



From Whole Genome  
to Single Gene

# Expression Profiling of Candidate Genes:

Assays-on-Demand Gene Expression products based on Taqman<sup>®</sup>  
MGB Chemistry.

Falko Kräusche, Manohar Furtado &  
Roland Wicki, Applied Biosystems, R & D

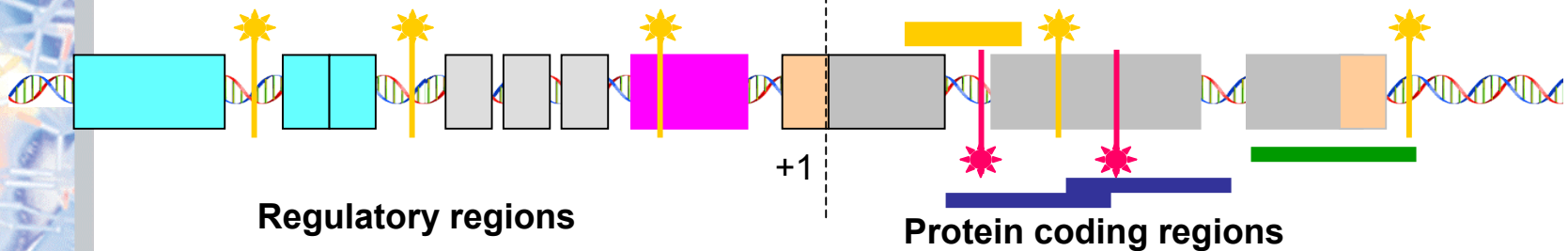
Session 8

# Applera Genome Initiative



**>150,000 SNP Assays**  
**Assays-on-Demand™**

**>40,000 Gene  
Expression Assays**  
**Assays-on-Demand™**



**>225,000 SNPs (75% novel)**

**> 65,000 Resequencing  
Assays**



# TaqMan® Assays-on-Demand™ Gene Expression Products

- Selection:
  - Off-the-Shelf Assays for Human, Mouse, and Rat Genes
    - >21,000 human assays
    - > 14,000 mouse assays
    - > 4,000 rat assays
- Convenience:
  - Single Tube Formulation (20X)
    - 1 TaqMan® Probe (250nM) + 2 Unlabeled Primers (900nM)
    - Universal Thermal Cycling Conditions
- Quality:
  - Analytical QC tested



# TaqMan® Assays-on-Demand™ Gene Expression Products

- Comprehensive collection of pre-designed, gene-specific primer and probe sets for quantitative gene expression studies of human, mouse, or rat genes.
- Built on 5' nuclease chemistry
- Each assay consists of 2 unlabeled PCR primers and 1 FAM™ dye-labeled TaqMan® probe formulated into a single tube (20X)
- Easy to Use: just add TaqMan® Universal PCR Master Mix and sample cDNA, and run on any Applied Biosystems Real Time PCR instrument



# Quantitative Gene Expression

- TaqMan® probe-based chemistry  
(“5’ nuclease chemistry”)
  - Large Dynamic Range (>5 orders of magnitude)
    - > Ability to detect both high expressers and low expressers in a single experiment
  - Sensitivity (detects 1 copy in 100 cells)
    - > Low sample requirement
    - > Most sensitive gene expression chemistry available
  - Closed tube reaction (no post-PCR processing)
    - > Minimizes contamination opportunities
  - Robust chemistry (not technique-dependent)
    - > Low coefficient of variation (CV)



# Complementary Gene Expression Technology

## Celera Discovery System

Identify Assays linked to results

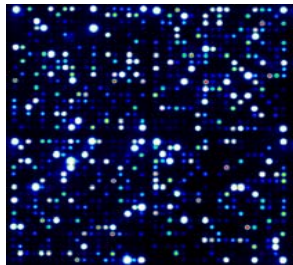
Assay ID	Assay Name	Gene Name	Assay Type	Assay Status	Assay Location	Assay Date	Assay Results
AB000001	AB000001	AB000001	AB000001	AB000001	AB000001	AB000001	AB000001
AB000002	AB000002	AB000002	AB000002	AB000002	AB000002	AB000002	AB000002
AB000003	AB000003	AB000003	AB000003	AB000003	AB000003	AB000003	AB000003
AB000004	AB000004	AB000004	AB000004	AB000004	AB000004	AB000004	AB000004
AB000005	AB000005	AB000005	AB000005	AB000005	AB000005	AB000005	AB000005
AB000006	AB000006	AB000006	AB000006	AB000006	AB000006	AB000006	AB000006
AB000007	AB000007	AB000007	AB000007	AB000007	AB000007	AB000007	AB000007
AB000008	AB000008	AB000008	AB000008	AB000008	AB000008	AB000008	AB000008
AB000009	AB000009	AB000009	AB000009	AB000009	AB000009	AB000009	AB000009
AB000010	AB000010	AB000010	AB000010	AB000010	AB000010	AB000010	AB000010

Sequence Detection System provides highly accurate, quantitative gene expression analysis

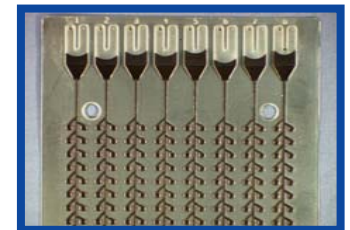


Order specific, pre-validated and ready-to-use TaqMan Assays...

....Or configure a custom Micro Card

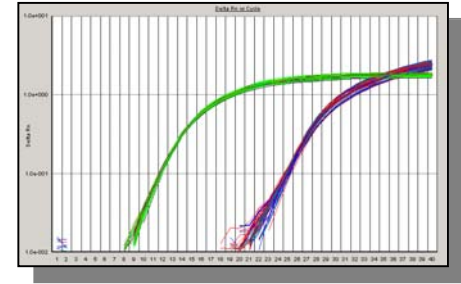
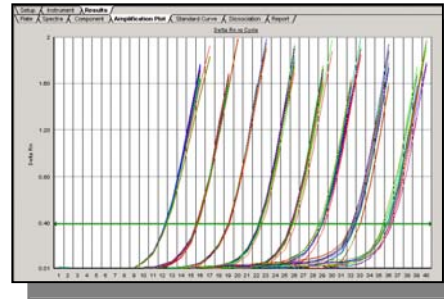


Broad scan using 1700 microarray

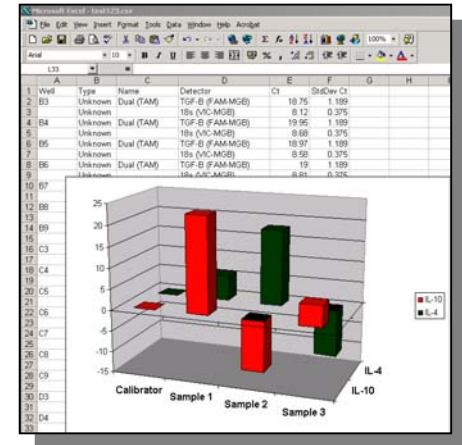
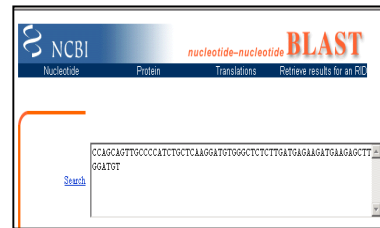
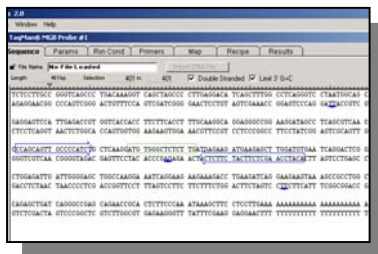
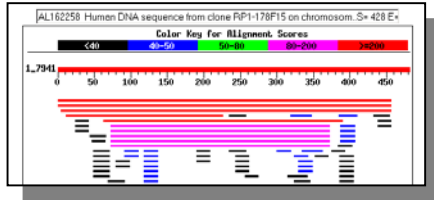
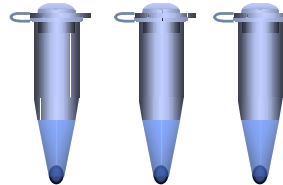


# Gene Expression - 2002

## Real-time PCR and its bottlenecks



**nM = ? pmol = ? μl**





# Gene Expression – 2003 & beyond

## Bottlenecks of PCR removed

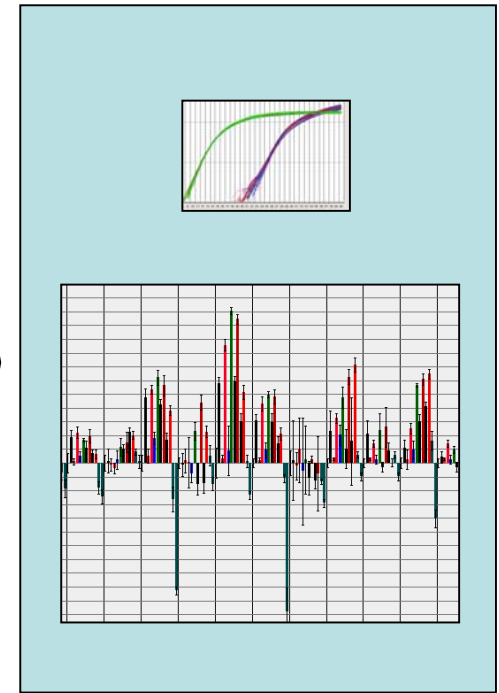
myScience  
research environment

Batch ID  
Classification  
Keyword  
Location

[www.appliedbiosystems.com](http://www.appliedbiosystems.com)

Microfluidic card with a vial and a multi-well plate.

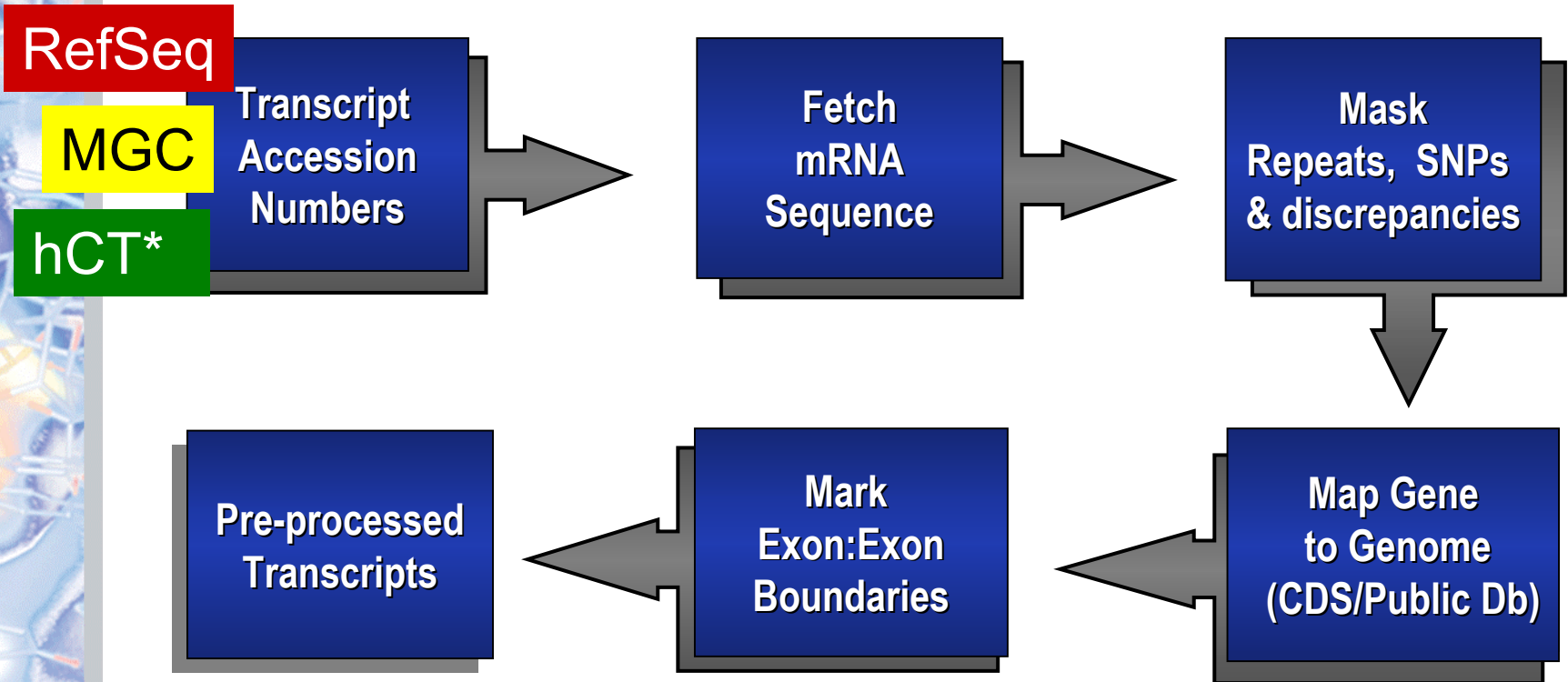
Assays-on-Demand™ Products  
Assays-by-Design<sup>SM</sup> Service  
Micro Fluidic Cards



ABI PRISM® 7900HT  
ABI PRISM® 7000



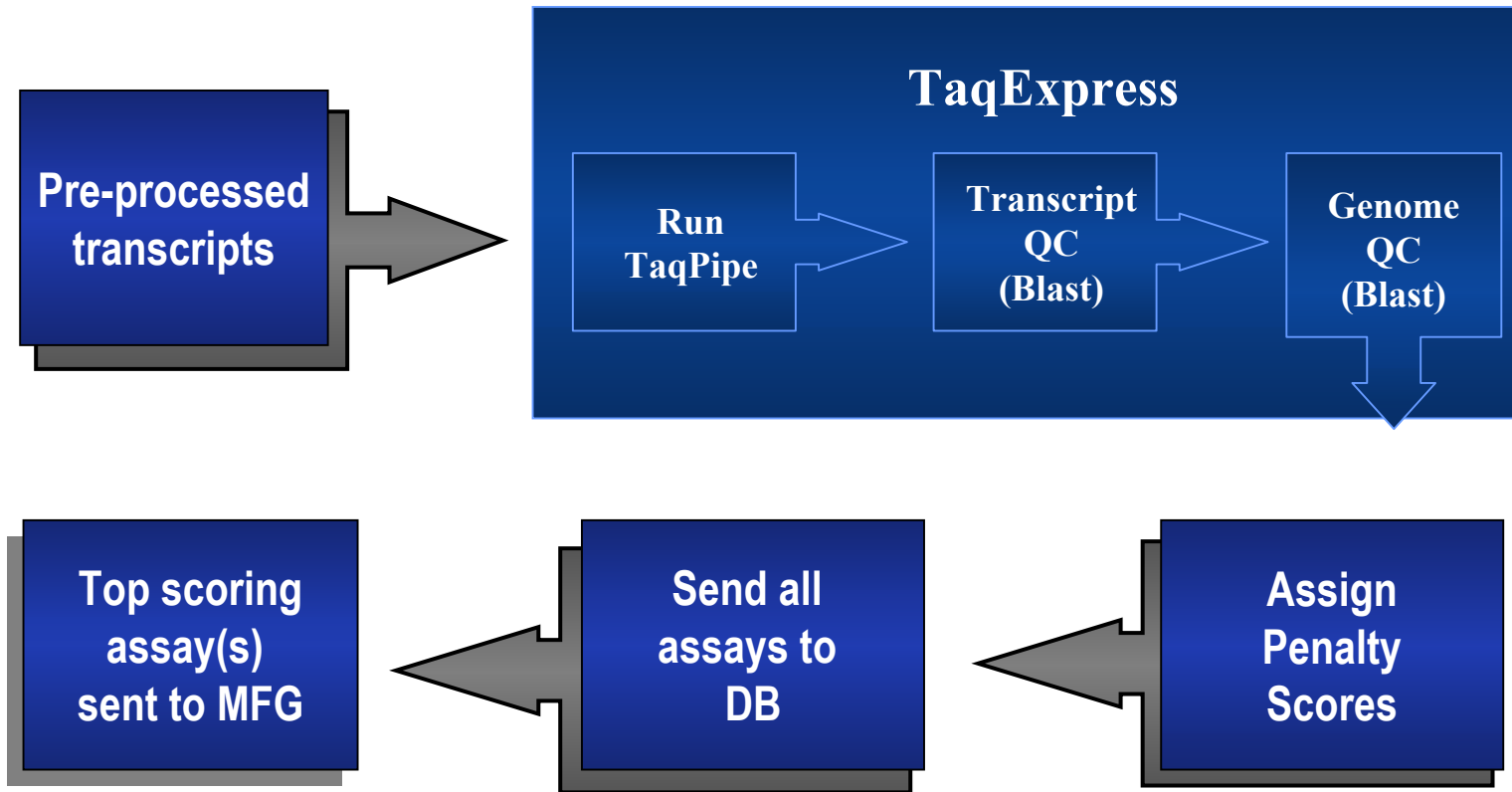
# TaqMan® Assays-on-Demand™ Gene Expression Products: Development Pipeline



\* hCT = human Celera Transcripts



# TaqMan® Assays-on-Demand™ Gene Expression Products: development pipeline





# Failure Analyses: Retrain

- Designed oligonucleotides that fail manufacturing QC
  - > Mass Spec
  - > quantitation
  - > genomic DNA amplification
- Both SNP and gene expression failed assays have shown the following:
  - > Some di-nucleotide sequences in probe create higher probability of probe failure
  - > Nearest-neighbor algorithms differ in the ability to precisely predict  $T_m$
  - > Choice of fluorescent reporter dye can affect  $T_m$  calculations



# Select Best Assay per Gene?

## 67 Exons = 66 Assays designed = 1 Chosen

**Top Scoring Assay is Chosen**

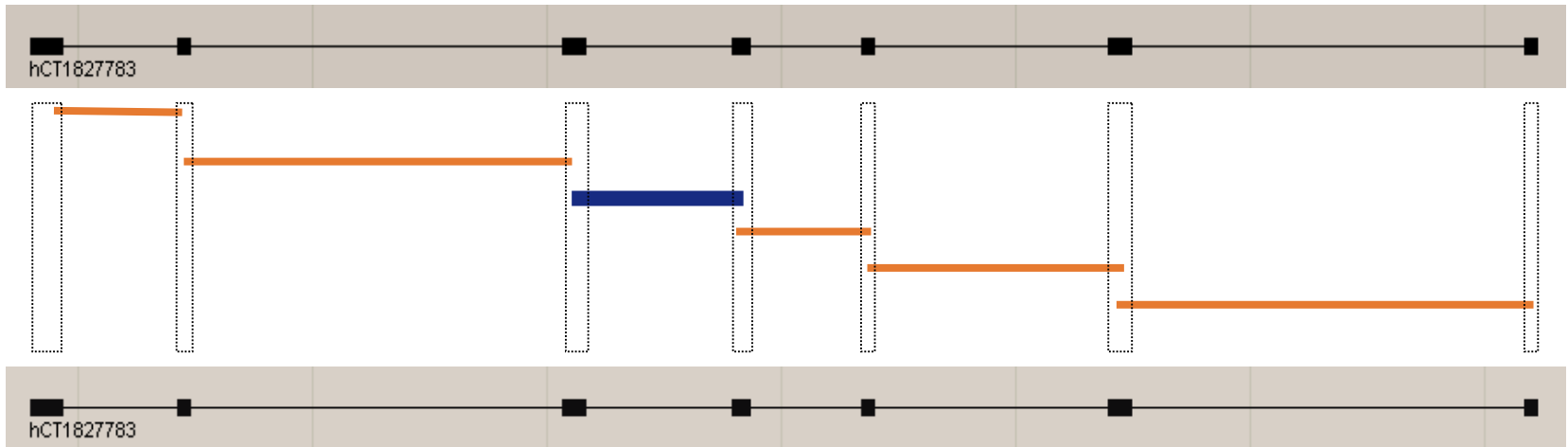
SeqAC	AssayID	Target Exon	F.Primer Position	Amplicon Length	Score	Status	Project	Sequence Description	PSet Score	Intron Penalty	Refseq Penalty	Genomic Penalty
<input type="checkbox"/>	153600	37	5549	63	3.84	Ordered	R&D		3.936	0.1	0.0	0.0
<input type="checkbox"/>	153601	38	5688	103	3.71	Ordered	R&D		3.792	0.08	0.0	0.0
<input type="checkbox"/>	153602	39	5898	105	4.0	Ordered	R&D		3.995	0.0	0.0	0.0
<input type="checkbox"/>	153604	40	6111	67	3.78	Ordered	R&D		3.78	0.0	0.0	0.0
<input type="checkbox"/>	153605	41	6267	75	3.91	Ordered	R&D		3.91	0.0	0.0	0.0
<input type="checkbox"/>	153606	42	6450	120	1.44	Ordered	R&D		3.931	0.89	0.0	1.6
<input type="checkbox"/>	153607	43	6623	92	4.0	Passed QC	Athena R&D		4.0	0.0	0.0	0.0
<input type="checkbox"/>	153608	44	6808	73	3.87	Ordered	R&D		3.95	0.08	0.0	0.0
<input type="checkbox"/>	153609	45	7000	61	2.39	Ordered	R&D		3.985	0.0	0.0	1.6
<input type="checkbox"/>	153610	46	7208	77	3.85	Ordered	R&D		3.995	0.14	0.0	0.0
<input type="checkbox"/>	153611	47	7342	71	2.69	Ordered	R&D		3.99	0.1	0.0	1.2
<input type="checkbox"/>	153612	48	7534	129	2.84	Ordered	R&D		3.639	0.0	0.0	0.8
<input type="checkbox"/>	153613	49	7694	98	3.52	Ordered	R&D		4.0	0.08	0.0	0.4
<input type="checkbox"/>	153615	50	7906	73	4.0	Ordered	R&D		4.0	0.0	0.0	0.0
<input type="checkbox"/>	153616	51	8053	75	3.95	Ordered	R&D		3.95	0.0	0.0	0.0
<input type="checkbox"/>	153617	52	8179	84	3.73	Ordered	R&D		3.9	0.17	0.0	0.0
<input type="checkbox"/>	153618	53	8404	129	3.89	Ordered	R&D		3.99	0.1	0.0	0.0
<input type="checkbox"/>	153619	54	8592	65	3.99	Ordered	R&D		3.99	0.0	0.0	0.0
<input type="checkbox"/>	153620	55	8745	69	3.94	Ordered	R&D		3.995	0.06	0.0	0.0
<input type="checkbox"/>	153621	56	8902	74	3.94	Ordered	R&D		3.936	0.0	0.0	0.0
<input type="checkbox"/>	153622	57	9082	97	4.0	Ordered	R&D		3.995	0.0	0.0	0.0
<input type="checkbox"/>	153623	58	9231	68	3.95	Ordered	R&D		3.945	0.0	0.0	0.0
<input type="checkbox"/>	153624	59	9443	73	3.99	Ordered	R&D		3.99	0.0	0.0	0.0

Example: Cubilin mRNA (NM\_001081)



# “Virtual Assays” – coming soon

Design Pipeline covers all viable exon/exon junctions





# TaqMan® Assays-on-Demand™ Products

## Label and 2D Barcode on Individual Tube



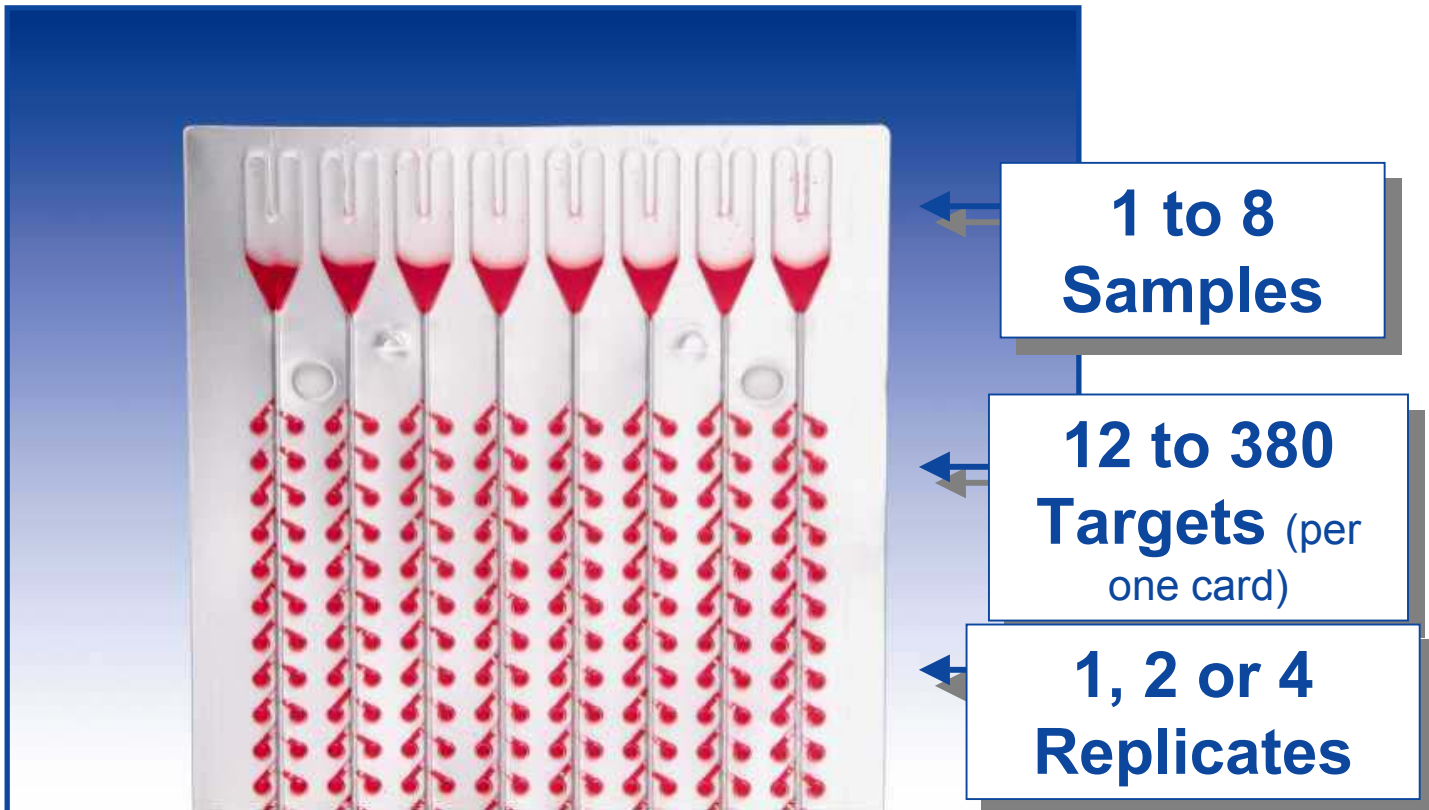
- Assay ID
- Volume (250µl)
- Formulation (20X)
  
- 1D barcoded Racks
- 2D barcoded Tubes
  
- CD-ROM containing annotation for each assay



# TaqMan® Low Density Array

## Micro Fluidic Channels

TaqMan® Assays-on-Demand Gene Expression Products also available in small volume format



**384 spots**



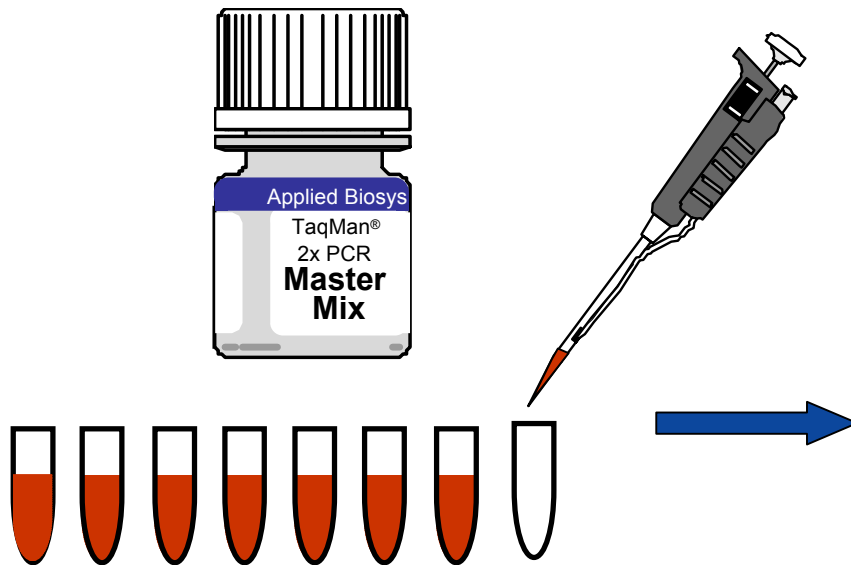
# TaqMan® Low Density Array Benefits

- Minimal pipetting steps
  - Streamline reaction set-up
  - Avoid expense of liquid handling robotics
- Highest accuracy even on 384-well plates
- Dramatic reduction of reagent consumption
- Standardized format
  - Direct comparison of results between labs
- Fixed Menu and Custom Cards
  - Pathway Research, Disease-specific Panels etc.



# Loading a TaqMan® Low Density Array

- 2 $\mu$ l reaction volume
- 384well format
- 1-8 samples
- easy loading





# TaqMan® Low Density Array Centrifuge for 2 minutes



Heraeus Multifuge 3S Centrifuge



Custom Heraeus Centrifuge Buckets  
(holds up to 3 Micro Fluidic Cards)



# How to Search for your Genes?

[www.myscience.appliedbiosystems.com](http://www.myscience.appliedbiosystems.com)

- Online assay catalog
- Graphical viewers of the Genome
- Free Access



[www.allgenes.com](http://www.allgenes.com)

- Product Information
- References

**It's 2004, why are you still designing your own primers and probes?**

**Gene Expression assays for human, mouse, and rat genes**

The Applied Biosystems Assays-on-Demand™ Gene Expression products are a comprehensive collection of pre-designed primer and probe sets for quantitative real-time PCR gene expression studies. Each assay is designed using Applied Biosystems sophisticated bioinformatics design pipeline, leveraging both public and private genome databases for increased specificity and optimal assay design.

**Assays-on-Demand™ Gene Expression Product Pipeline**

```

    graph TD
      A[Database of Transcripts (RefSeq)] --> B[Map transcript to Genome, Mark Exon Boundaries]
      B --> C[Primer & Probe Design]
      C --> D[Computational QC & Quality Scoring]
      D --> E[Formulation & Analytical QC]
      E --> F[Primer & Probe Synthesis]
      F --> G[Select Highest Scoring Assay]
      G --> H[Genomic Assays Catalog]
      H --> I[Browse Online Catalog Now]
  
```

**Assays Available**

Human	21 998	<input type="checkbox"/>
Mouse	15 716	<input type="checkbox"/>
Rat	4 040	<input type="checkbox"/>

**Product Introduction**  
[Product Page](#)

**Product Literature**

**Publications**

- [5' Nuclease Assays for Validating Hits from Fluorescent Microarrays July 2003: Application Note](#)
- [Using Real-Time RT-PCR to Measure Gene Silencing by RNAi](#)
- [Assays-on-Demand Gene Expression product bulletin](#)
- [Mouse Assays-on-Demand Gene Expression product bulletin](#)
- [Rat Assays-on-Demand™ Gene Expression Products: Product Bulletin](#)

"Delivering the Genome: Off-the-Shelf Assays for Candidate Gene Expression Applications" scientific poster

- [ABI PRISM® 7000 Sequence Detection System](#)
- [ABI PRISM® 7900HT Sequence Detection System](#)

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STEFAN SCHREIBER  
Christian-Abrechts University, Kiel, Germany

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GENOMIC ASSAYS | SNP Genotyping

**4000 Q TRAP<sup>™</sup>**  
LC/MS/MS SYSTEM

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[New Mouse Gene Expression Assays](#)



# myScience: home of genome-based tools

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research environment

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genomic products

SELECT REAGENTS FOR EXPERIMENTS

Genomic Products

Gene Expression

- > Search Assays-on-Demand™ Gene Expression Products
- > Create Micro Fluidic Cards

Genotyping

- > Search Assays-on-Demand™ SNP Genotyping Products

biobeat

ONLINE MAGAZINE

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gene search

SEARCH LOCUSLINK GENES AND VIEW GENOMIC REAGENTS

Keyword Batch ID Location Classification

Keyword Query

search by: All Text

enter terms:   use wild

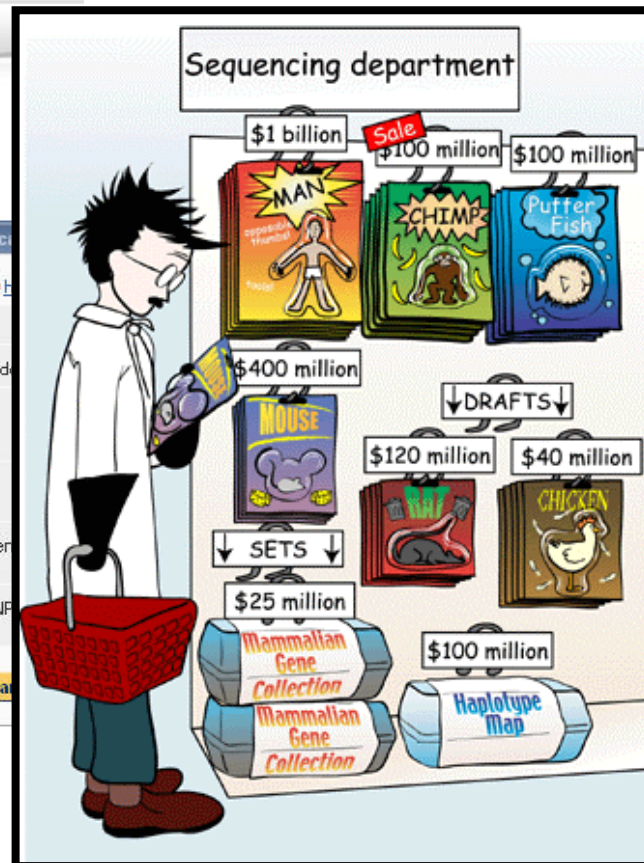
choose species:  H. sapiens  M. musculus

R. norvegicus

choose set membership:  All Genes (default)

Genes with Assays-on-Demand™ Gene Expression Assays

Genes with Assays-on-Demand™ SNP Genotyping Assays



Keyword

**Batch ID**

Location

Classification

## Batch ID query

[help](#)

**reset**

enter ID(s):

separate IDs with a space - [supported IDs](#)

upload IDs:

Browse...

- [file format](#)

select file type :

- ID list
- Previously exported Gene search results

choose species:

- H. sapiens       M. musculus
- R. norvegicus

**search**

Keyword

Batch ID

Position

Classification

## Browse for Genes

 [help](#)

species:  H. sapiens  M. musculus  R. norvegicus

(changing species here will refresh the page)

**update species**

 [Classification](#) Molecular Functions

**Category Name  
(# of assigned subcategories)**

**# of public genes**

 [Receptor](#) (7)

[7109](#)

A molecular structure within a cell or on the cell surface characterized by selective binding of a specific substance and a specific physiologic effect that accompanies the binding.

 [Signaling molecule](#) (6)

[2026](#)

A molecule that transduces a signal between cells.

 [Kinase](#) (5)

[2027](#)

An enzyme that catalyzes the transfer of a phosphate from ATP to a second substrate.

Keyword	Batch ID	Position	Classification
---------	----------	----------	----------------




## Browse for Genes [help](#)

species:  H. sapiens  M. musculus  R. norvegicus  
 (changing species here will refresh the page)

**update species**

 [Classification](#) : Biological Processes

Category Name (# of assigned subcategories)	# of public genes
--	-------------------

 <a href="#">Cell cycle</a> (4) A series of events between mitotic divisions. The cycle is conventionally divided into G0, G1, (G standing for gap), S (Synthesis phase during which the DNA is replicated), G2 and M (Mitosis).	<a href="#">1642</a>
 <a href="#">Blood circulation and gas exchange</a> (2) The process and regulation of circulating blood through the arteries and veins, delivering oxygen to the tissues and removing carbon dioxide.	<a href="#">159</a>
 <a href="#">Apoptosis</a> (4) An active process of cell death which requires metabolic activity by the dying cell. It is often characterized by cleavage of the DNA into fragments that give a so-called laddering pattern on gels. It is also called programmed cell death. It should not be confused with senescence.	<a href="#">999</a>


# gene search

SEARCH LOCUSLINK GENES AND VIEW GENOMIC REAGENTS

- Keyword
- Batch ID
- Location
- Classification

## Keyword Query

 [help](#)

search by:  

enter terms:   use wildcard

choose species:  H. sapiens  M. musculus  
 R. norvegicus

choose set membership:

- All Genes (default)
- Genes with Assays-on-Demand™ Gene Expression Assays
- Genes with Assays-on-Demand™ SNP Genotyping Assays

**search**

Assays-on-Demand  
SNP Genotyping

Celera Gene ID

Panther Classification:  
Function and Process

Assays-on-Demand  
Gene Expression

results list

LOCUSLINK

help

Display: 30 [Update] Hits 1-27 of 27 [ page: (1) ]  
[Export Results](#) [Send To Workspace](#) [Gene Expression Assays](#) [SNP Genotyping Assays](#)

<input type="checkbox"/>	<a href="#">Locus Link Id</a>	<a href="#">Species</a>	<a href="#">Locus Link Gene Name</a>	<a href="#">Locus Link Gene Symbol</a>	<a href="#">Panther Molecular Function</a>	<a href="#">Panther Biological Process</a>	<a href="#">Public Location</a>	<a href="#">Celera Gene ID</a>	<a href="#">Gene Expression Assays</a>	<a href="#">SNP Genotyping Assays</a>	<a href="#">RefSeq/Genbank mRNA</a>
<input type="checkbox"/>	<a href="#">200539</a>	Homo sapiens	diabetes related ankyrin repeat protein (Interim)	<a href="#">DARP</a>	<a href="#">Molecular function unclassified</a>	<a href="#">Biological process unclassified</a>	chr. 2 96,971,578 - 96,991,598	-	<a href="#">Hs00329135_m1</a>	-	<a href="#">5 transcripts</a>
<input type="checkbox"/>	<a href="#">2820</a>	Homo sapiens	glycerol-3-phosphate dehydrogenase 2 (mitochondrial)	<a href="#">GPD2</a>	<a href="#">2 more...</a>	<a href="#">2 more...</a>	chr. 2 157,255,990 - 157,403,797	<a href="#">hCG40096</a>	<a href="#">Hs00264937_m1</a>	<a href="#">15 assays</a>	<a href="#">6 transcripts</a>
<input type="checkbox"/>	<a href="#">4760</a>	Homo sapiens	neurogenic differentiation 1	<a href="#">NEUROD1</a>	<a href="#">2 more...</a>	<a href="#">6 more...</a>	chr. 2 182,505,218 - 182,509,393	<a href="#">hCG1776881</a>	<a href="#">Hs00159598_m1</a>	<a href="#">2 assays</a>	<a href="#">4 transcripts</a>
<input type="checkbox"/>	<a href="#">3667</a>	Homo sapiens	insulin receptor substrate 1	<a href="#">IRS1</a>	<a href="#">2 more...</a>	<a href="#">6 more...</a>	chr. 2 227,563,957 - 227,628,494	<a href="#">hCG18361</a>	<a href="#">Hs00178563_m1</a>	<a href="#">7 assays</a>	<a href="#">2 transcripts</a>
<input type="checkbox"/>	<a href="#">11132</a>	Homo sapiens	calpain 10	<a href="#">CAPN10</a>	<a href="#">32 more...</a>	<a href="#">16 more...</a>	chr. 2 241,545,962 - 241,576,932	-	<a href="#">3 assays</a>	<a href="#">6 assays</a>	<a href="#">21 transcripts</a>
<input type="checkbox"/>	<a href="#">5468</a>	Homo sapiens	peroxisome proliferative activated receptor, gamma	<a href="#">PPARG</a>	<a href="#">12 more...</a>	<a href="#">36 more...</a>	chr. 3 12,321,215 - 12,467,696	-	<a href="#">Hs00234592_m1</a>	<a href="#">20 assays</a>	<a href="#">11 transcripts</a>
<input type="checkbox"/>	<a href="#">6514</a>	Homo sapiens	solute carrier family 2 (facilitated glucose transporter), member 2	<a href="#">SLC2A2</a>	<a href="#">2 more...</a>	<a href="#">3 more...</a>	chr. 3 172,116,164 - 172,146,795	<a href="#">hCG14921</a>	<a href="#">Hs00165775_m1</a>	<a href="#">7 assays</a>	<a href="#">2 transcripts</a>
<input type="checkbox"/>	<a href="#">6690</a>	Homo	serine protease	<a href="#">SPNK1</a>	<a href="#">3 more...</a>	<a href="#">2 more...</a>	chr. 5	<a href="#">hCG1979926</a>	<a href="#">Hs00162154_m1</a>	<a href="#">6 assays</a>	<a href="#">4 transcripts</a>

LocusLink ID  
and Gene Symbol

Public Location

Public RefSeq and  
GenBank Transcripts

# Calpain 10

## Three Assays

<a href="#"><u>Hs00225048</u></a> <a href="#"><u>m1</u></a>	<a href="#"><u>7 RefSeqs</u></a> <a href="#"><u>11 GenBank mRNAs</u></a>	calpain 10
<a href="#"><u>Hs00256661</u></a> <a href="#"><u>m1</u></a>	<a href="#"><u>NM_023087</u></a> <a href="#"><u>AF089093</u></a>	calpain 10
<a href="#"><u>Hs00225050</u></a> <a href="#"><u>m1</u></a>	<a href="#"><u>NM_023089</u></a> <a href="#"><u>AF089095</u></a>	calpain 10

Applied Biosystems myScience research environment

Right-click on an assay or gene to open assay list or gene list [help](#)

Human - chr. 2 NCBI Build 33 assembly 241,544,058 241,578,739

Human - chr. 2  
241,545K 241,550K 241,555K 241,560K 241,565K 241,570K 241,575K

Assays-on-Demand™ Gene Expression: NCBI Assembly Human  
Hs00225050\_m1

Assays-on-Demand™ SNP Genotyping: NCBI Assembly Human  
Hs00225048\_m1, CAPNTU

Locus Link Genes Human  
11132

NCBI RefSeq Human

- NM\_023084
- NM\_023087
- NM\_023088
- NM\_023086
- NM\_023085
- NM\_023089
- NM\_023083
- NM\_021251

**Selected GEx Assay #2**

**Transcripts targeted by selected Assay**



# TaqMan® Endogenous Controls

- Human, Mouse, Rat
  - Available Formats
    - > FAM / MGB Controls
    - > VIC / MGB Controls
    - > VIC / TAMRA Controls
  - Available Scales
    - > VIC – labeled assays: 2500 rxns.
    - > FAM – labeled assays: 125 rxns and 500 rxns



# TaqMan® Assays-by-Design<sup>SM</sup> Service

Custom made ready-to-use assays

- Any gene
- Any organism
- Any transcript
- Directed probe placement
  - a) Specific exon-exon junction
  - b) Transcript - specific vs. gene - specific
  - c) Positional bias ability (5' vs. 3')
  - d) Design determined optimal position



# TaqMan® Assays-by-Design<sup>SM</sup> Service

Free Software to submit sequences





# TaqMan® Assays-by-Design<sup>SM</sup> Service

## Enter Sequences and Order

Enter Sequence

Target Sequence  
CTCTTCCTAAGCATATGCCGTATACTGCGATCATCATTATA  
GCGCGCGCGCGCGTGCATACGATCGTGTGAAATATCTATAGC  
ACAGCGGCGCATGCATATATTTATATACGATCGATGCA

Check for Errors  
in sequence

Validate

Target Coordinates

23=EX4 65=EX5

TaqMan® MGB  
probe location  
e.g. Ex/Ex junctions

Upload sequence(s)  
and order assay(s)

File Upload

Error Message Log:  
9014 : Target site error. For SNP Genotyping, annotated SNP sites must be at the end of the target sequence. For Gene Expression, the target site position must be at least 30 bases from the 5' and 3' ends of the target sequence.

File :

> Roland Wicki 0041417997735 4331348 wickim@eur.appliedbiosystems.com



# Custom TaqMan® Probes

- Known primer/probe sequence design
- Large scale synthesis
- Flexible component optimization
  - Individual tubes
- Multiple dyes available
  - FAM, VIC, TET
- Multiple Quenchers
  - MGB
  - TAMRA



# Summary

- TaqMan® Assays-on-Demand™ Gene Expression Products: **over 40,000 available**
  - Pre-designed, gene-specific, off-the-shelf assays
  - Available in single tube format, or dried down on TaqMan® Low Density Array
  - TaqMan® Endogenous Controls – available in large scale and multiple dyes
- TaqMan® Assays-by-Design<sup>SM</sup> Service
  - User submitted target sequence
  - Single tube format
  - Free File Builder Software on the web
- Custom TaqMan® Probes
  - User defined sequences
  - Primers and probes in separate tubes