Session 14 michela.colombo@studio.unibo.it

Analysis of RNA stability in pig *post mortem* skeletal muscle with Affymetrix GeneChip® Genome Arrays: implications for gene expression studies

M. Colombo[1], G. Galimberti[2], D.G. Calò[2], A. Astolfi[3], V. Russo[1] and L. Fontanesi[1]

[1]DIPROVAL, Sezione di Allevamenti Zootecnici, University of Bologna, Reggio Emilia, Italy
[2]Department of Statistics, University of Bologna, Bologna, Italy
[3]Dep. of Hematology and Oncology sciences "L. A. Seragnoli", Sant'Orsola-Malpighi Hospital, University of Bologna, Italy

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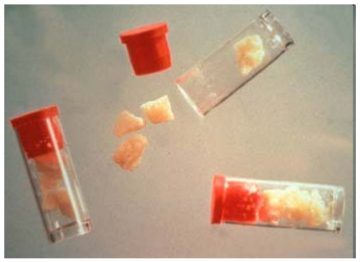
POST MORTEM DELAY AND RNA QUALITY



SAMPLING

STORAGE IN
LIQUID
NITROGEN

CAN WE CONSIDER POST MORTEM SKELETAL MUSCLE (MEAT) AN USEFUL TISSUE FOR GENE EXPRESSION STUDIES ?

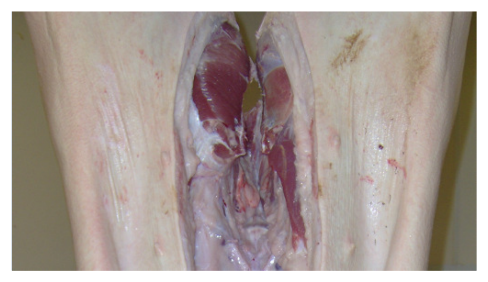
AIM

TO EVALUATE THE RNA STABILITY in *POST MORTEM* SKELETAL MUSCLE FROM PIGS and USE IT IN TRANSCRIPTOMIC ANALYSIS

PILOT STUDY

Materials and Methods
SAMPLING/1

- *Semimembranosus* muscle was sampled from 4 legs of different commercial heavy pigs
- The first sampling was carried out at 20 min *post mortem*
- Following the cold chain of the slaughterhouse other 4 samples, from the same legs, were collected at 2, 6, 24, 48 and 72 hours *post mortem*.



Material and Methods

RNA EXTRACTION AND QUALITY CONTROLS

Samples were stored at -80 °C

Total RNA was extrated using the RNAEasy Midi kit (Qiagen)

Quality controls by:

1. **Agarose gel**
2. **Agilent Byoanalyzer and RIN**
3. **28S/18S**
4. **GAPDH amplification**

Matherials and Methods

Microarray analysis

GENE CHIP® PORCINE GENOME ARRAYS were hibridized with RNA obtained from:

3 pigs

at 20 min, 2, 6 and 24 h *post mortem*

using the Affymetrix recommended protocol

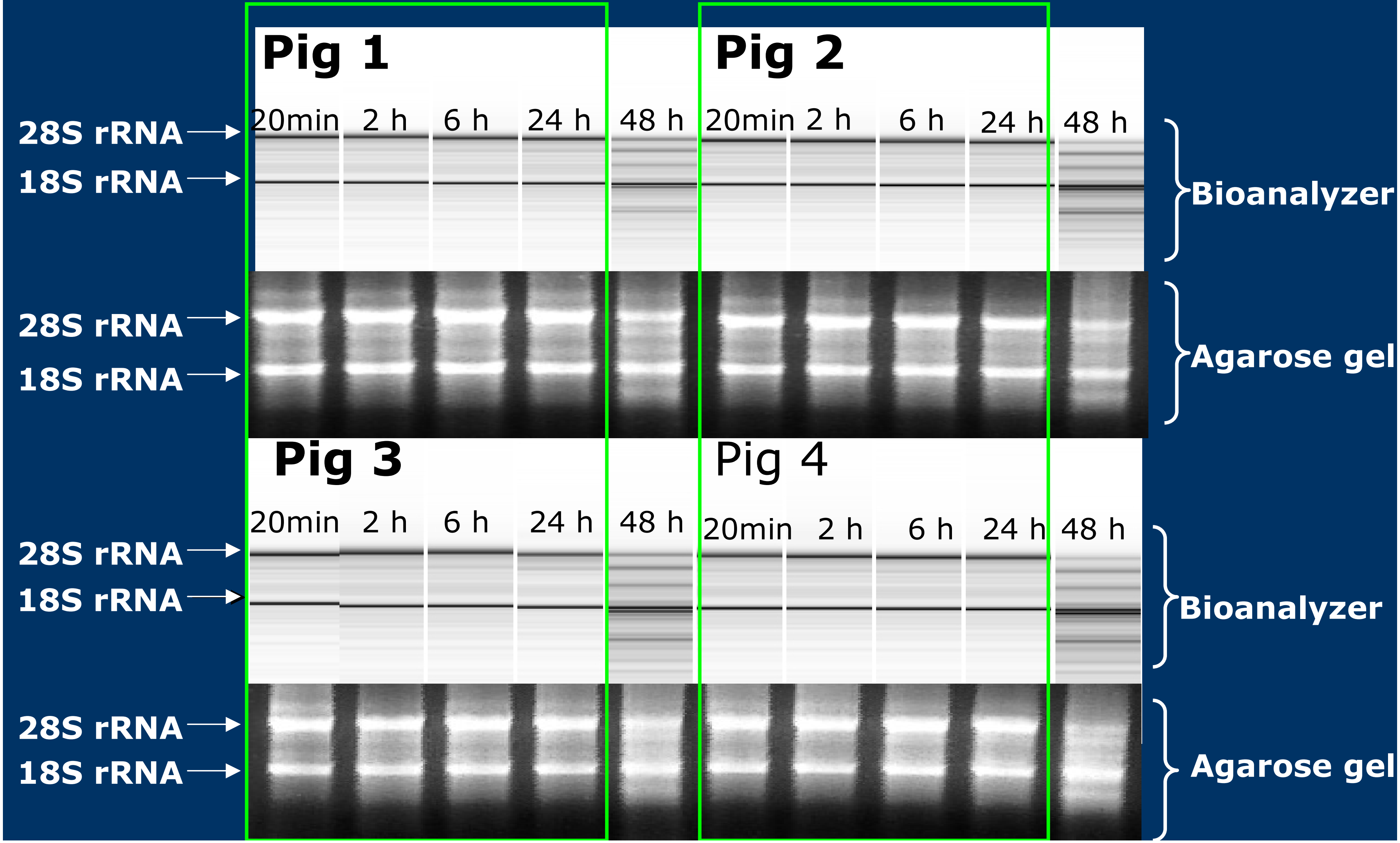
Material and Methods
DATA ANALYSIS

1. ***affy*** **package was used to normalize expression values (RMA) in R**
2. **Application of non-specific filtering**
3. **EDGE software (Storey et al., 2005)was used for significance analysis of time course variation**
4. **Additional RNA quality controls were calculated with R using hybridization data**

RESULTS

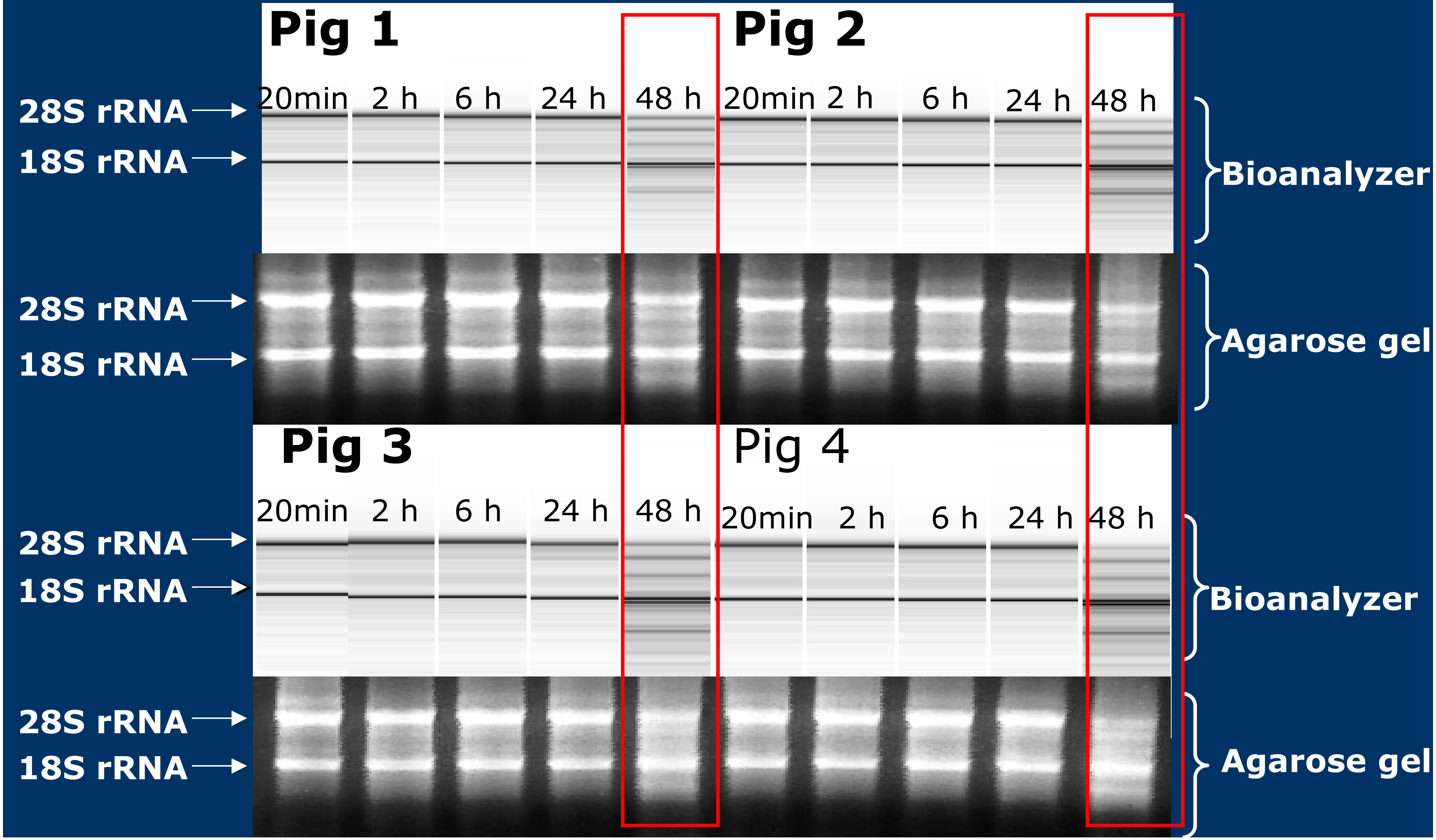
RESULTS/1

RNA quality control/Agarose gel and Bioanalyzer



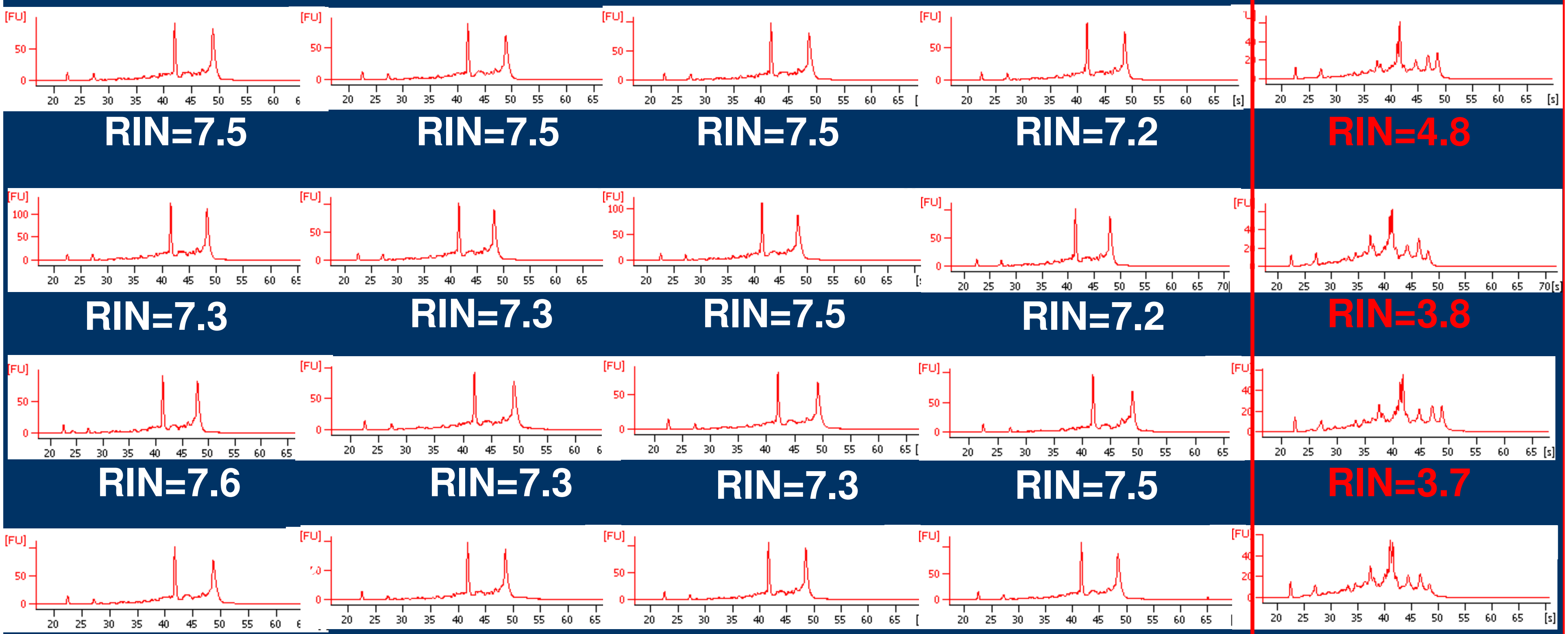
RESULTS/1

RNA quality control/Agarose gel and Bioanalyzer



RESULTS/2

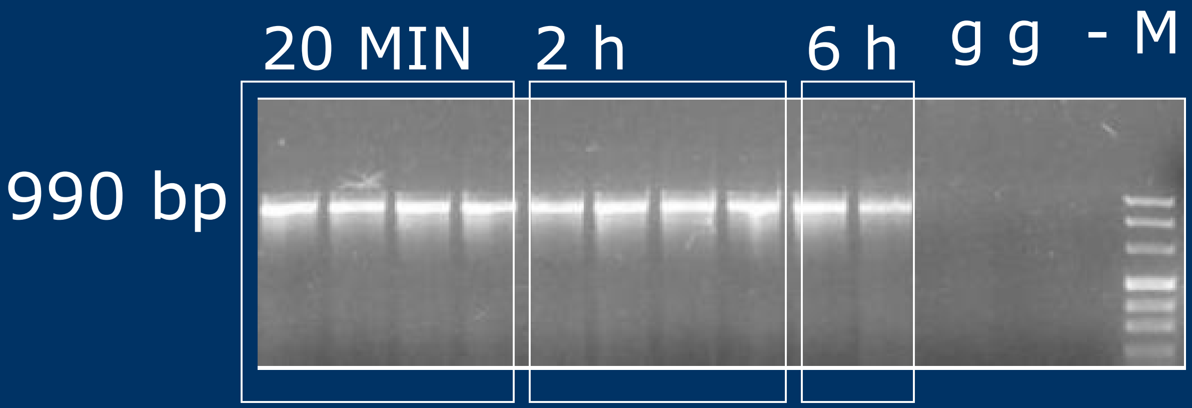
RNA quality control/RIN and 28S/18S

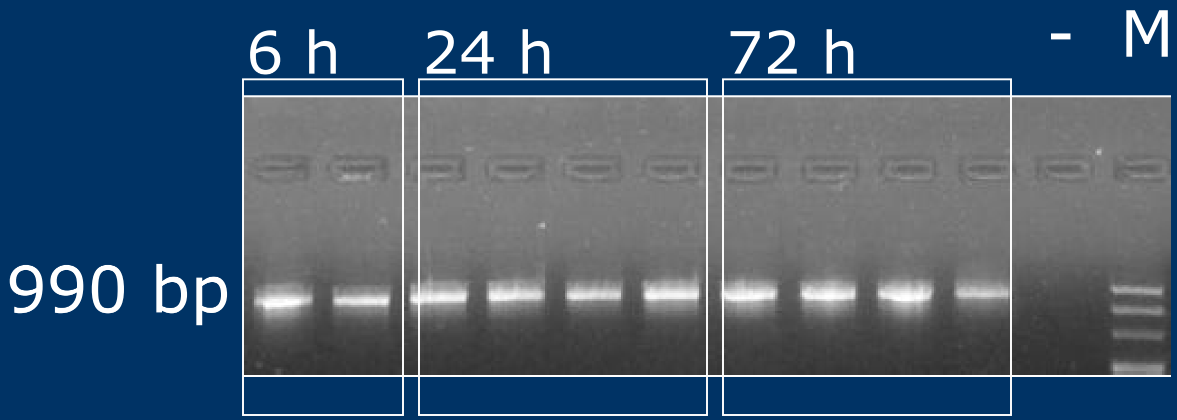


20 min	2 h	6 h	24 h	48 h
RIN=7.5	RIN=7.5	RIN=7.5	RIN=7.2	RIN=4.8
RIN=7.3	RIN=7.3	RIN=7.5	RIN=7.2	RIN=3.8
RIN=7.6	RIN=7.3	RIN=7.3	RIN=7.5	RIN=3.7
RIN=7.4	RIN=7.6	RIN=7.5	RIN=7.4	RIN=3.5

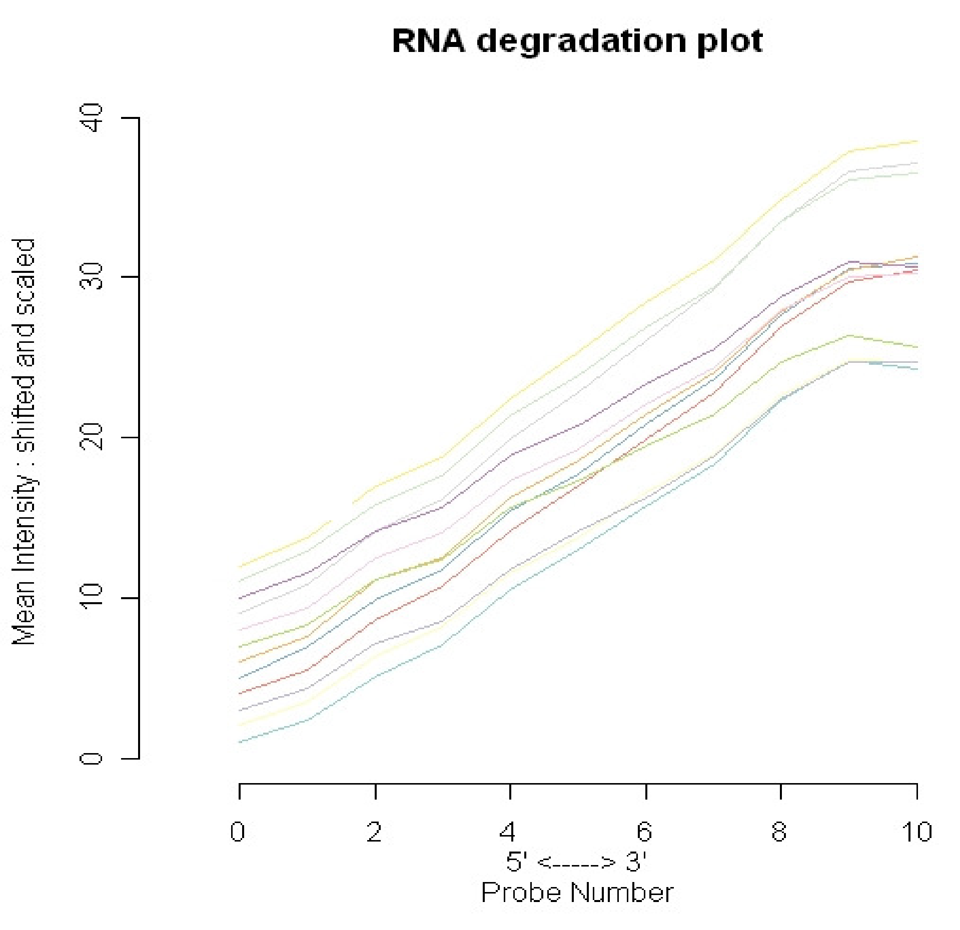
RESULTS/3

RNA quality control/GAPDH amplification





RESULTS/4
RNA DEGRADATION PLOT



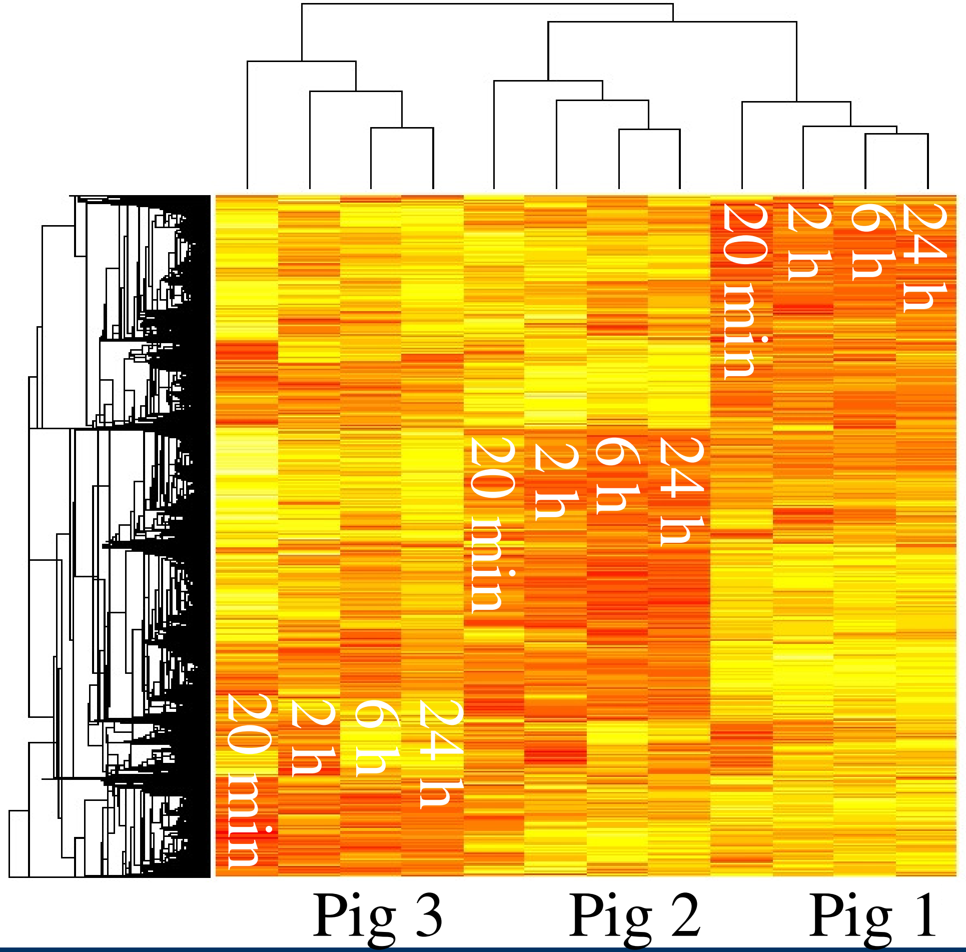
RESULTS/5

CHIP QUALITY CONTROL

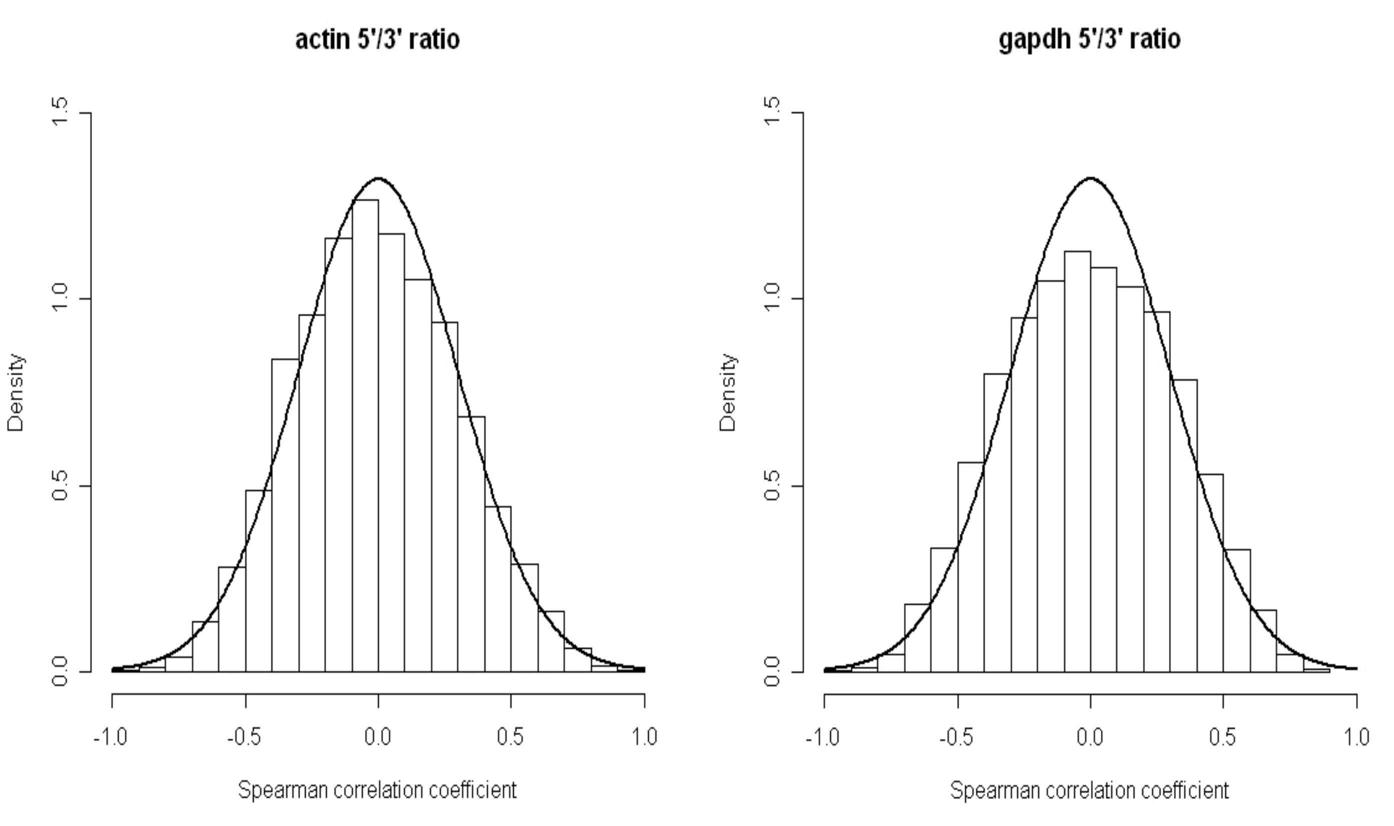
CHIP/PM interval	% Present Call	Average background	Scaling Factor
1A/20 min	53.88	54.81	0.66
1B/2 h	54.39	66.69	0.50
1C/6 h	54.69	61.21	0.54
1D/24 h	54.22	56.55	0.56
2A/20 min	53.64	49.1	0.69
2B/2 h	53.07	52.5	0.67
2C/6 h	53.04	64.77	0.56
2D/24 h	53.08	58.32	0.65
3A/20 min	55.99	46.14	0.66
3B/6 h	54.95	60.26	0.51
3C/6 h	55.18	53.0	0.59
3D/24 h	53.31	46.94	0.69

RESULTS/6

Cluster analysis of 5203 genes retained after filtering

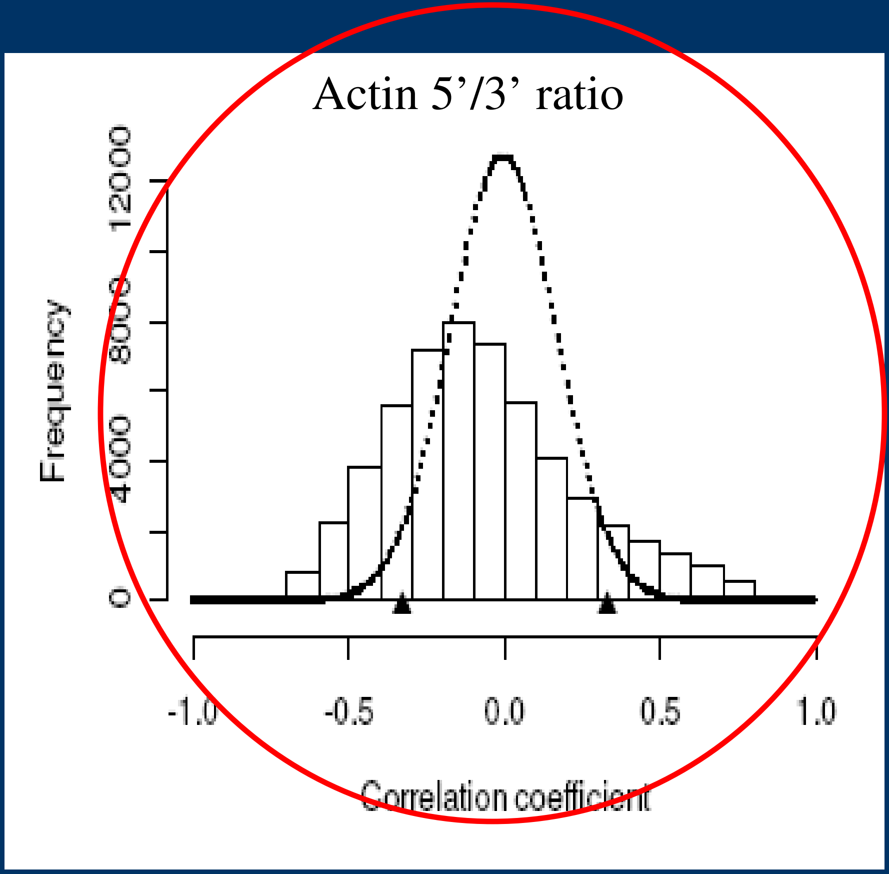


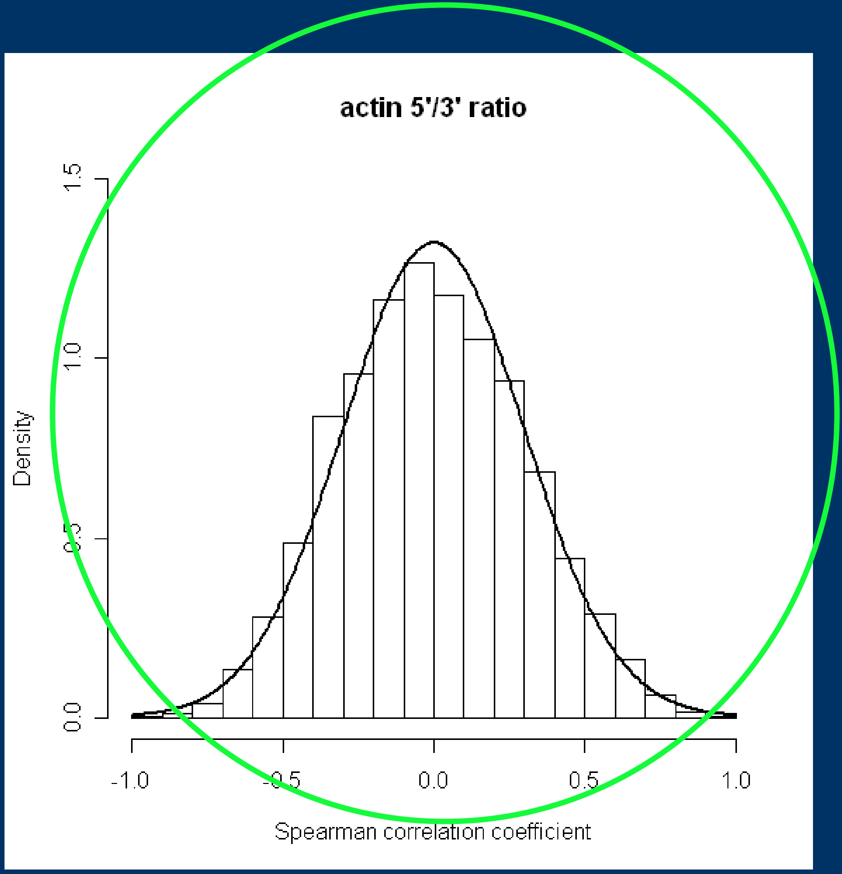
RESULTS/7
Correlation structure caused by RNA quality for actin and GAPDH



NO CORRELATION
(POPOVA ET AL. 2008)

our data





Number of RNA quality dependent transcripts

	Pvalue <0.05 (Expected 5%)	**Pvalue <0.01 (Expected 1%)**
ACTIN	**4.93**	**0.93**
GAPDH	**5.3**	**0.73**

IN MUSCLE PIG

24 hours

CONCLUSION/1

- IT IS POSSIBLE TO OBTAIN RNA OF GOOD QUALITY TILL 24 HOURS POST MORTEM
- THIS RNA CAN BE USED FOR GENE EXPRESSION ANALYSIS
- TISSUE SAMPLING CAN BE SIMPLER

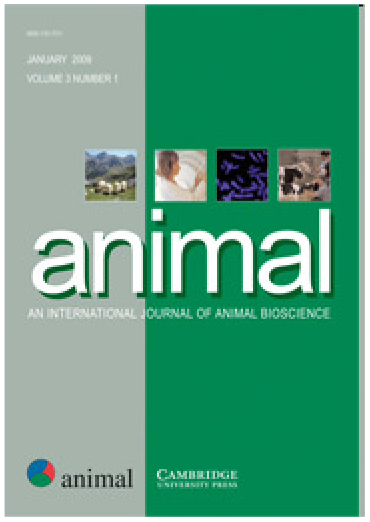
CONCLUSION/2

NEW PERSPECTIVES OF APPLICATION OF GENE EXPRESSION ANALYSIS FOR

- AUTHENTICATION AND TREACEBILITY OF MEAT PRODUCTS
- IDENTIFICATION OF BIOMARKERS FOR MEAT QUALITY AND PRE-SLAUGHTERING CONDITIONS

ACKNOWELEDGEMENTS

ANIMAL JOURNAL COMMISSION FOR THE SCOLARSHIP



MONTANA'S AWARD FOR FOUNDING

THANKS FOR YOUR ATTENTION