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Diagnostics

ProbeLibrary

“One Transcriptome - One Kit”

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Problem in real-time PCR: Specificity or flexibility ?

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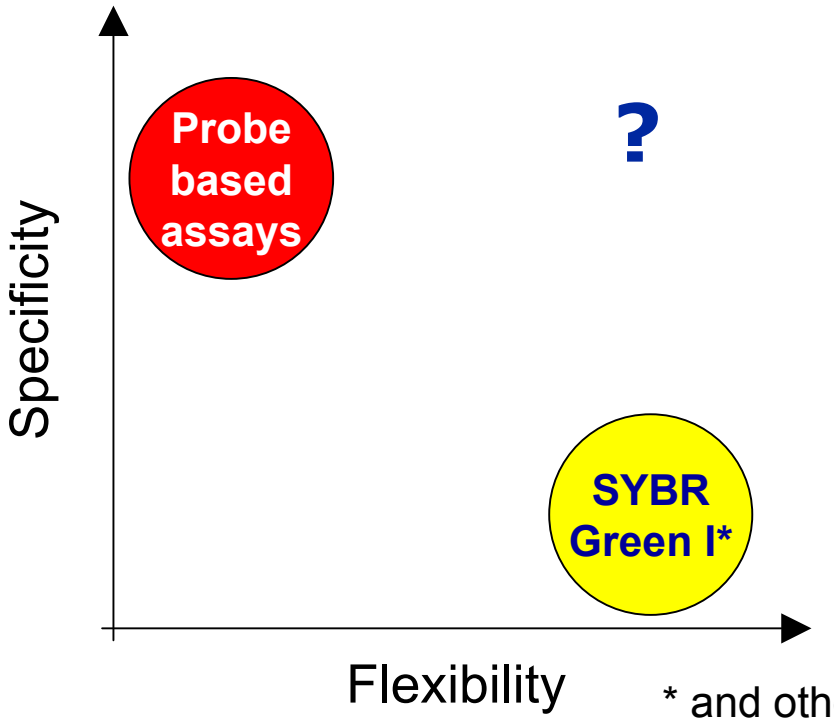
Today you have to decide whether using a specific probe-based assay or a flexible SYBR Green I assay !

What about having both characteristics together in one assay ?



* and other intercalating dye based assays

How to solve the problem of combining flexibility and specificity ?
Use the ProbeLibrary*

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- Kit with 90 probes and 250 rxns (50 µl) each
- Very short dual-labeled probes (8 and 9 mers)
- Probes are modified with LNA to ensure compatibility with standard real-time PCR methods
- Each transcript matches with ~ 16 probes, each probe matches with ~ 7.000 transcripts
- Specificity of assays through specific primers and assisted by probes

* ProbeLibrary is a trademark of Exiqon A/S

Advantages of the ProbeLibrary against other detection formates

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- **Advantage against SYBR Green I:**
  - No primer dimers or side products detectable due to detection with specific probes
- **Advantage against probe based assays:**
  - Time to result within 2 days (instead of 1 week)
  - Less expensive than regular probe assays
- **Customer workflow:**
  - Order kit
  - Design assay with ProbeFinder design software via web
  - Order primers at oligohouse
  - Run experiment

What are the properties of the ProbeLibrary ?

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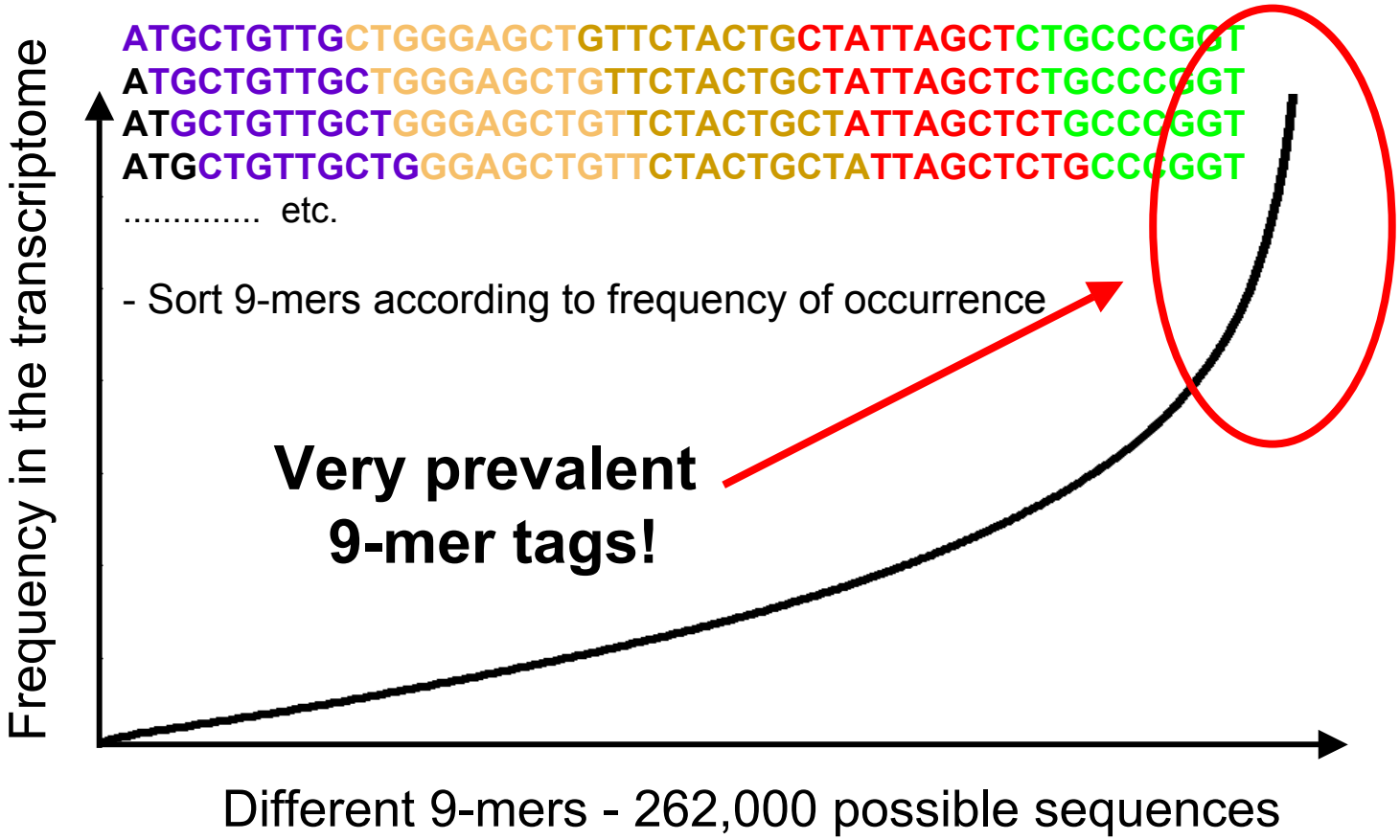
- Real-time PCR assays for Expression Profiling
- Complete transcriptome of one organism is covered by 98%
- Assay design is very fast and easy with web-based ProbeFinder design software
- Organisms available so far are human, mouse, rat, arabidopsis, drosophila and C. elegans
- The product is developed and already launched by Exiqon since May 2004

Why using 9-mers as probes ? Occurence of 9-mers in human transcripts



90 probes cover entire transcriptome

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Bioinformatic analysis of the genome reveals:

- Some 8 & 9 mer motifs are very prevalent in the trancriptome
- The same probes can be used for the same motifs in several genes

ProbeLibrary

Only 90 pre-validated probes are sufficient to cover the entire human transcriptome !

ProbeLibrary:
High flexibility and specificity

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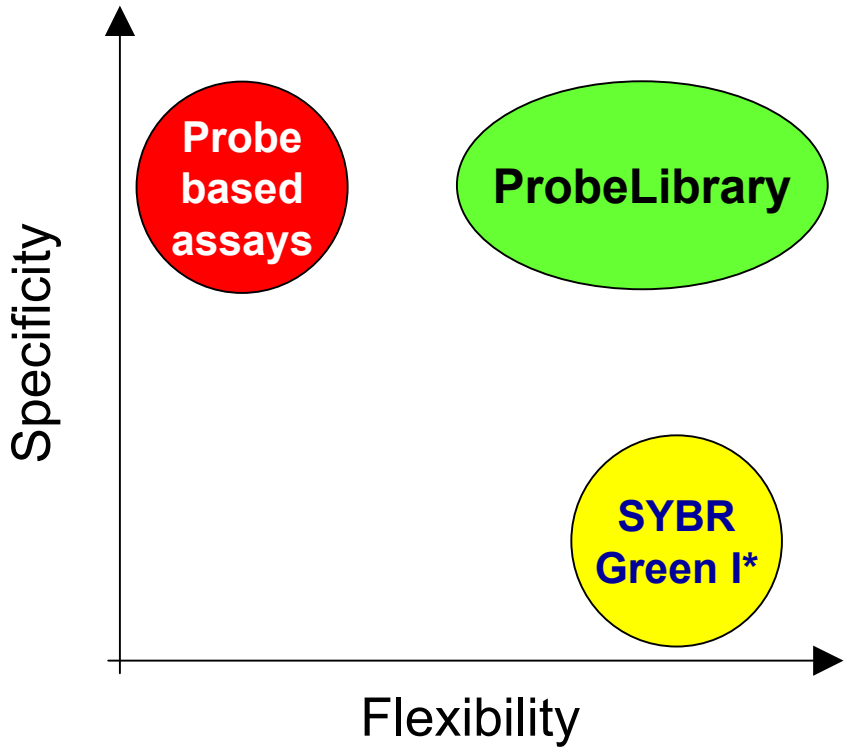
Just 90 Probes but ...

- 99 % of mRNA transcripts in RefSeqHum are targeted
- 90 % of Ensembl mRNA transcripts are targeted with intron spanning assays
- Targeting 999 of the 1000 most cited human genes in RefSeq db with at least one assay



* and other intercalating dye based assays

Workflow of the ProbeLibrary: From assay design to results within 2 days

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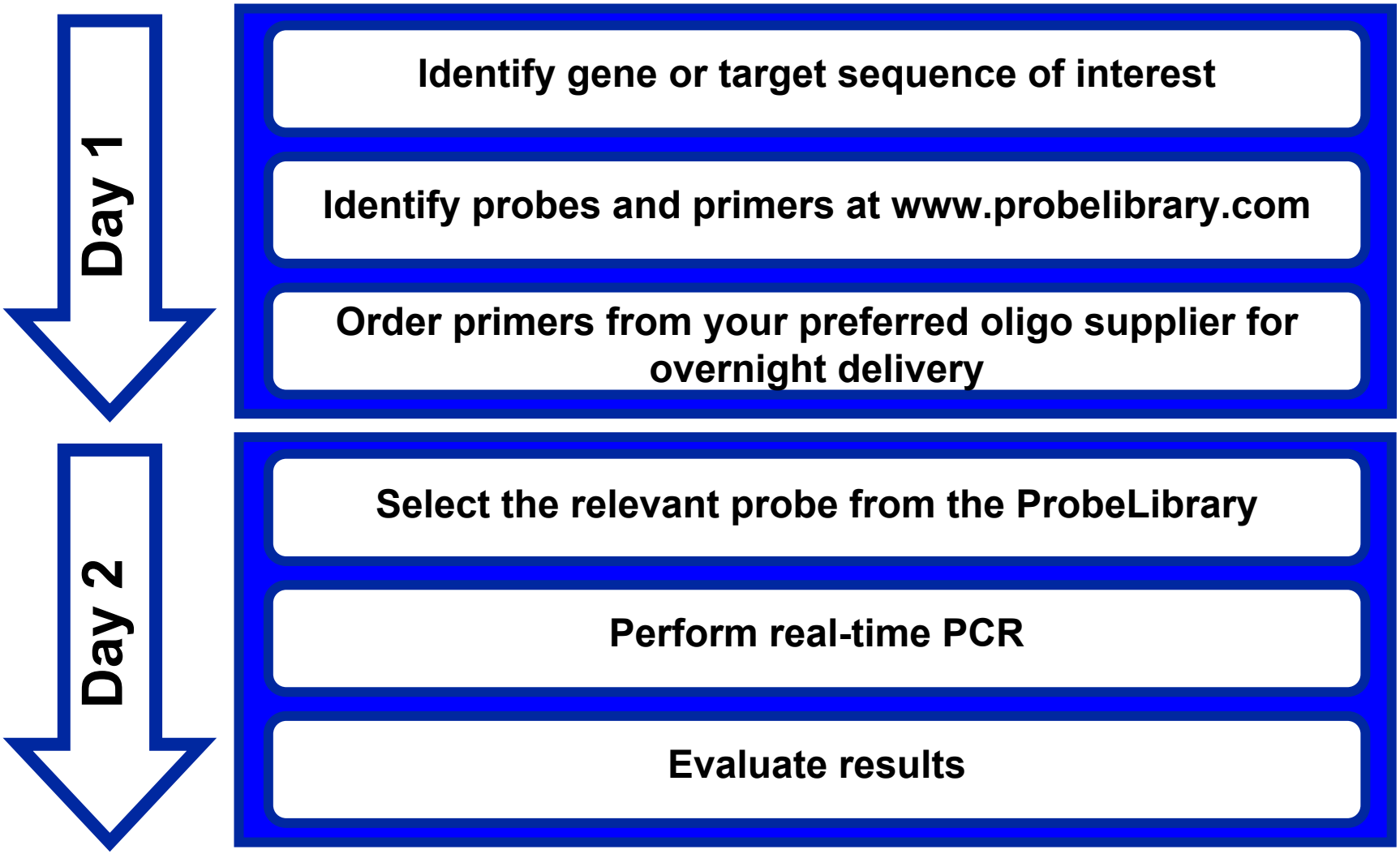

Assay design, step 1: Choose an organism

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Real-Time PCR Assay Design Center

The Assay Design Center allows you to fully benefit from the power of the ProbeLibrary , by quickly and easily designing your real-time PCR assays.

The core component of the Assay Design Center is the ProbeFinder software: a free-access, web-based tool that provides you with clear and detailed real-time PCR assay designs for your genes of choice.

- **Save Time**
  - Design your assays in seconds
- **Simplicity**
  - Just enter your target gene and you will get optimal design of your real-time PCR primers and a ProbeLibrary probe selection
- **Design Assays You Can Trust**
  - *In silico* PCR feature
  - Intron-spanning options
  - 96% assay design success rate

Start the Assay Design Software by choosing an organism:

Human

Mouse

Rat

Arabidopsis

Drosophila

C. elegans

Assay design, step 2:
Enter your sequence / gene of interest

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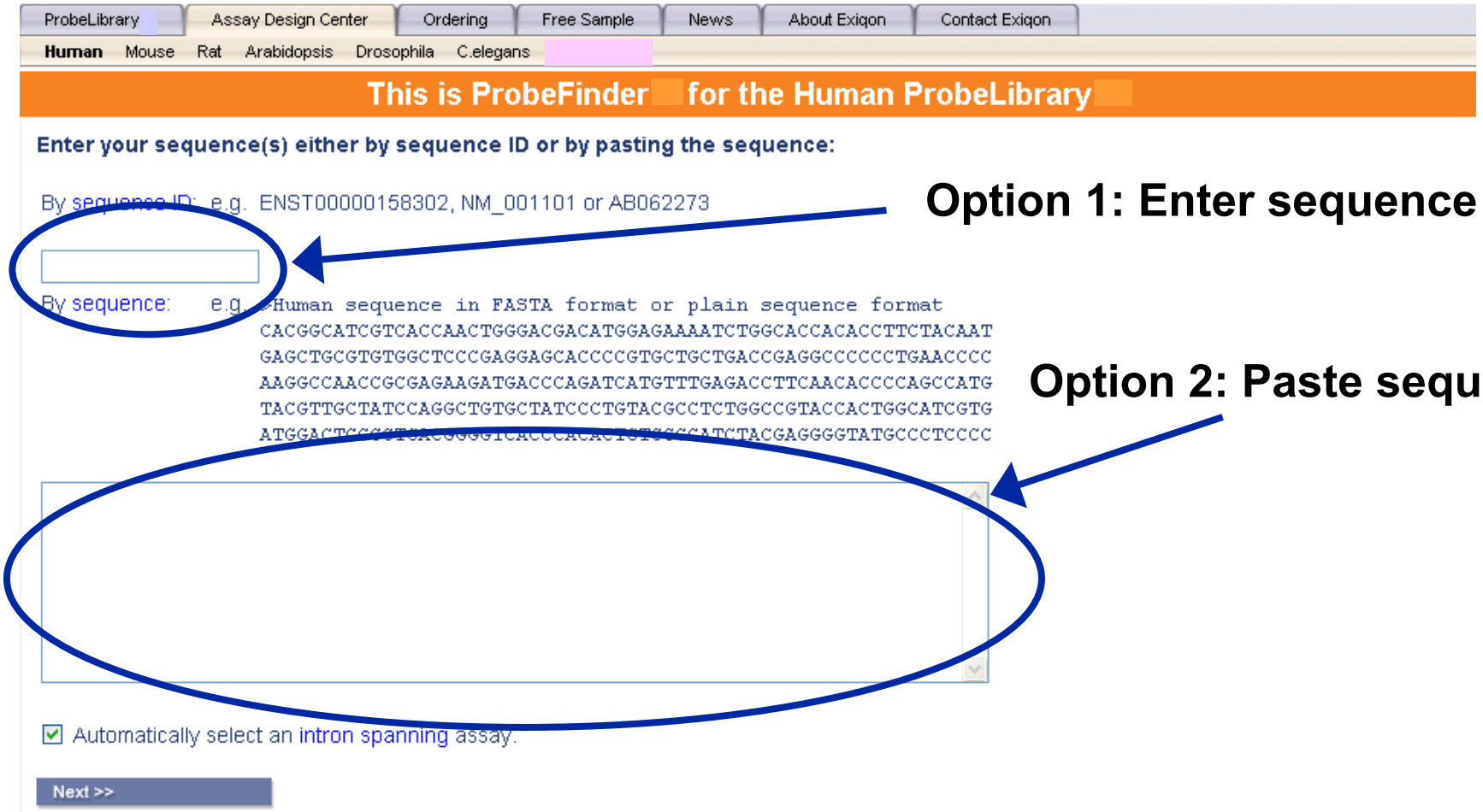

Option 1: Enter sequence ID

Option 2: Paste sequence

Assay design, step 3: Output information of optimal assay

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Human Mouse Rat Arabidopsis Drosophila C.elegans

This is ProbeFinder for the Human ProbeLibrary

ProbeFinder has designed the optimal real-time PCR assay for:

NM_001101.2 Homo sapiens actin, beta (ACTB), mRNA.

Assay details:

Use ProbeLibrary probe: **Human#64**

Primer	Length	Position	Tm	%GC	Sequence
Left Primer	18	414 - 431	60	56	ccaaccgcgagaagatga
Right Primer	20	491 - 510	59	60	ccagaggcgtacagggatag

Amplicon (97 nt)

ccaaccgcgagaagatgacccagatcatgtttgagaccttcaacaccccagccatgtacg
ttgctatccaggctgtgctatcccctgtacgcctctgg

Download report | Order Probes or Kit

Primer information (sequence, Tm, %GC)

Amplicon information

Assay design, step 4 (optional): Choose another assay from the list

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Human Mouse Rat Arabidopsis Drosophila C.elegans

Additional good real-time PCR assays designed for:

NM_001101.2 Homo sapiens actin, beta (ACTB), mRNA.

Transcript overview:

8 intron spanning assays

6 introns detected

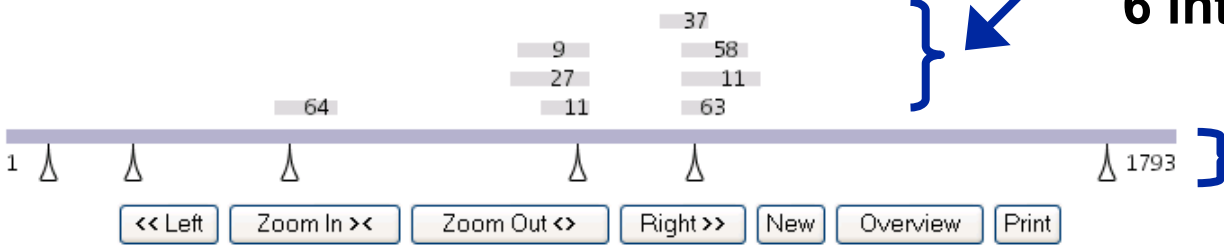

Figure legend ?

LibraryProbe	Rank	Position	Intron spanning	InSilicoPCR test	Score	Class
Human#64	1	481	+	+		Good
Human#11	2	856	+	+		Good
Human#27	3	796	+	+		Good
Human#09	4	814	+	+		Good
Human#63	5	1065	+	+		Good

Comparison HybProbe* vs ProbeLibrary assays

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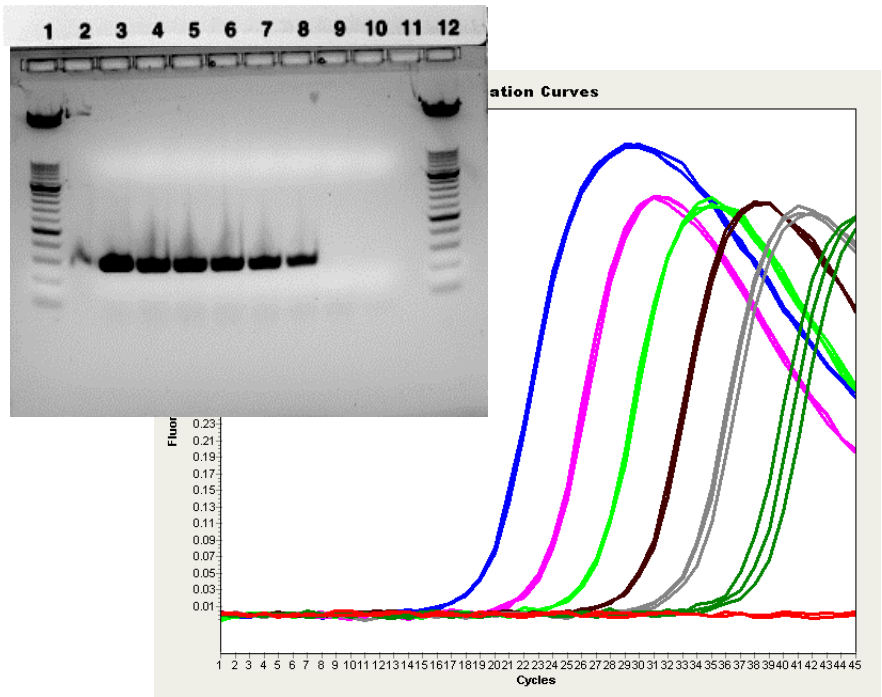
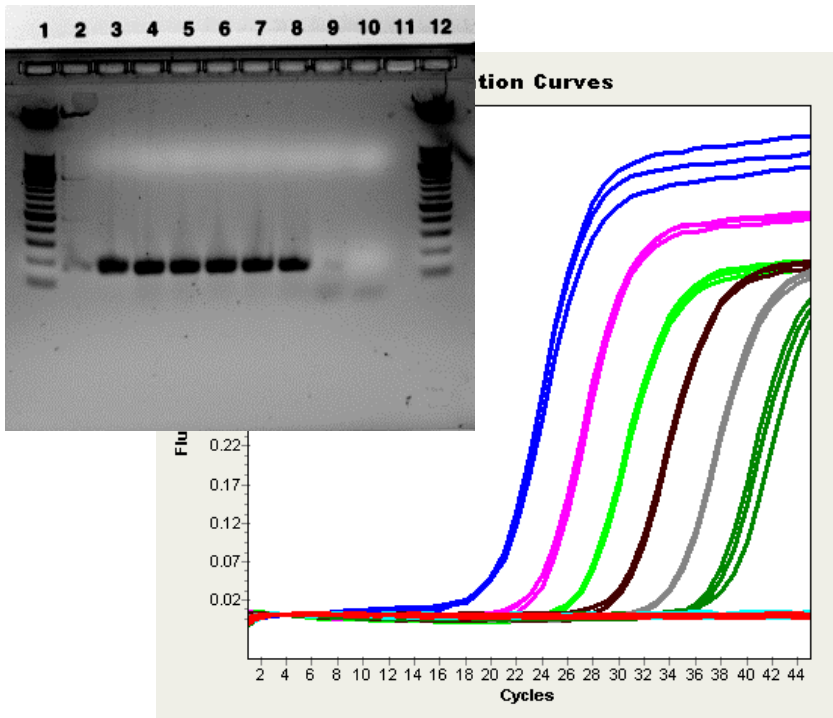
HybProbe* assay



ProbeLibrary assay



Target: ß2M; Template: In-vitro RNA-Transcript; Dilution series of 10e6 (blue line, slot3), 10e5 (pink line, slot4), ….,10e0 (red line, slot 9) copies of transcript, negativ control (NTC, turquoise), all samples in triplicates; Instrument: LightCycler 2.0; PCR Kit: LightCycler TaqMan Master

* HybProbe is a trademark of a Member of the Roche Group

Comparison of different detection formates due to linearity - 1

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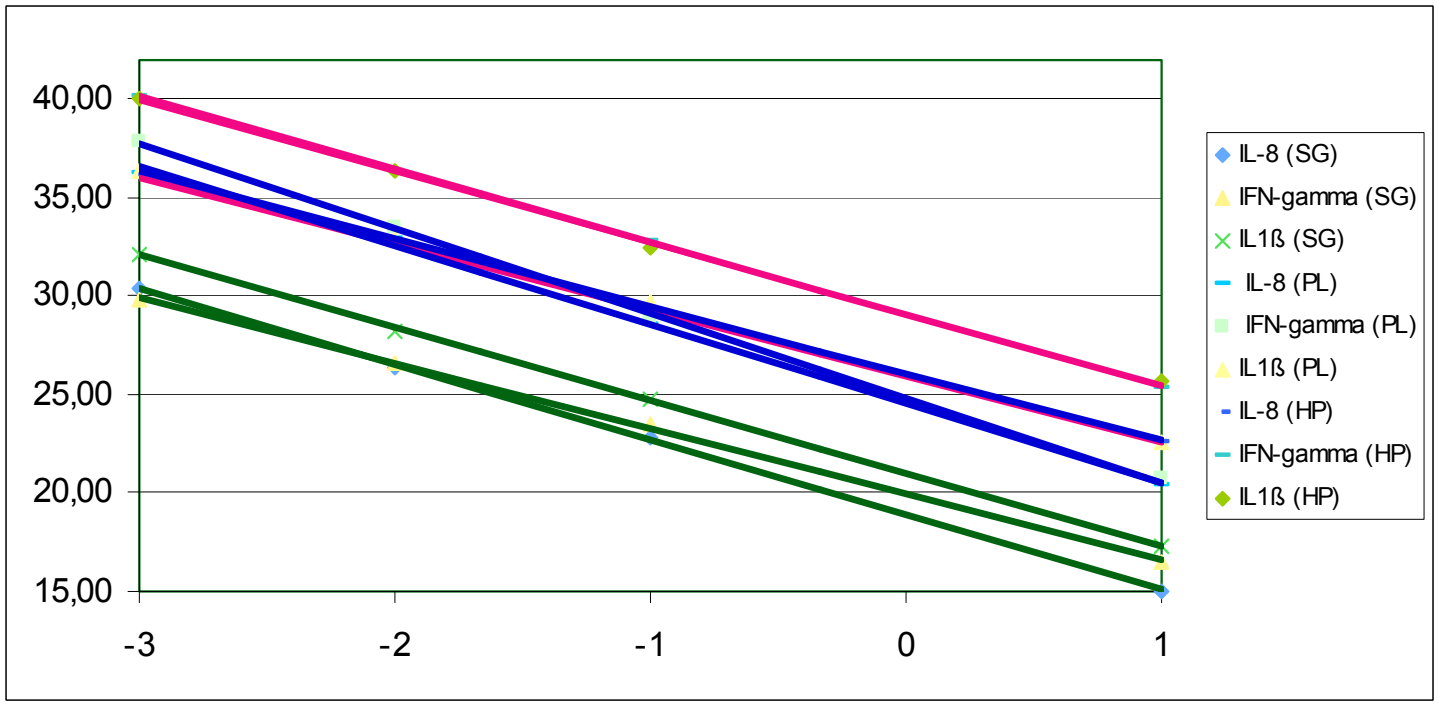
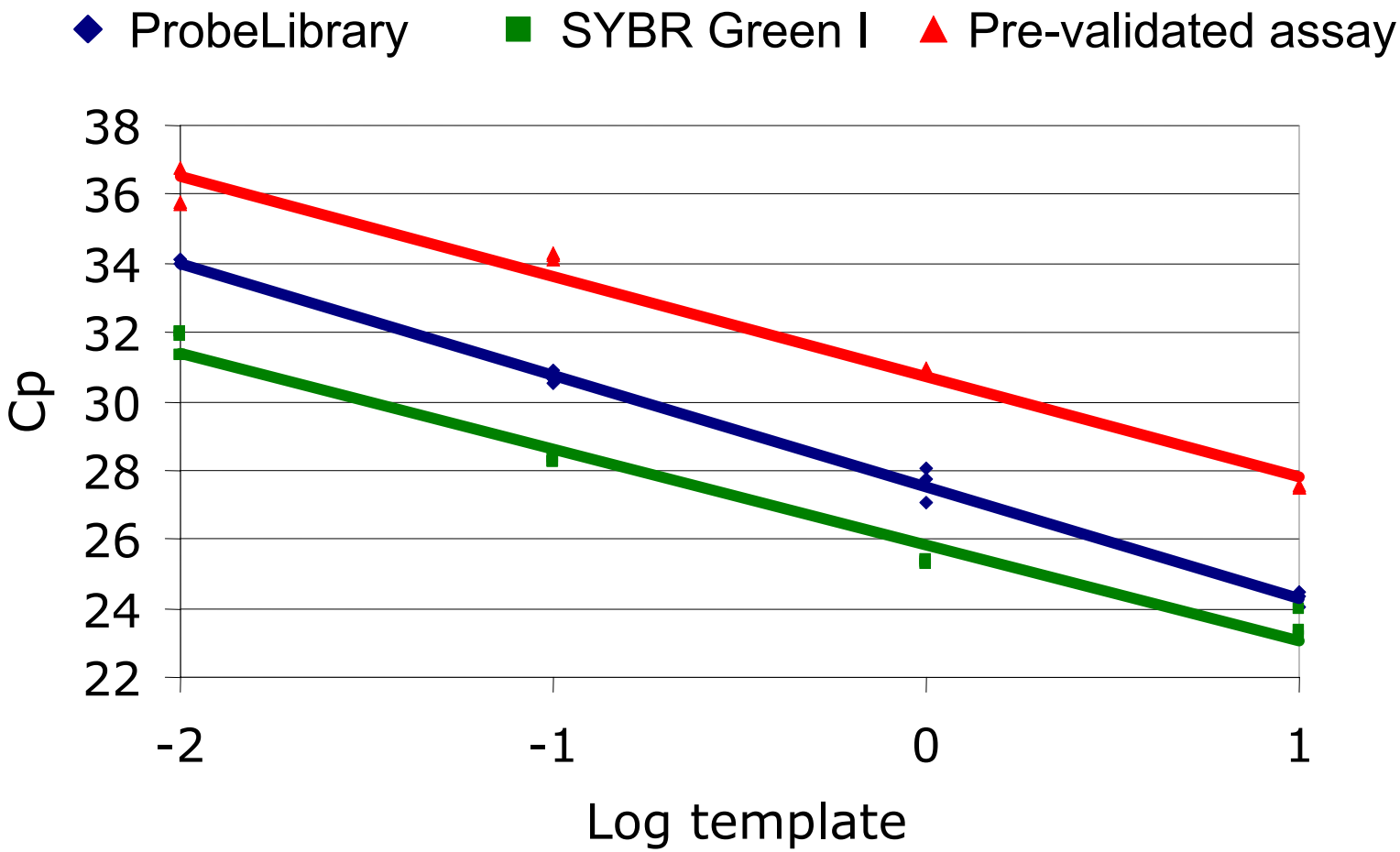
◆ ProbeLibrary ■ SYBR Green I ▲ HybProbe assay



3 assays (IL-8, IL1ß, IFN-gamma) for each detection formate

Comparison of different detection formates due to linearity - 2



Conclusion 1

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What is the benefit by using the ProbeLibrary:

- Custom real-time PCR assays
- Flexible and specific assays
- Fast and easy assay design by web-based ProbeFinder software
- Faster time to result (Design one day, run PCR the next day)
- Probes are compatible with any real-time PCR instrument
- Cost sensitive assays in the range of SYBR Green I assays

Conclusion 2

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Performance:

- Equal sensitivity of ProbeLibrary against SYBR Green I and other probe-based assays
- Advantage against SYBR Green I: No primer dimers or side products detectable due to detection with specific probes
- Advantage against probe based assays: Time to result within 2 days (instead of 1 week), less expensive than regular probe assays
- Reproducible and reliable results

Take home message

- You can quantify just about any human gene using only 90 pre-validated real-time PCR probes
- You can actually design assays one day and run them the next
- You can save money as costs for ProbeLibrary assays are similar to SYBR Green I assays

This is what we call:

One Transcriptome – One Kit

or just:

The ProbeLibrary