

Genomic Assays

Past, Present, and Future

Steven Shak

Genomic Health

2nd TBCI Correlative Sciences Workshop 2/23/09

- What is the Genomic Health RT-PCR Assay and how does it perform?
- What is the experience in using the assay?
- What are some challenges in performing “discovery” studies?
- Should NCI increase efforts to investigate the genomics of tissue samples from clinical trials?

What is the Genomic Health RT-PCR Assay and how does it perform?

Genomic Health RT-PCR Assay Process



Accession Sample



Pathology Review and Manual Microdissection

RNA Extraction

RNA Quantification

DNA Contamination Assay

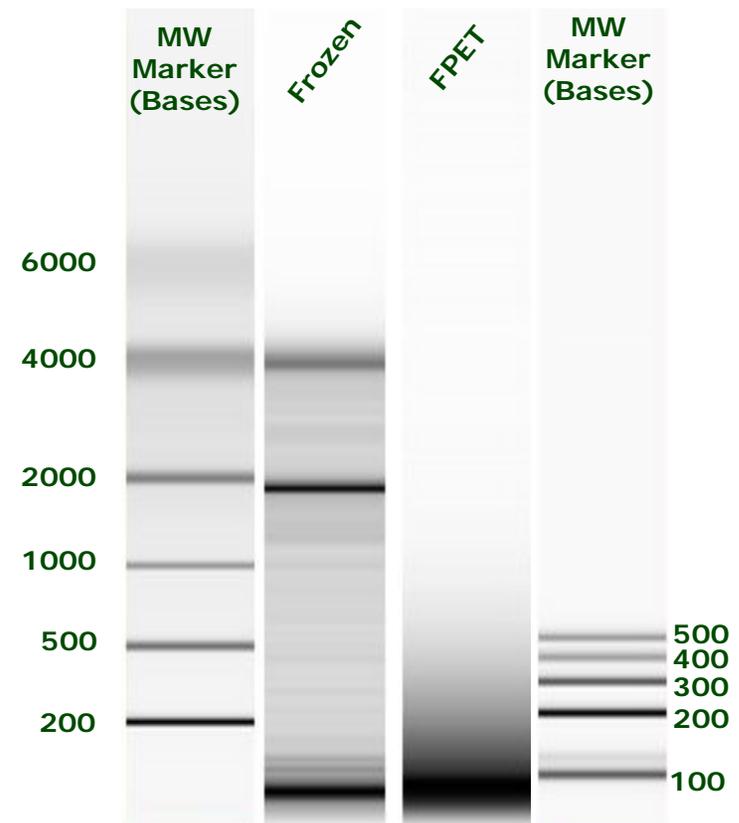
Reverse Transcription

Quantitative PCR – Three Wells for Each Gene

Genomic Health RT-PCR Assay Process*



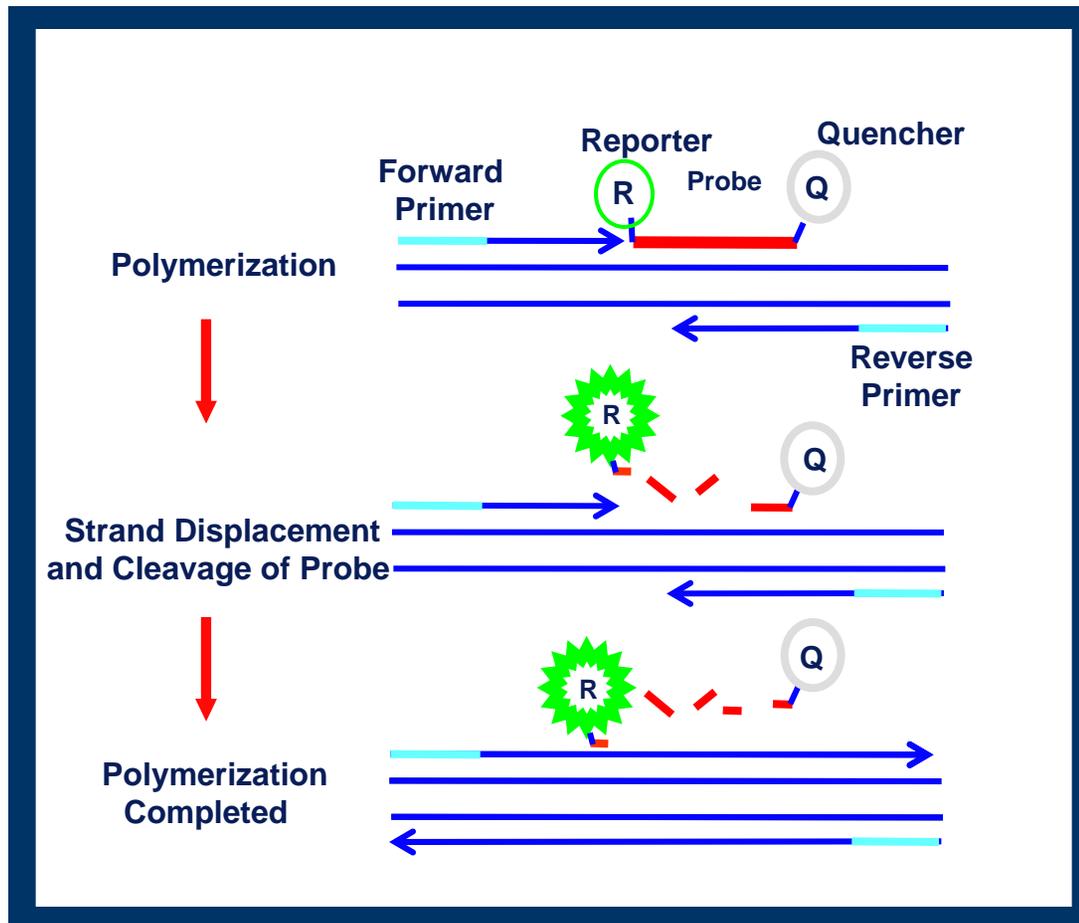
- Standardized process
 - Optimized for the small RNA fragments in fixed paraffin embedded tissue (FPET)
 - Optimized to be robust with regard to sources of pre-analytic variability such as
 - Time from surgery to fixation
 - Tumor size
 - Fixative type and duration
 - Block age: >20yrs
 - Tissue heterogeneity



*Cronin et al. *Am J Pathol.* 2004;164:35-42

Real-time RT-PCR for RNA Quantification*

- Sensitive
- Specific
- Wide dynamic range
- Reproducible
- Success with FPET depends on design of primers/probes for small amplicons



*Cronin et al. *Am J Pathol.* 2004;164:35-42

Publication: Analytical Validation of the Oncotype DX Assay*



Clinical Chemistry 53:6
000–000 (2007)

Cancer Diagnostics

Analytical Validation of the Oncotype DX Genomic Diagnostic Test for Recurrence Prognosis and Therapeutic Response Prediction in Node-Negative, Estrogen Receptor–Positive Breast Cancer

MAUREEN CRONIN,* CHITHRA SANGLI, MEI-LAN LIU, MYLAN PHO, DEBJANI DUTTA, ANH THU NGUYEN, JENNIE JEONG, JENNY WU, KIM CLARK LANGONE, and DREW WATSON

Background: Oncotype DX™ is a clinically validated, high-complexity, multianalyte reverse transcription–PCR genomic test that predicts the likelihood of breast cancer recurrence in early-stage, node-negative, estrogen receptor–positive breast cancer. The Recurrence Score™ (RS) provides a more accurate, reproducible measure of breast cancer aggressiveness and therapeutic responsiveness than standard measures. Individualized patient

contributed by instrument, operator, reagent, and day-to-day baseline variation was low, with SDs of <0.5 C_T. **Conclusion:** The analytical and operational performance specifications defined for the Oncotype DX assay allow the reporting of quantitative RS values for individual patients with an SD within 2 RS units on a 100-unit scale.

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*Cronin et al. *Clin Chem*. 2007;53:1084-91

Analytical Validation Characterization of Linearity*



Official gene symbol	Linear RNA range, ng	Minimum linear range	C _T , 8 ng RNA
<i>BAG1</i>	2 ⁻¹⁰ to 2 ³	16 384-fold	27.4
<i>BCL2</i> ^a	2 ⁻¹⁰ to 2 ³	16 384-fold	27.9
<i>CCNB1</i>	2 ⁻⁸ to 2 ³	4096-fold	29.0
<i>CD68</i>	2 ⁻¹⁰ to 2 ³	16 384-fold	26.5
<i>SCUBE2</i> ^a	2 ⁻¹⁰ to 2 ³	16 384-fold	27.7
<i>CTSL2</i>	2 ⁻⁷ to 2 ³	2048-fold	30.0
<i>ESR1</i>	2 ⁻¹⁰ to 2 ³	16 384-fold	26.6
<i>GRB7</i> ^a	2 ⁻¹⁰ to 2 ³	16 384-fold	26.2
<i>GSTM1</i>	2 ⁻⁸ to 2 ³	4096-fold	28.5
<i>ERBB2</i> ^a	2 ⁻¹⁰ to 2 ³	16 384-fold	23.7
<i>MKI67</i>	2 ⁻⁹ to 2 ³	8192-fold	28.5
<i>MYBL2</i> ^a	2 ⁻⁸ to 2 ³	4096-fold	28.8
<i>PGR</i>	2 ⁻⁹ to 2 ³	8192-fold	28.1
<i>AURKA</i> ^a	2 ⁻⁸ to 2 ³	4096-fold	29.1
<i>MMP11</i> ^a	2 ⁻¹⁰ to 2 ³	16 384-fold	24.8
<i>BIRC5</i> ^a	2 ⁻⁸ to 2 ³	4096-fold	29.3
<i>ACTB</i>	2 ⁻¹⁰ to 2 ³	16 384-fold	21.9
<i>GAPDH</i> ^a	2 ⁻¹⁰ to 2 ³	16 384-fold	24.0
<i>GUSB</i>	2 ⁻⁷ to 2 ³	2048-fold	29.7
<i>RPLP0</i>	2 ⁻¹⁰ to 2 ³	16 384-fold	22.8
<i>TFRC</i>	2 ⁻¹⁰ to 2 ³	16 384-fold	27.2

*Cronin et al. *Clin Chem.* 2007;53:1084-91

Analytical Validation Characterization of Reproducibility*



Official gene symbol	SD, reference normalized C _T
<i>ACTB</i>	0.01
<i>BAG1</i>	0.03
<i>BCL2</i>	0.09
<i>CCNB1</i>	0.09
<i>CD68</i>	0.10
<i>SCUBE2</i>	0.11
<i>CTSL2</i>	0.11
<i>ESR1</i>	0.05
<i>GAPDH</i>	0.25
<i>GRB7</i>	0.22
<i>GSTM1</i>	0.04
<i>GUSB</i>	0.03
<i>ERBB2</i>	0.07
<i>MKI67</i>	0.06
<i>MYBL2</i>	0.30
<i>PGR</i>	0.08
<i>RPLP0</i>	0.05
<i>AURKA</i>	0.10
<i>MMP11</i>	0.02
<i>BIRC5</i>	0.05
<i>TFRC</i>	0.04

*Cronin et al. *Clin Chem.* 2007;53:1084-91

What is the experience in using the assay?

Breast Cancer Clinical Studies



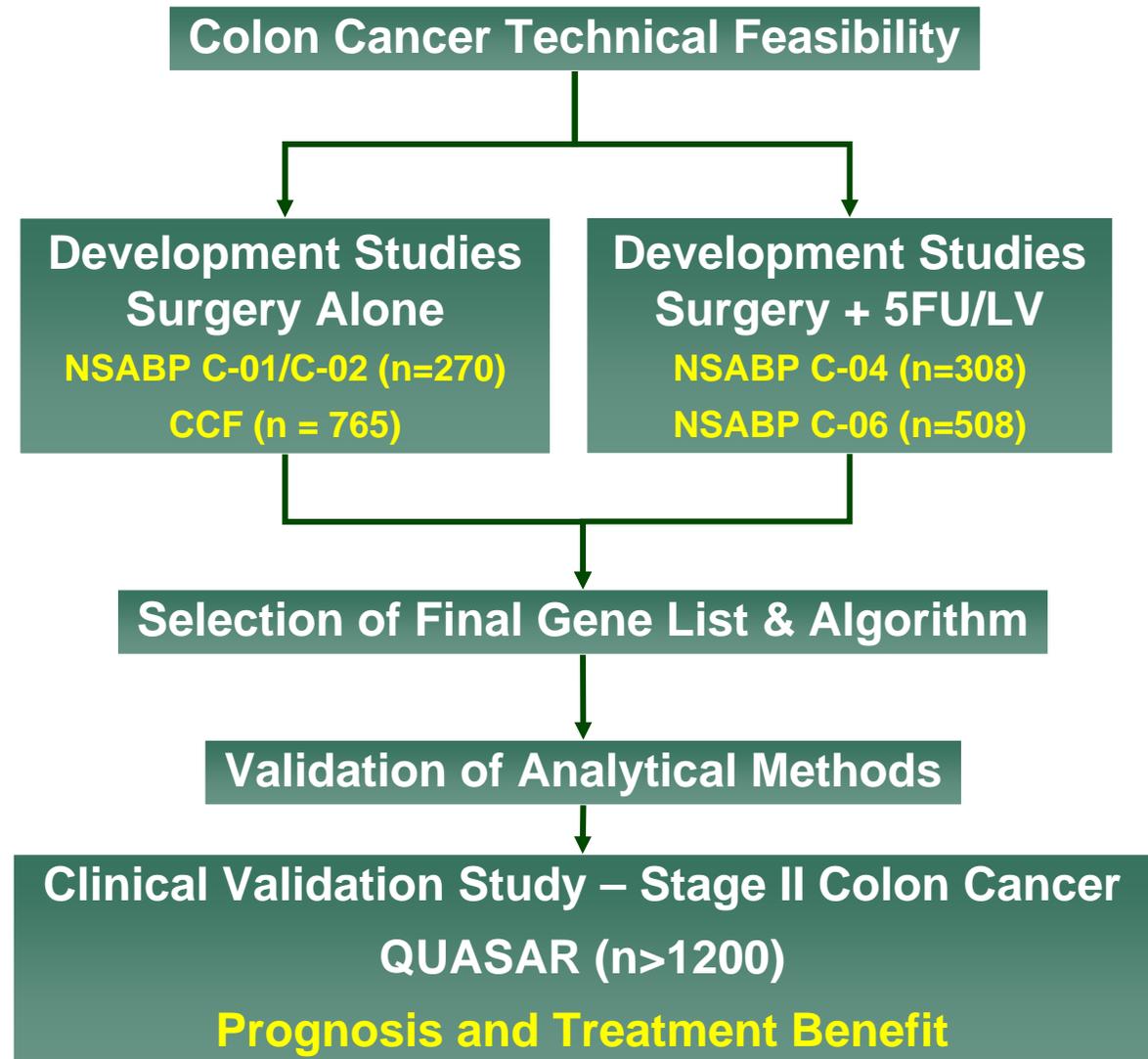
Study	Type	No. Pts	No. Genes	Nodal Status
Providence	Exploratory	136	250	Neg
Rush*	Exploratory	78	250	Pos
NSABP B-20	Exploratory	233	250	Neg
NSABP B-14*	Prospective	668	21	Neg
MD Anderson*	Prospective	149	21	Neg
Kaiser Permanente*	Prospective Case-Control	790 Cases/Controls	21	Neg
NSABP B-14	Prospective Placebo vs Tam	645	21	Neg
Milan*	Exploratory	89	384	Neg/Pos
NSABP B-20*	Prospective Tam vs Tam+Chemo	651	21	Neg
ECOG 2197*	Exploratory and Prospective	776	371	Neg/Pos
SWOG 8814	Prospective Tam vs Tam+Chemo	367	21	Pos
ATAC	Prospective Tam vs AI	1231	21	Neg/Pos
TAILORx	Prospective	Target 10,000+	21	Neg

*Published studies

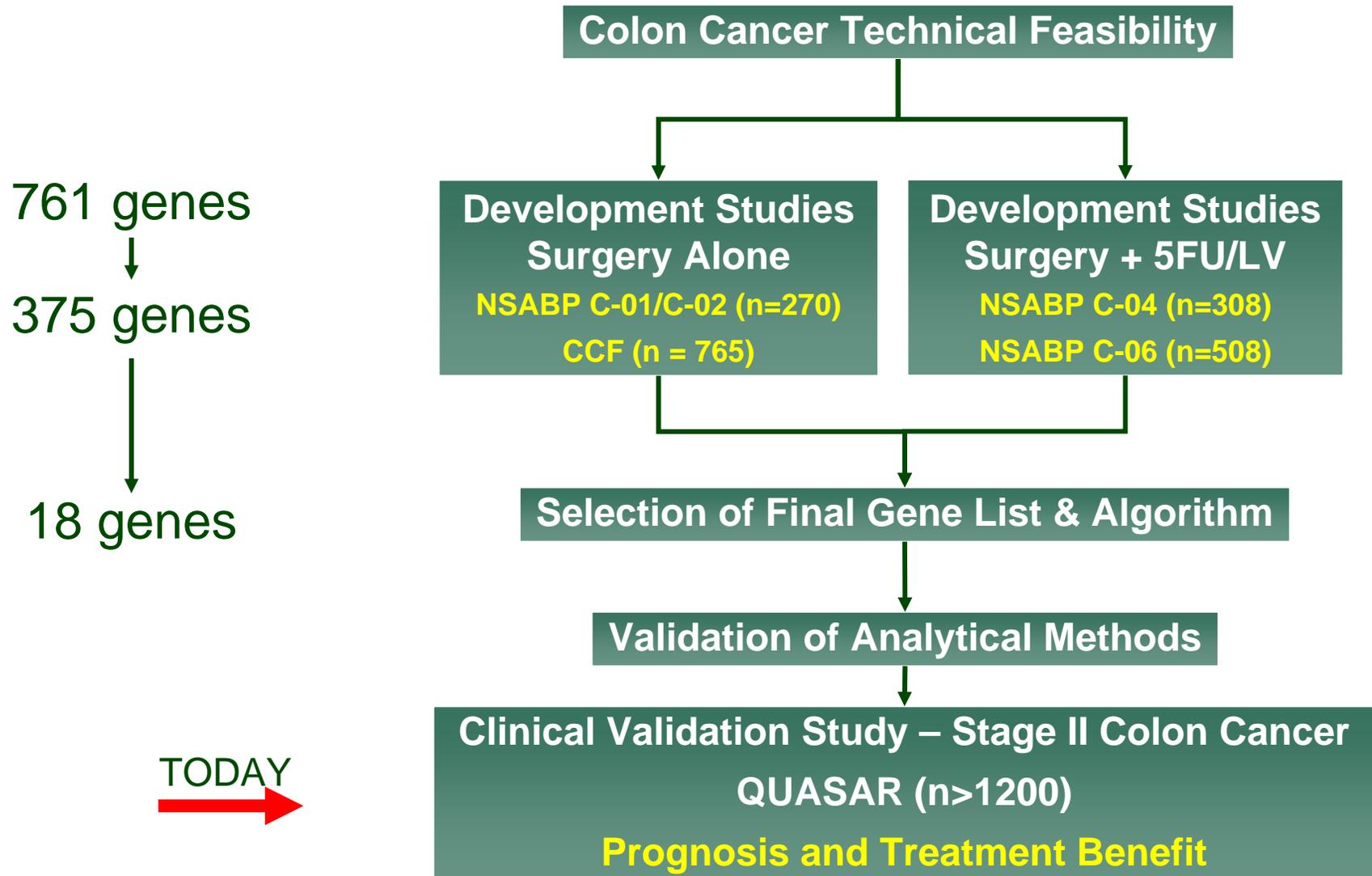
Development and Validation of an 18-Gene RT-PCR Colon Cancer Assay



- Parallels strategy used for 21-gene Oncotype DX breast cancer assay
- Larger size and number of Development Studies (total n=1,851) enable identification of genes for prognosis and genes for treatment benefit
- Clinical Validation of final assay in a large, prospectively designed independent study



Development and Validation of an 18-Gene RT-PCR Colon Cancer Assay



Colon Cancer Clinical Studies



Study	Type	No. Pts	No. Genes	Stage
NSABP C-01/C-02	Exploratory	270	761	II/III
NSABP C-04	Exploratory	308	761	II/III
CCF	Exploratory	765	375	II/III
NSABP C-06	Exploratory	508	375	II/III
QUASAR	Prospective	>1200	18	II
Erbix Study (BMS/Imclone)	Exploratory Erbix Treatment	645	103 + K-Ras Mutations	IV

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RT-PCR Assay Throughput



- Currently have >3,000 in-house assays
- Documented performance from older and recent FPET; successful RT-PCR in >95%
- Can readily prepare assays for new candidate genes, for specific gene mutations, for microRNAs, and for splice variants
- Improved methods allow testing of 1,526 candidate genes

What are some challenges in performing “discovery” studies?

"Discovery" Challenge: Some Pre-Analytic Issues

- Time to fixation
- Fixatives
- Block age (degree of RNA degradation)
- Tissue heterogeneity
 - e.g., biopsy cavities must be manually microdissected – SEE USCAP 2009, Baehner et al

“Discovery” Challenge: Some Analytic Issues

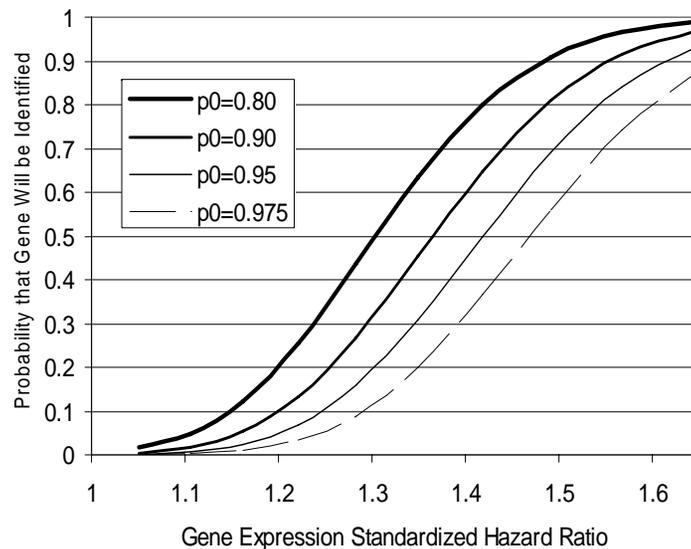


- Non-standardized assays (e.g. HER2)
- Discordances between platforms (e.g. arrays vs RT-PCR)
- Limits of quantitation – assay performance depends on level of RNA
- Intra- and inter-laboratory variability
- Control of reagents, instrumentation, and processes
- Simultaneous QC control of multiple analytes (multiple comparisons)

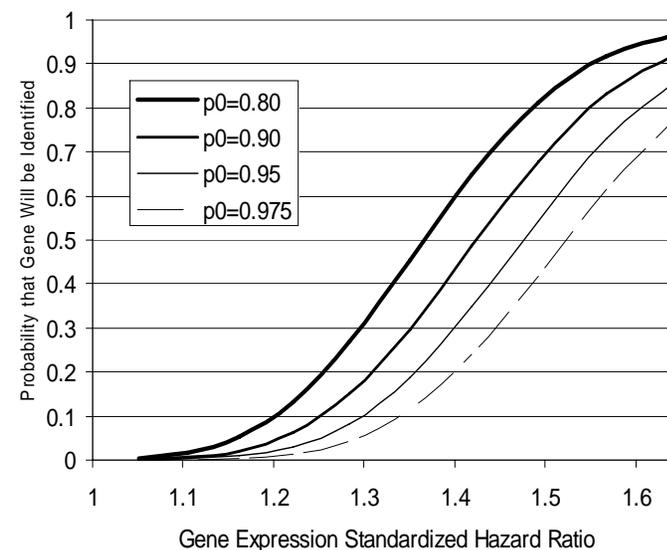
“Discovery” Challenge: The Effect of Many Low-Association Genes on the Power to Identify High-Association Genes*

- Lots of no-association/low-association genes hurt identification power!
- Lower power means low probability of finding the “winners”
- Proportion of null genes impacts identification power

FDR = 0.2



FDR = 0.1



*M. Crager. Prospective calculation of identification power for individual genes in analyses controlling the false discovery rate

“Discovery” Challenge: Resources and Incentives



- Talent – a “new” science
- Time
- Money
- Teamwork – The Most Important Ingredient

“Discovery” Challenge: Not Losing Sight of Clinical Relevance



- “Discovery” studies must not only find genes, they must also provide evidence concerning clinical relevance
 - Comparison to standard assays and co-variates
 - Not just a p-value; must be relevant to clinical decision-making
- Some clinical studies may be better suited to use for clinical validation than for “discovery”
- Avoiding the temptation to quickly do what is convenient is hard

“Discovery” Challenge and Opportunity: Speed of Technology Innovation



2001 Slide: Cost to Sequence an Individual Genome?



The Whole Genome

- 2000 \$300,000,000
- 2010 \$9,375,000
- 2020 \$292,968
- 2030 \$9,155
- 2040 \$286

If costs cut in half every 24 months

Starting from \$0.10 per base

2009 Slide: Cost to Sequence an Individual Genome?



The Whole Genome

• 2000	\$300,000,000
• 2010	\$9,375,000
• 2020	\$292,968
• 2030	\$9,155
• 2040	\$286
• 2015	\$100-1000

Should NCI increase efforts to investigate the genomics of tissue samples from clinical trials?

Should NCI increase efforts to investigate the genomics of tissue samples from clinical trials?

YES