



WAGENINGEN UNIVERSITY

HITChip

Human Intestinal Tract Chip

A comprehensive microchip tool targeting human intestinal microbiota

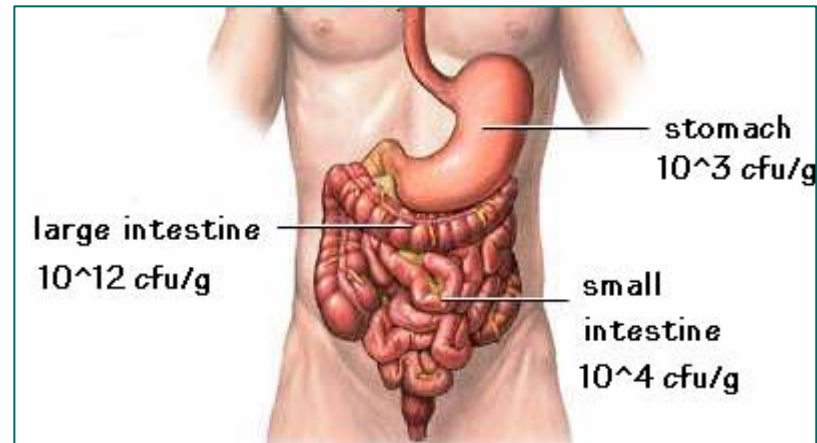
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Erwin Zoetendal
Willem M de Vos

- ❖ Human intestinal tract microbiota composition
- ❖ Currently available data about HIT microbiota

- ❖ Probe design
- ❖ Validation of the approach
- ❖ Results
- ❖ Conclusions

- ❖ Questions

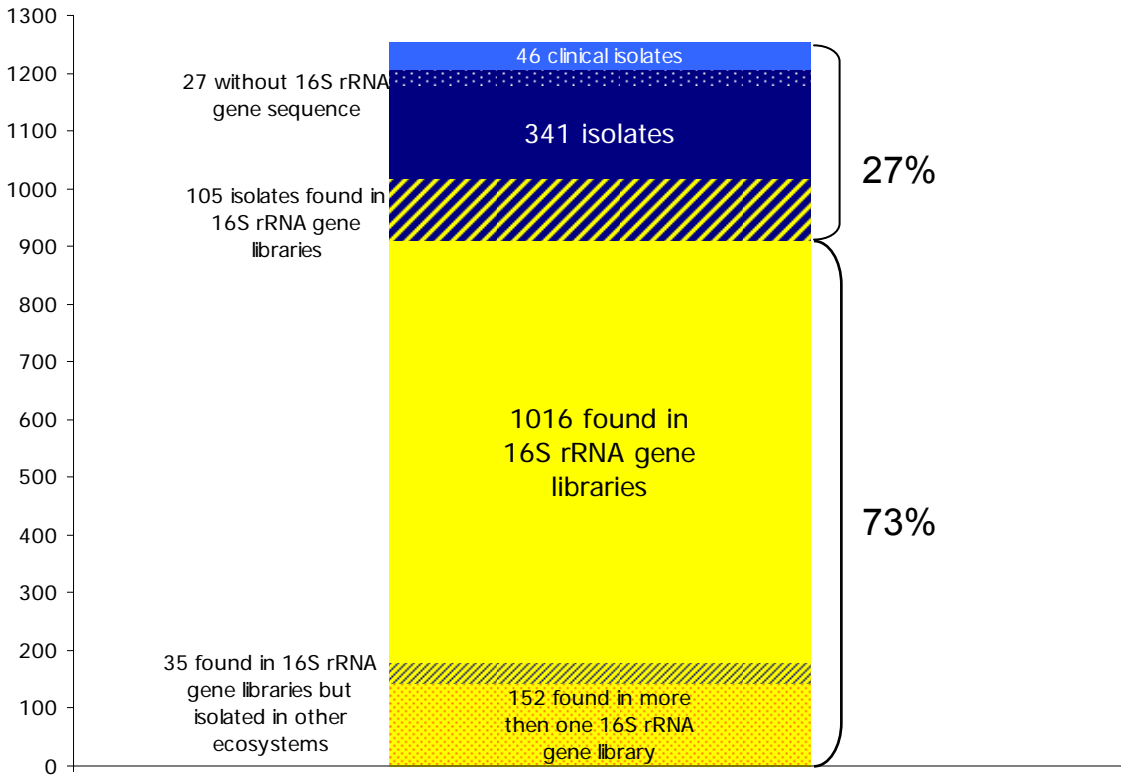
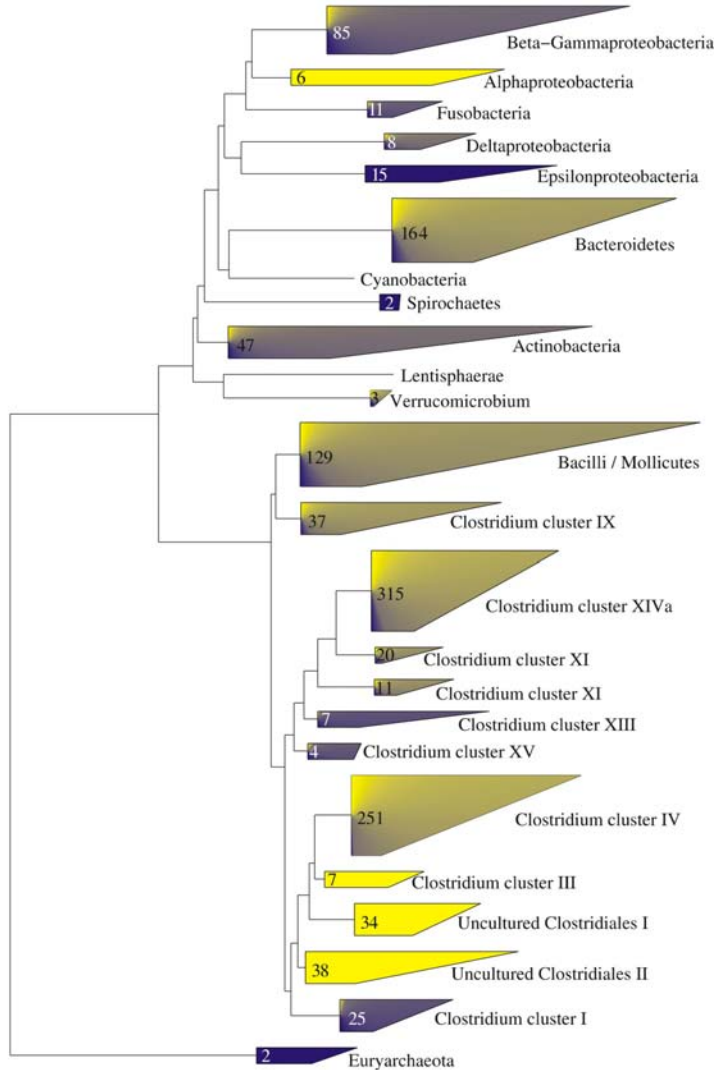


95% of the cells in the human body are bacterial
~ 1kg of total biomass (an “organ”)
~ 500 different bacterial species in each individual
the HIT microbiota composition influenced by genetics and environment
“unique fingerprint”

Digests 100g of food per day
Provides SCFA for the growth of the gut epithelial cells
Produces vitamins (K and B)
Makes the first line of protection against pathogens
Modulates hosts immunity

Introduction

Uncultured Cultured



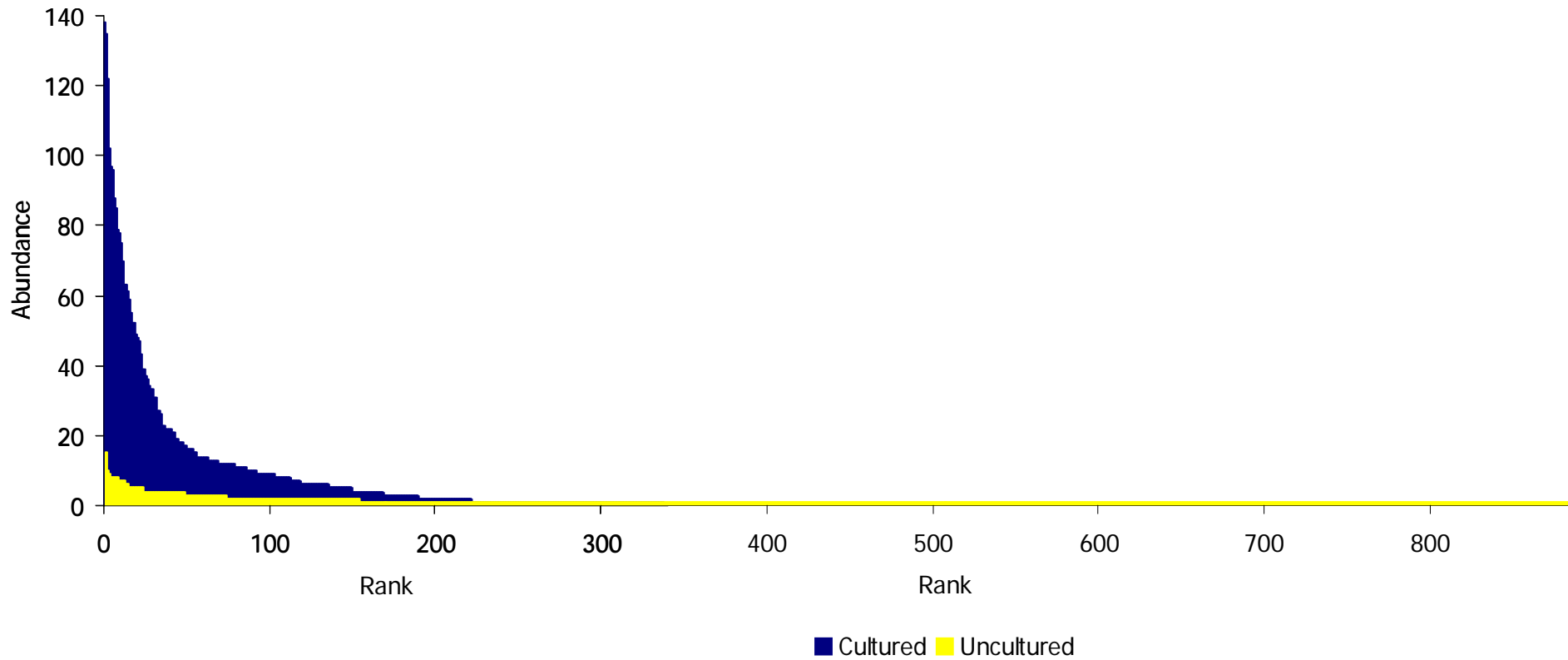
For majority of phylotypes
16S rRNA gene sequence – the only available information

0.10

Introduction

341 phylotypes found
~ 800 individuals analysed

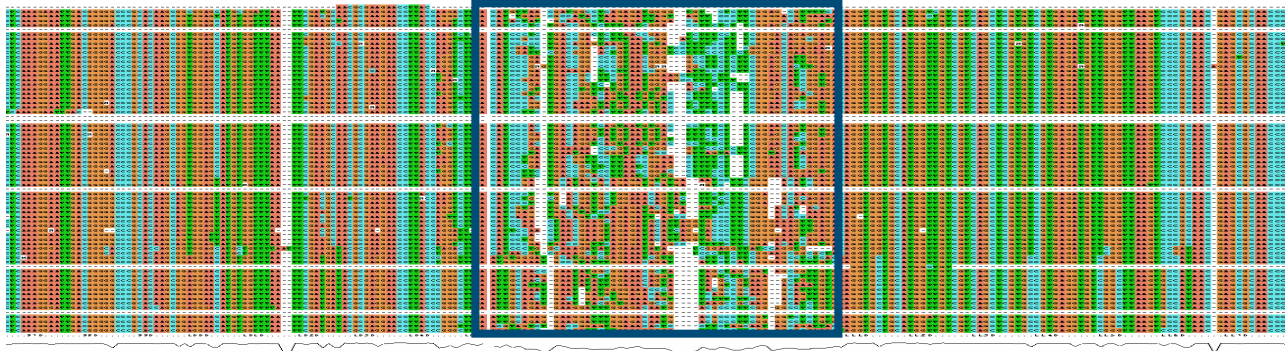
1016 phylotypes found
~ 50 individuals analysed



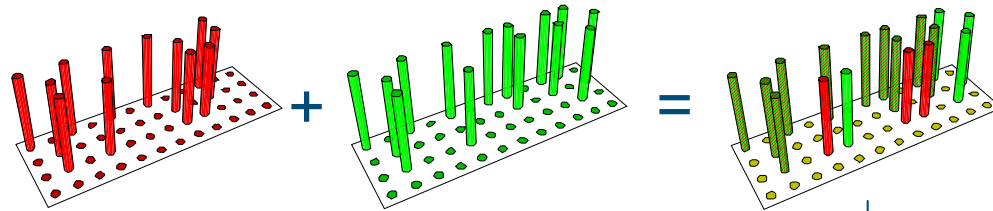
~ 500 phylotypes expected

~ 4,500 phylotypes expected

Flexible array design desired



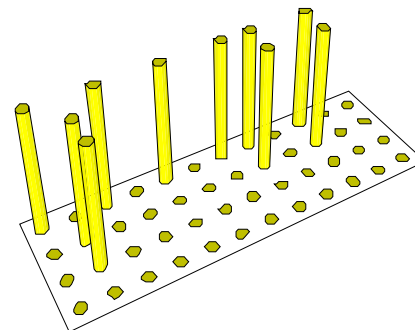
Hypervariable regions identified

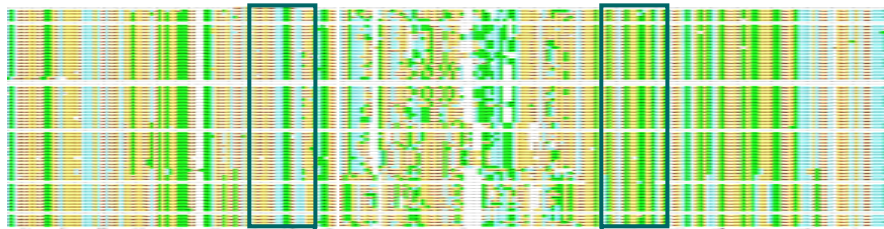


V1 probes –12 signals

V6 probes –14 signals

In combination –10 signals
SPECIFIC HYBRIDISATION





V1 and V6 region 24-72bp

CAAGTCTTGACATCCCACTGAAAACACTTTAACCGTGATCCCTCTTCGGAGCACTGGAGACA

Reverse complement

tgtctccactgctccgaagagggatcacggtaaagtgttttcagtgggatgtcaagacttg

Sequence from the left side, length 24 bp

tgtctccactgctccgaagaggga

Sequence around middle nucleotide, length 24 bp

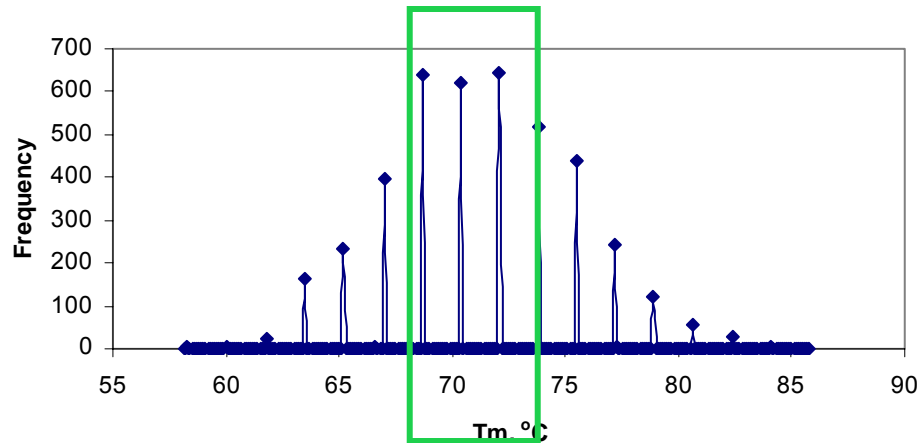
gagggatcacggtaaagtgtttt

Sequence from the right side, length 24 bp

ttttcagtgggatgtcaagacttg

Prediction of the Melting Temperatures of Probes ($T_m = 81.5 + 16.6 \times \log(Na^+) + \%GC - 600/N$)

Distribution of predicted melting temperatures for the microarray probes (24bp)



Probes should fit into a narrow range of Tm

Sequence from the left side, length 24 bp

```
tgtctccactgctccgaagaggga
```

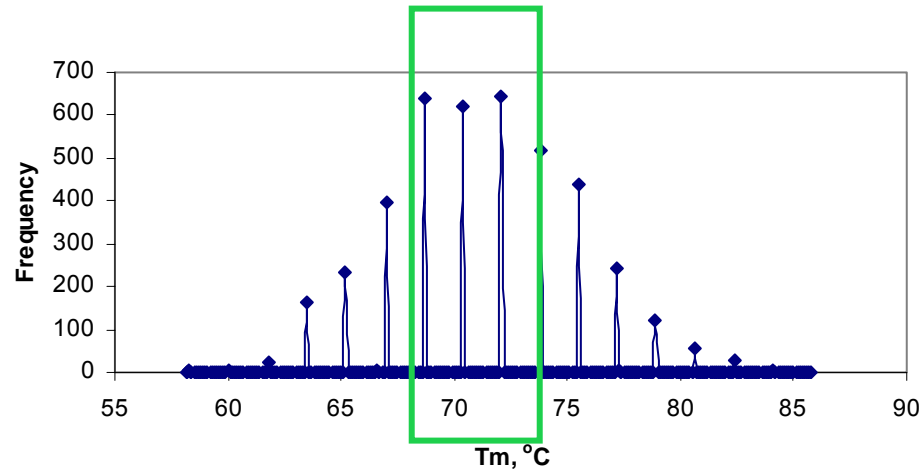
Sequence around middle nucleotide, length 24 bp

```
gagggatcacggttaangtgtttt
```

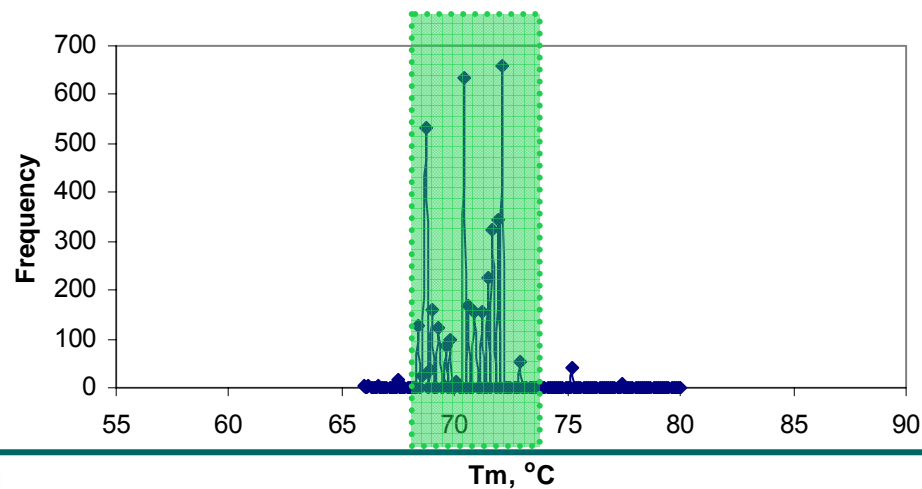
Sequence from the right side, length 24 bp

```
gtgttttcagtgggatgtcaagacttg
```

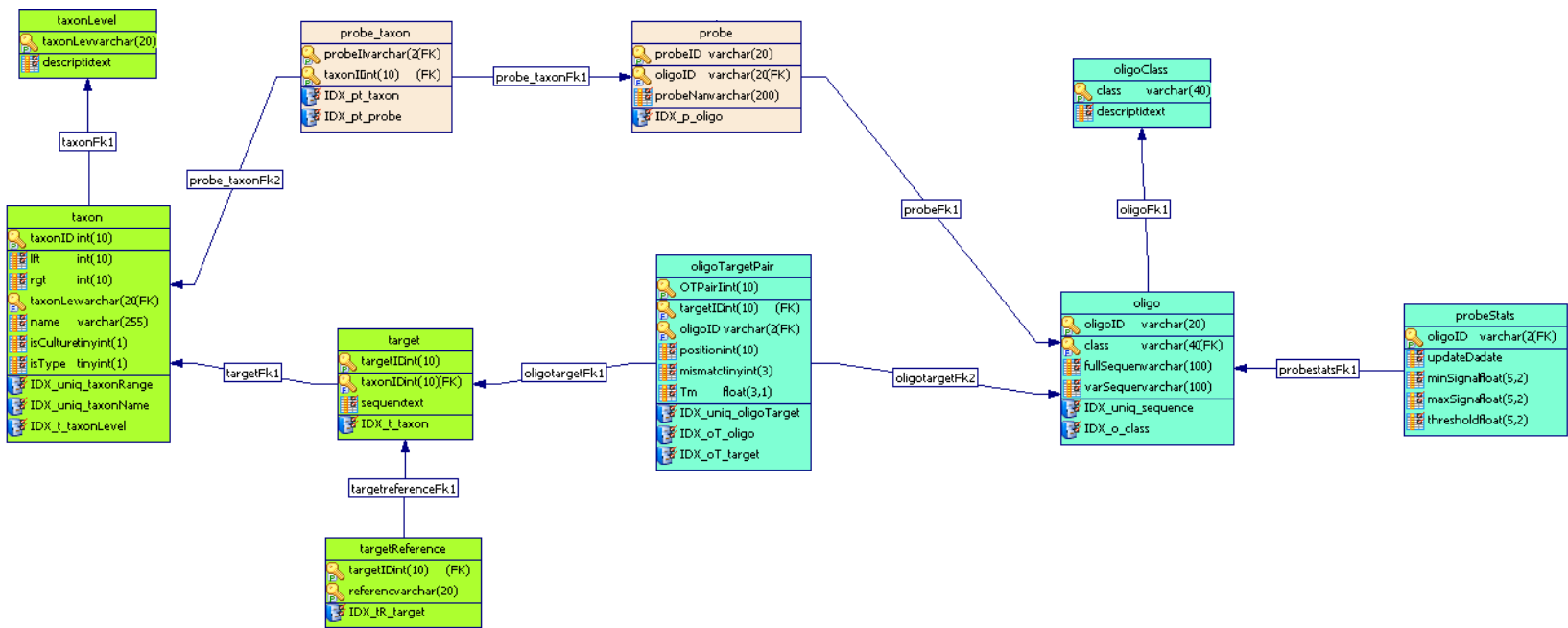
Distribution of predicted melting temperatures – original design (24 bp)



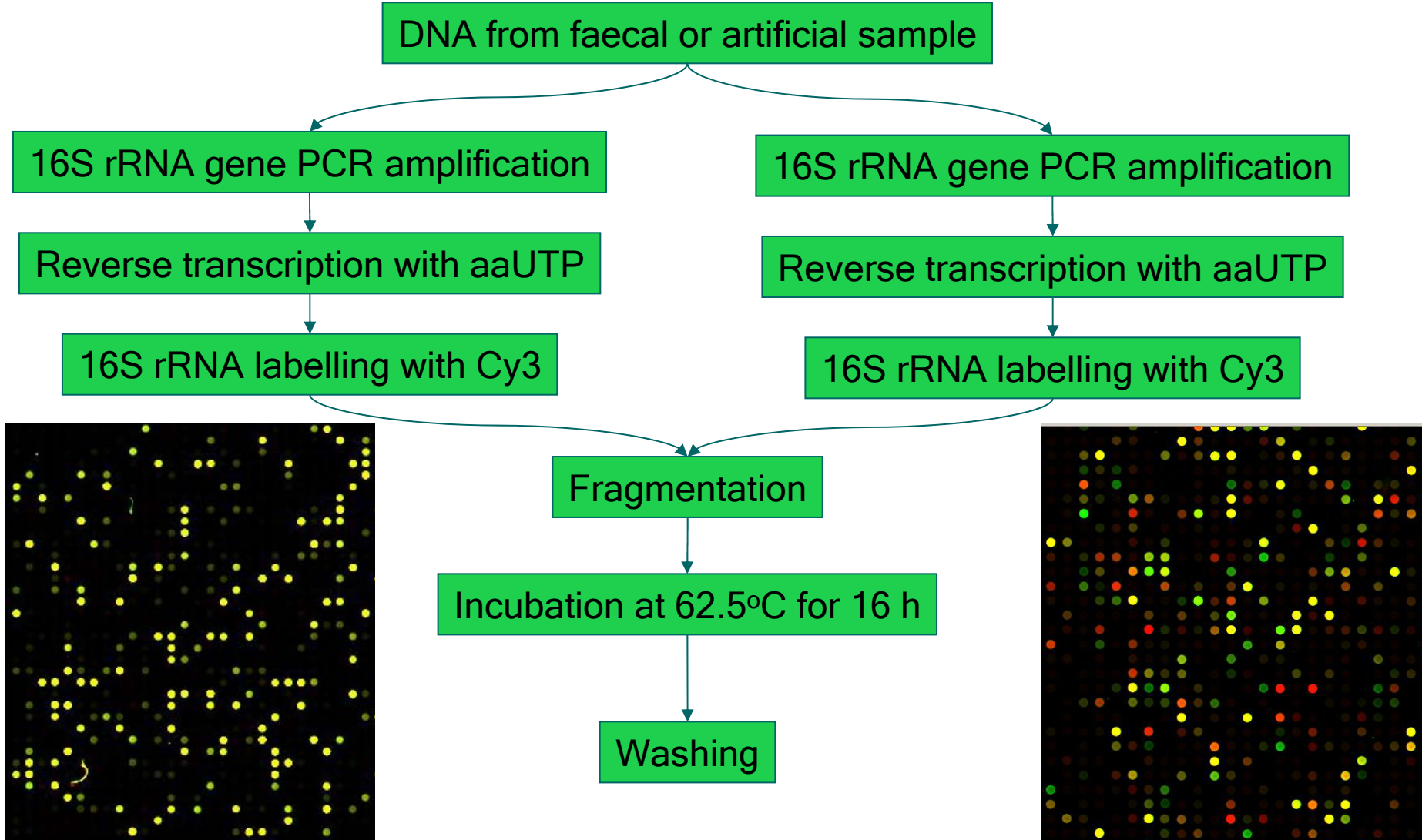
Distribution of predicted melting temperatures after redesign (24 +/- 6 bp)



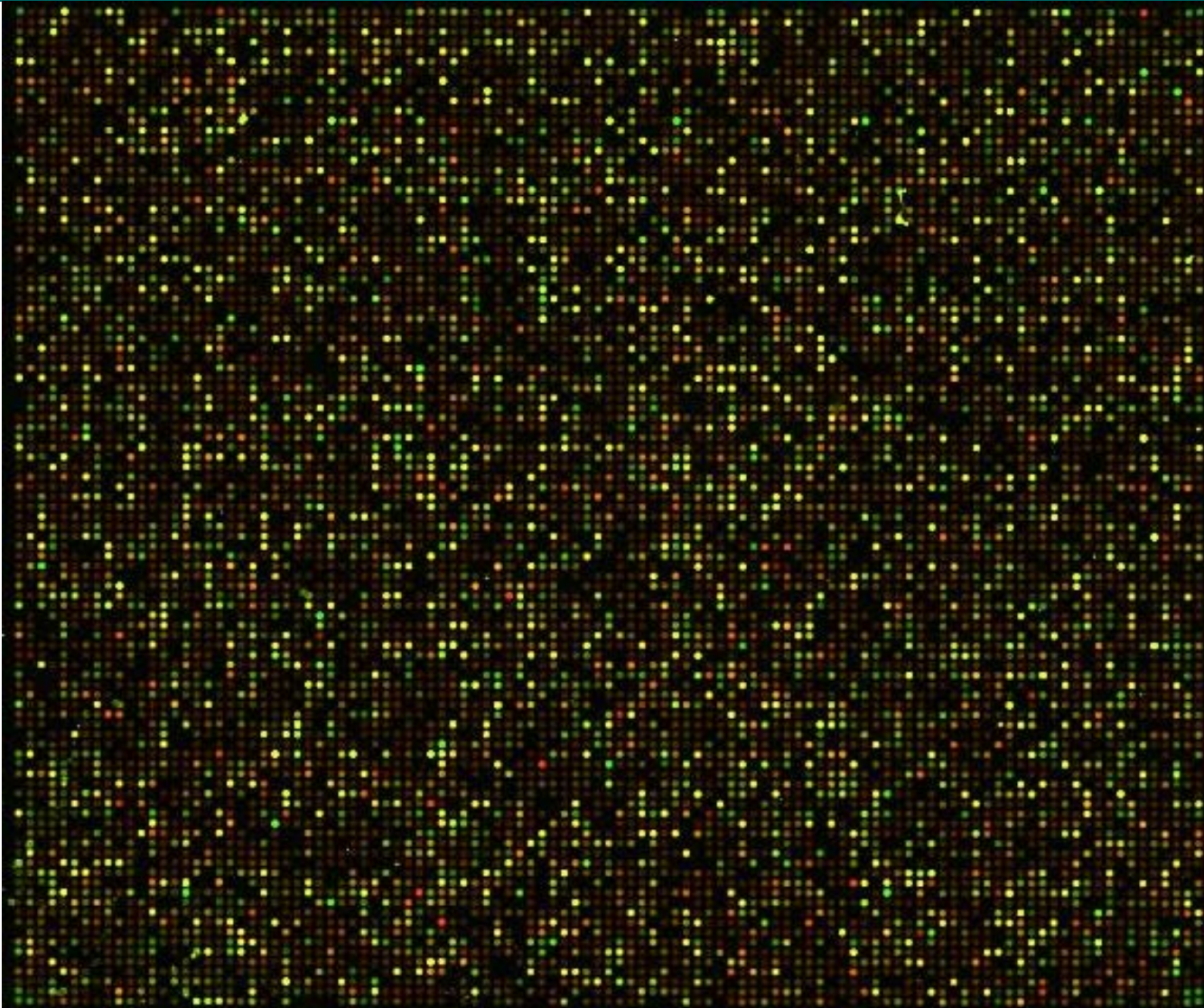
~ 5 500 probes printed in duplo
~ 1 100 organisms targeted



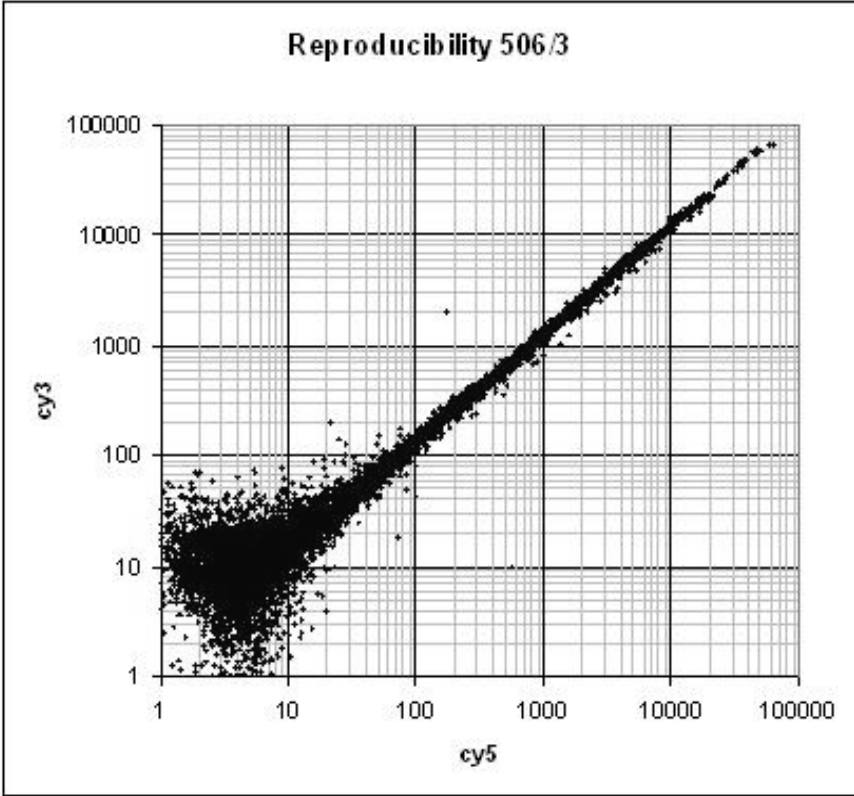
MySQL database constructed



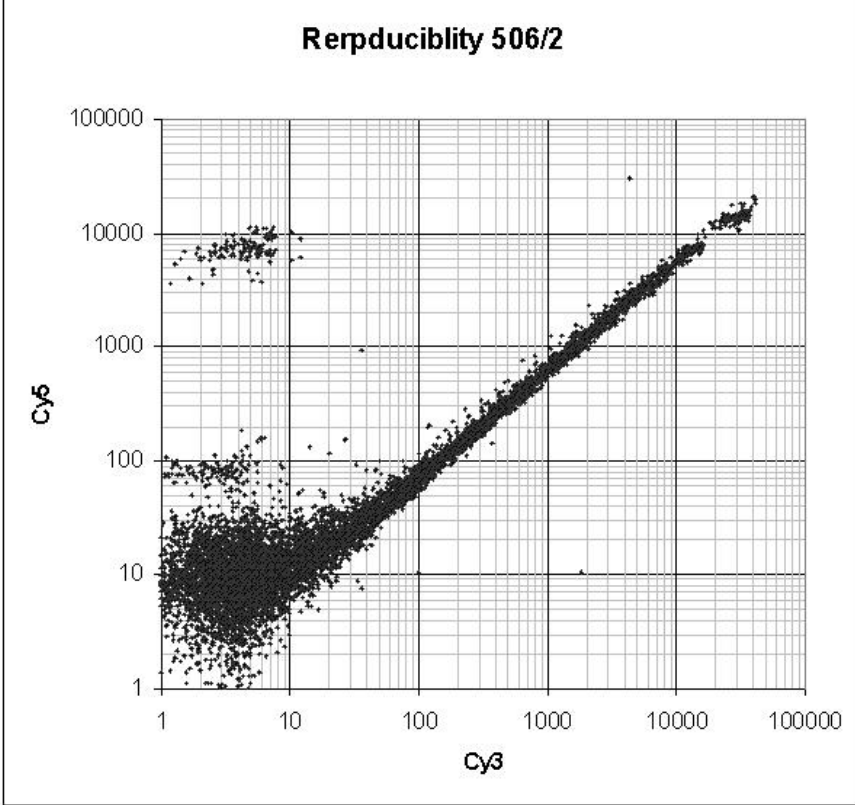
Results



Agilent *in situ* synthesized arrays – good quality of reading

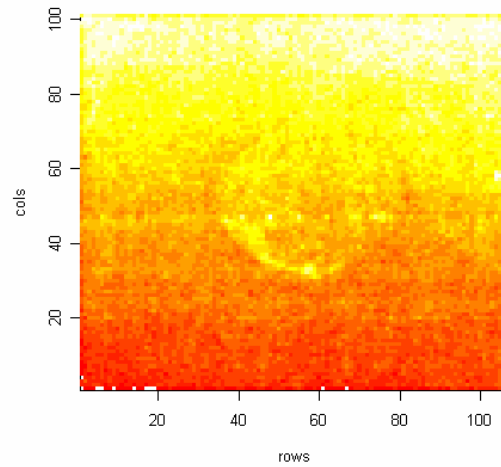
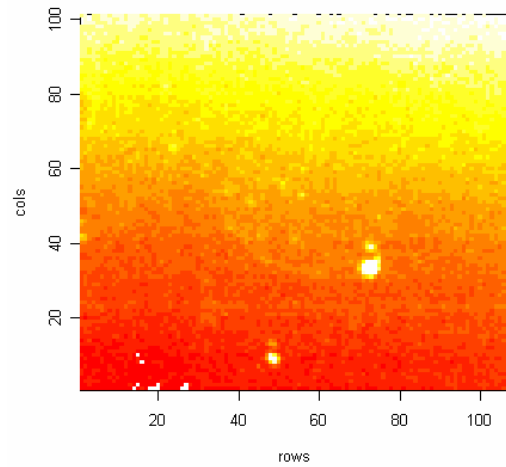


Pearson's R = 0.998

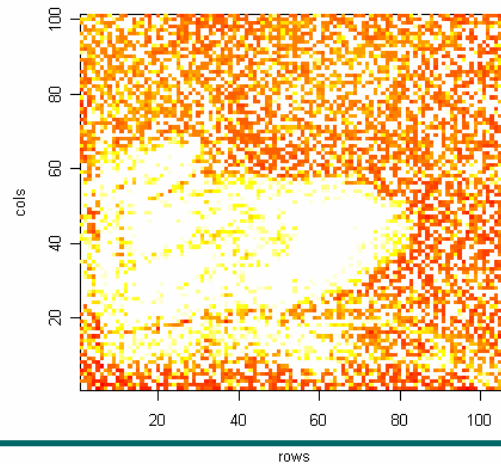
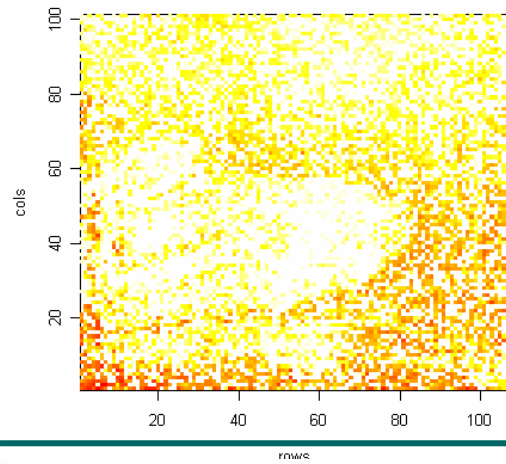


Pearson's R = 0.890

In silico obtained images of the HITChip signals for:

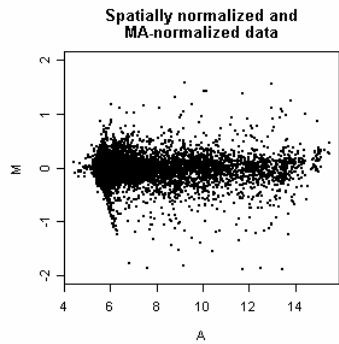
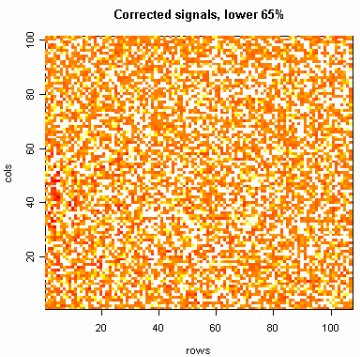
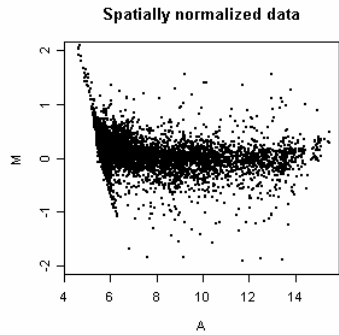
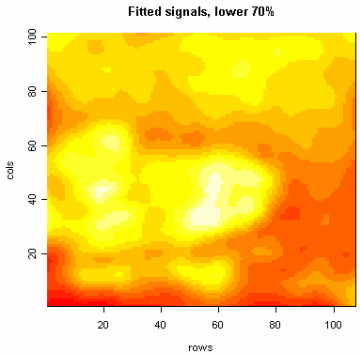
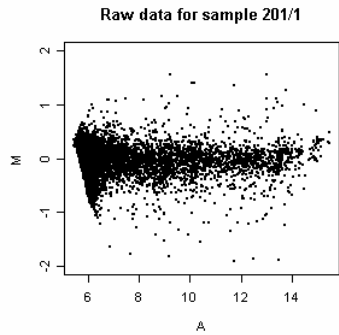
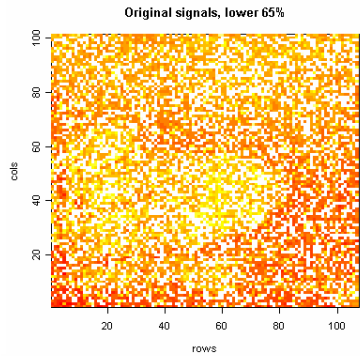


Background



Probes with low signal intensity (< 120)

Results



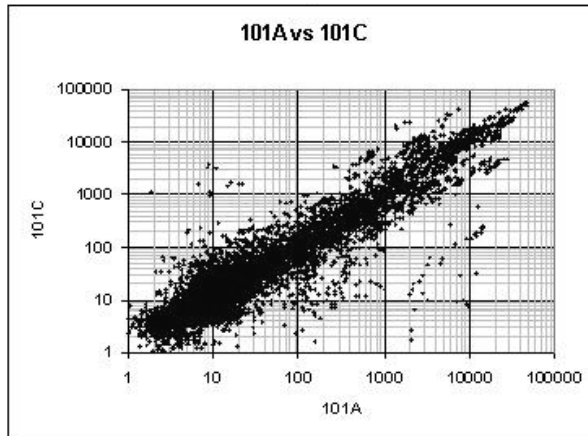
Pearson's R = 0.890

normalisation

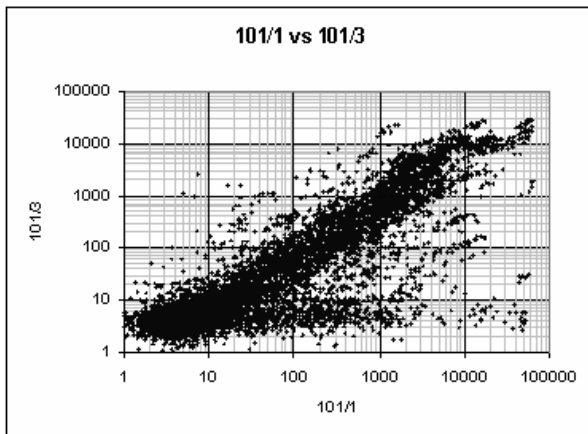
Pearson's R = 0.987

Highly reproducible experiments

Different DNA extraction	~97%
Different PCR amplification	~99%
Different hybridisation	~100%



Pearson's R = 0.89



Pearson's R = 0.62

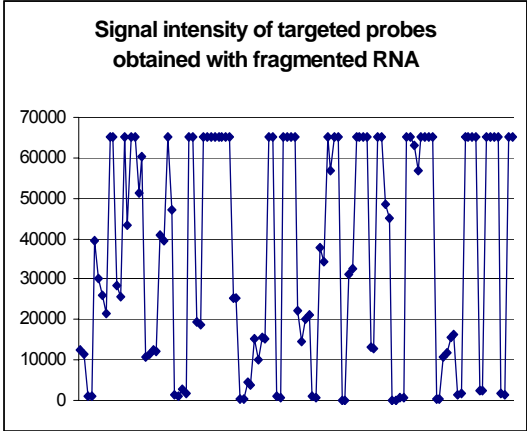
Pearson's correlation – indication of stability

Value of the correlation factor influenced by

- quantitative changes
- qualitative changes

Principle: both variable regions responding > organism present

6 probes hybridising > organism identified



Large signal variation

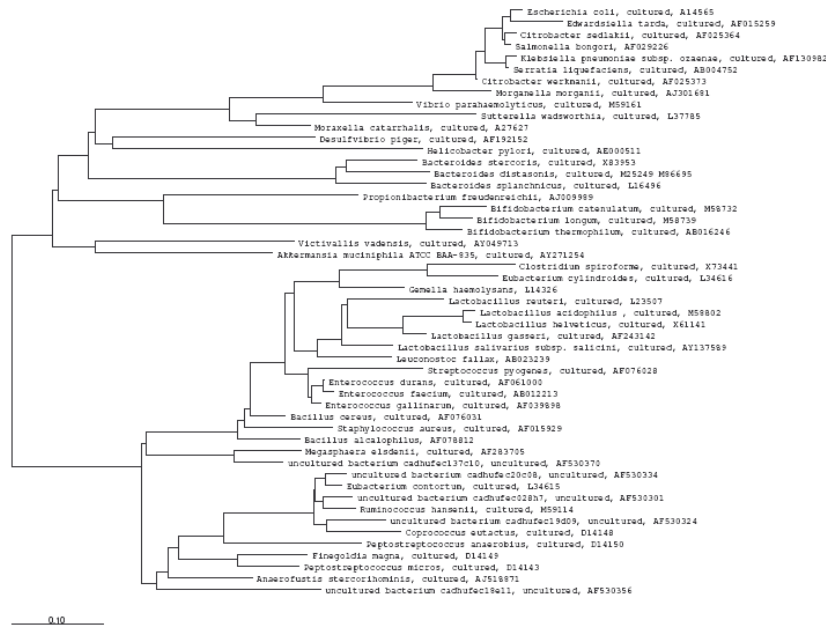
CON
Narrow range of T_m
The same targeted region

PRO
Secondary structure of a probe
Multiple prints of a sequence

Impossible to correct for
Not-responding probes empirically determined and excluded

Identification

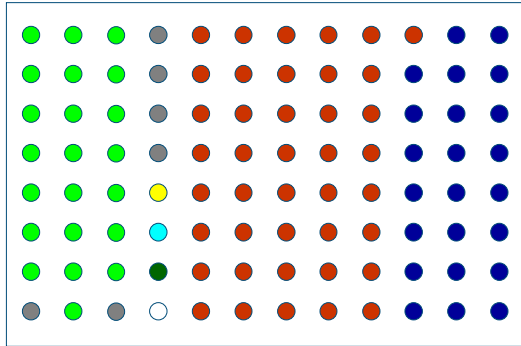
6 (or less) probes hybridising > organism present



Name	Phylum	Order	Family	Genus	16S level 1	16S level 2
bacterium adhufec355	<i>Bacteroidetes</i>	<i>Bacteroidales</i>	<i>Bacteroidaceae</i>	<i>Bacteroides</i>	<i>Bacteroidetes</i>	<i>Bacteroides fragilis</i> et rel.
<i>Bacteroides ovatus</i>	<i>Bacteroidetes</i>	<i>Bacteroidales</i>	<i>Bacteroidaceae</i>	<i>Bacteroides</i>	<i>Bacteroidetes</i>	<i>Bacteroides ovatus</i> et rel.
<i>Ruminococcus gnavus</i>	<i>Firmicutes</i>	<i>Clostridiales</i>	<i>Lachnospiraceae</i>	<i>Ruminococcus</i>	<i>Clostridium</i> cluster XIVa	<i>Ruminococcus gnavus</i> et rel.
uncultured bacterium G170	<i>Firmicutes</i>	<i>Clostridiales</i>	<i>Peptostreptococcaceae</i>	Unclassified	<i>Clostridium</i> cluster XIII	<i>Peptostreptococcus micros</i> et rel.

Group quantification

Results

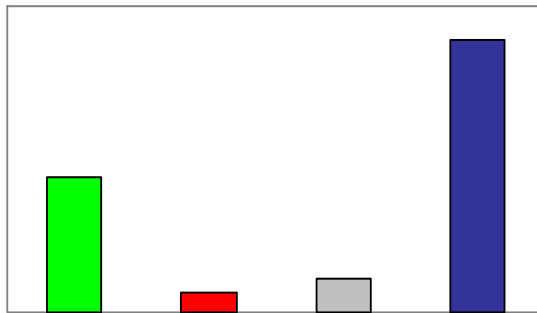
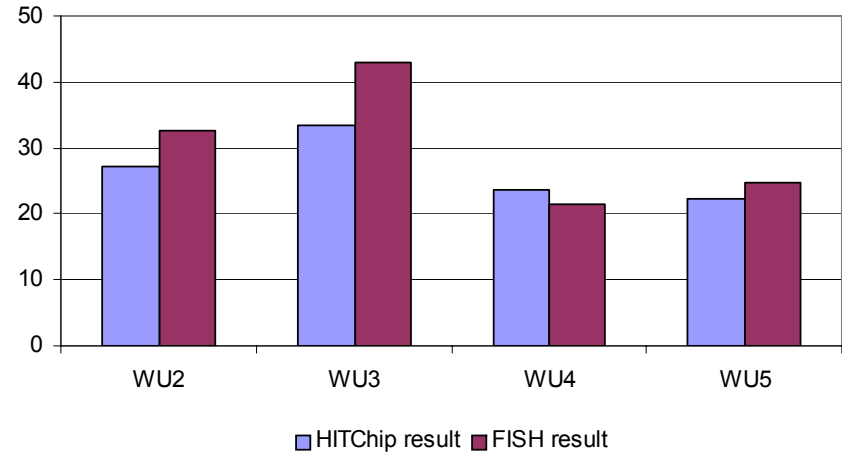


Probe signal - not quantitative

Variation in signal intensity stochastic

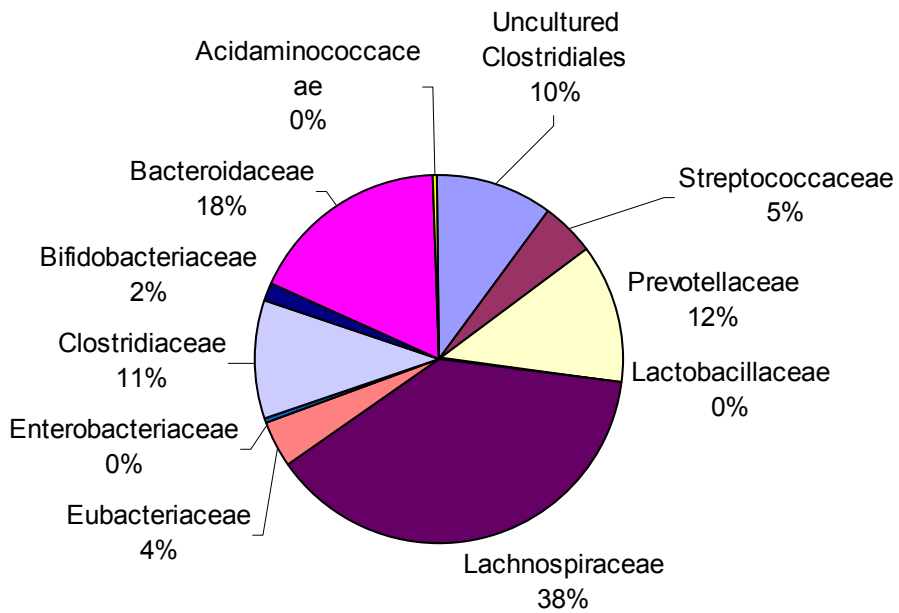
Σ (signal per group) - quantitative

Percentage of C.leptum group in the faeces

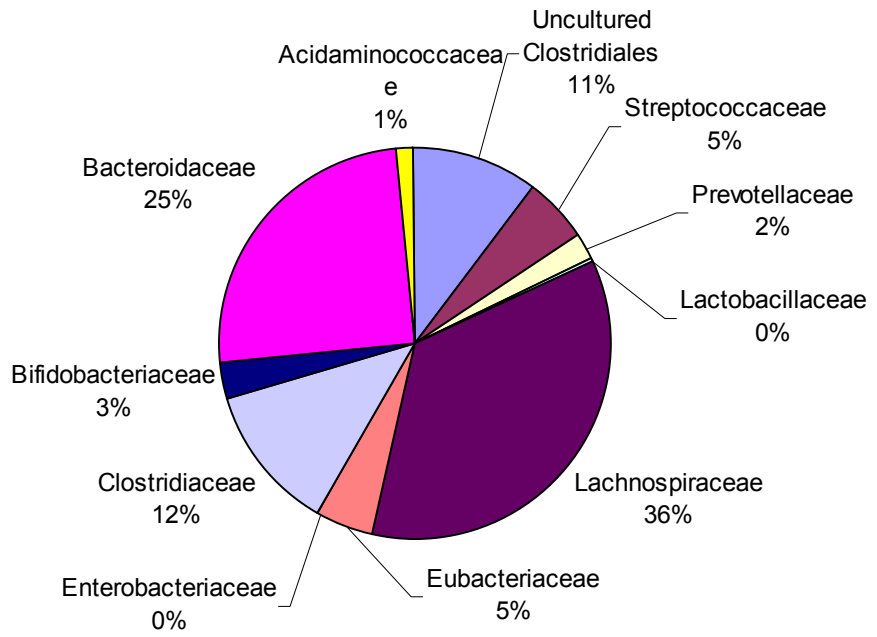


The same trends as with other techniques

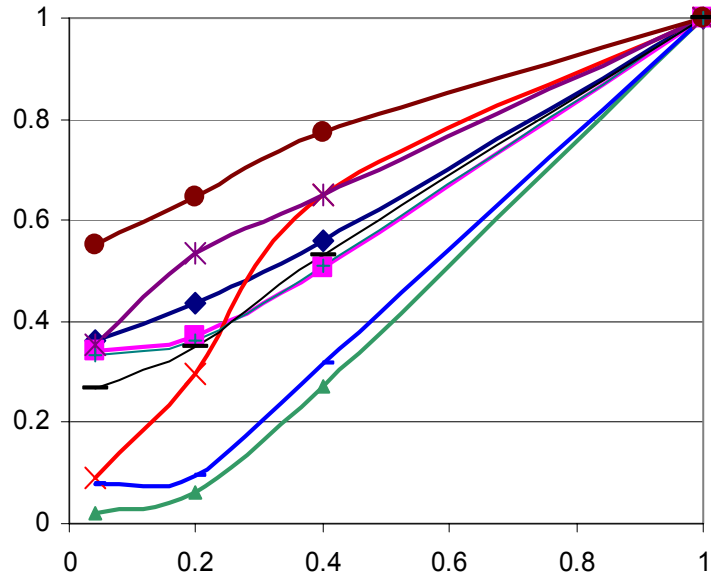
WU2



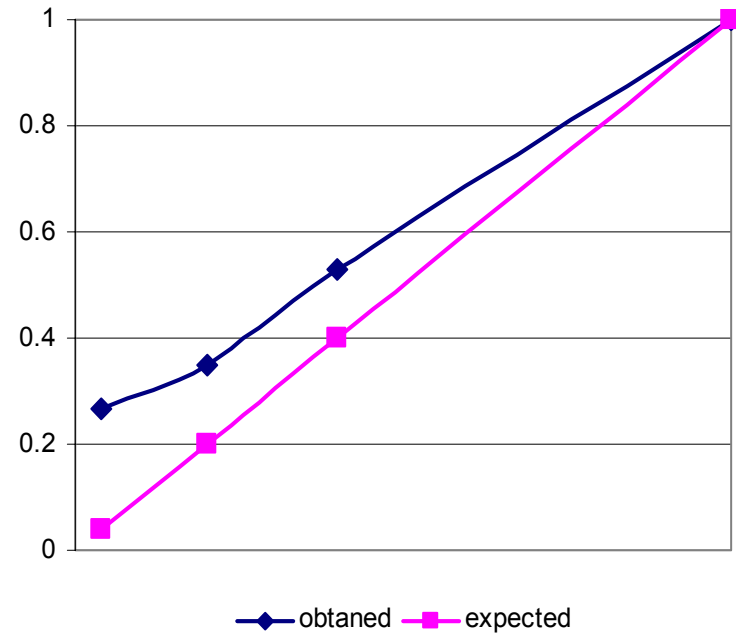
WU4



Relative quantification obtained for 10 OTU's in range 0.1 to 3%



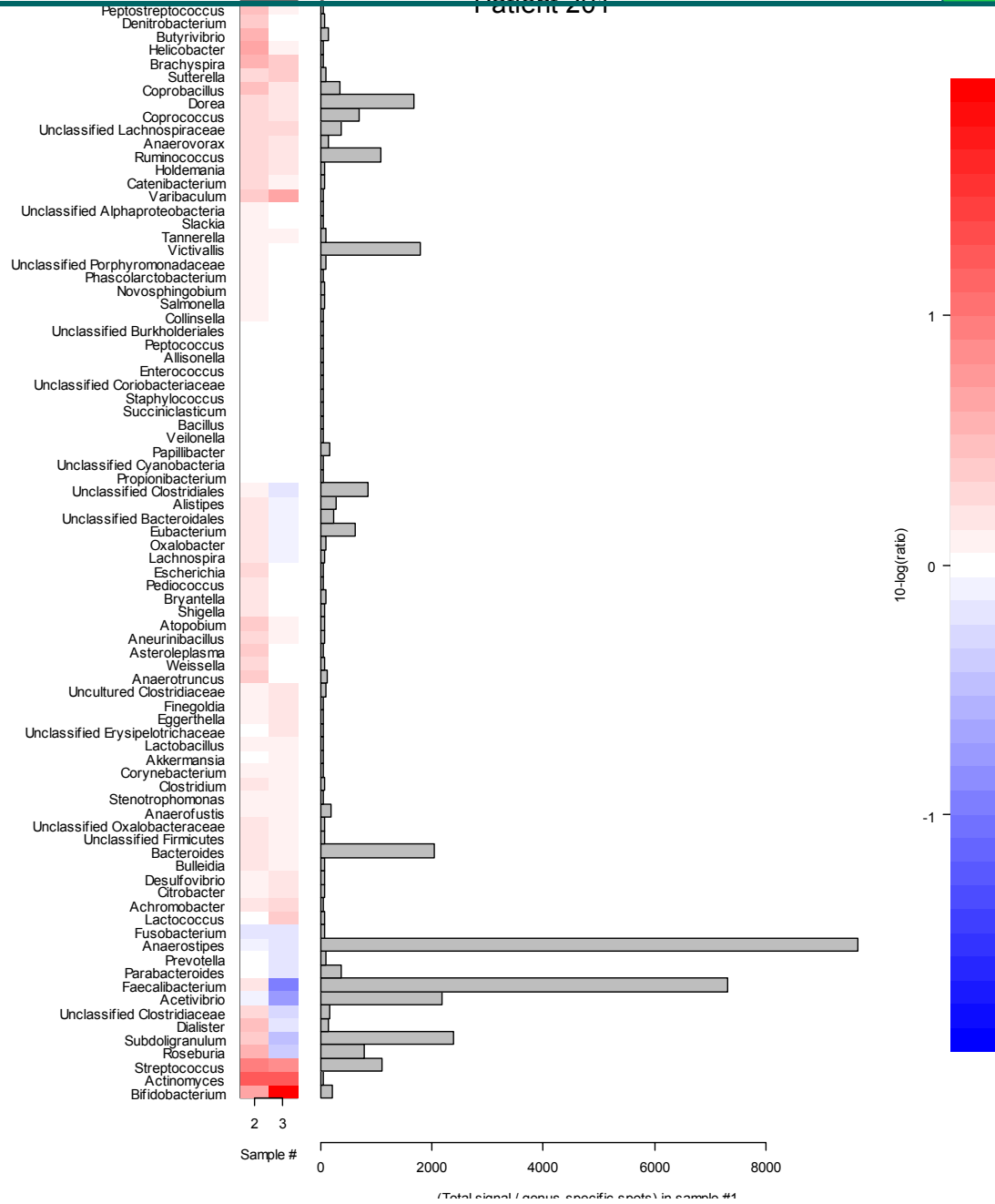
Relative quantification for 10 clones summarised



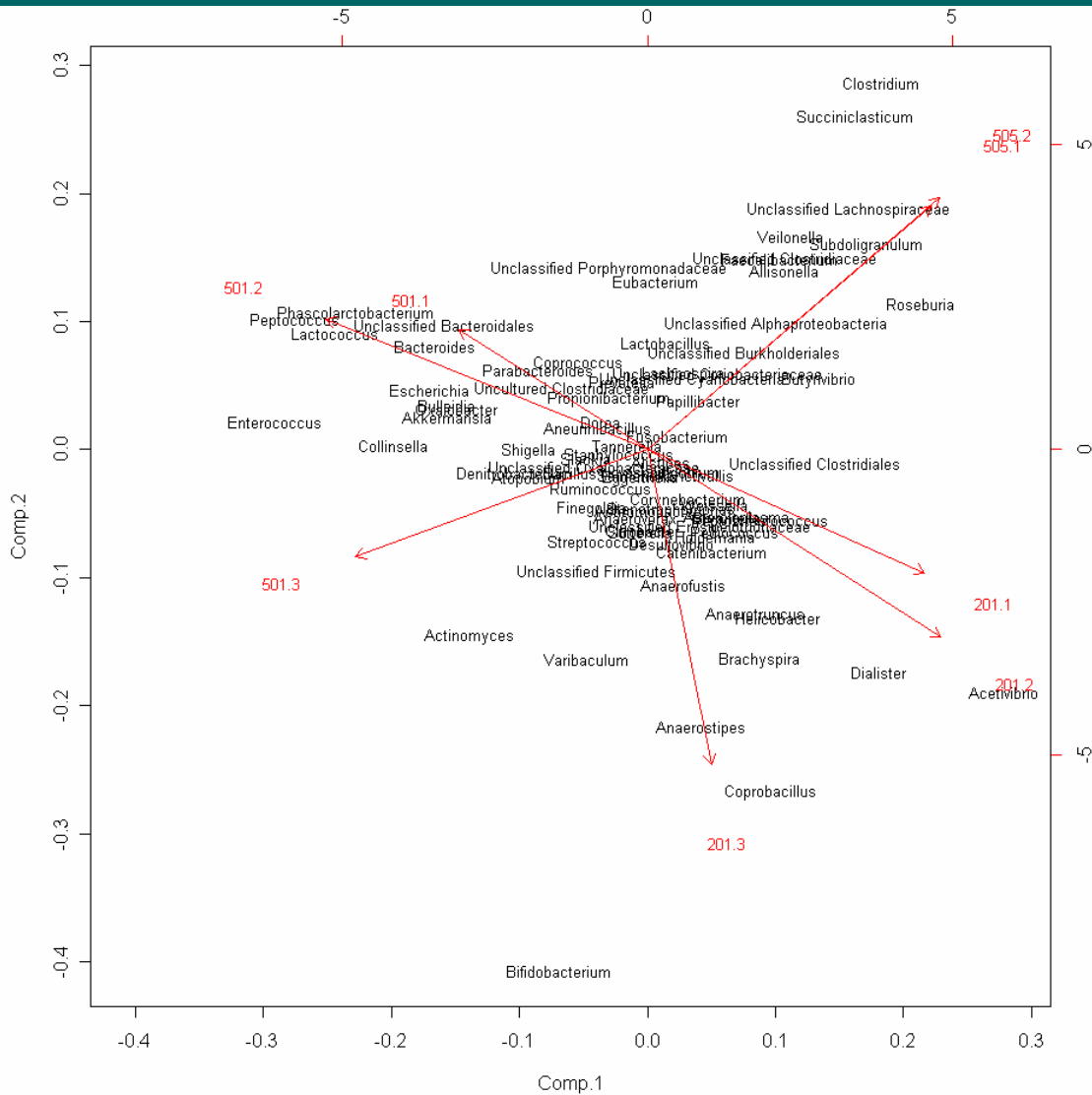
All 6 probes per targeted organism used
Not all probes are species specific

Analysis should be limited only on the probes specific for the taxonomic level

Patient 201



Results



❖ PCA with absolute signals

❖ PCA with relative signals

- ❖ HITChip – diversity microchip that covers all up to now known human intestinal microbiota was developed and validated
- ❖ Probe design is flexible and allows easy addition of new probes for novel organisms of the ecosystem
- ❖ Results of the HITChip are of good quality and reproducibility
- ❖ MySQL support database for the chip for normalisation and processing of the results was designed
- ❖ HITChip allows rapid identification, group quantification, relative quantification and stability check – superior tool for intestinal microbiota analysis



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THANKS FOR YOUR ATTENTION !

QUESTIONS?