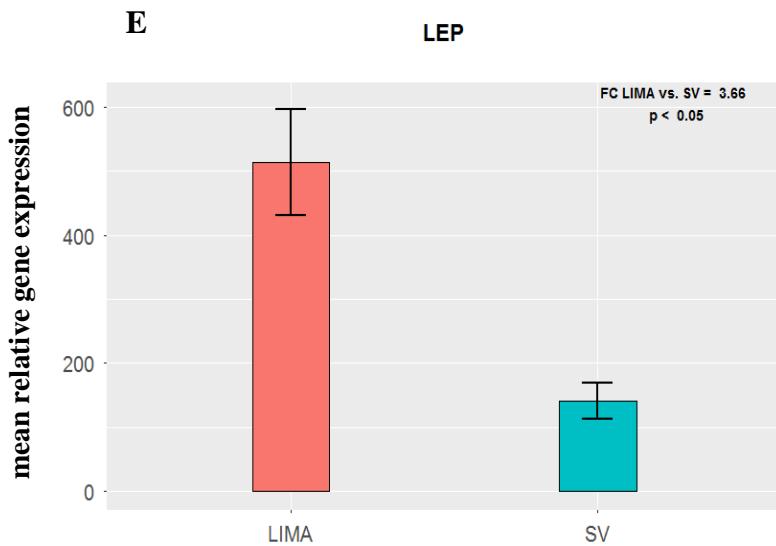
# Microarrays ~ Q-PCR



# Microarrays ~ Q-PCR

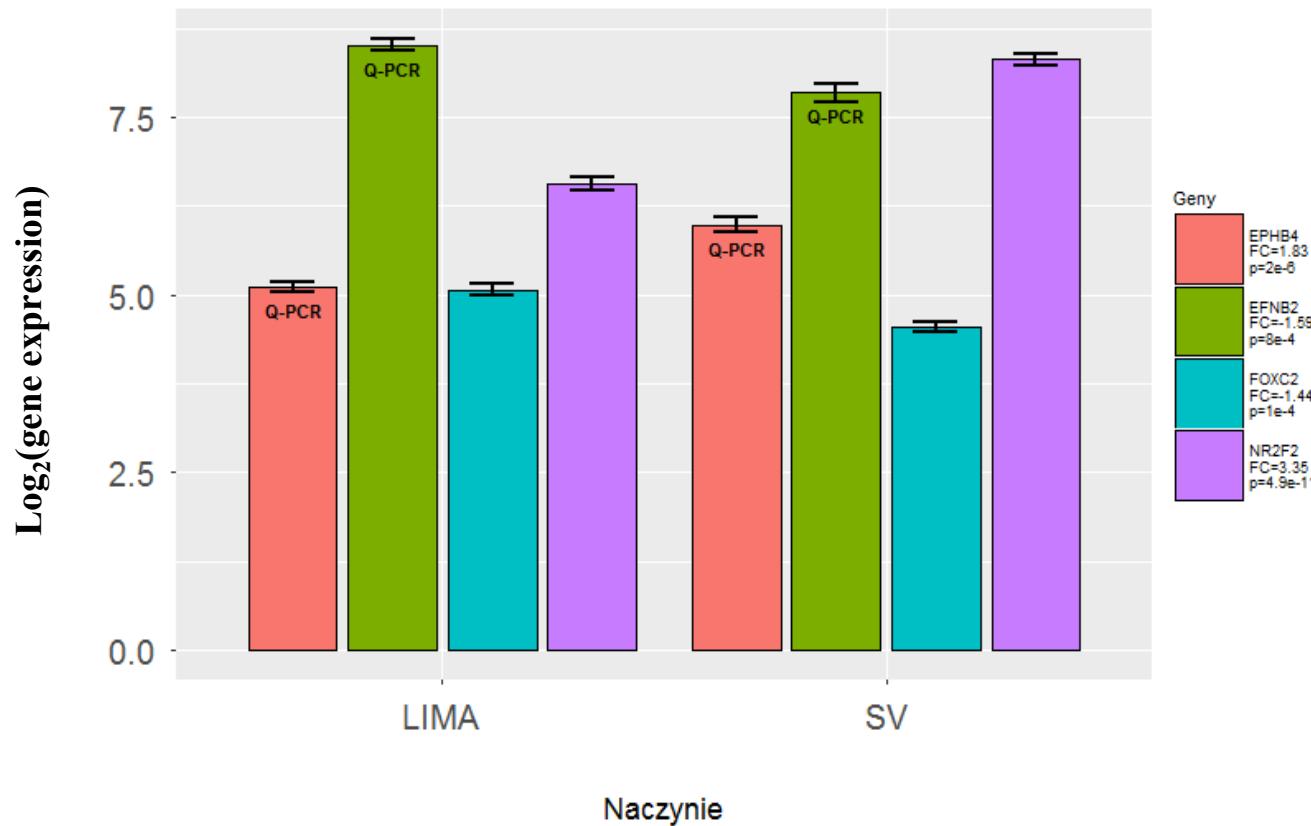


# Microarrays ~ Q-PCR



# Venous and arterial markers

Ekspresja wybranych markeów genetycznych żył oraz tętnic



# Conclusions

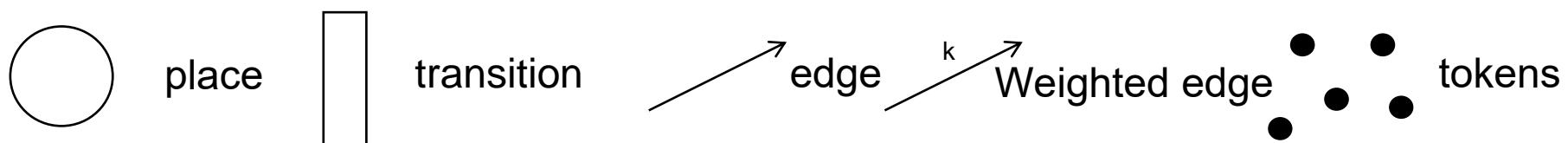
1. The investigated molecular profile of the saphenous vein and internal mammary artery largely justifies the selection based on clinical data.
2. Assessment of global gene expression provides an invaluable source of information on the processes involved in candidate bridging vessels and provides a strong starting point for further in-depth analyses.
3. Both vessels were characterized by the enrichment of many ontological groups. It should be noted, however, that most of these were sets or subsets of processes that are angiogenic or could lead to angiogenesis.
4. The enrichment of proangiogenic processes in SV is indicative of the potentially greater exposure of this vessel to pathological changes observed in the clinical setting.

5. Mature venous and arterial vessels are characterized by preservation of markers typical for both vessels: EPHB4 and NR2F2 - venous vessels, and EFNB2 and Foxc2 - arterial vessels. Their role in mature vessels may determine vessel preservation after coronary artery bypass grafting.
6. The enrichment of the immune response group, especially the HLA genes in the SV may reflect the reduced utility of this vessel when used as an allograft.
7. Confirmation of gene expression by QPCR allows an in-depth analysis of the problem and verification of the results obtained from microarray techniques, which demonstrates the reliability of the results obtained throughout the experiment.
8. Currently, there are many methods for analyzing results from high-throughput techniques. Using linear gene expression analysis (R language with the Bioconductor and LIMMA package) and independent gene set enrichment analysis, similar results were obtained, allowing additional conclusions to be drawn and confirming the nature of the results obtained.

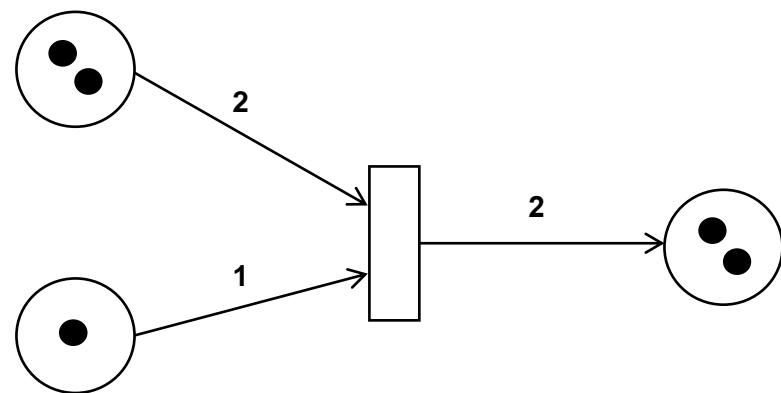
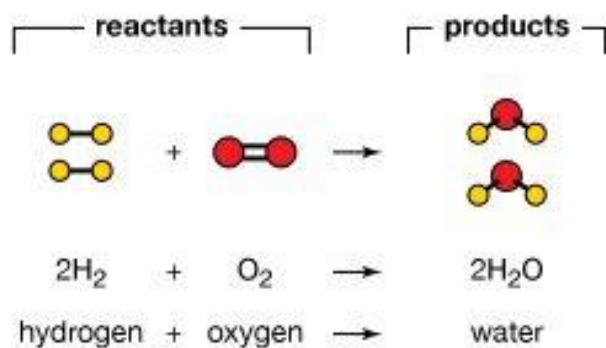
Thank you for your attention

# Bonus

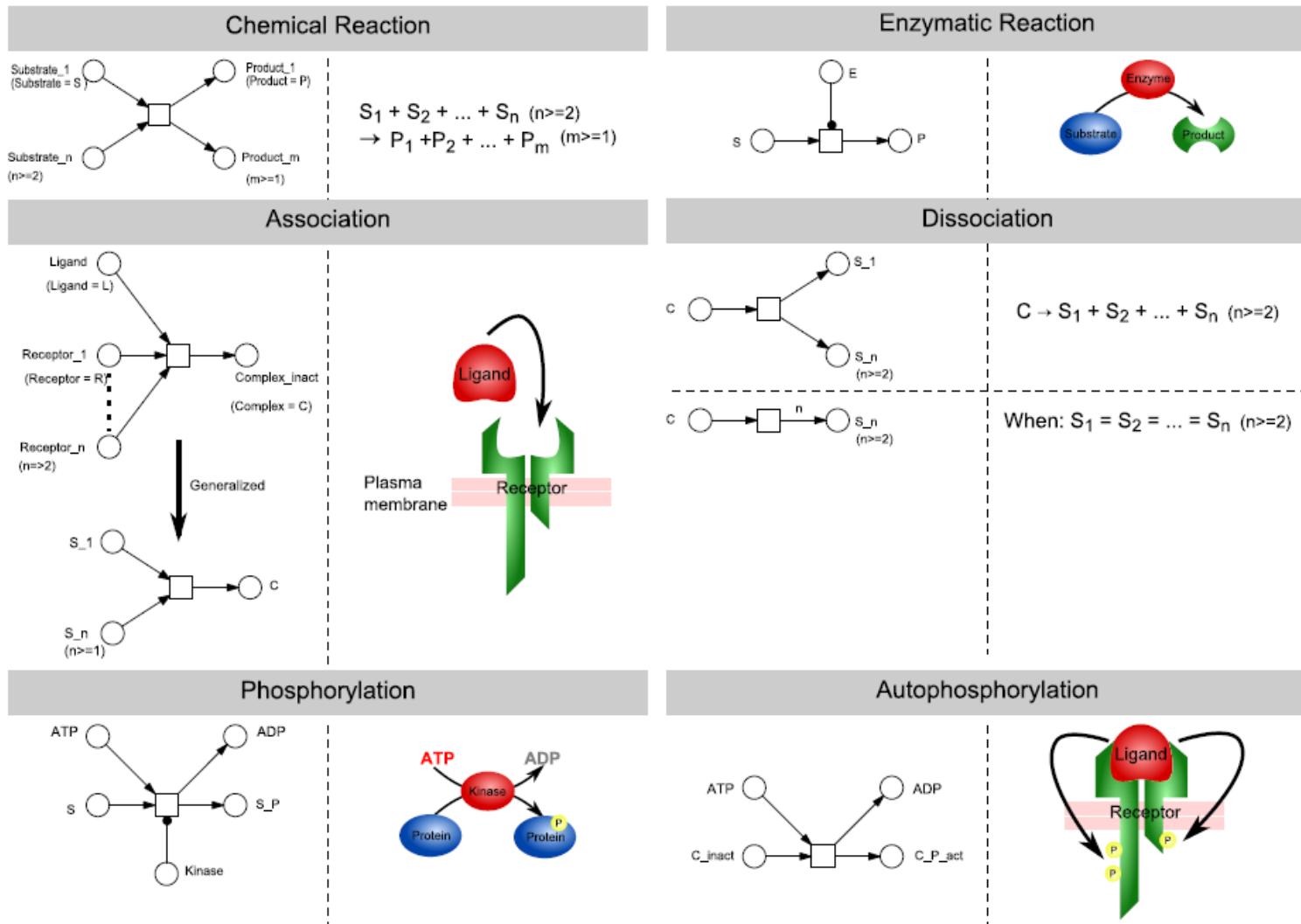
# Petri Nets



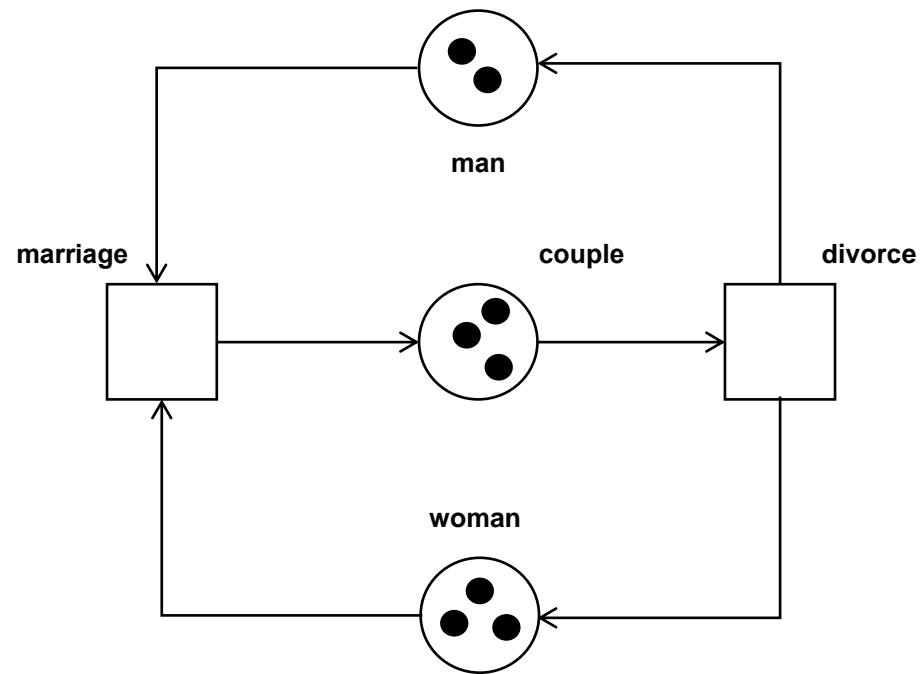
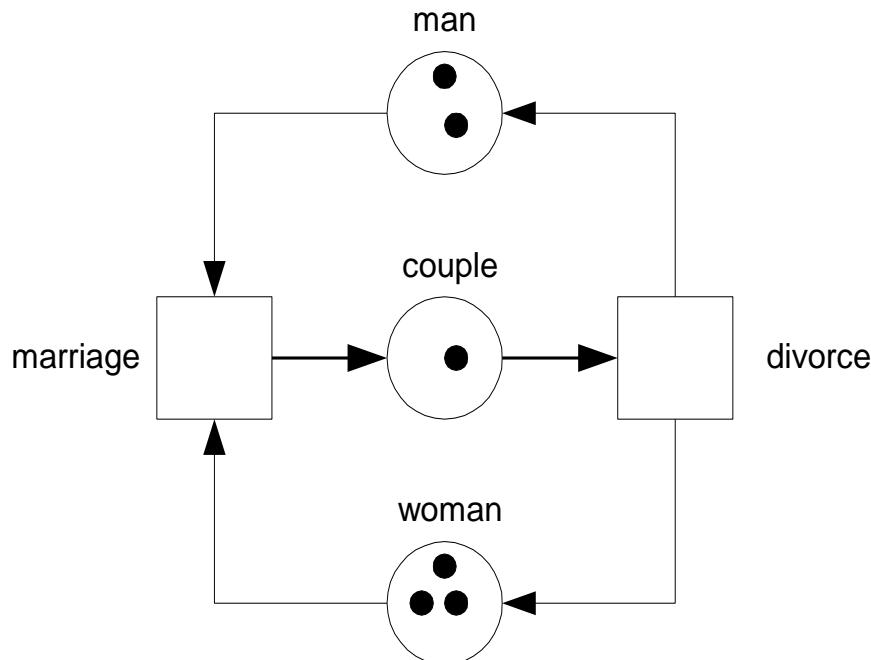
## Water synthesis reaction



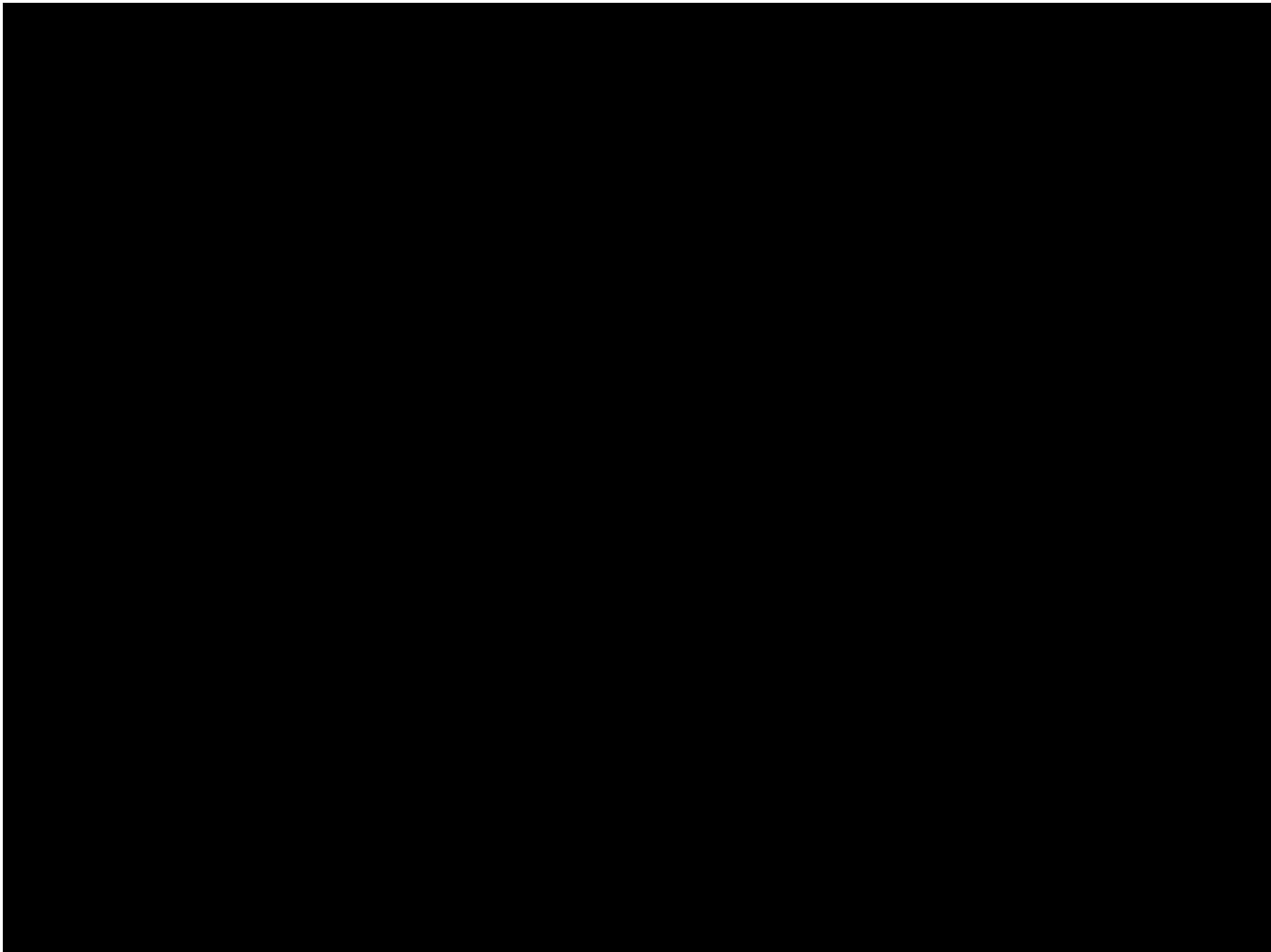
# Petri nets - examples



# Petri nets - invariant transitions



2 marriage + 2 divorce



## RESEARCH ARTICLE

 Wycinek prostokątny

# Petri net-based approach to modeling and analysis of selected aspects of the molecular regulation of angiogenesis

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