Gene expression profiling for meat quality in swine

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56th Annual Meeting of the European Association for Animal Production, June 5-8 2005, Uppsala, Sweden

Swine meat in Italy

5,7% of the total European pigs are reared in Italy

“heavy” pig breeds selected for ham production

Large white (LW)

Landrace (LA)

Genetic selection

Pigs are selected for:

- Average daily gain
- Lean cut weight
- Ham fat thickness
- Curing loss after salting

Meat quality is affected both by genetic and environmental factors

Aim of the work

The Suppression Subtractive hybridisation (SSH) technique was employed to indentify genes differentially expressed in skeletal muscle tissue of pigs with different meat quality

Sampling

Pigs → 100 individuals of LW and LA

same rearing conditions

butchered in the same slaughterhouse

same age

same weight

Tissue → Skeletal muscle, preserved in “RNA later®” at –20°C

Phenotipic data

Seven linear raw ham phenotypes (average of the two ham per individual) were evaluated

1. Muscle compactness
2. Marbling
3. Colour uniformity
4. Fat covering
5. Colour
6. Dorsal fat thickness
7. Ham fat thickness

Statistical analysis

SAS/STAT software

A Principal Component Analysis (PCA) was first performed on the seven previously defined variables

Multivariate analysis was performed on the first two principal components to adjust for effect of sex and sampling data

A new “meat quality” index was associated to each individual, combining the residuals for the first and the second principal component genetic index

16 + 16 animals, with extreme meat quality index, were selected for the following molecular analyses

Molecular analysis

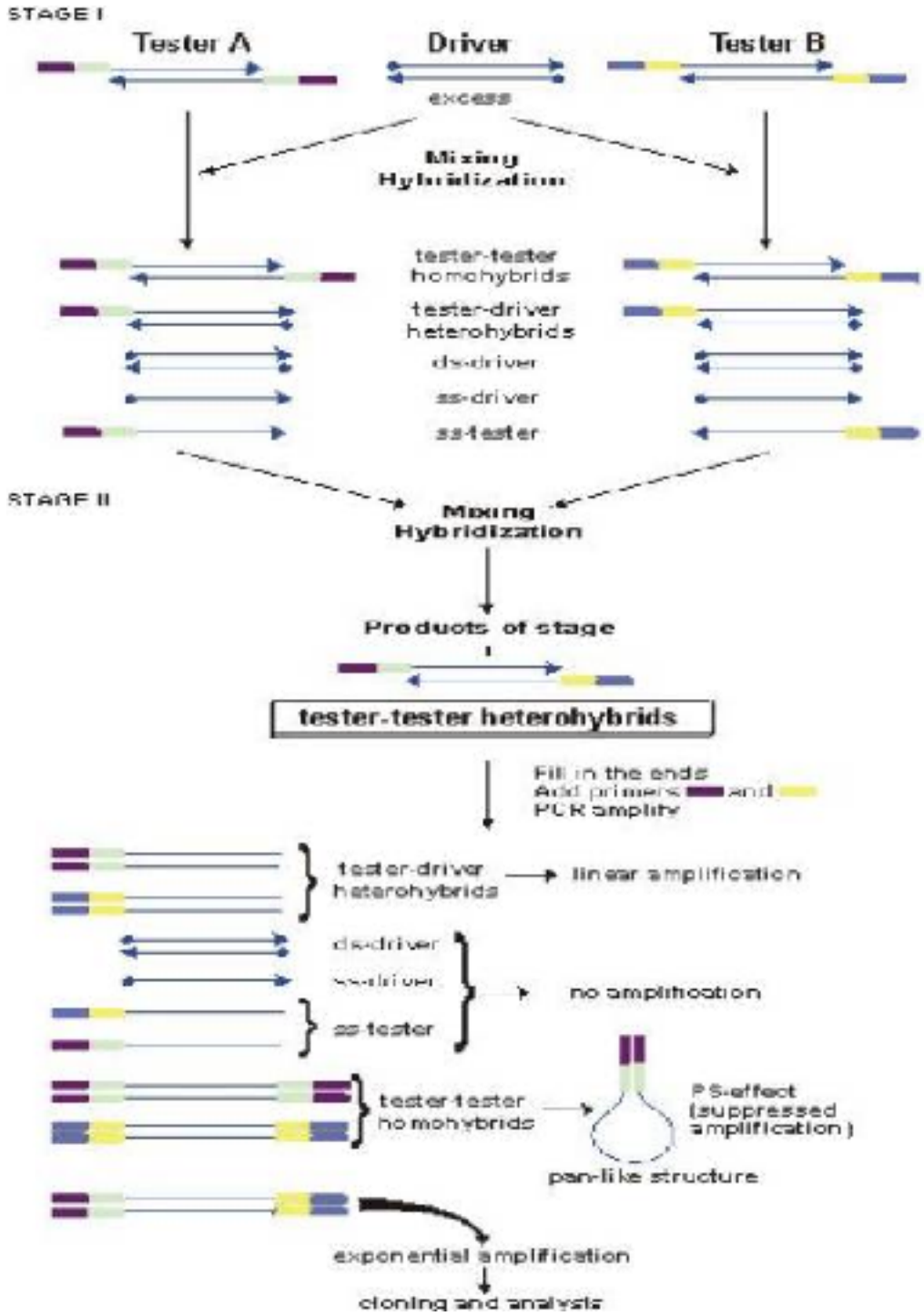
The mRNA of the 32 individuals was extracted from skeletal muscle tissue

The mRNAs were pooled in two groups of 16, based on the index value of the correspondent individuals

The 2 pools (F and R) were analysed using the SSH technique to create 2 libraries (named F and R) of differentially expressed genes

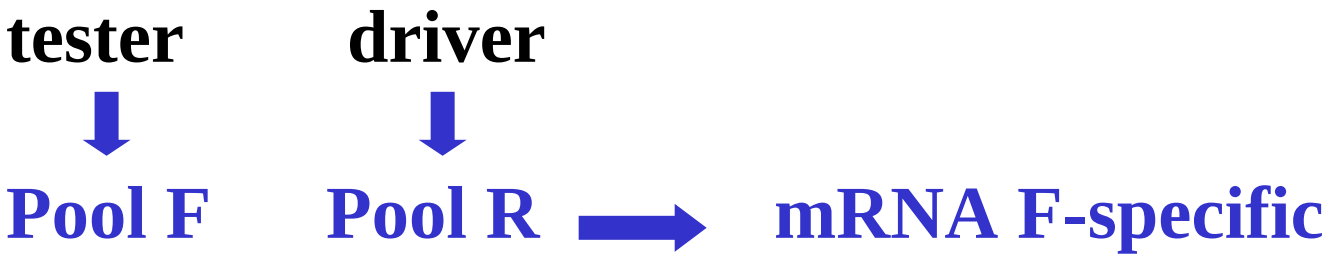
SSH technique

BD Clontech PCR-Select™ cDNA Subtraction Kit

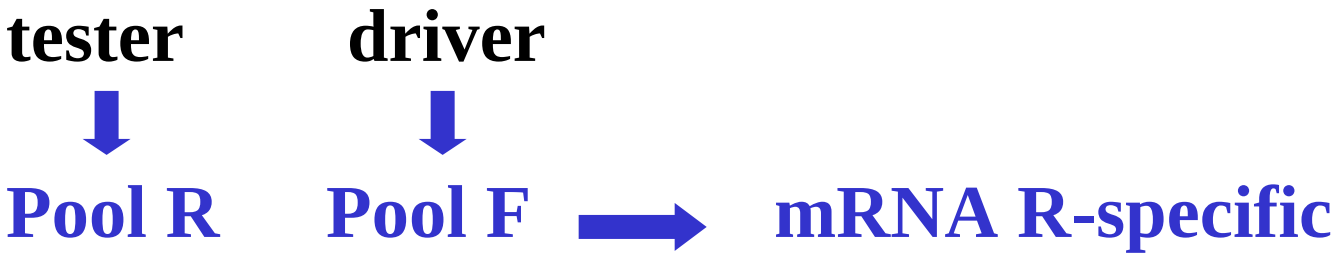


SSH technique

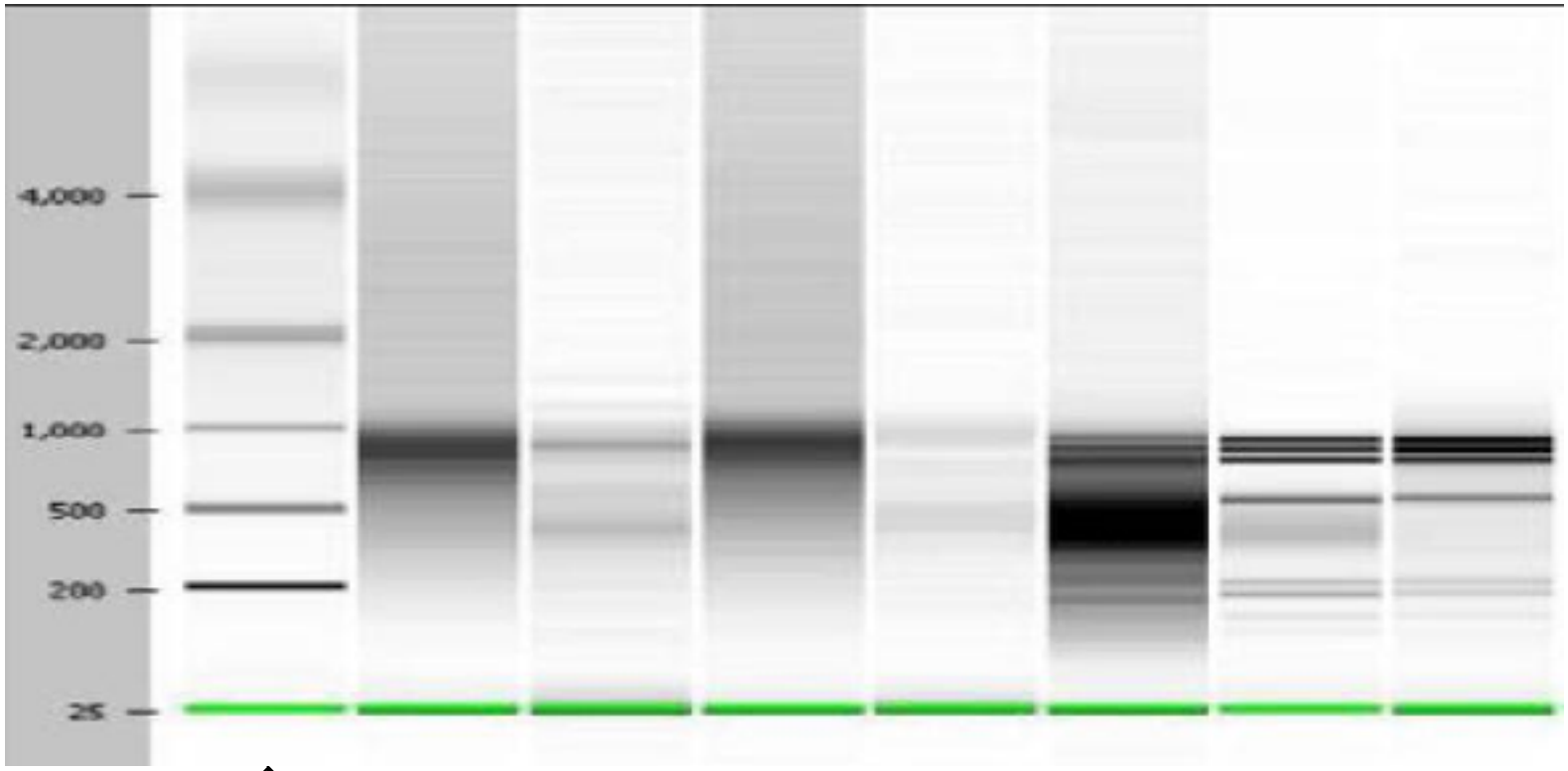
F library



R library



SSH analysis



Library analysis

Plasmidic DNA extraction

Screening by PCR

Isolation of inserts

Sequencing

Sequence/Library analysis

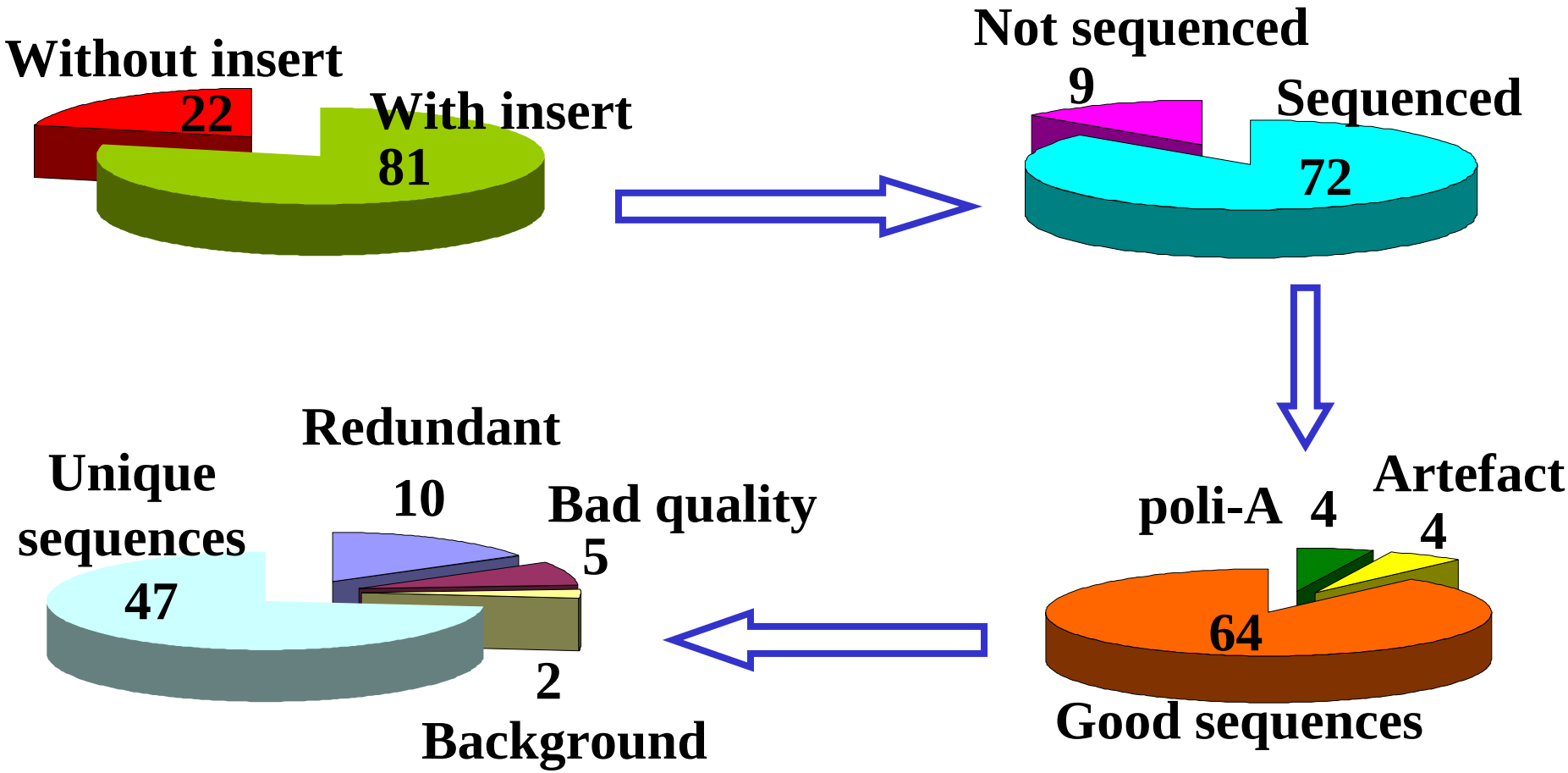
Sequence quality → Presence of artefacts (by SSH technique or by cloning)

Library redundancy → Identification of multiple copy sequences in the library

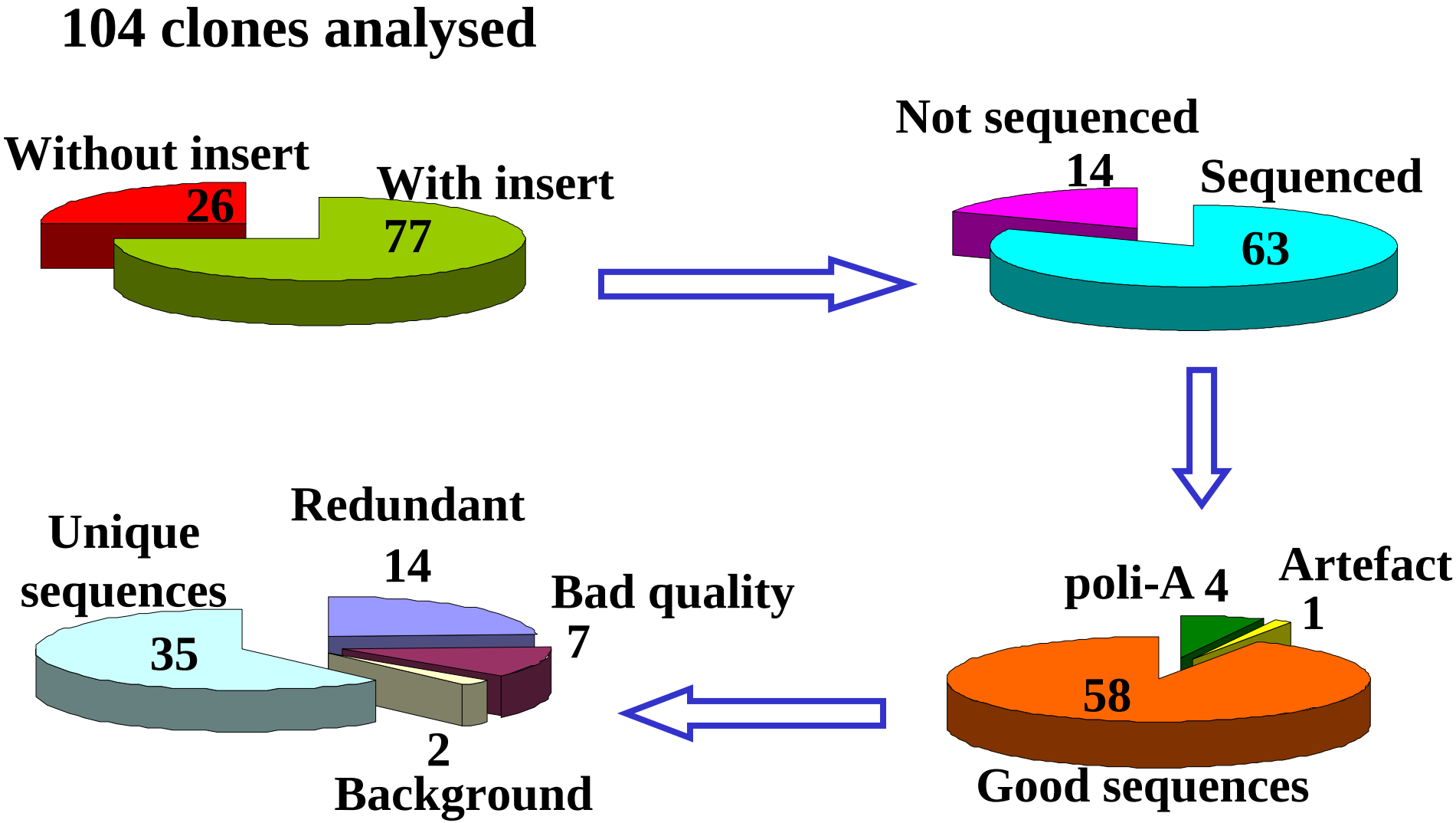
Library background → Identification of sequences present in both libraries

Results, F library

103 clones analysed



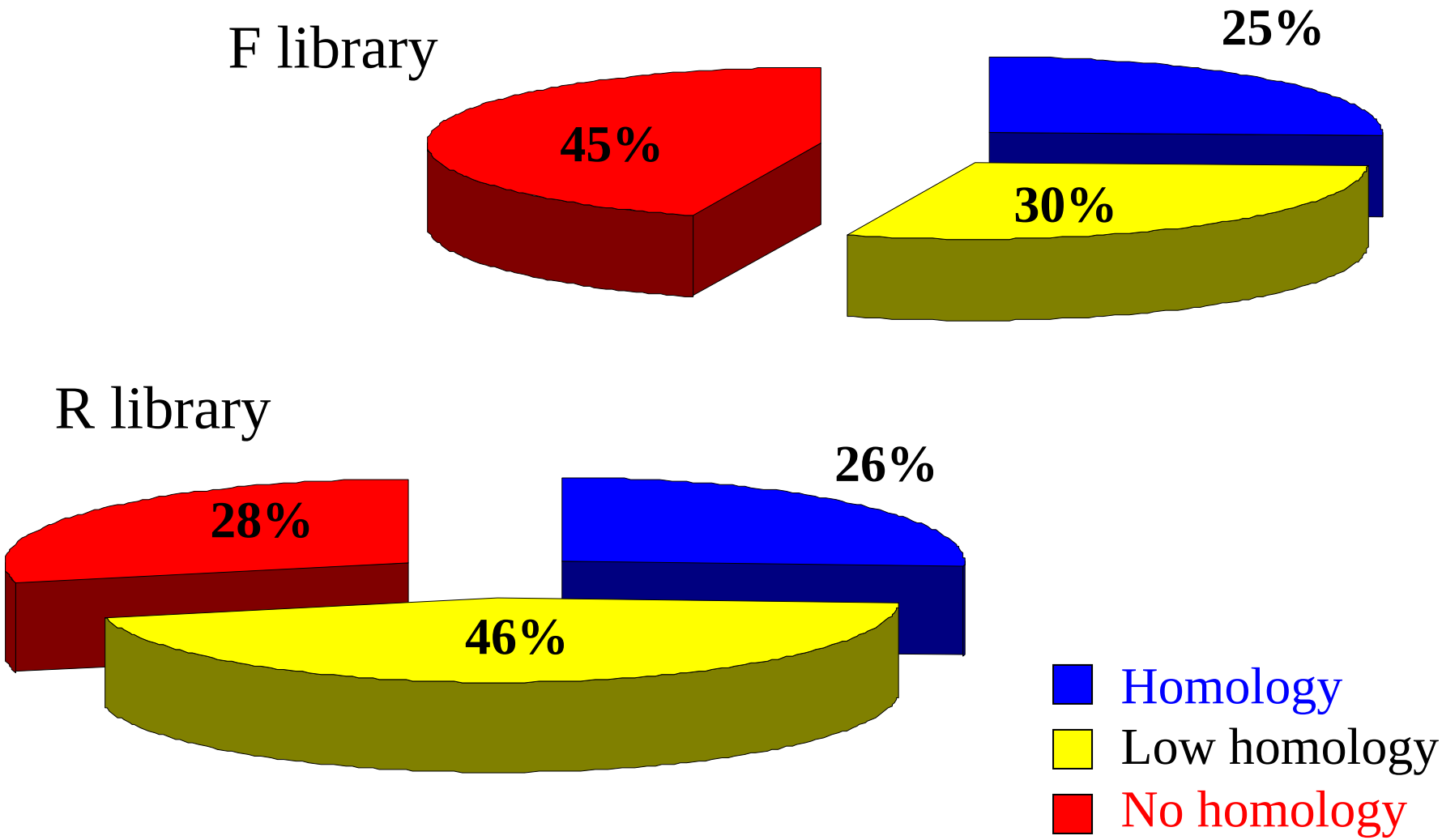
Results, R library



In silico analysis

Sequence homology (Pig, Human; EST and DNA)	• GenBank • EMBL • DDBJ • TIGR • PEDE
Putative map position based on human-pig sinteny	• literature • TIGR • INRA
QTLs previously mapped in these regions	• literature • Pig QTL (NCBI)

Homology results



Sinteny and QTLs

F library

4 sequences, putatively mapped basing on human-pig sinteny

Previously identified QTLs for fat (dorsal and subcutaneous), carcass weight and post-mortem pH (de Koning *et al.*, 2001; de Koning *et al.*, 2000; Rattink *et al.*, 2000)

R library

5 sequences, putatively mapped basing on human-pig sinteny

Previously identified QTLs for fat (dorsal), meat colour and post-mortem pH (Milan *et al.*, 2002; de Koning *et al.*, 2001; Rohrer *et al.*, 1998)

Conclusions

SSH libraries to investigate the “meat quality” phenotype in pig

82 differentially expressed sequences identified

25% of these sequences showed significant homology with sequences published in databases

Preliminary map results indicate that QTLs are present in the same regions where the F and R sequences are located

Perspectives

Differential expression of the sequences in the F and R individuals (16 per pool) can be confirmed by Real-time PCR

Mapping of the sequences to confirm/identify their chromosome position

Investigate the presence of polymorphisms and test their association with the “meat quality” phenotype

Acknowledgments

This work was funded by Ministry of Instruction, University and Research (FIRB MIUR RBNE01MMHS)

Samples and phenotipic data were collected at 

slaughterhouse