



# Human Transcriptome ProbeLibrary™

- Detecting 37000 transcripts with 90 probes -

**1st International qPCR Symposium**

3rd-6th March, 2004

Freising-Weihenstephan – Germany

Peter Mouritzen

# Outline

- Explaining the Human ProbeLibrary™ concept
  - Human transcript coverage
  - Comparison to other Real Time PCR technologies
- How can 90 probes enable detection of 37000 transcripts?
- Workflow – Finding the probe and performing the assay
- Examples & biological applications
  - Exploiting probe recurrence
  - Performance compared to other Real Time PCR technologies
  - Validation of microarray results

# Introducing the Human ProbeLibrary™

- 90 prevalidated 8 & 9-mer probes



- Target short recurring 8- & 9-mer sequences

- Web tool designs the assays

EXIQON ProbeLibrary™

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EM\_000126.1 Homo sapiens estrogen receptor 1 (ESR1), mRNA.

Lib\_hum\_A\_005

	Position	TM	Sequence
Left Primer	1819 - 1859	69	atccacgctgctgacac
Right Primer	1810 - 1830	60	tgagtaaacagagctgagc

Genomic map showing exons (green) and introns (grey) with primer binding sites (blue arrows) and a 44C1 probe site (blue arrow).

LibraryProbe Rank Position Intron spanning Score Class

Lib_hum_A_015	1	1858	+	94.76	94.76
Lib_hum_A_005	2	1876	+	94.60	94.60
Lib_hum_B_101	3	1876	+	94.60	94.60
Lib_hum_A_0109	4	1879	+	91.4	91.4
Lib_hum_A_0109	5	1206	+	91.4	91.4

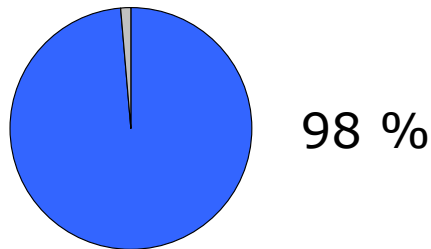
More Candidates return to solution

version: 0.35

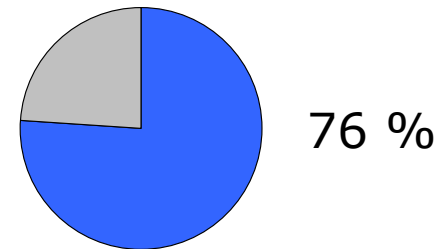
- Determines probe target sites and designs intron-spanning assays

# Coverage of the 90 ProbeLibrary™ Probes

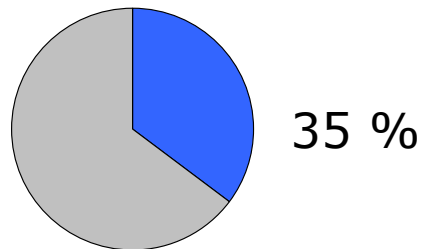
% of mRNA transcripts targeted in RefSeqHum



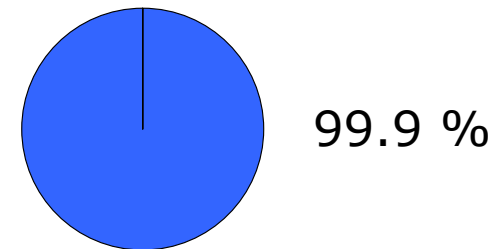
% Ensembl mRNA transcripts with intron spanning assays



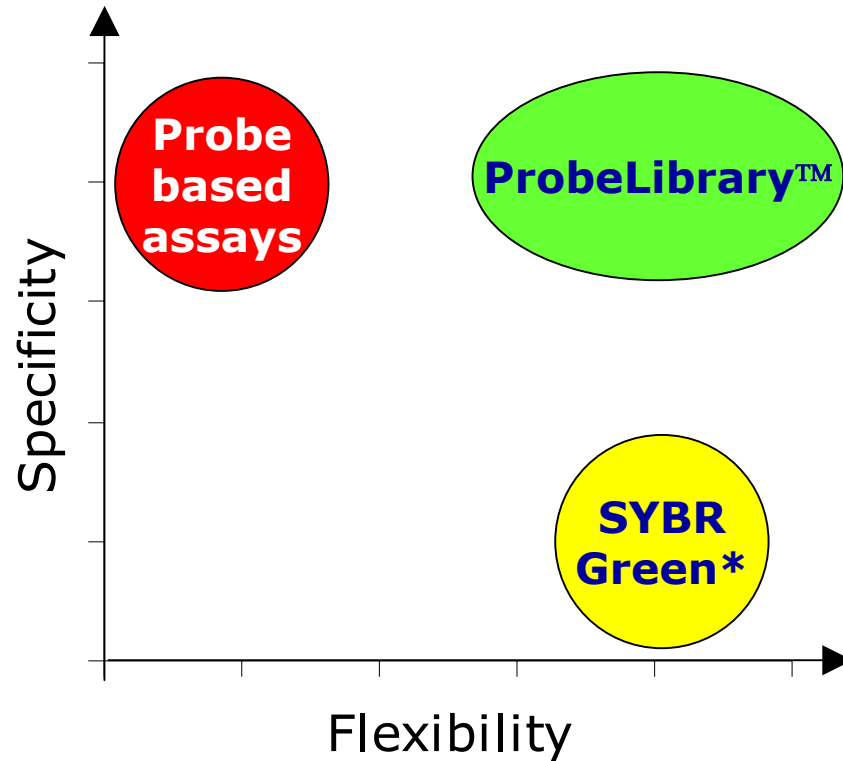
% of Ensembl exon junctions targeted



Targeting the 1000 most cited refseqs



# High Flexibility and Specificity

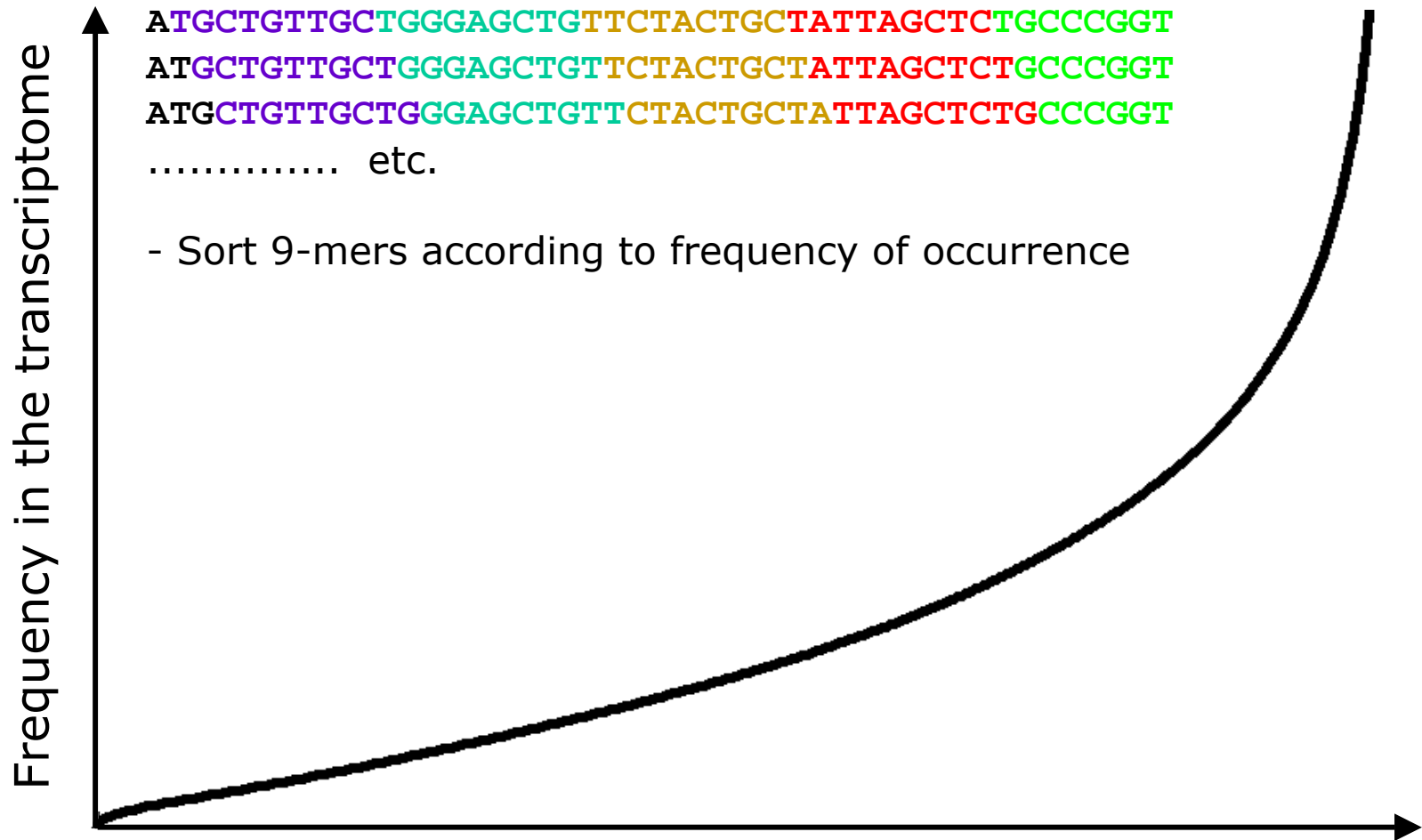


**\* and other intercalating dye based assays**

# Occurrence of 9-mers in Human Transcripts

ATGCTGTTGCTGGGAGCTGTTCTACTGCTATTAGCTCTGCCCGGT  
ATGCTGTTGCTGGGAGCTGTTCTACTGCTATTAGCTCTGCCCGGT  
ATGCTGTTGCTGGGAGCTGTTCTACTGCTATTAGCTCTGCCCGGT  
ATGCTGTTGCTGGGAGCTGTTCTACTGCTATTAGCTCTGCCCGGT  
..... etc.

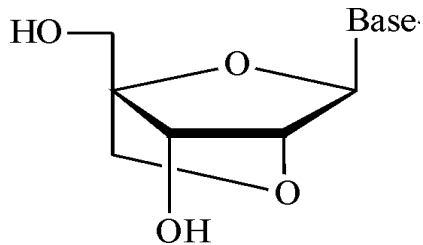
- Sort 9-mers according to frequency of occurrence



Different 9-mers - 262,000 possible sequences

# T<sub>m</sub> in short probes maintained by LNA™

## Locked Nucleic Acid (LNA™)



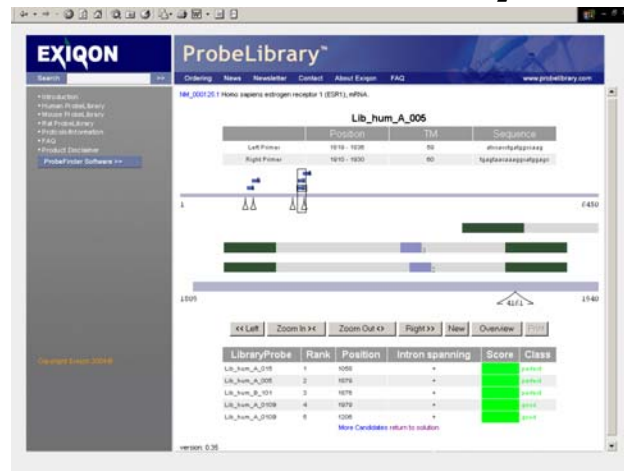
- High affinity duplex formation ( $\Delta T_m = +2 - 8^\circ\text{C}$  per base)
- Improved base-pairing specificity

# Workflow using the Human ProbeLibrary™

**1. Identify the gene(s) of interest**



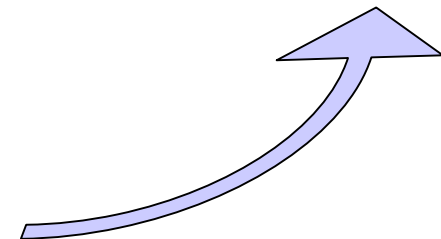
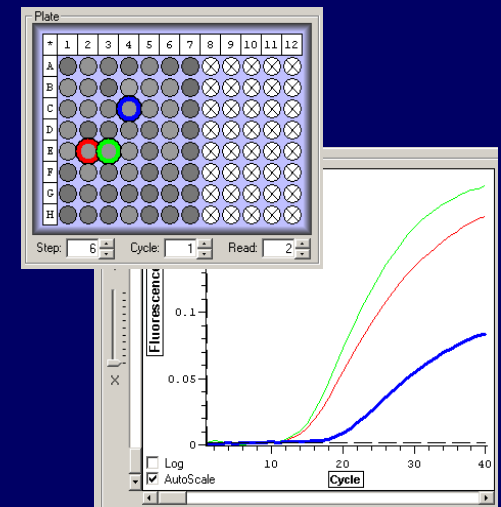
**2. Identify probes and primers at [www.ProbeLibrary.com](http://www.ProbeLibrary.com)**



**3a. Order primers at your favourite oligo house for overnight delivery**

**3b. Pull out probe from the ProbeLibrary™ in your freezer**

**4. Perform qPCR & Evaluate results**

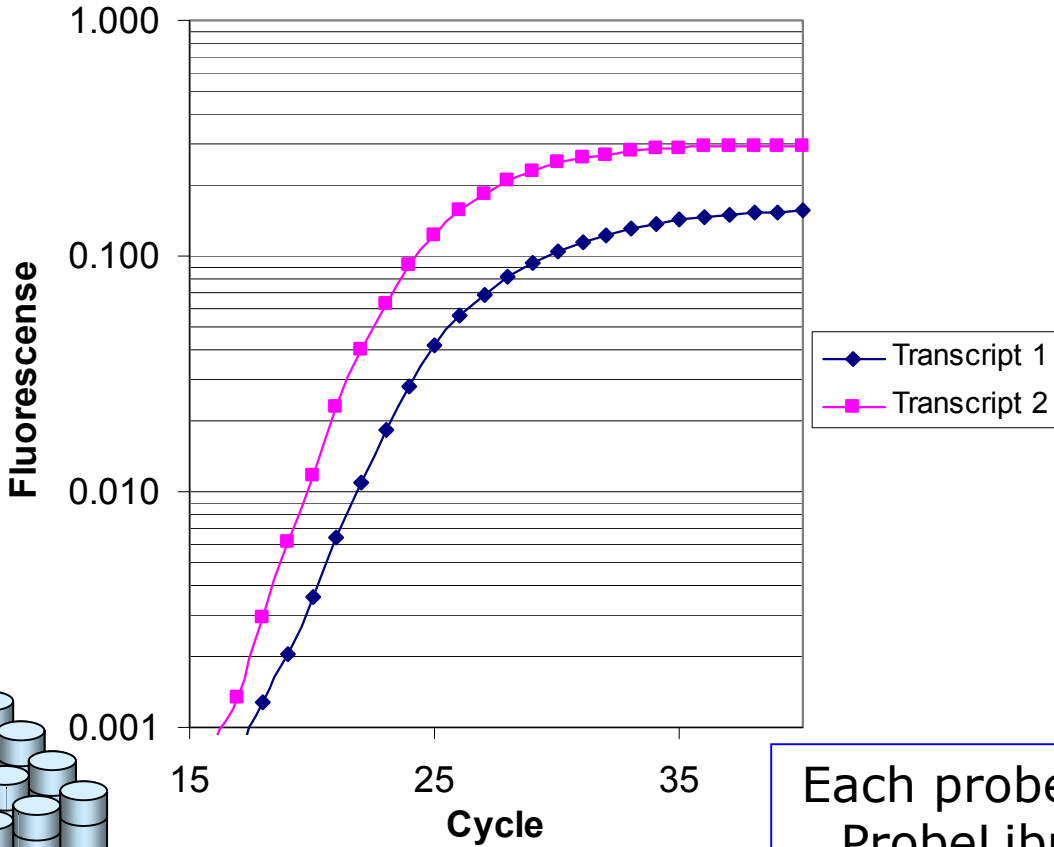
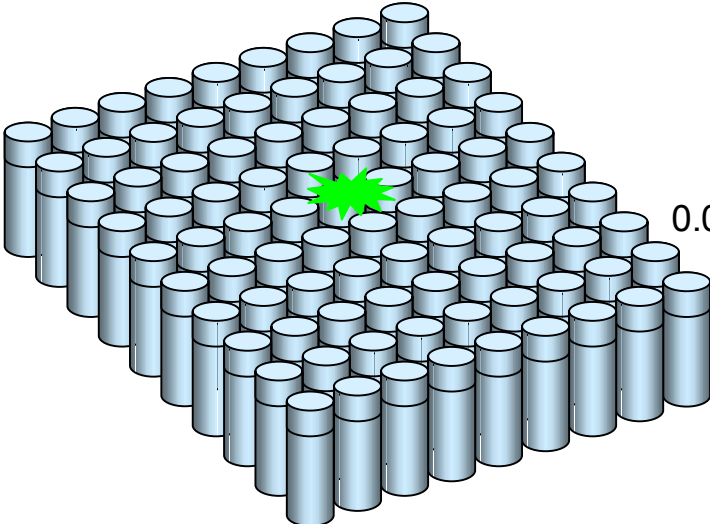


# One Probe Multiple Transcripts

Exploiting recurrence



One Probe



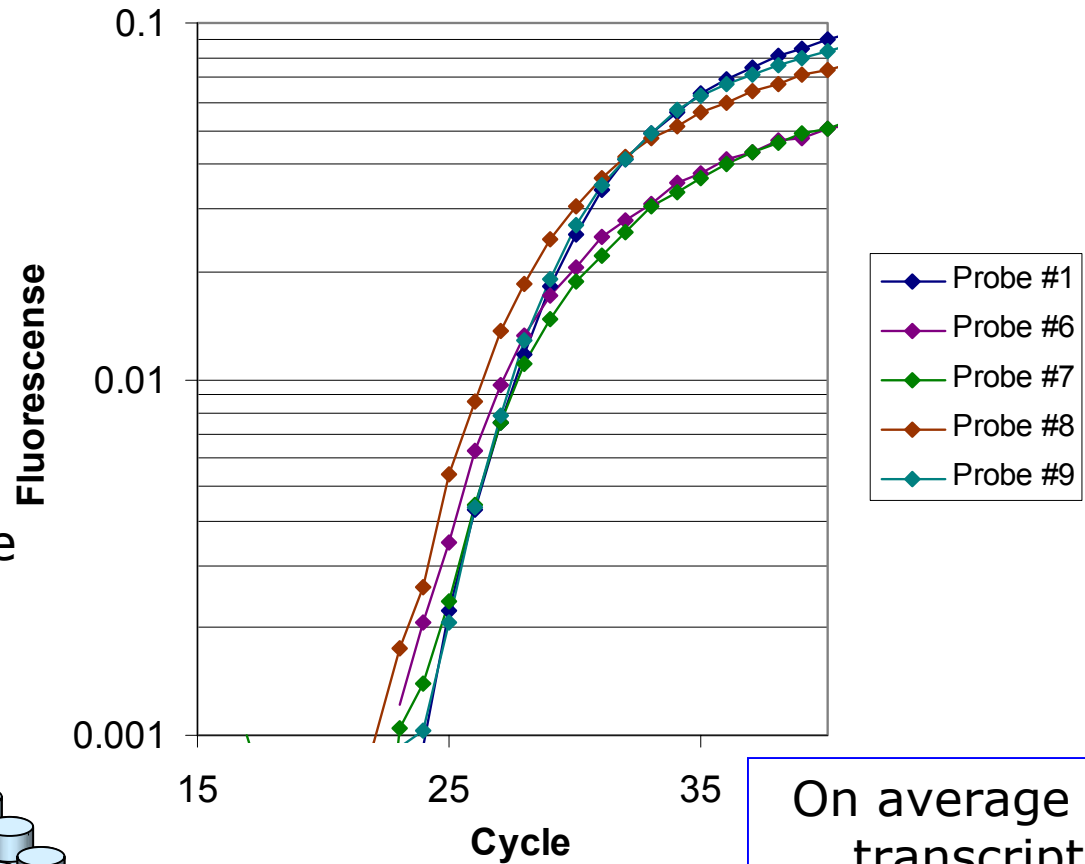
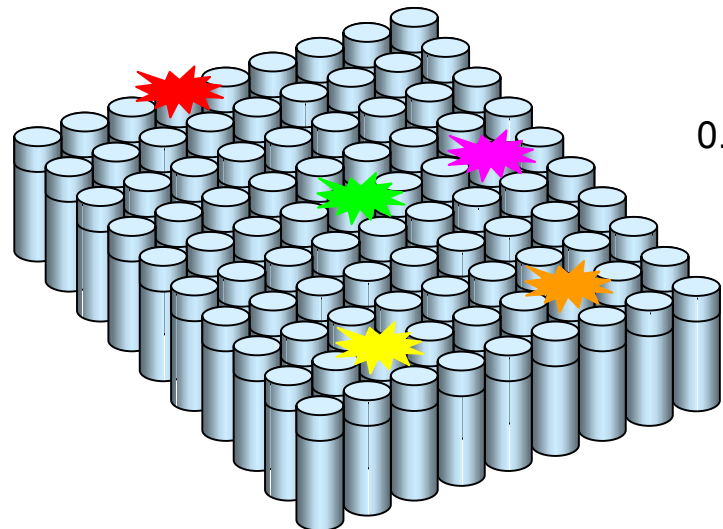
Each probe in the ProbeLibrary™ target 7000 transcripts on average

# Multiple Probes per Transcript

Confirming results



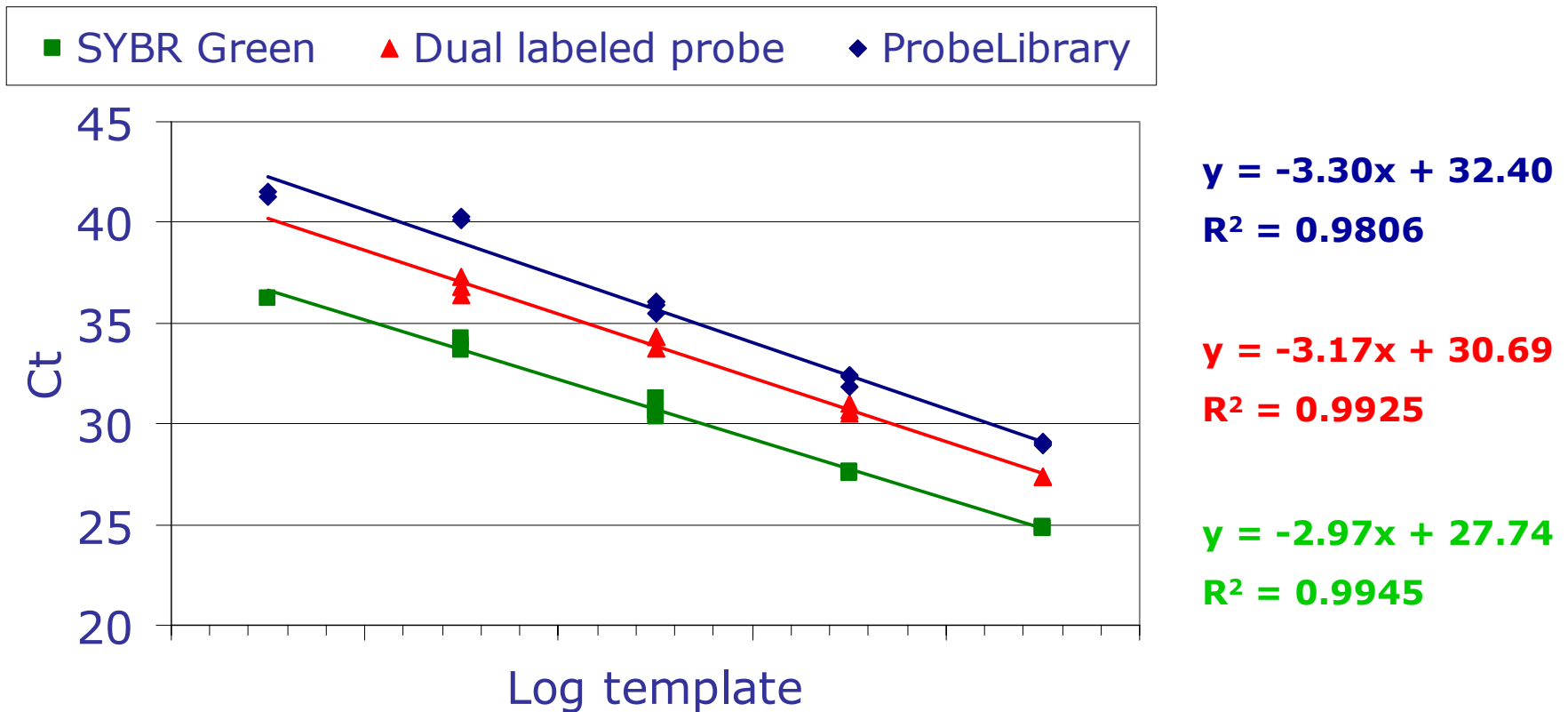
with more than one probe



On average each transcript is targeted by 17 different probes in the ProbeLibrary™

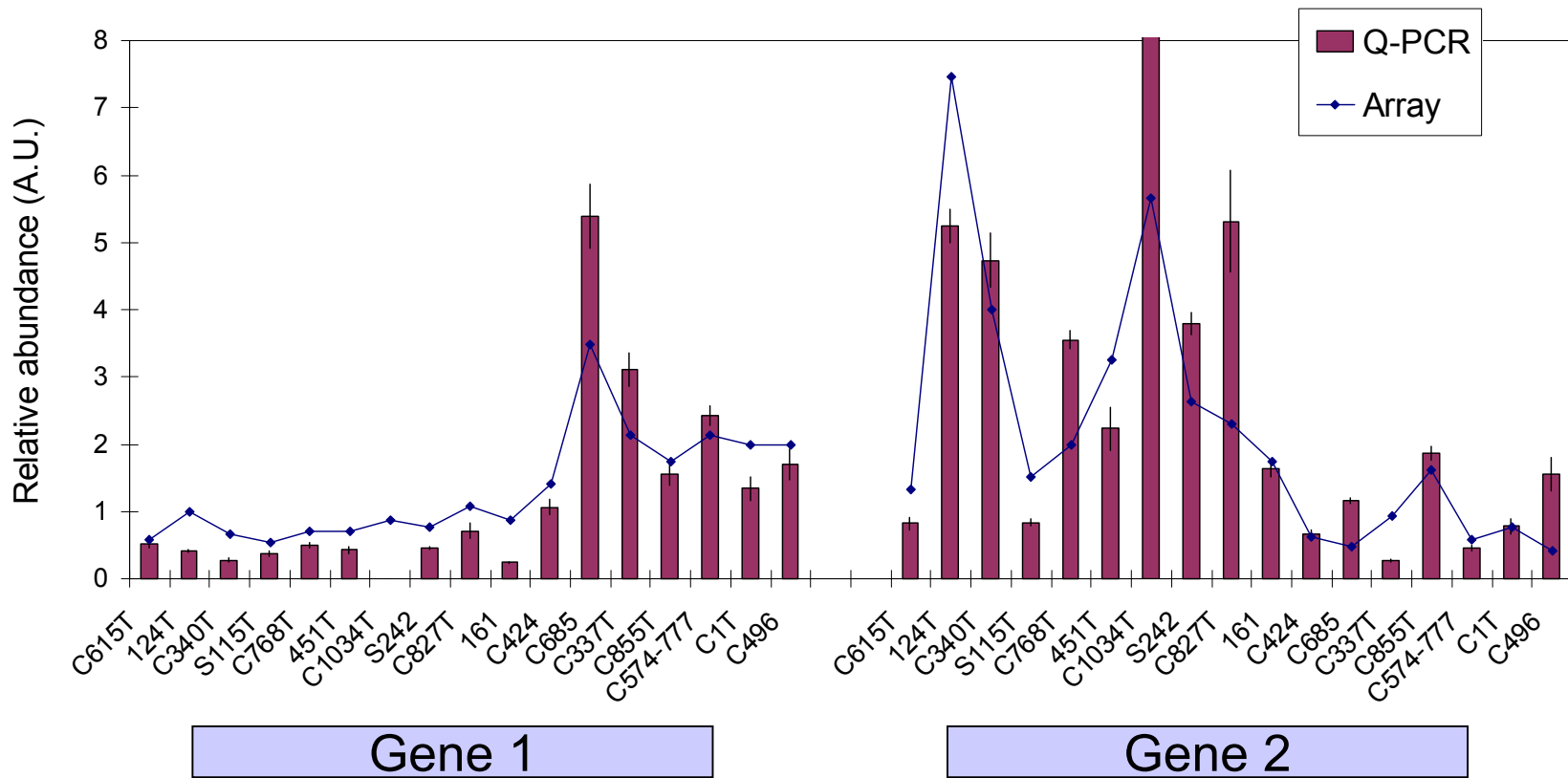
# Performs as other qPCR Technologies

Example: ALOX5AP (arachidonate 5-lipoxygenase-activating protein)



# Good correlation to Microarray data

Kruhøffer M. et al. Aarhus University Hospital - Skejby



# Summery

- The 90 probes in the ProbeLibrary™ are prevalidated
- The web tool at [www.probelibrary.com](http://www.probelibrary.com) designs the assays
- The assays are specific
- High flexibility
  - the 90 probes enable detection of 98% of human transcripts in RefSeq
- $\frac{3}{4}$  of the gene transcripts are targeted near an intron
- $\frac{1}{3}$  of exon-exon junctions are targeted
- Assay performance comparable to other Real Time PCR technologies
- Good concordance with microarray data
- Rat ProbeLibrary™ to be launched this summer

# Poster Overview - ProbeLibrary™

## **Comparison to other Real Time PCR technologies**

- Nielsen PS - Poster Abstract P53

## **Applications in Biology**

- Kruhøffer M - Poster Abstract 125 - Microarray
- Kauppinen S - Poster Abstract 119 - Target Validation

## **Description of the ProbeFinder Software**

- Tolstrup N - Poster Abstract P113

## **General**

- Mouritzen P - at the Exiqon boot

# Acknowledgements

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