



TaqMan[®] Assays

...not just Primers and Probes

Dr. Uta Möllers
Support

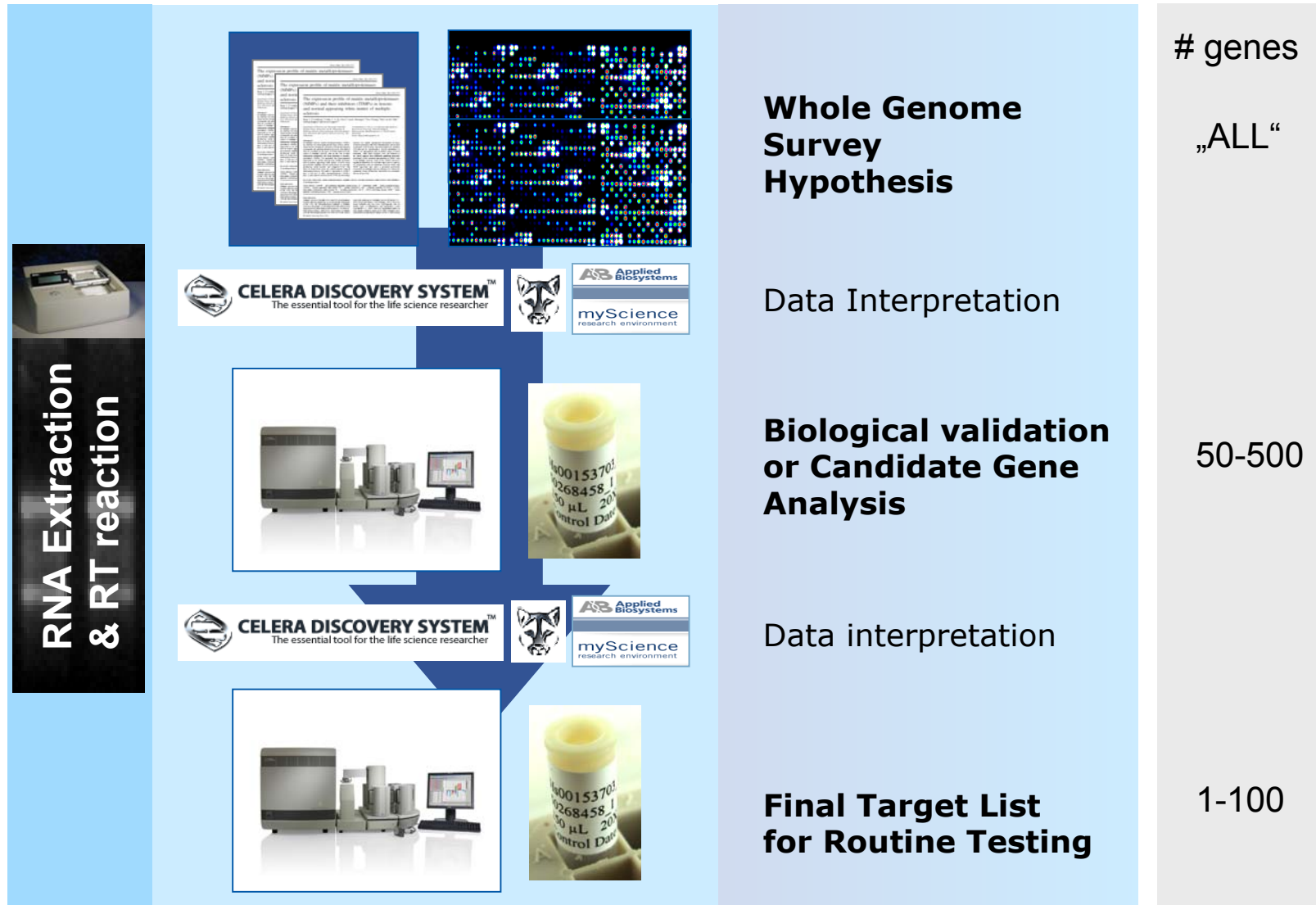


The Applera Genomic Initiative



***Integrating high value genomic content with
state of the art technology solutions***

Integrated Workflow



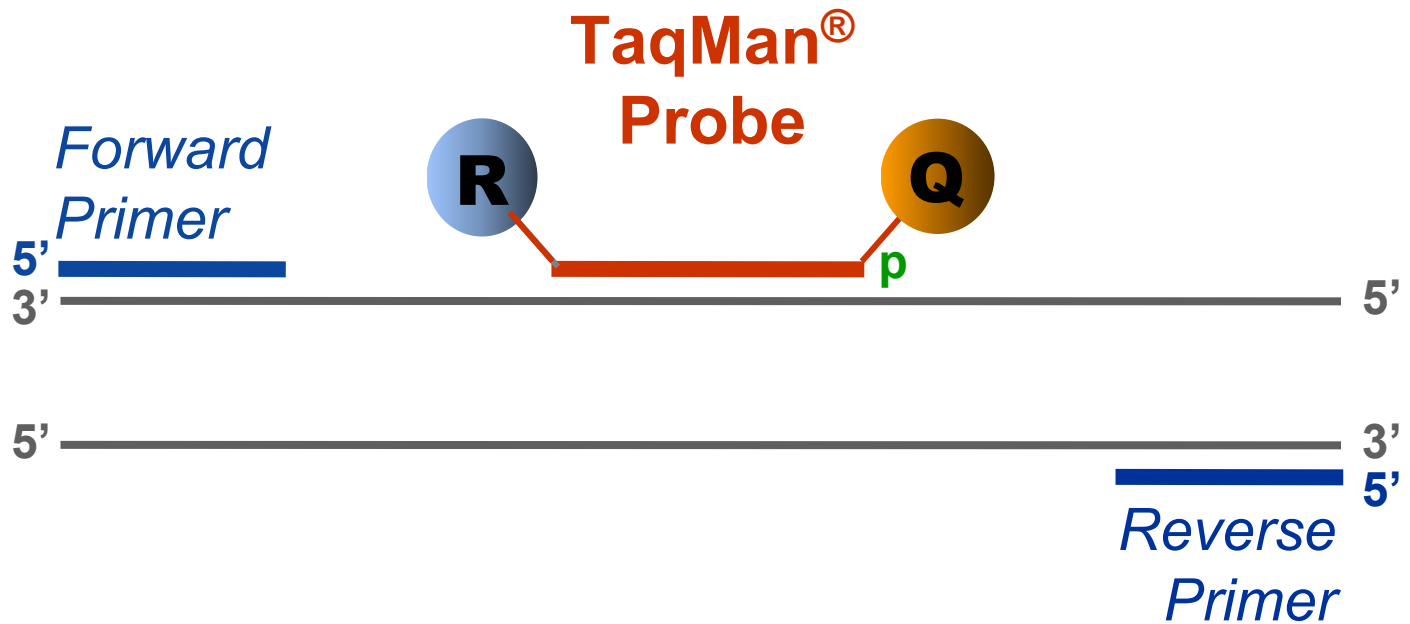


Real Time PCR Chemistry Basics

Signal generation with
TaqMan[®] MGB Probes

5' Nuclease Assay

using TaqMan[®] probes



Why short Amplicons?

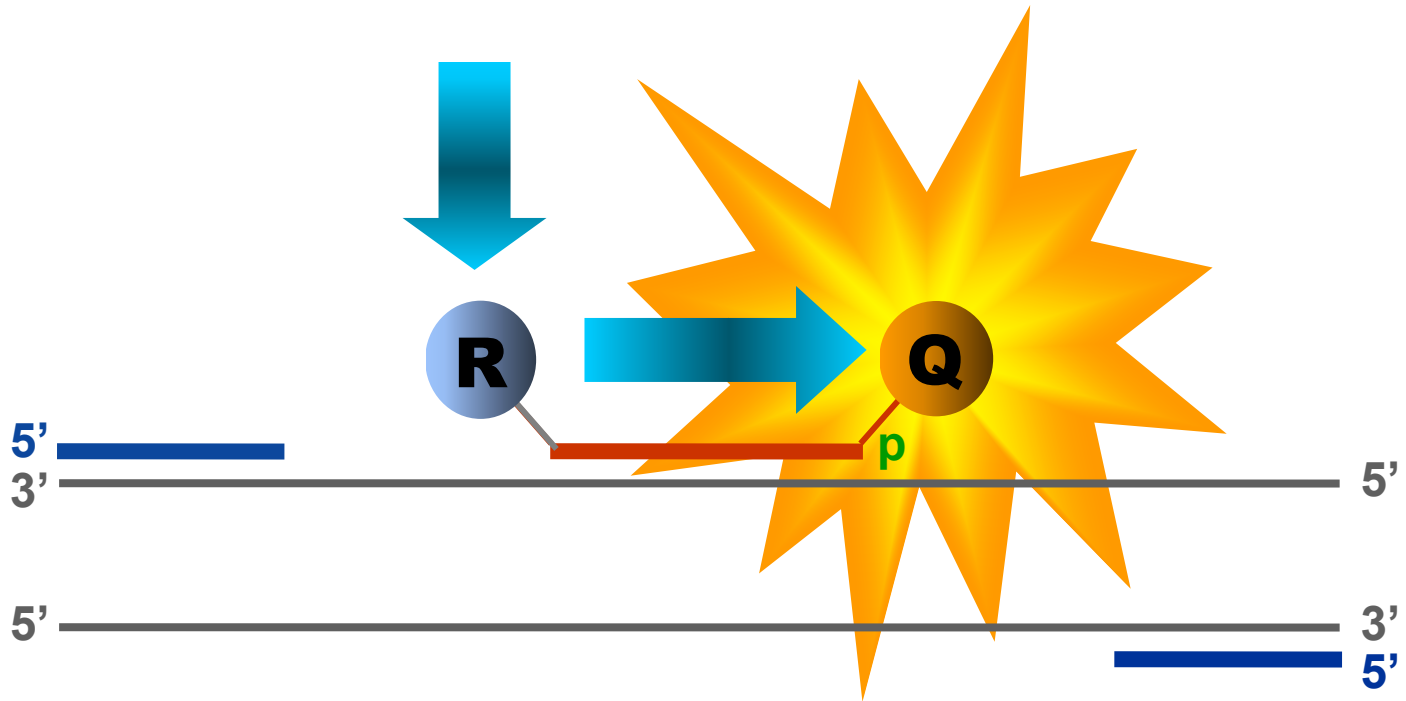
- Recommendation: Assays with short Amplicons
 - robust PCR-Reactions = high sensitivity!
 - high efficiency = no standard curve necessary!

but...

- Also long Amplicons can be used
 - ☹ PCR-efficiency of longer Amplicons drops
- Quantification requires standard curves

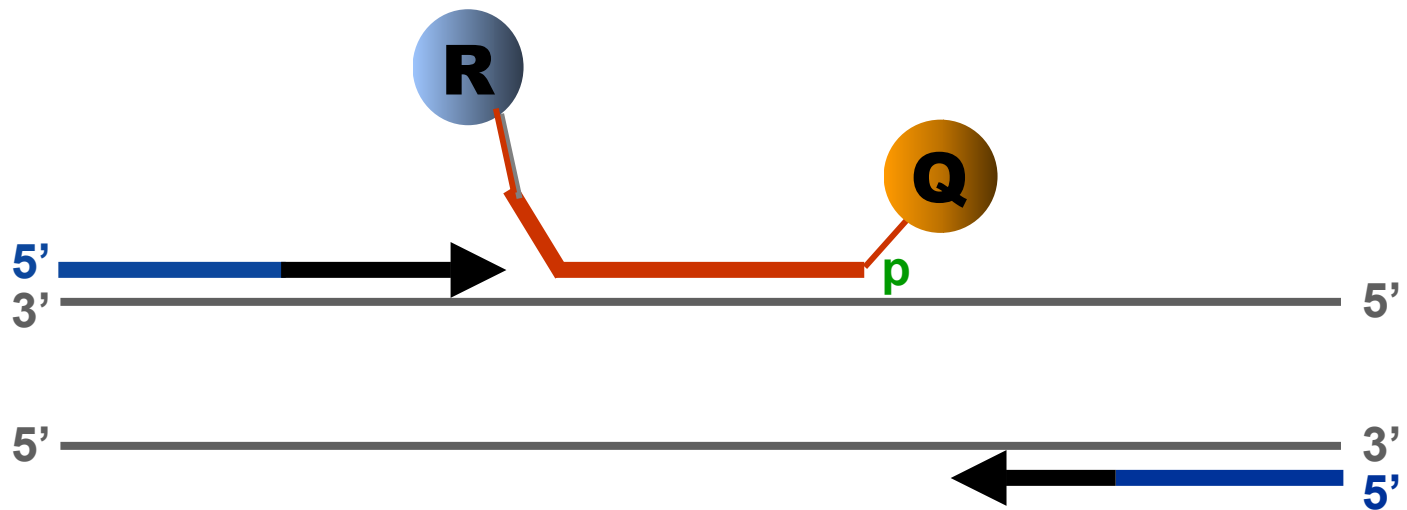
5' Nuclease Assay

using TaqMan[®] probes



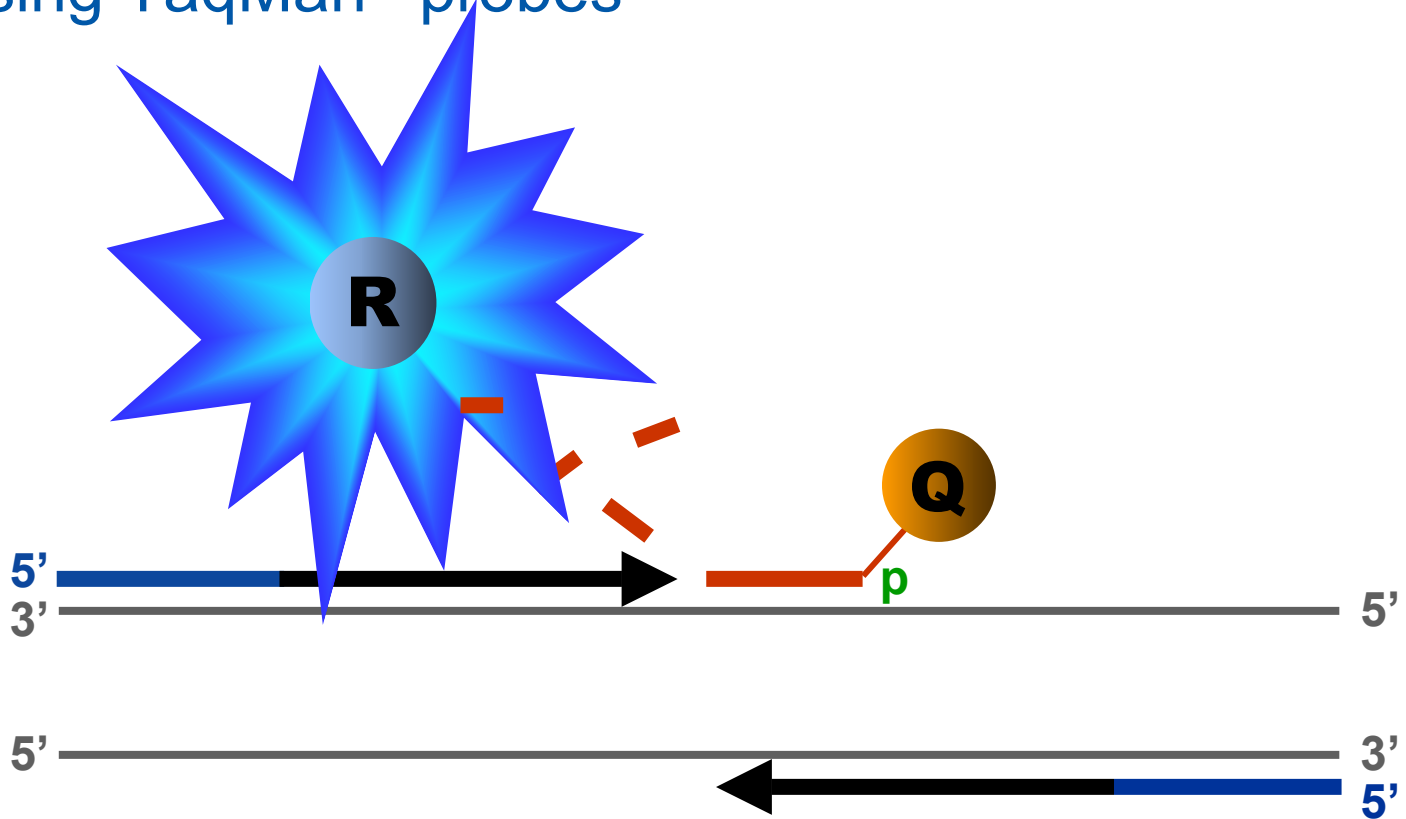
5' Nuclease Assay

using TaqMan[®] probes



Strand Displacement

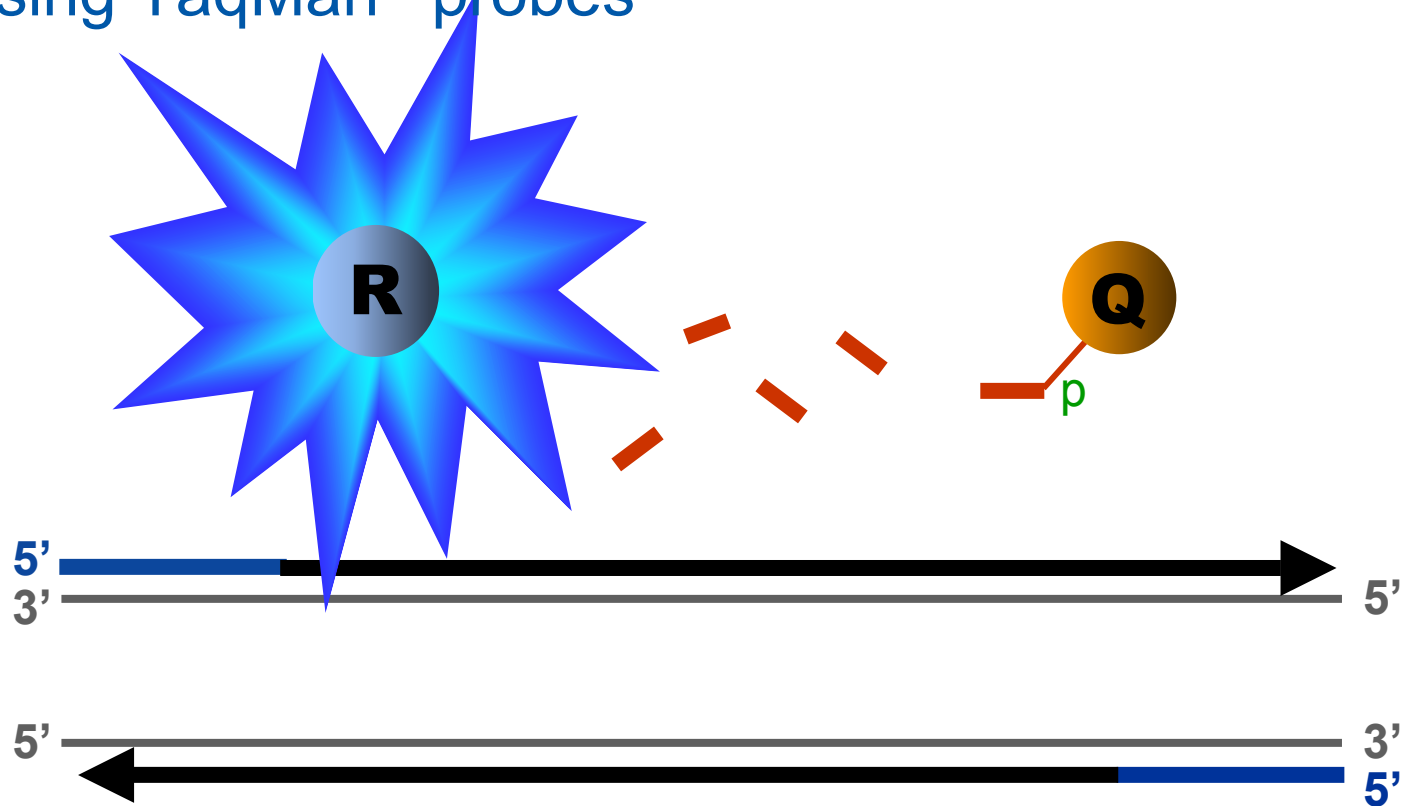
5' Nuclease Assay using TaqMan[®] probes



Strand Displacement and Cleavage

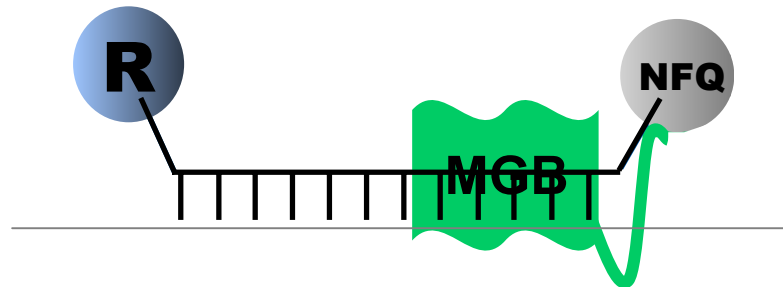
5' Nuclease Assay

using TaqMan[®] probes



Finishing Synthesis

TaqMan[®] MGB probes



MGB: Minor Groove Binder

NFQ: Non-Fluorescent Quencher

Stabilization of last 5-6 bp on 3' end

- ⇒ **Short and more specific** probes for a given T_m
- ⇒ high signal intensity



TaqMan[®] Assays

How to get the assay for YOUR gene?

Manual Approach in the Past





TaqMan[®] Assays remove Bottlenecks

**Gold Standard
Quantitation Technology
Effort and cost decreased**

www.appliedbiosystems.com

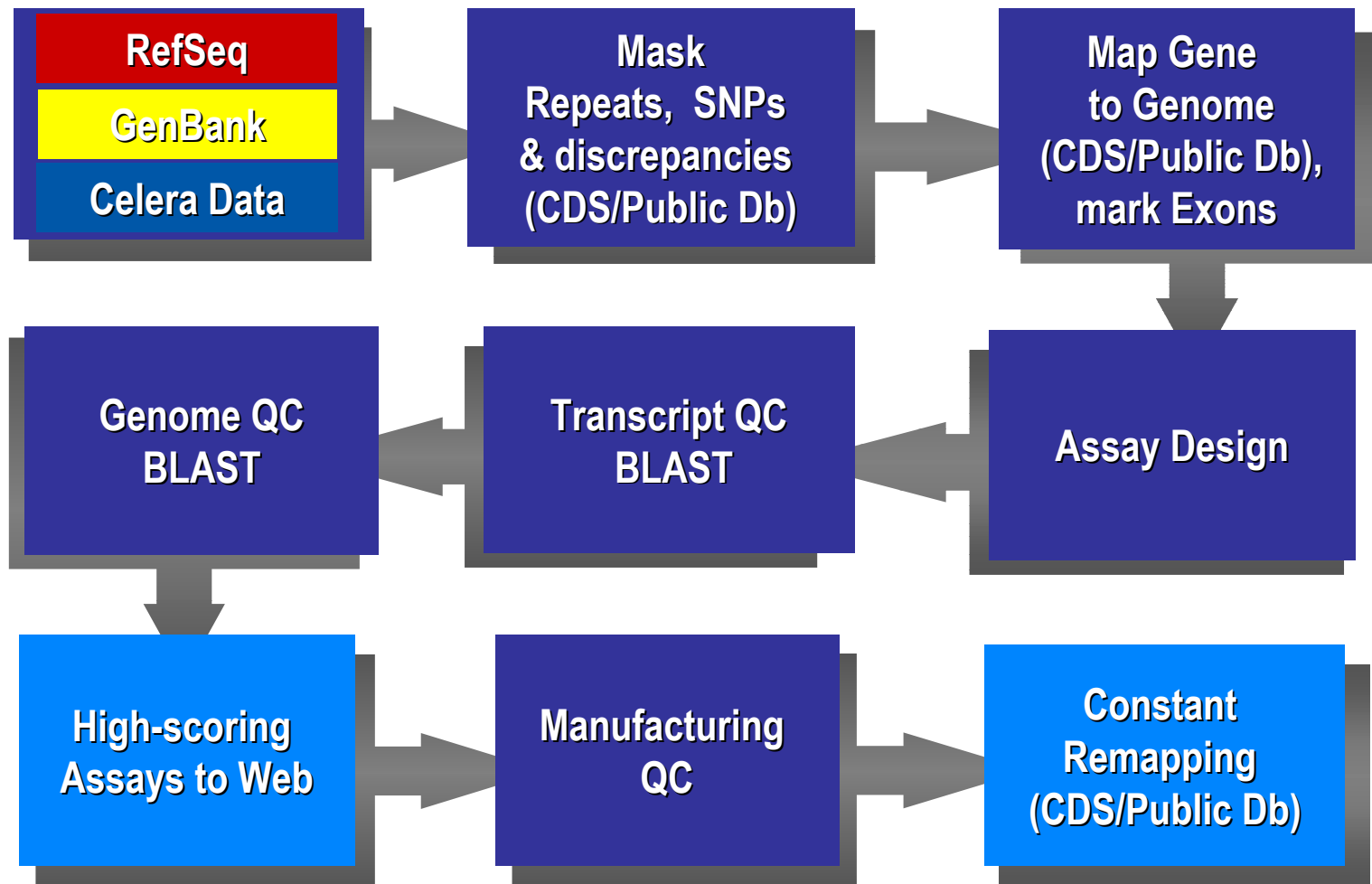
TaqMan[®] Assays
Custom TaqMan[®] Assays
TaqMan[®] Low Density Array

Applied Biosystems
7900HT, 7500 and 7300
Real-time PCR Systems



TaqMan[®] Assays

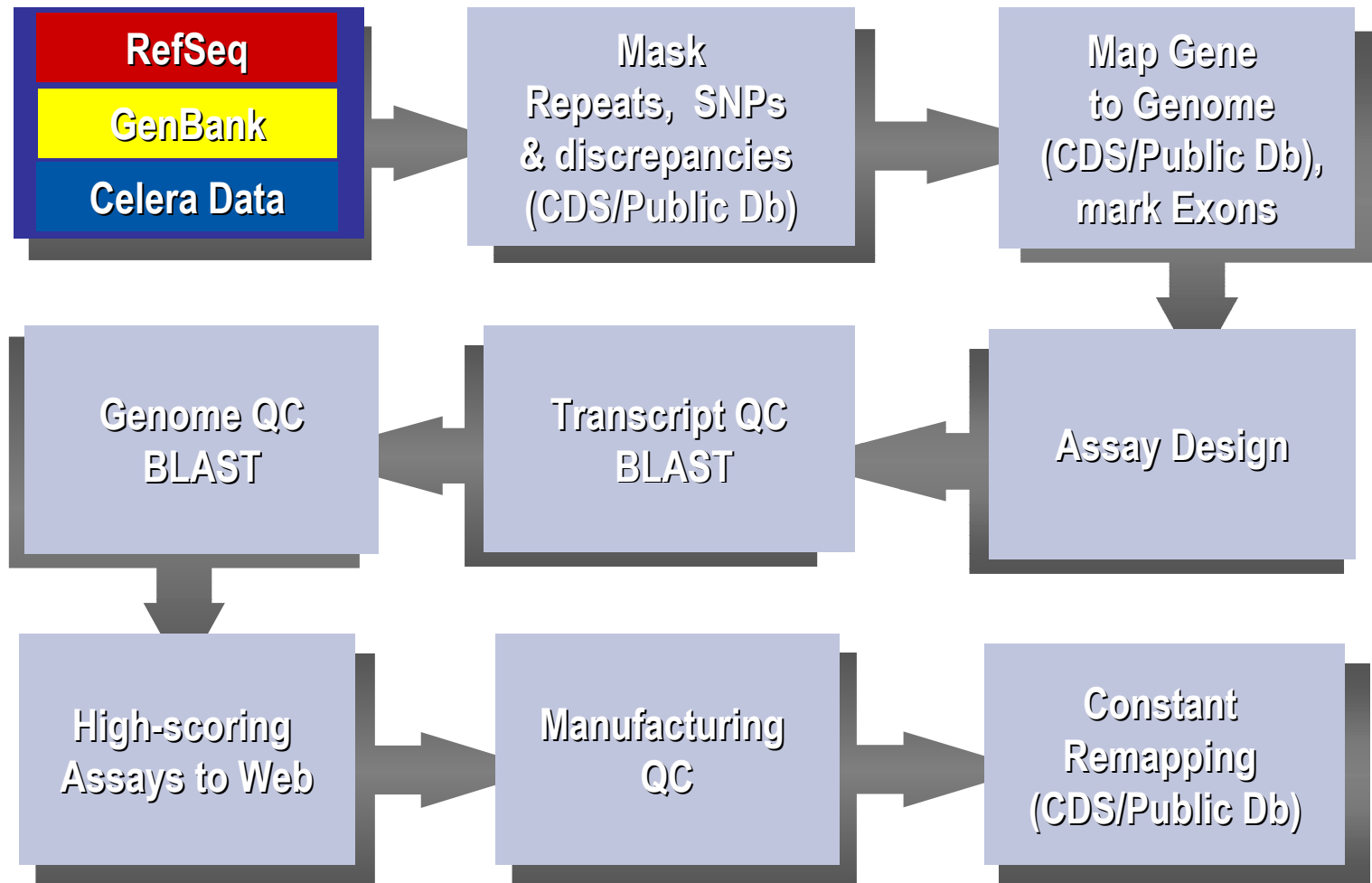
Massive Bioinformatics Pipeline - High Content





TaqMan[®] Assays

Massive Bioinformatics Pipeline - High Content





TaqMan[®] Assays

Source of transcripts

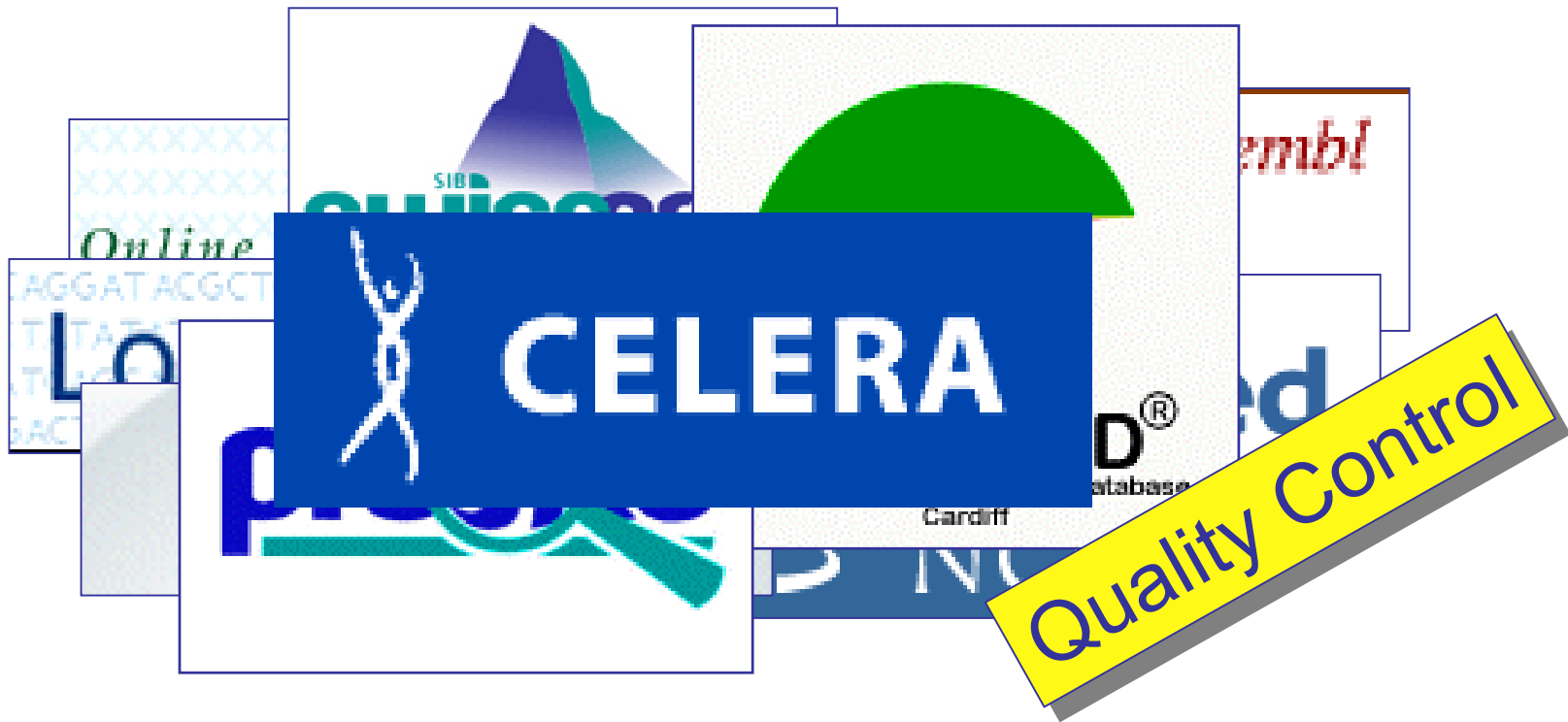
- Start with **NCBI Reference Sequence** transcripts

(<http://www.ncbi.nlm.nih.gov/LocusLink/refseq.html>)

- Best known, highly curated and non-redundant transcripts in the public domain
- Manually curated by NCBI scientist
- Good evidence available: cDNA clones



TaqMan[®] Assays



Regularly updated data from upto
40 total databases,
both proprietary and public

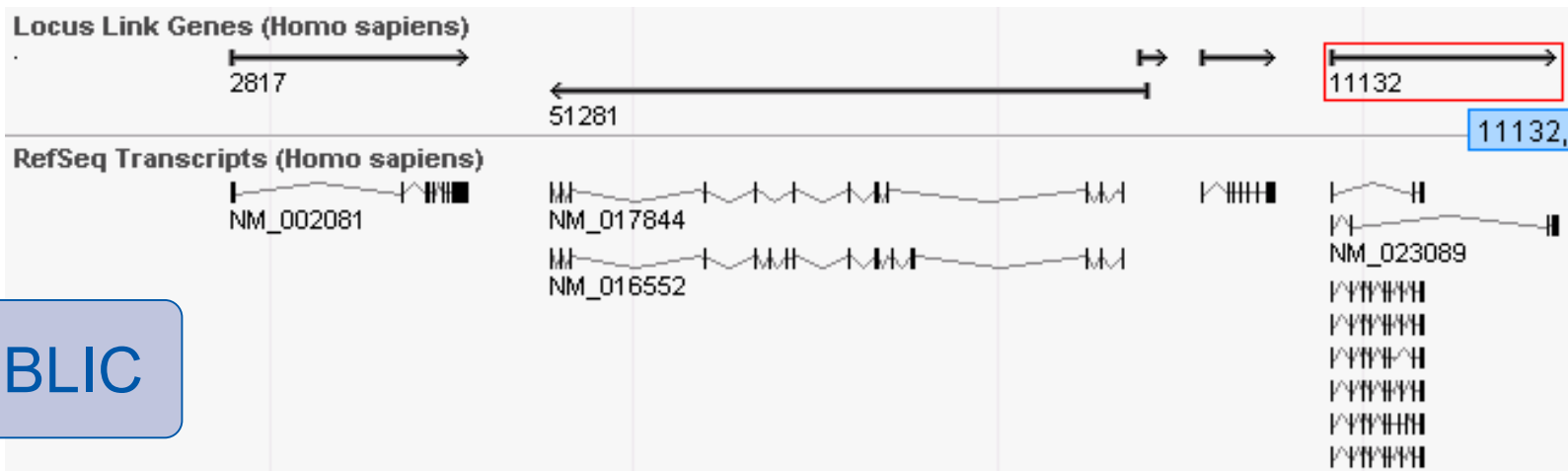


CELERA DISCOVERY SYSTEM™
The essential tool for the life science researcher

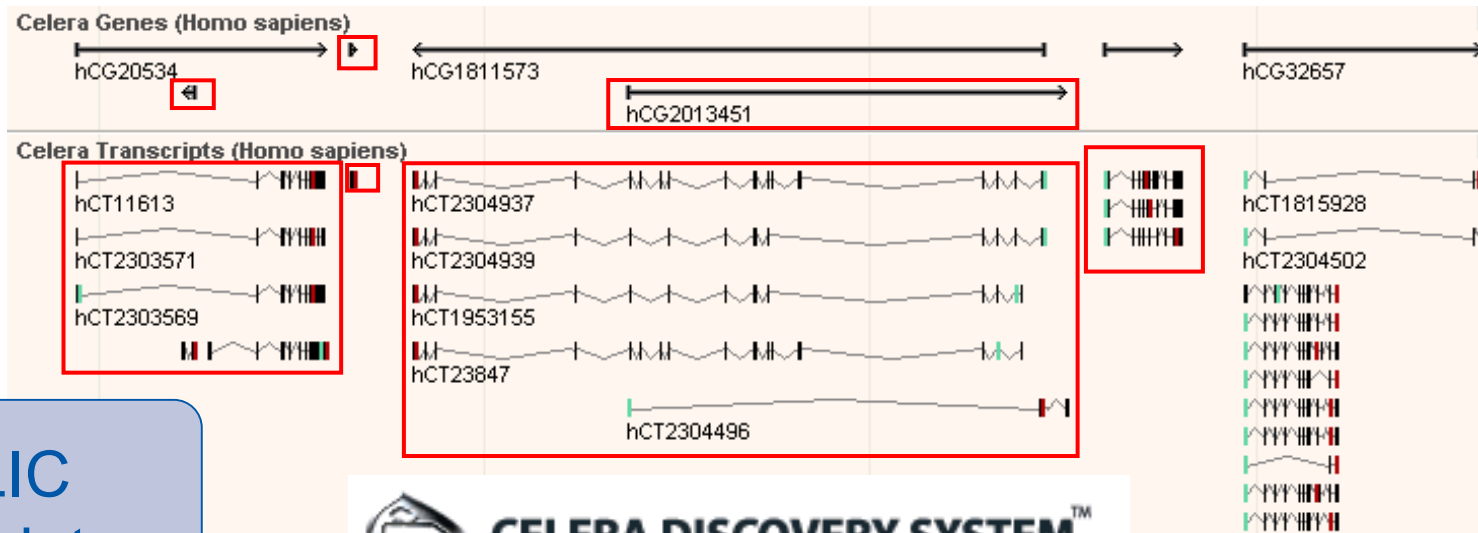
AB Applied Biosystems



Value of the Celera Discovery SystemSM Platform in the Assay Pipeline



PUBLIC



PUBLIC
plus proprietary



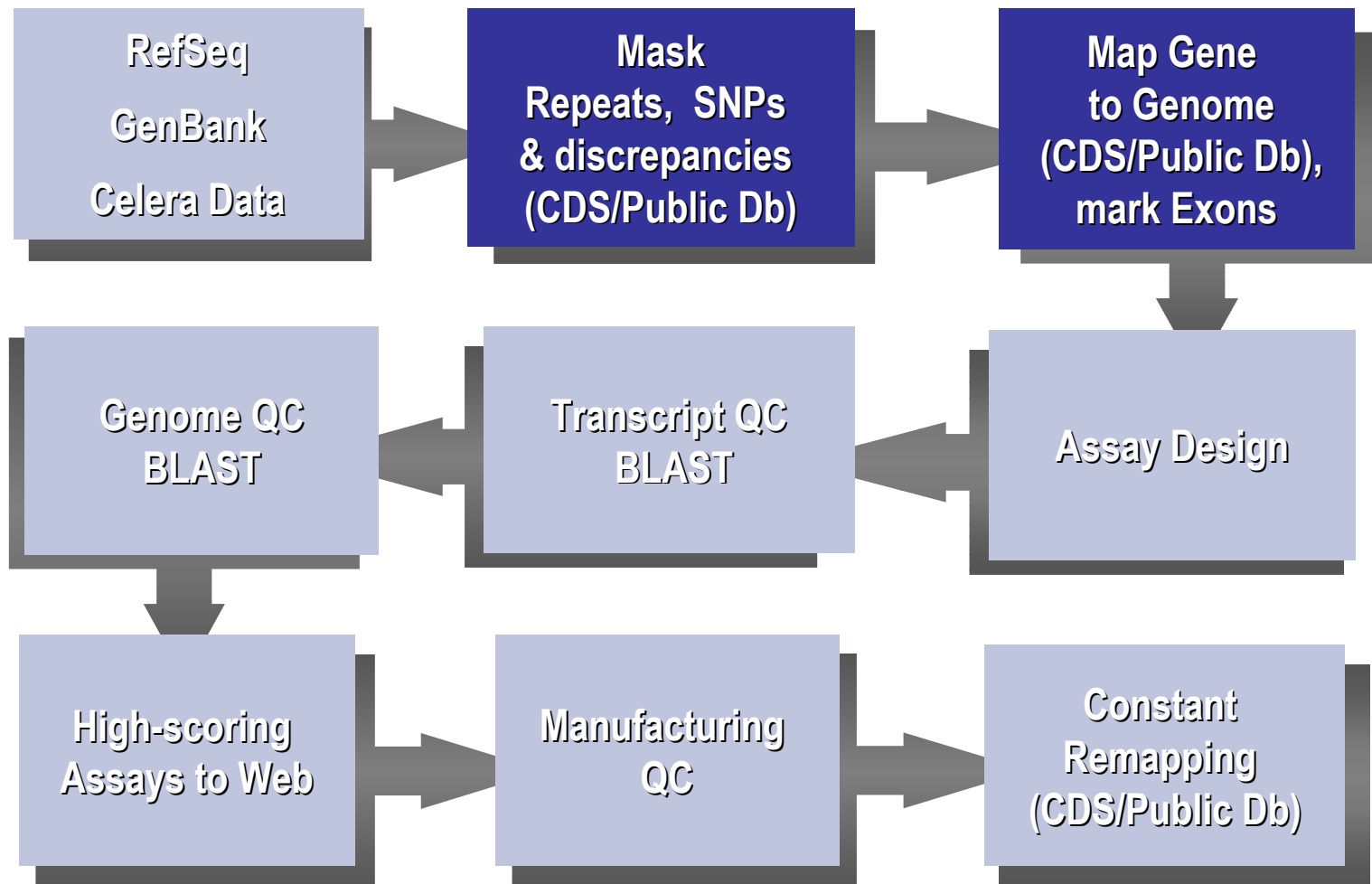
CELERA DISCOVERY SYSTEMTM
The essential tool for the life science researcher

AB Applied Biosystems



TaqMan[®] Assays

Massive Bioinformatics Pipeline - High Content



Transcript Pre-Processing 1

- Design only in a region of unambiguous sequence
 - > No repeats (poly-nucleotide repeats, ALU repeats etc)
- Mapping the masked transcripts to the Celera and Public Genome assembly
 - Sequence discrepancies
 - BLAST against Celera SNP database
 - > Most comprehensive SNP database, integrates both public and Celera-proprietary SNPs
 - > Over 4 million known human SNPs
 - > More than 3 million mouse SNPs



Transcript Pre-Processing 2

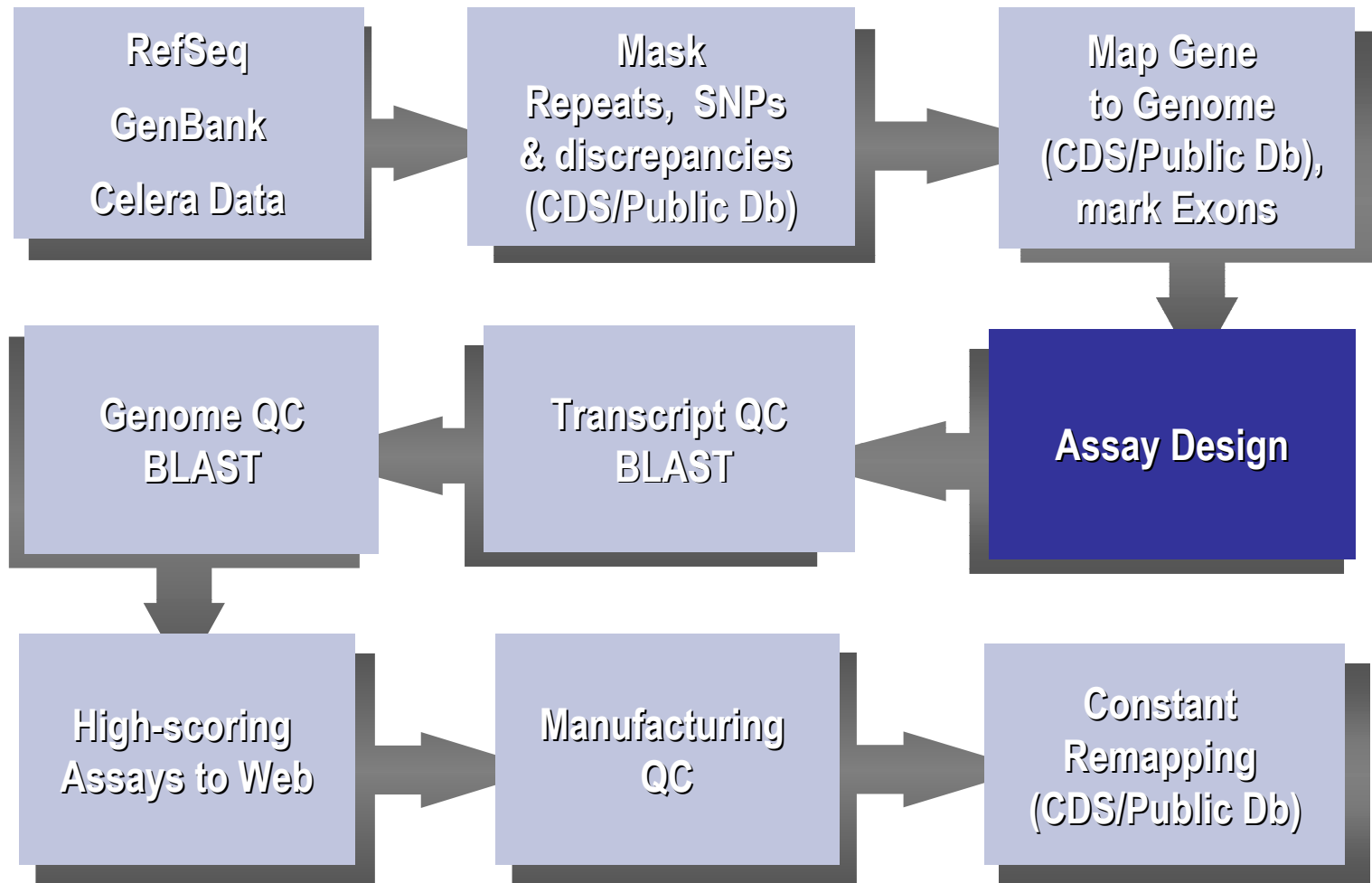
- Exon-Exon boundaries of multi-exon genes are marked
- Identification of single-exon transcripts

cDNA specific Assays!



TaqMan[®] Assays

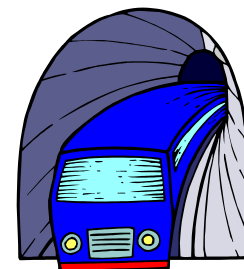
Massive Bioinformatics Pipeline - High Content





Assay Design

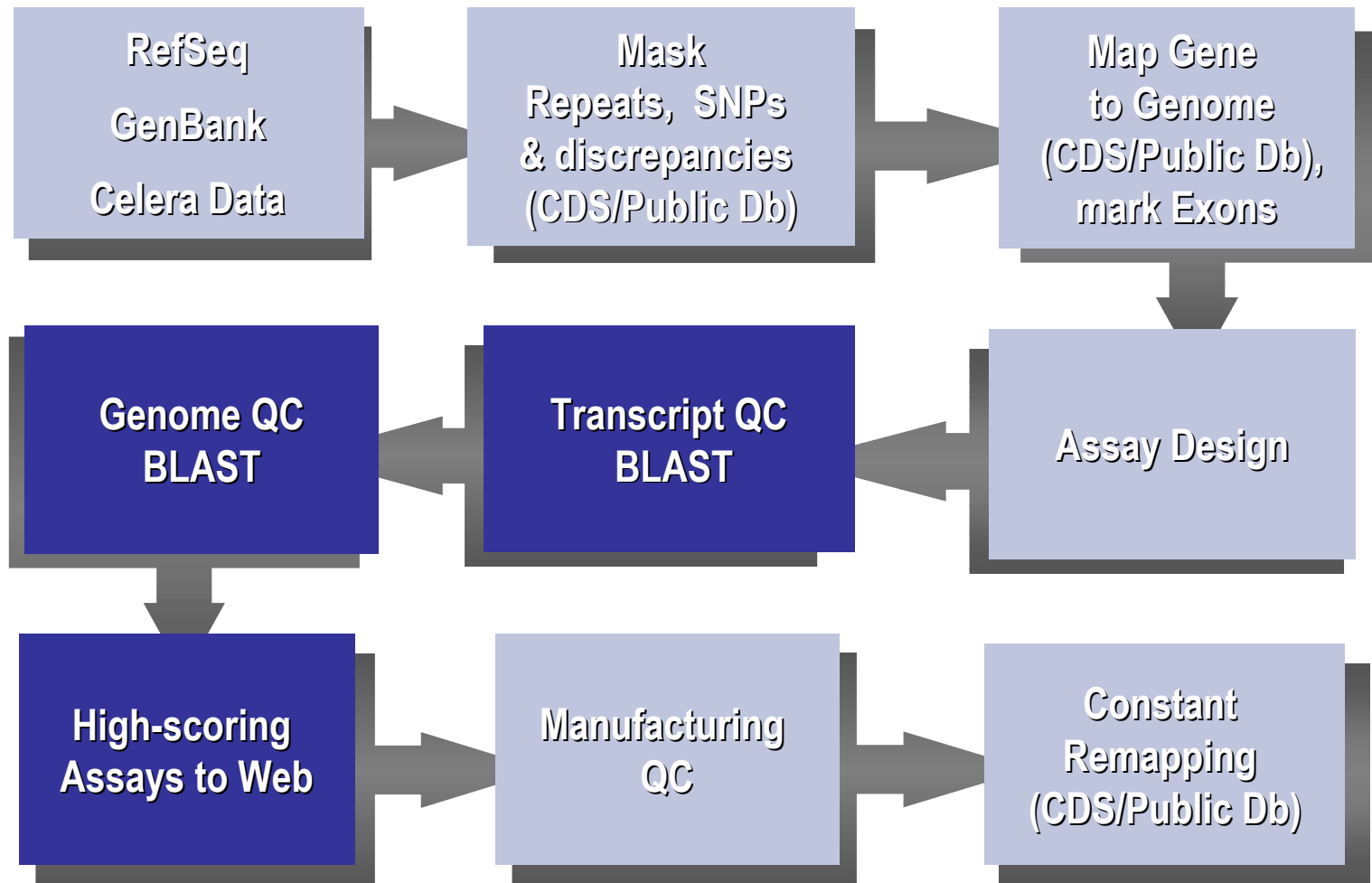
- Long experience with probe and primer design of 5' nuclease assays
- TaqExpress
 - Automated process
 - Significant enhancement of the algorithms resident in AB Primer Express[®] Software





TaqMan[®] Assays

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In Silico Quality Scoring 1

Penalty score for

- Assay designs that are not highly specific for the gene of interest
- Assay designs that may not accurately report the quantitative expression results for a particular target



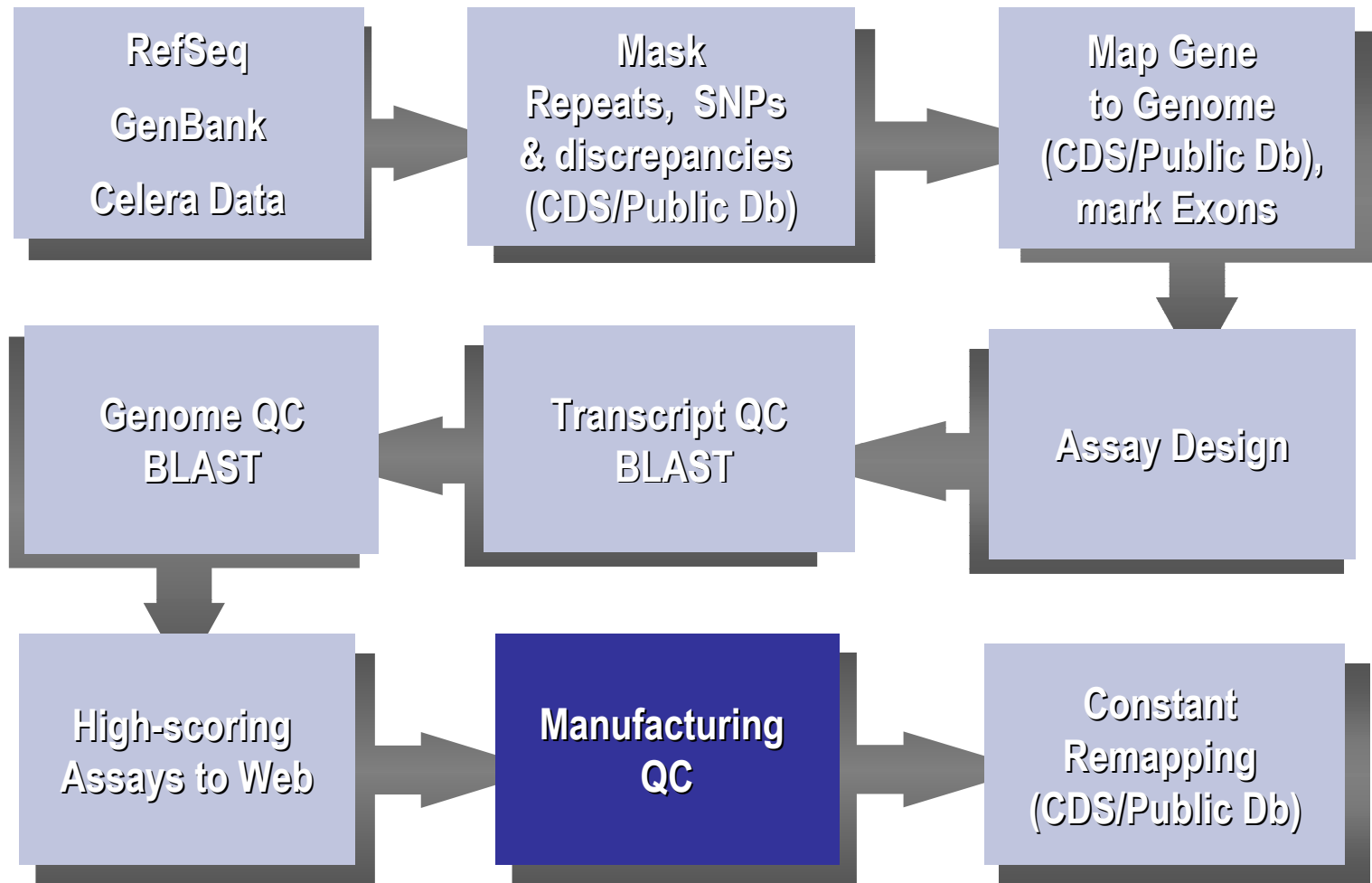
In Silico Quality Scoring 2

- Transcript BLAST scoring
 - Penalty is assigned if an assay detects any closely homologous transcript(s)
- Genome BLAST scoring
 - Penalty is assigned if an assay hits a second location on the genome
- Intron Size scoring
 - Penalty is assigned if an assay is designed across an exon-exon boundary that spans a small intron (<2kb)



TaqMan[®] Assays

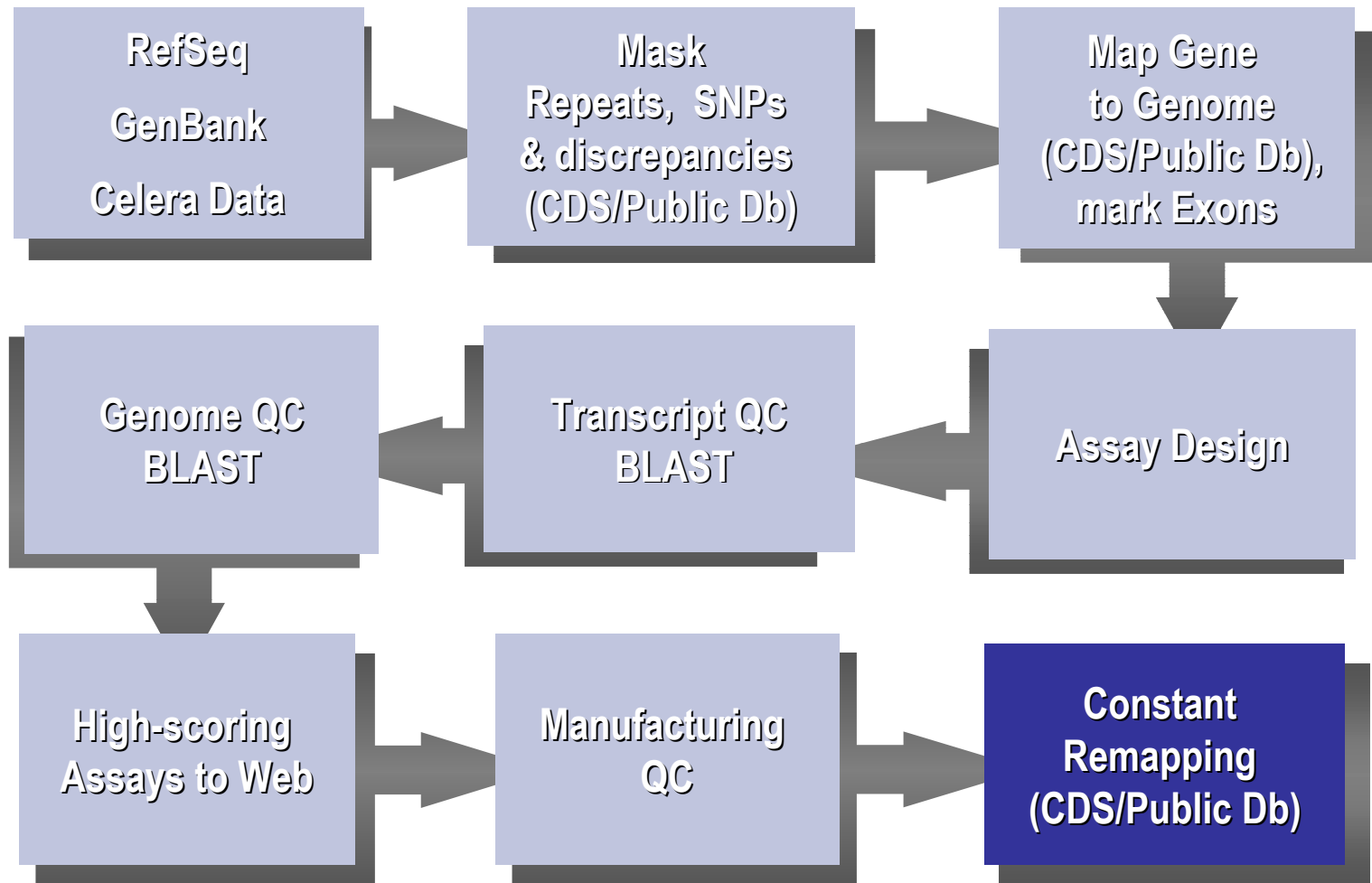
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Remapping

- Genomic information is still changing
- New transcripts are being discovered
- Constant remapping after a new transcript database is released (i.e. when RefSeq is updated, approx. Every 4 weeks)



TaqMan[®] Assays

Assays Available!

Human

		2	0	4	0	7	8
--	--	---	---	---	---	---	---

 Gene Expression

**Universal conditions,
no optimization!**

Rat

		1	4	7	0	3	5
--	--	---	---	---	---	---	---

 Gene Expression

www.allgenes.com



TaqMan[®] Assays

How to find the ones I need



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results list LOCUSLINK

Display: 30
Export Results

LocusLink ID
and Gene Symbol

Panther Classification:
Function and Process

Public Location

	Locus Link Id	Species	Locus Link Gene Name	Locus Link Gene Symbol	Panther Molecular Function	Panther Biological Process	Public Location
☐	1. 4318	Homo sapiens	matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	MMP9	2 more...	2 more...	chr. 20 45,322,969 - 45,330,621

Celera Gene ID

Celera Gene ID	Gene Expression Assays	siRNAs	SNP Genotyping Assays	Resequencing Sets	RefSeq/Genbank mRNA
hCG40015	11 assays	Ambion siRNAs	31 assays	3 RSS IDs	3 transcripts

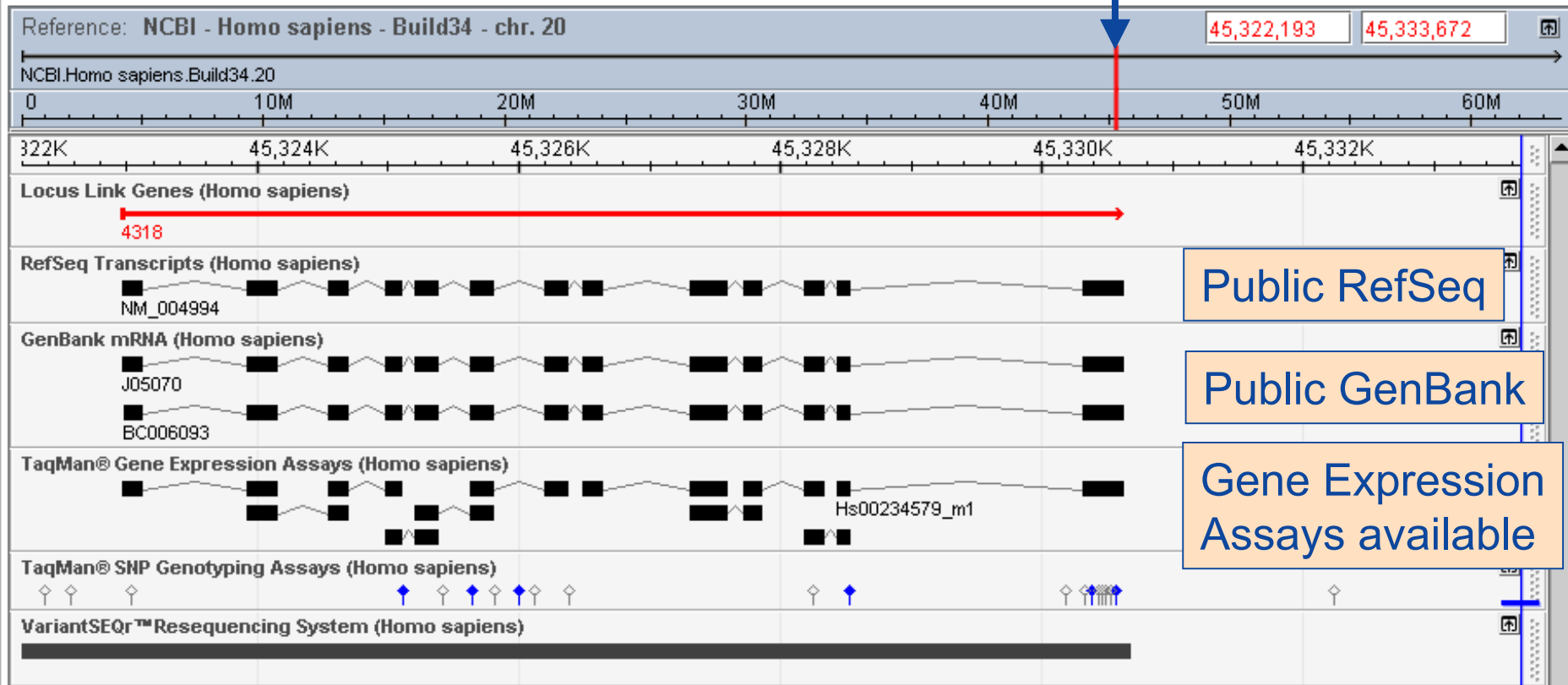
Public RefSeq and GenBank Transcripts

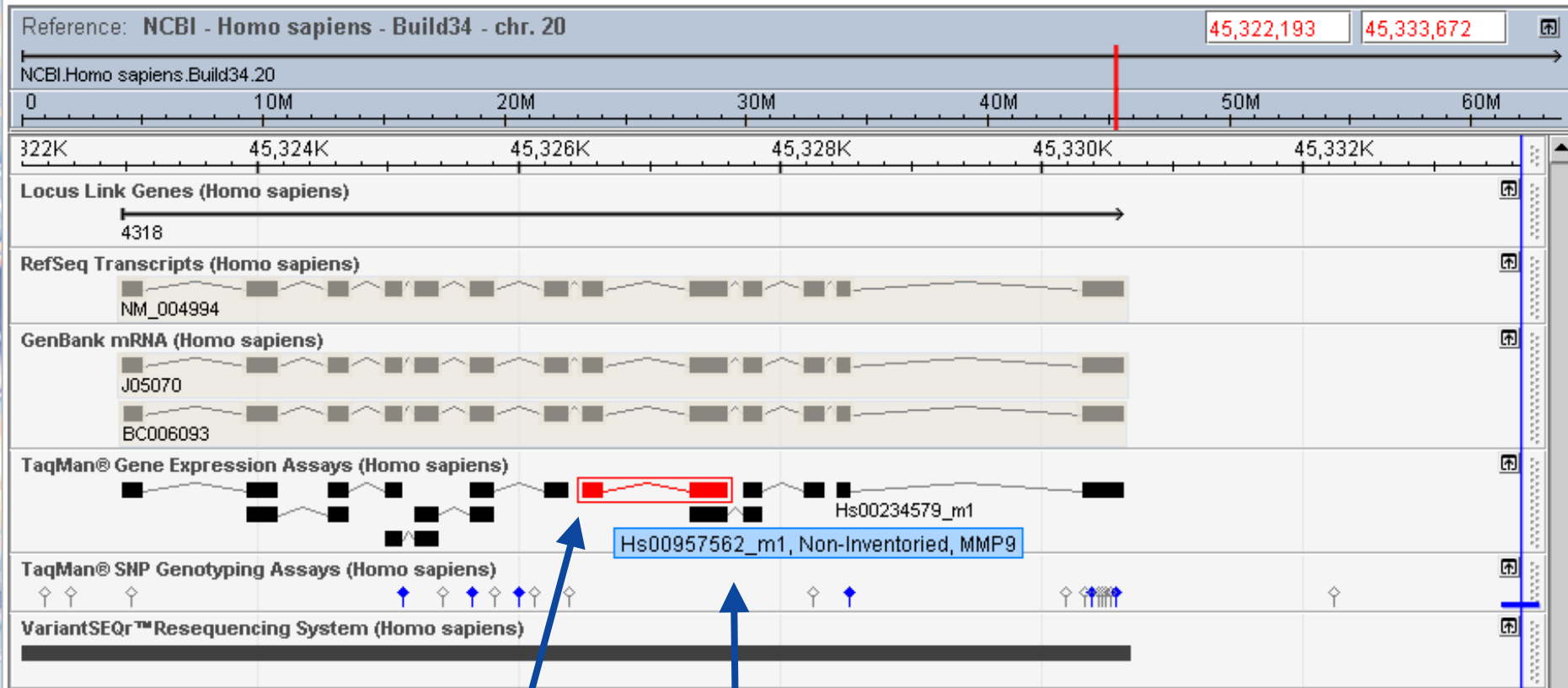
TaqMan Assays
Gene Expression

siRNAs

TaqMan Assays
SNP Genotyping

human Chromosome 20
Location displayed graphically





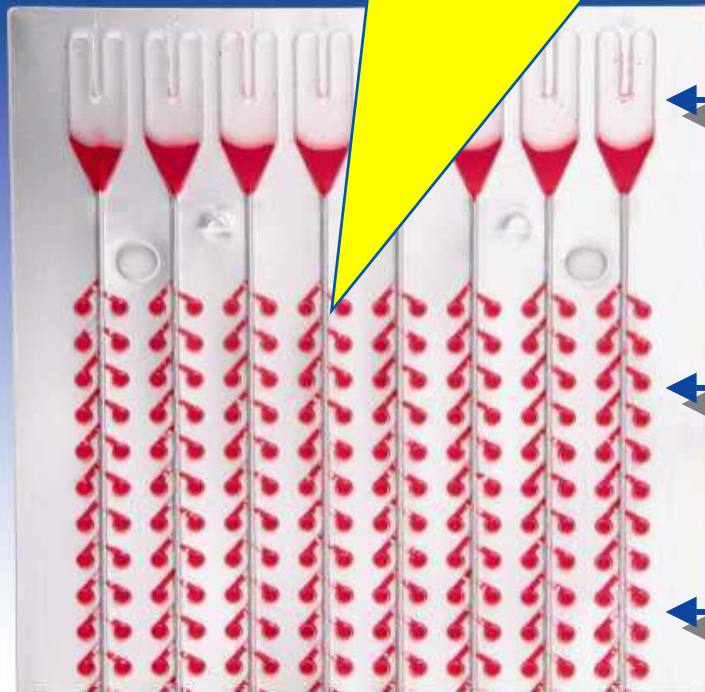
Click on assay to see which sequences are interrogated

Mouse over to get Assay information



TaqMan[®] Low Density Array

TaqMan[®] Assays pre-loaded



1 to 8 Samples

12 to 380 Targets
(per one array)

No. of Replicates

384 spots

Configuring my Custom Array

Number of Genes	Number of Replicates	Number of Samples per Array
12	4	8
16	3	8
24	2	8
32	3	4
48	1	8
64	3	2
96	1	4
96	2	2
381	1	1

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
B	1	1	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12	13
C	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	13
D	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	13
E	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	13
F	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	13
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H	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	13
I	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	13
J	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	13
K	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	13
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P	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	



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