



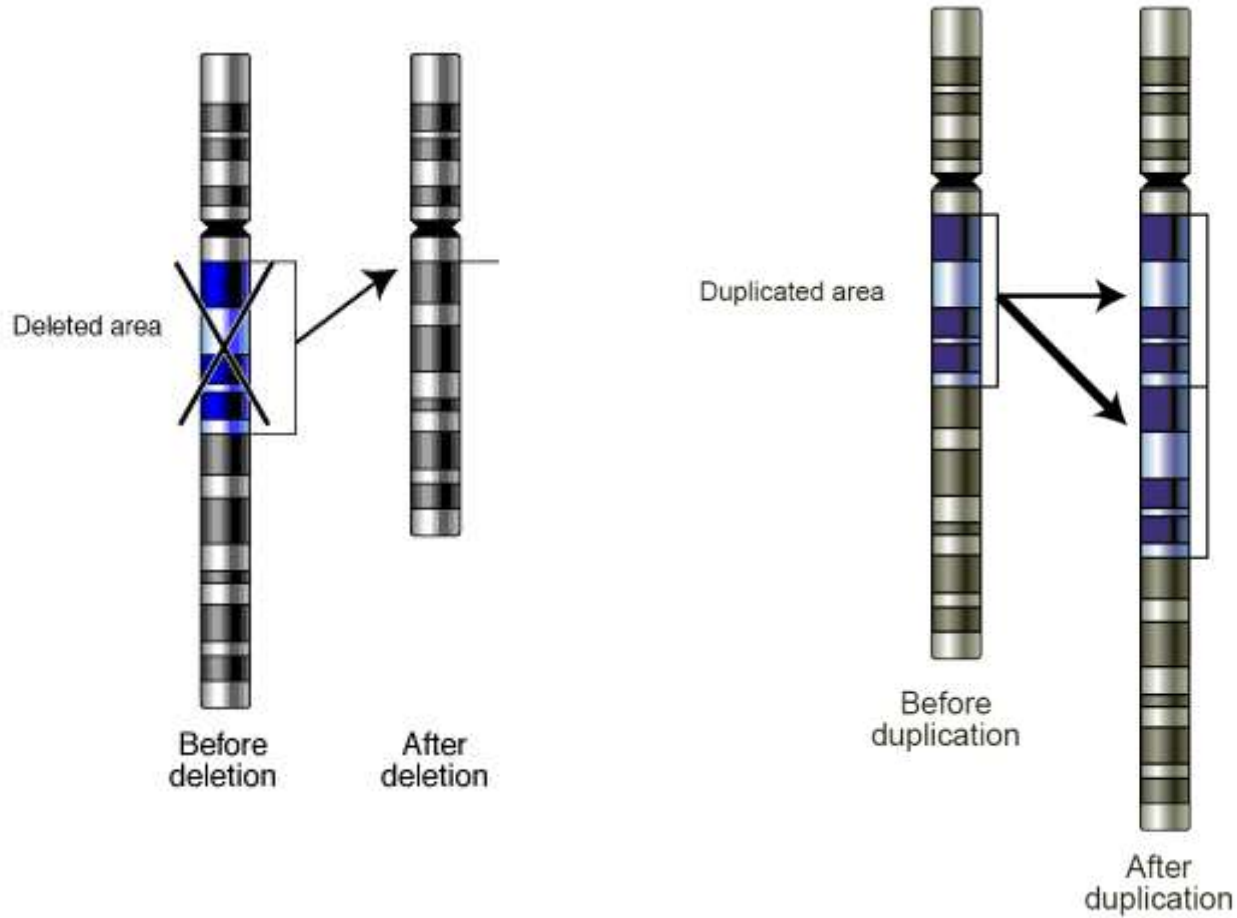
Copy Number: Variation and Alteration Analysis Strategies



- Overview of Copy Number
- Experimental Strategies
 - Techniques Used
 - Discovery
 - Validation
- Controls for qPCR
- qBiomarker Assay Pipeline
- Disease-focused Copy Number Profiling
 - qBiomarker Copy Number Arrays
- Data Analysis
- Ordering Information
- Summary & Questions

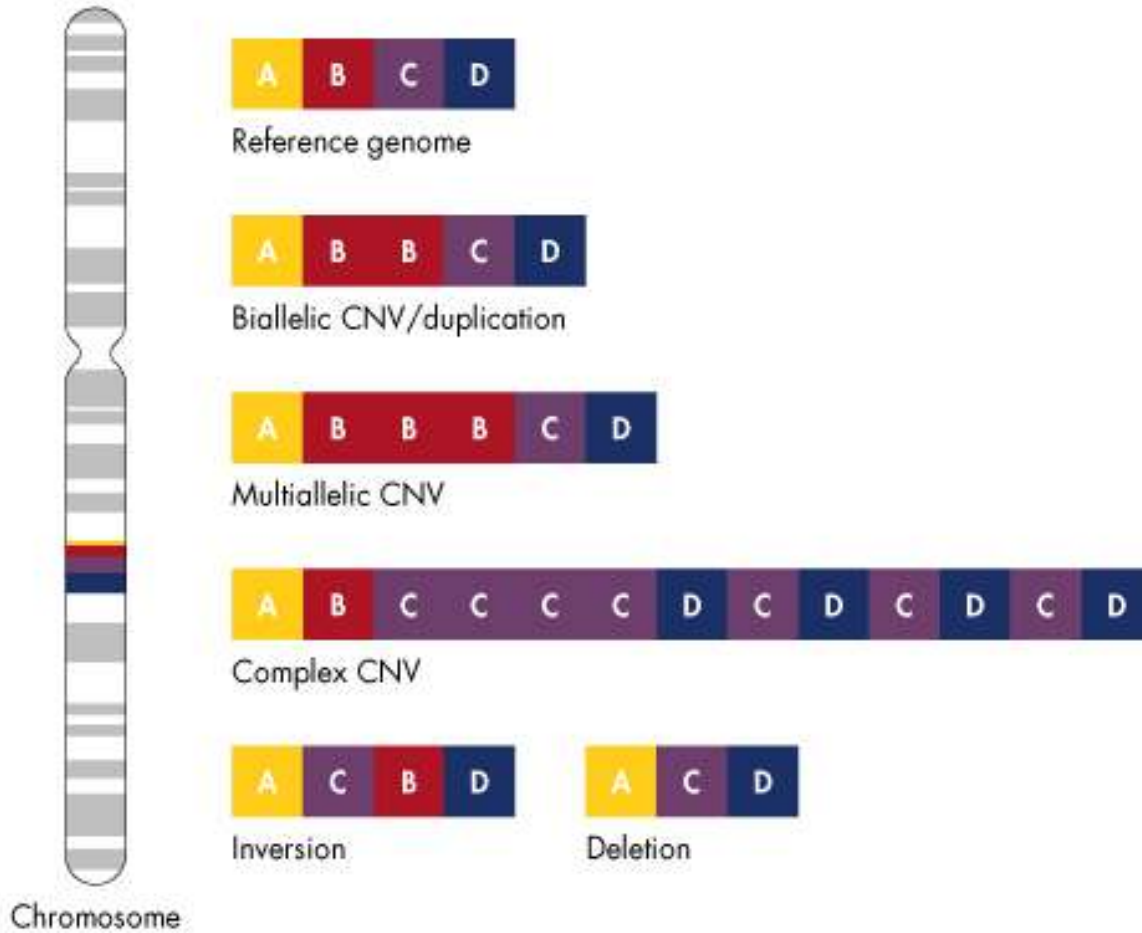
What is Copy Number?

Copy Number changes are Genomic Structural Variations



What is Copy Number?

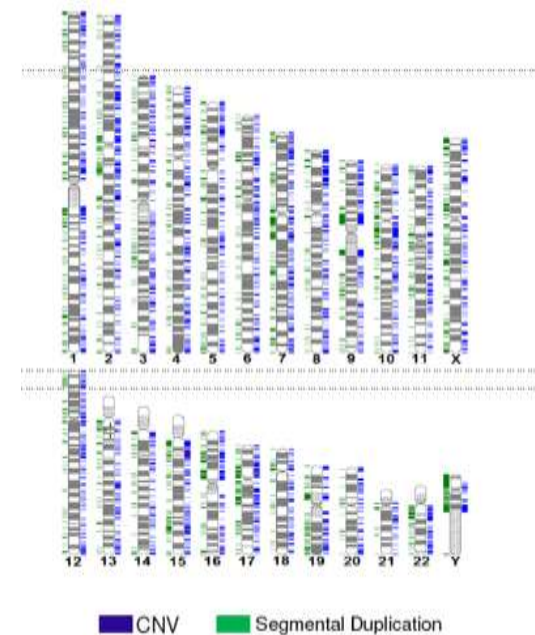
Copy Number changes are Genomic Structural Variations



Increasing Number of CN being detected

CNVs represent a significant source of genetic diversity

- ❑ In August 2011, 81,000 CNVs described
- ❑ In April 2012, 179,000 CNVs described
 - ❑ [Nat Rev Genet.](#) 2011 Aug 18;12(9):628-40
- ❑ Copy number influence Gene Expression & Human Health
 - ❑ CNVs/CNAs associate with Cancer
 - ❑ Prostate
 - ❑ Breast
 - ❑
 - ❑ CNVs associate with complex diseases
 - ❑ Autism
 - ❑ Schizophrenia
 - ❑ Intellectual disability
 - ❑ Crohn´s disease
 - ❑ Obesity
 - ❑



Facts about Copy Number

- Changes in copy number are frequent and occur semi-randomly throughout the genome.
- Variation accounts for ~ 10% of all observed changes in gene expression
- This variation accounts for roughly 12% of human genomic DNA and each variation may range from about one kilobase (1,000 nucleotide bases) to several megabases in size.
- CNV is not always negative. Example: human salivary amylase gene (AMY1). This gene is typically present as two diploid copies in chimpanzees. Humans average over 6 copies and may have as many as 15. This is thought to be an adaptation to a high-starch diet that improves the ability to digest starchy foods.
- Copy Number Variations (CNV) occur in all cells including the germ line and are heritable.
- Copy Number Alterations (CNA) are acquired changes that occur in somatic cells.

Increasing or decreasing the number of copies of a gene have been associated with pathophysiological conditions.

Interesting Examples:

Breast Cancer:

In some cases primary breast cancer tumor cells show no evidence of HER2/neu amplification, but metastatic circulating tumor cells in those patients do show increased expression.

Copy number as a biomarker for mechanism?

Autism Spectrum:

Phelan-McDermid Syndrome (PMS) is a rare genetic syndrome in which one copy of the q13 portion of chromosome 22 is missing. The neurological and psychiatric phenotypes are due to loss of the SHANK3 gene contained in the q13 portion of chromosome 22. Mutations in the SHANK3 gene also produce a syndrome with the neurological and behavioral aspects of PMS.

Copy Number for Patient Stratification?



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Different Methods for Different Experimental Questions

Pre-Screen



Discovery

- Array CGH
- SNP Chips
- NGS



Validation

- FISH
- qPCR

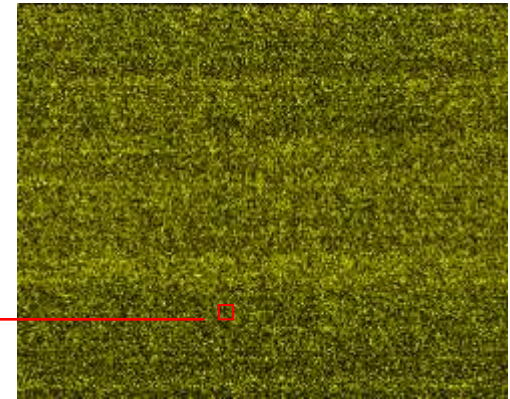


Diagnostic Test

Tumor



Normal



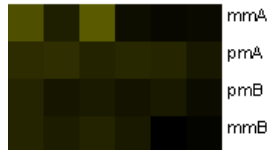
Affymetrix Mapping
250K Sty-I chip
~250K probe sets
~250K SNPs
probe set (24 probes)



CN=1



CN=0



CN>2



Deletion

Deletion

Amplification

CN=2

CN=2

CN=2

Greater DNA copy number ↔ more DNA hybridization ↔ Increased intensity

qPCR Measurement of Copy Number

In this “concept” experiment, a user wishes to measure the number of copies of a gene in three samples. The user orders a primer assay for gene B. Using the qBiomarker Copy Number Assays or Arrays, the user will be able to state the relative change in copy number for the three samples.

Sample 1



1 x

Sample 2



2x

Sample 3



3 x

Most often applied for verification/validation of CNVs/CNAs

High reliability of copy number calling results

- Easy and fast method
- Majority of verification/validation method (>50 %)

How is it applied to Copy Number research?

- The copy number of a reference gene (Ref) is presumed to be consistent in different samples
 - Copy Number of Gene of Interest (GOI) is normalized to reference gene by $\Delta\Delta C_T$ method, with need of
 - Control assay remaining unchanged with CNA of GOI(s)
 - Assay for GOI(s)
 - Control sample(s) with known copy number of GOI(s)
 - Sample of interest with unknown copy number for GOI(s)

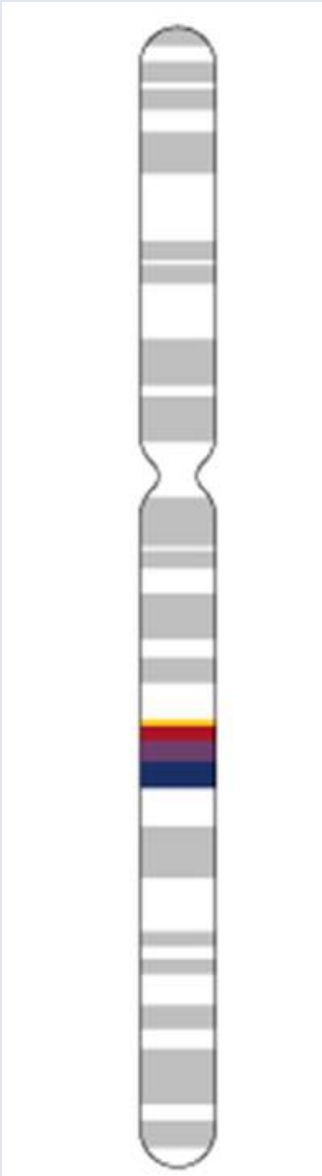


Calculation of copy number gain or loss in sample of interest



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Traditional Control/Normalization Assay

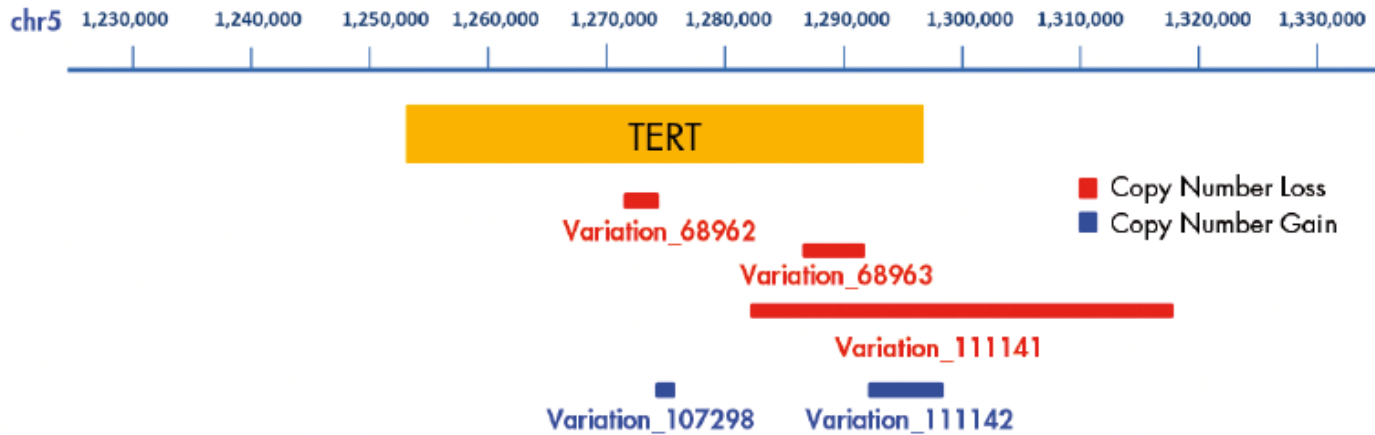


- Control Assay used for normalizing DNA input
 - Greater # of Genomes added = Lower C_T values for GOI
- Control Assay Counts the number of Genomes Present
- Traditional Assays are single-copy genes such as:
 - RNase P
 - TERT (Telomerase)
- General recommendation for using these requires quantifying the amount of DNA in your samples by testing these genes against your samples before your GOI..... Why?
- Potential Pitfalls of Single Copy Reference Assays:
 - 1) Copy Number Events
 - 2) SNP-related effects on qPCR efficiency
 - 3) Unstable Genomes

Single-copy genes are less reliable reference assays

Variations don't discriminate based on you experiment.

TERT, a single copy gene that is commonly used as reference gene for qPCR-based CNV validation, is subject to copy number variation (3x loss, 2x gain, according to the Database of Genomic Variants; DGV).



Different genes don't help escape this experimental consideration

BETA
Database of Genomic Variants
BETA

A curated catalogue of human genomic structural variation

File ▾ Help ▾

Genomic Variants in Human Genome (Build 36: Mar. 2006, hg18): 340 bp from chr14:19,881,070..19,881,410

Browser [Select Tracks](#) [Custom Tracks](#) [Preferences](#)

Search

Landmark or Region:

 Examples: [chr7:71890181..72690180](#), [CFTR](#), [AC108171.3](#), [dgv_3535539](#).
 Data Source:

Scroll/Zoom: << < - Show 341 bp + > >> Flip

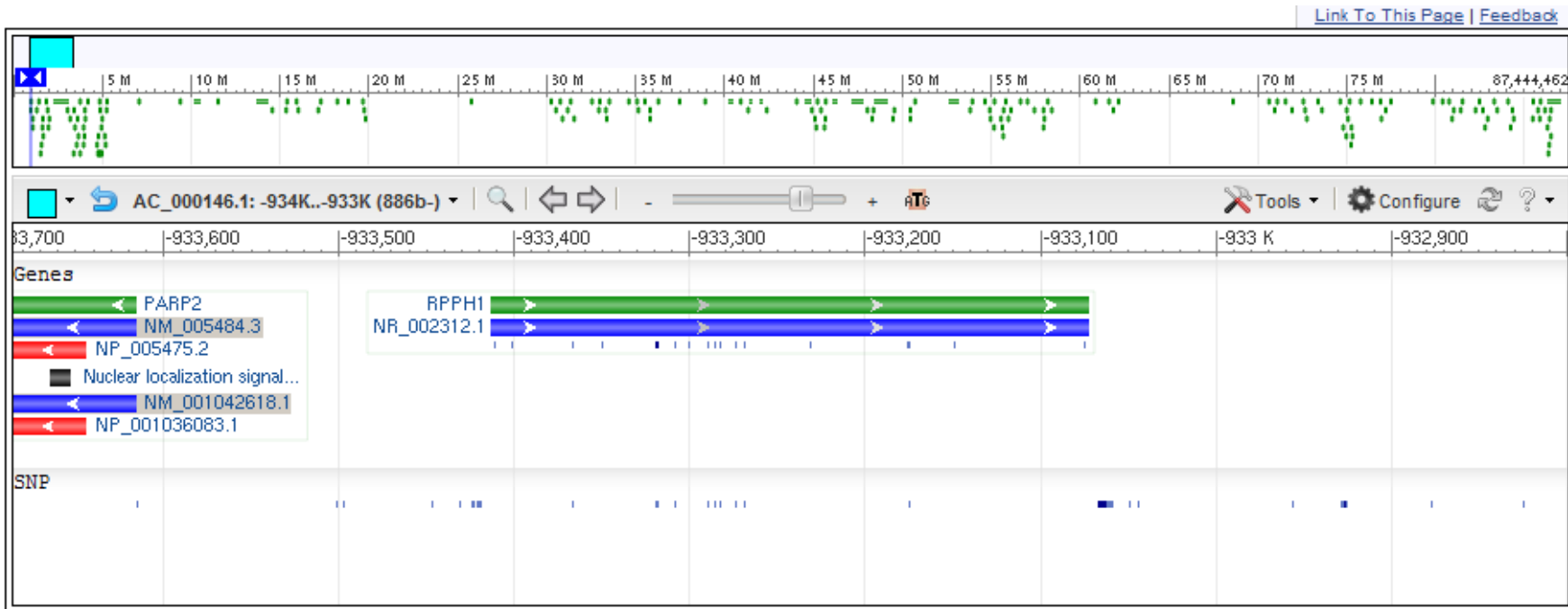
Filter variants
 = + -

Overview

Details

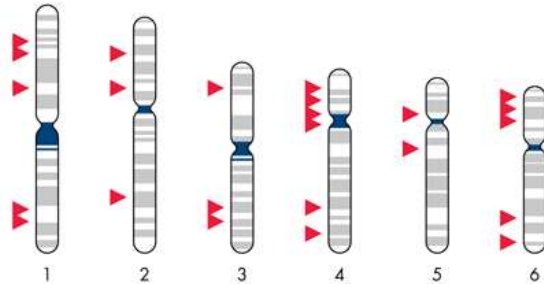
Reference Assay	Copy Number of Reference Gene	C_T change (reference assay)	GOI copy number (real)	GOI copy number (calculated)	Copy Number Call
RNase P	2	0	2	2.00	No Change
	2 + 1	-0.58	2	1.33	Loss
	2 - 1	+1	2	4.00	Gain

SNPs may effect qPCR efficiency and lead to altered C_T values



Genomic map of RNase P (RPPH1) has at least 17 documented SNPs over 341 bases

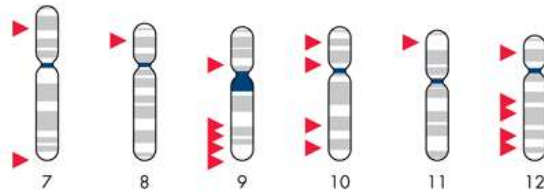
Superior Assay For Input Normalization



The ideal reference assay should fulfill the following criteria:

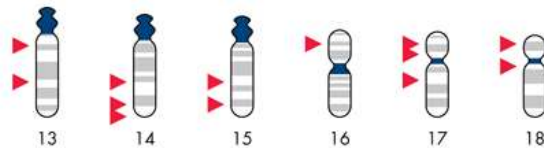
Not be affected by a local change in the genome

-Copy Number or SNP



Copy number:

>20 copies in a diploid genome

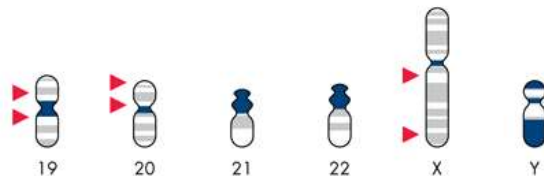


Location distribution:

Located on different chromosomes

≤ 10% copies concentrated on a single chromosome

For copies on the same chromosome, preferably on different arms

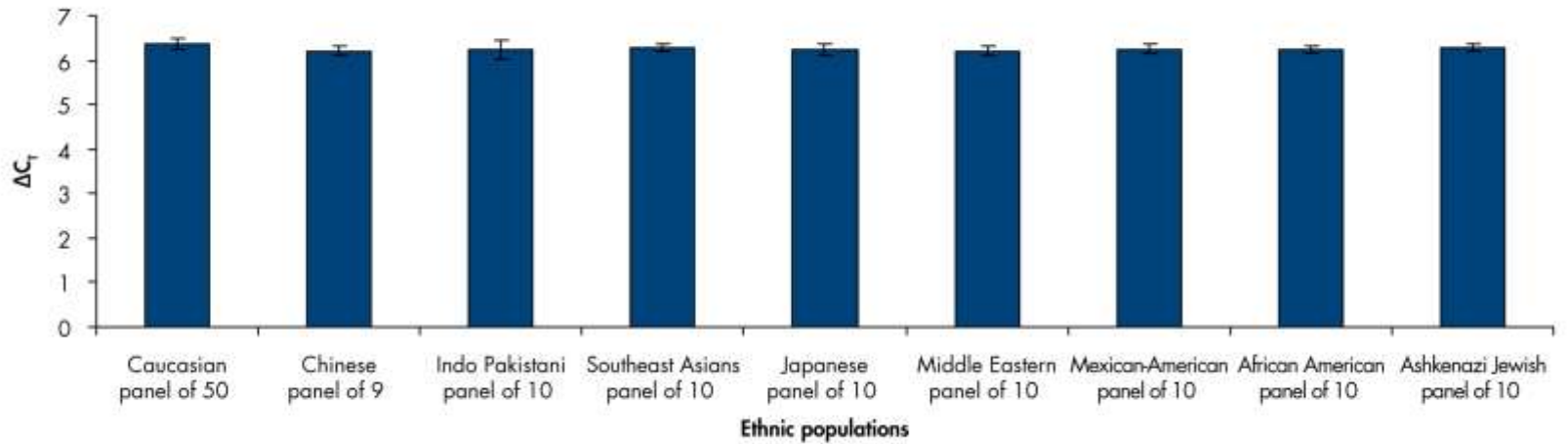


Sequence:

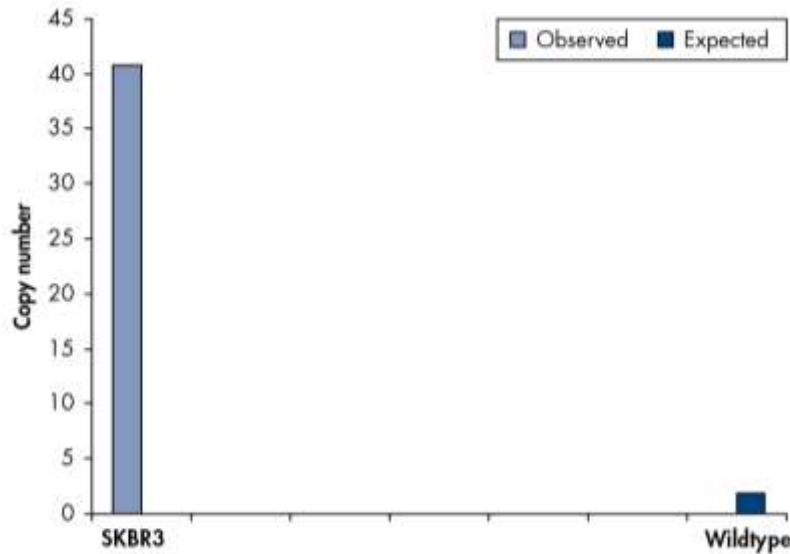
Sequence stable in human genome

Reference Assay	Copy Number of Reference Gene	C_T change (reference assay)	GOI copy number (real)	GOI copy number (calculated)	Copy Number Call
RNase P	2	0	2	2.00	No Change
	2 + 1	- 0.58	2	1.33	Loss
	2 - 1	+ 1	2	4.00	Gain
Multicopy Reference Assay	40	0	2	2.00	No Change
	40 + 1	-0.035	2	1.96	No Change
	40 -1	+ 0.035	2	2.04	No Change

Studies must take into account various human populations



Using a Single Copy Gene as a Reference (RNase P)



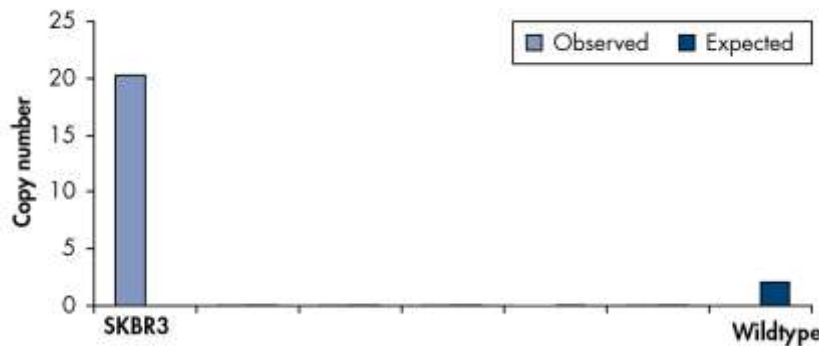
Typical CNA Experimental Setup:

Genomic DNA Samples
 Wildtype (Calibrator sample)
 SKBR3 cells

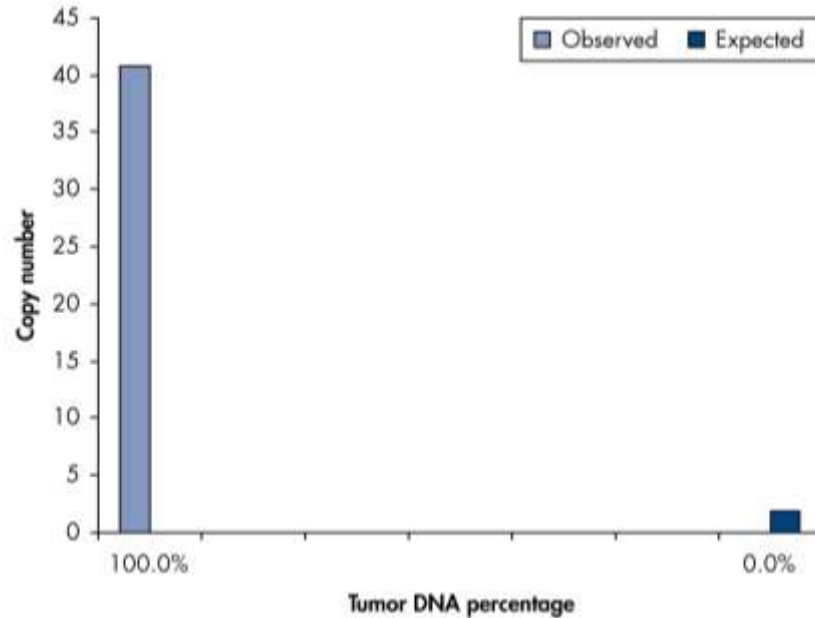
Measure GRB7 (qBiomarker Assay)
 and either RNaseP or Multicopy
 Reference Assay

Result: Calculated Copy Number are
 almost 2-fold different

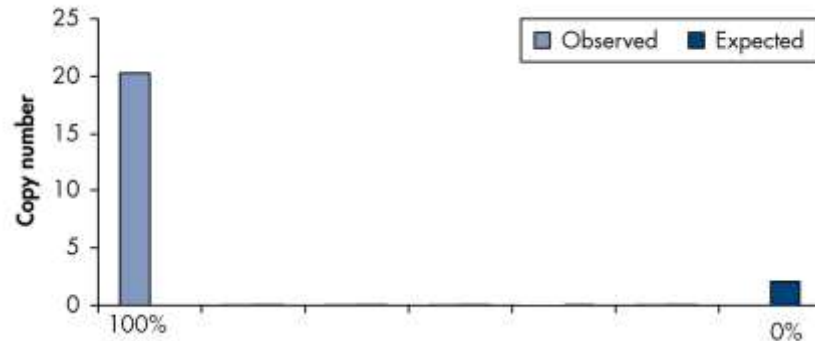
Using Multicopy Reference Assay



Using a Single Copy Gene as a Reference (RNase P)



Using Multicopy Reference Assay



Typical CNA Experimental Setup:

Genomic DNA Samples
 Wildtype (Calibrator sample)
 SKBR3 cells

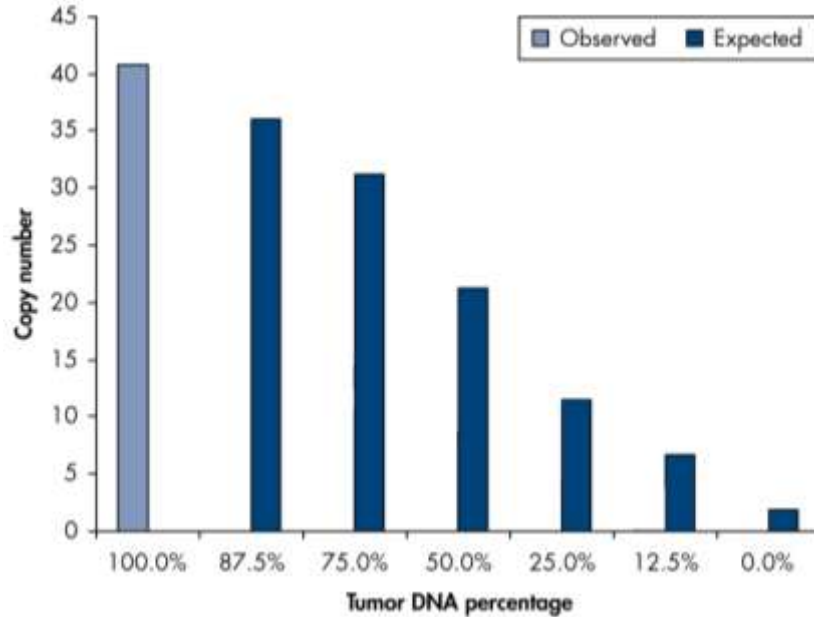
Measure GRB7 (qBiomarker Assay)
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 Reference Assay

Result: Calculated Copy Number are
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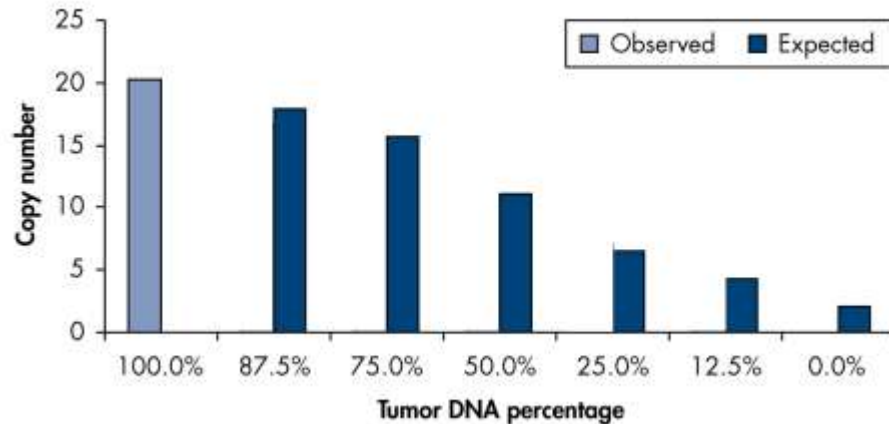
Validation Experiment:

Blend DNA samples at various ratios

Using a Single Copy Gene as a Reference (RNase P)



Using Multicopy Reference Assay



Typical CNA Experimental Setup:

Genomic DNA Samples
 Wildtype (Calibrator sample)
 SKBR3 cells

Measure GRB7 (qBiomarker Assay)
 and either RNaseP or Multicopy
 Reference Assay

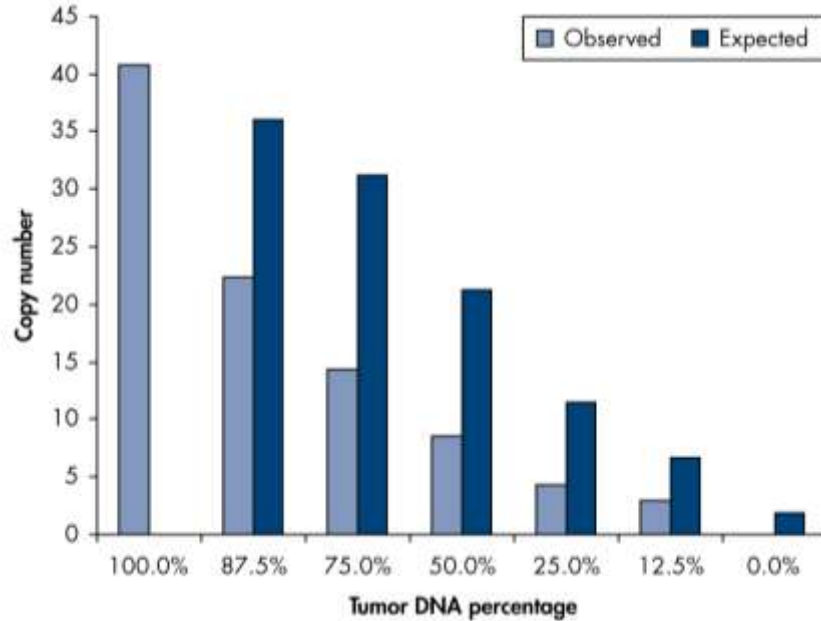
Result: Calculated Copy Number are
 almost 2-fold different

Validation Experiment:

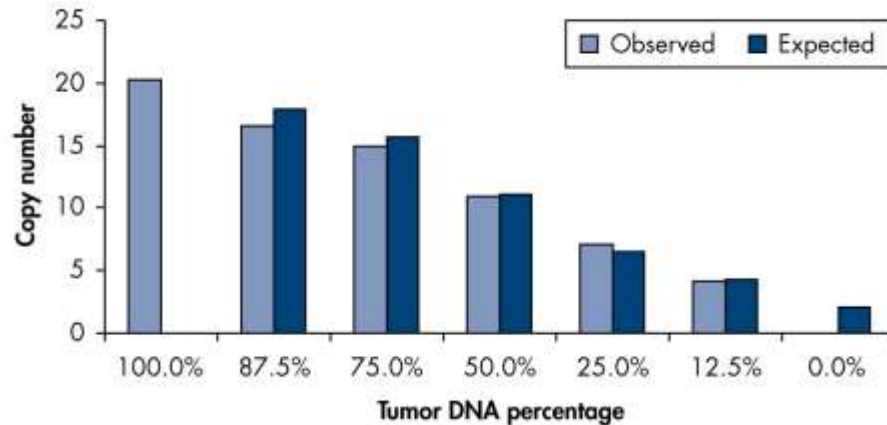
Blend DNA samples at various ratios

Calculate Expected Copy Number

Using a Single Copy Gene as a Reference (RNase P)



Using Multicopy Reference Assay



Typical CNA Experimental Setup:

Genomic DNA Samples
 Wildtype (Calibrator sample)
 SKBR3 cells

Measure GRB7 (qBiomarker Assay)
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 Reference Assay

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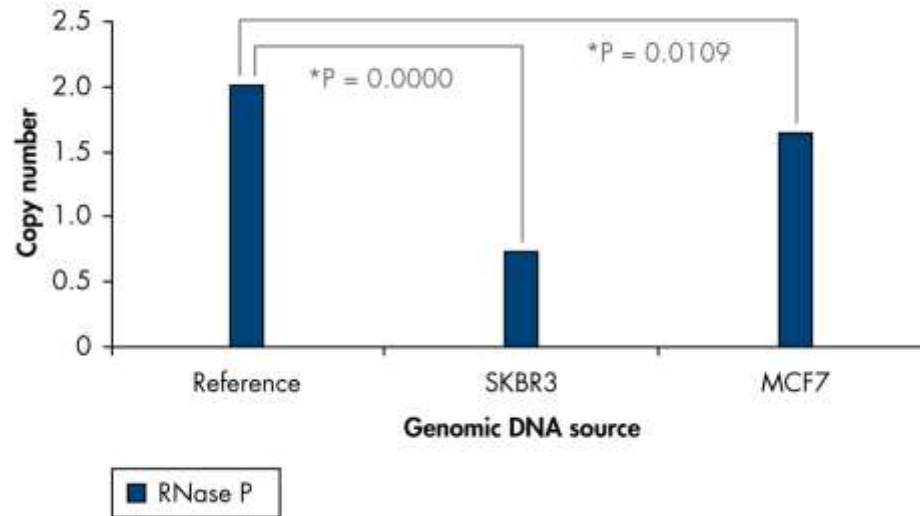
Validation Experiment:

Blend DNA samples at various ratios

Calculate Expected Copy Number

Measure GRB7 (qBiomarker Assay)
 and either RNase P or Multicopy
 Reference Assay

Compare Expected Copy Number to
 Observed Copy Number

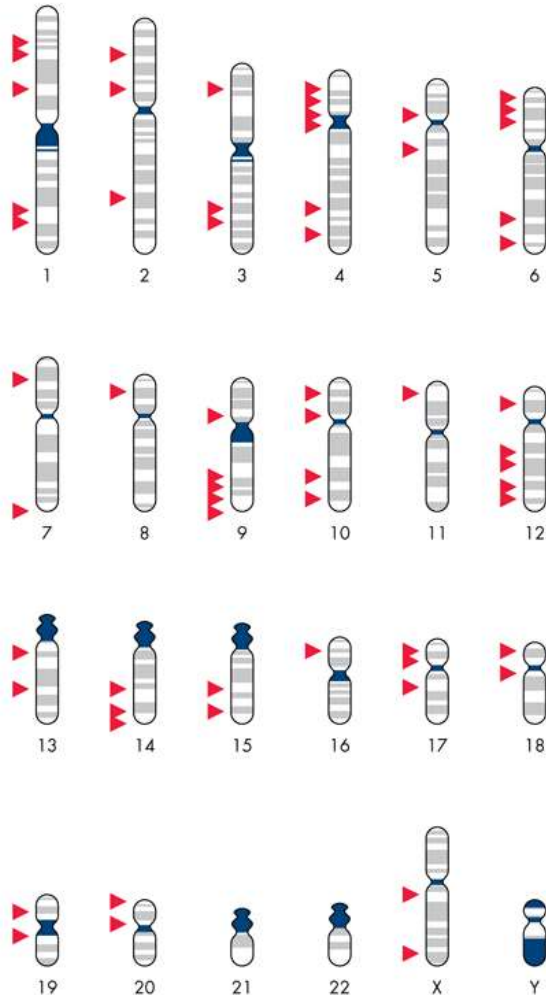


Above experiment uses MRef for Normalization of DNA Input and tests RNase P as the GOI

With cancer cells having increased genomic content (sometime 80 or more chromosomes), single copy genes “appear” like deletions because they are diluted.

Since RNase P, was the denominator in the last experiment the Copy Number value appears artificially high, while the multicopy reference assay better mirrors the amount of DNA input.

Superior Assay For Input Normalization

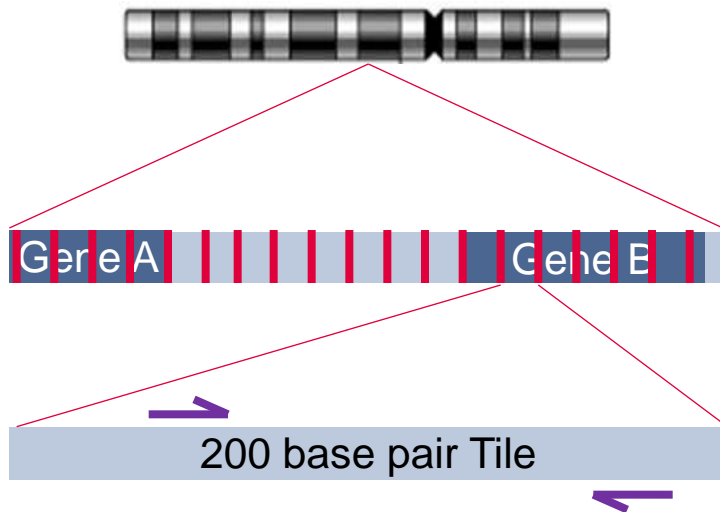


- Multicopy Reference yields stable C_T values in spite of local changes
- SNPs do not significantly effect C_T values of Multicopy Reference Assay
- Large amplifications or deletions of DNA are more consistently normalized
- Stable Normalization Assay across human populations
- Relative DDC_T calculation yields calculated Copy Number



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Tiling the human genome at 200 base pair resolution



Assay Pipeline

1. “Virtually-Cut” the Genome into 200 bp tiles
 - Yielded ~11.6 million designable tiles
2. Design a Primer assay within the tile
3. Lab-test the Primer assay for performance
4. Available as single assays or array content



The screenshot shows the SABiosciences website interface. At the top, there is a navigation bar with links for 'Products and Services', 'Catalog', 'Support', 'Resources', 'Order', and 'About'. A search bar is located in the top right corner. Below the navigation bar, the page title is 'qBiomarker Copy Number Products'. The main content area features a search form with the following fields and options:

- Species: Human, Mouse, Rat
- Gene Symbol:
- Refseq / Transcript:
- NCBI Gene ID:
- DGV ID:
- Chromosome: Start: End:
- Assay ID:
- Search:

Users can search by:

1. Gene Symbol
TP53
2. Refseq/Transcript:
NM_001126116
3. NCBI Gene ID:
7157
4. DGV ID:
Database like COSMIC
5. Chromosome Position:
Chr. 17
7571801-7572001
6. Assay ID:
VPH117-1234567A

Testing Gene Dosage with Aneuploidy Samples

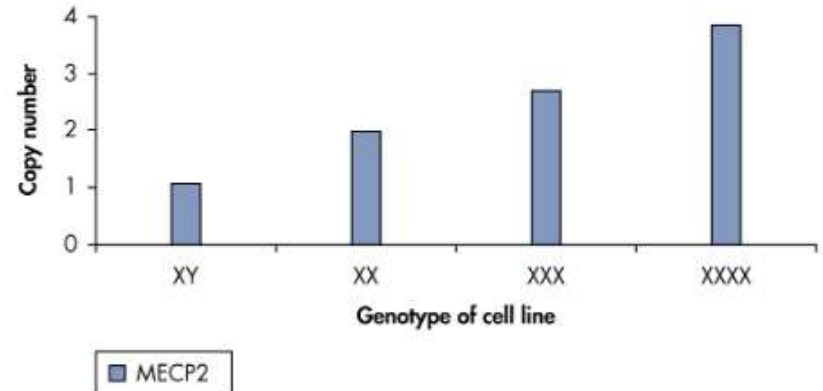
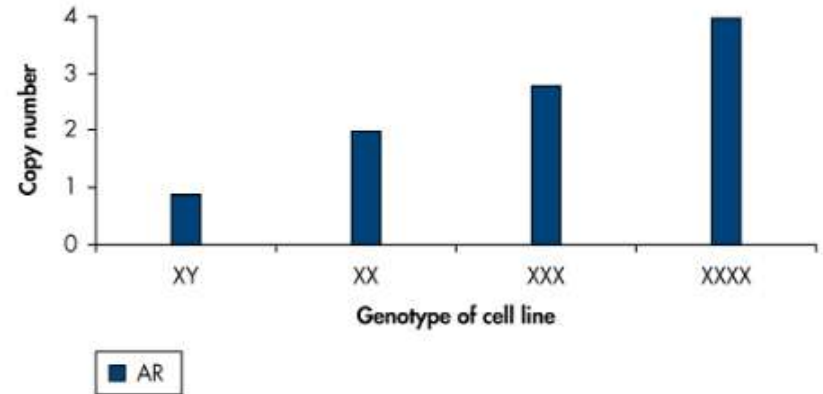
Aneuploidy samples that have different numbers of X chromosomes

Samples acquired from Coriell Cell Repositories

Use qBiomarker Copy Number Assays for Androgen Receptor (AR) and Methyl CpG binding Protein 2 (MECP2) that are single copy genes on the X chromosome

Use qBiomarker Multicopy Reference Assay to normalize for DNA Input

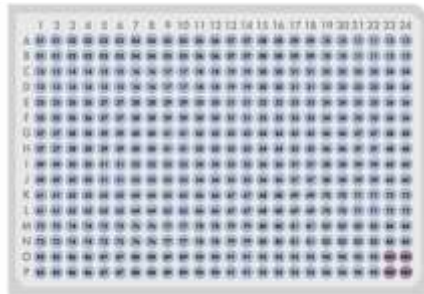
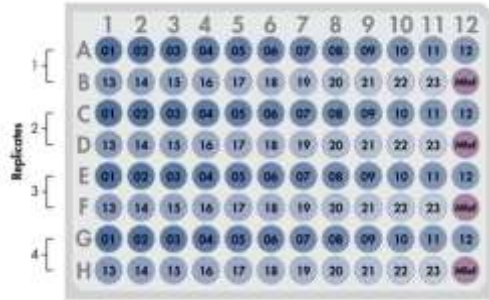
Use DDC_T to calculate relative copy number changes compared to the XX sample





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Profile disease or pathway-focused copy number profiling



Platform-independent

- Compatible with almost any qPCR instrument
- Choose appropriate mastermix for instrument type

23 or 95-genes/loci tested per array

1 sample per PCR plate/ring

Gene Selection

- Arrays by disease
- Arrays by pathway
- Arrays by functional gene classes

Diseases/Disorders Associated with CNA	Diseases/Disorders Associated with CNV	Pathway-Focused	High Content Arrays
<u>Breast Cancer</u>	<u>Birth Defects</u>	<u>Kinases & Phosphatases</u>	<u>Oncogenes & TSGs</u>
<u>Lung Cancer</u>	<u>Intellectual Disability</u>	<u>WNT signaling Pathway</u>	
<u>Ovarian Cancer</u>			
<u>Prostate Cancer</u>			
<u>Gastric Cancer</u>			
<u>Glioma</u>			
<u>Pancreatic Cancer</u>			

Custom Copy Number Arrays are also available



qPCR Primer Assays are pre-plated

1 sample per PCR plate/ring

23 or 95-genes/loci tested per array

- each # in a well represents a different gene

1 qBiomarker Copy Number Assay per Gene

- Assays cover exons
- Assay positions are close to the center of a gene

1 qBiomarker Multicopy reference assay (MRef)

Used for DNA input normalization

Calculations by DDC_T

Assay in technical quadruplicate for accuracy

Description for the content of each array

Breast Cancer Copy Number PCR Array

Human

Human Breast Cancer Copy Number PCR Array

[Price & Ordering](#)

HOW IT WORKS

[Data Analysis](#)

The Human Breast Cancer qBiomarker Copy Number PCR Array profiles the copy number of 23 genes reported to undergo frequent genomic alterations in human breast tumor DNA. DNA copy number changes in breast cancer cells have prognostic impact. For example, HER2 amplification and overexpression defines the HER2+ subgroup of breast cancer patients and is both a prognostic marker for poor outcome and a predictive marker for response to anti-HER2 targeted therapies. The genes on the array encode receptors, kinases, phosphatases, and transcription factors that regulate processes such as the cell cycle, growth factor signaling, and cell adhesion. Genes were chosen from the most frequently amplified or deleted genes relevant to oncogenic pathways and breast cancer biology based on the primary literature and public databases. This array may serve as a useful tool to help classify breast cancer samples by genotype and help verify breast cancer phenotypic biomarkers. The array analyzes each gene in each sample in quadruplicate and includes a stable multi-copy reference assay for accurate copy number determination via appropriate DNA input normalization. The simplicity of the product format and operating procedure allow routine and reliable copy number profiling in any research laboratory with access to a real-time PCR instrument.

The qBiomarker Copy Number PCR Arrays are intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

96-well Plate, 384-well (4 × 96) Plate, and 100-well Disc formats are available.

Functional Gene Grouping [How It Works](#) [Manual & Resources](#) [Reagents & Software](#)

[Modify this Array](#) [Gene Table](#)

Functional Gene Grouping [How It Works](#) [Manual & Resources](#) [Reagents & Software](#)

[Modify this Array](#) [Gene Table](#)

ER+PR+, HER2: CCND1

Gain in ER+ Tumor: AURKA

HER2+: ERBB2

Inflammatory Breast Cancer: ERBB2, MTDH, MYC, PTK2, RB1

Lapatinib Sensitivity: CDKN2A, EGFR, ERBB2

AKT & PI-3-Kinase Signaling: AKT1, PP4PDC1B, PTEN

Apoptosis: BCL2L1, MTDH

Cell Adhesion & Cytoskeleton: CSDM1, PAK1, PTK2

Cell Cycle: AURKA, BCL2L1, CCND1, CDK4, CDKN2A, RB1

DNA Repair: C11orf93 (EMSY), TOP2A

Drug Metabolism: BCHE

NFkB Signaling: MTDH

Receptor Tyrosine Kinases: EGFR, ERBB2, FGFR1, FGFR2

Transcription Factors and Co-Factors: MTDH, MYC, NCOA3, RB1, TFDP1

[Modify this Array](#) [Gene Table](#)

Technical Assay Details for each array are provided in Gene Table



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qBiomarker Copy Number PCR Array Human Breast Cancer (VAHS-0055Z)

Need more information about qBiomarker Copy Number PCR Arrays? Please [Visit qBiomarker Copy Number Home Home](#) or [Email Technical Support](#).

Array Layout											
AKT1	AURKA	BCHE	BCL2L1	C11orf30	CCND1	CDK4	CDKN2A	CSMD1	EGFR	ERBB2	FGFR1
FGFR2	MTDH	MYC	NCOA3	PAK1	PPAPDC1B	PTEN	PTK2	RB1	TFDP1	TOP2A	Mref
AKT1	AURKA	BCHE	BCL2L1	C11orf30	CCND1	CDK4	CDKN2A	CSMD1	EGFR	ERBB2	FGFR1
FGFR2	MTDH	MYC	NCOA3	PAK1	PPAPDC1B	PTEN	PTK2	RB1	TFDP1	TOP2A	Mref
AKT1	AURKA	BCHE	BCL2L1	C11orf30	CCND1	CDK4	CDKN2A	CSMD1	EGFR	ERBB2	FGFR1
FGFR2	MTDH	MYC	NCOA3	PAK1	PPAPDC1B	PTEN	PTK2	RB1	TFDP1	TOP2A	Mref
AKT1	AURKA	BCHE	BCL2L1	C11orf30	CCND1	CDK4	CDKN2A	CSMD1	EGFR	ERBB2	FGFR1
FGFR2	MTDH	MYC	NCOA3	PAK1	PPAPDC1B	PTEN	PTK2	RB1	TFDP1	TOP2A	Mref

Gene Table						
Position	Entrez Gene ID	Gene Symbol	Chromosome	Start	End	Assay Cat #
A01	207	AKT1	14	105239588	105239716	VPH114-0543285
A02	6790	AURKA	20	54962801	54963000	VPH120-0274815
A03	590	BCHE	3	165548601	165548800	VPH103-0827744
A04	598	BCL2L1	20	30309647	30310021	VPH120-0316977
A05	56946	C11orf30	11	76157983	76158052	VPH111-0683422
A06	595	CCND1	11	69456082	69456279	VPH111-0682610
A07	1019	CDK4	12	58145283	58145500	VPH112-0675846
A08	1029	CDKN2A	9	21994001	21994200	VPH109-0109971
A09	64478	CSMD1	8	4851854	4851938	VPH108-0732005
A10	1956	EGFR	7	55224452	55224525	VPH107-0798903
A11	2064	ERBB2	17	37871993	37872192	VPH117-0412044
A12	2260	FGFR1	8	38318401	38318600	VPH108-0191593



Need more information about qBiomarker Copy Number PCR Arrays? Please [Visit qBiomarker Copy Number Home Home](#) or [Email Technical Support](#).

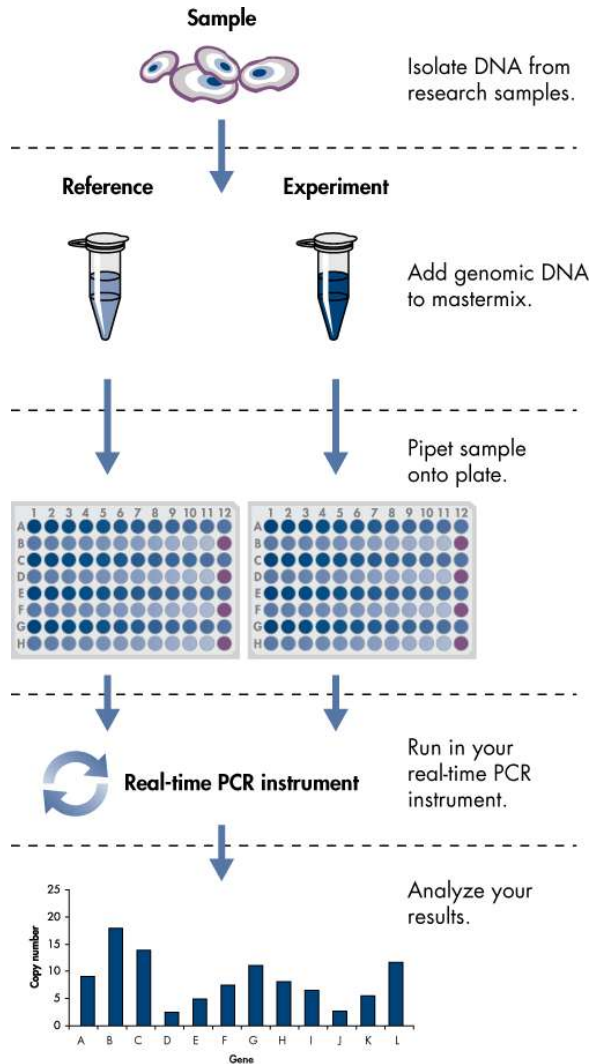
Array Layout



Gene Table

Position	Entrez Gene ID	Gene Symbol	Chromosome	Start	End	Assay Cat #
A01						
A02						
A03						
A04						
A05						
A06						
A07						
A08						
A09						
A10						
A11						
A12						

Profile disease or pathway-focused copy number profiling



1

Isolate genomic DNA from fresh, frozen or FFPE samples using QIAamp or DNeasy kits recommended in the handbook.

2

Add qBiomarker SYBR mastermix to genomic DNA.

- 400 – 1000 ng fresh/frozen DNA
- 800 – 1200 ng FFPE DNA

3

1 sample goes on 1 plate
Assays in technical quadruplicate

4

Standard 40 cycle PCR on most real-time Thermocyclers.

5

Upload C_T values to Data Analysis Webportal

Initial Screen for Copy Number Changes

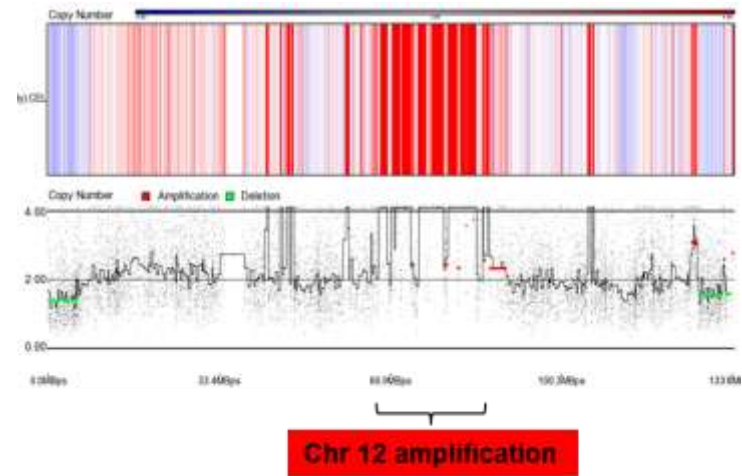
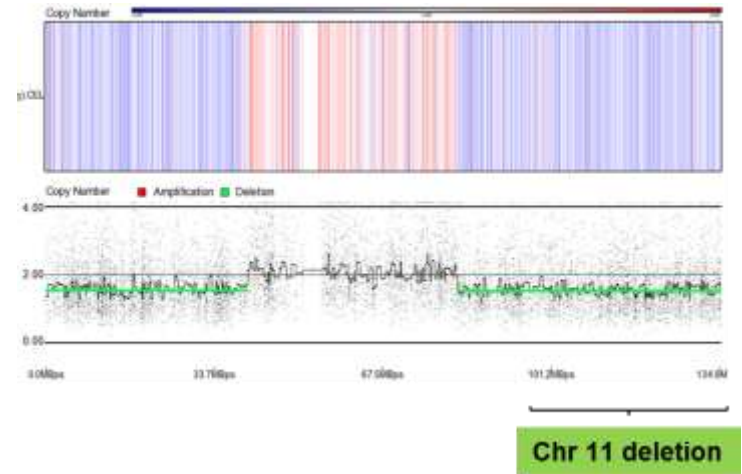
Thirty (30) liposarcoma samples were tested by aCGH for copy number events

Results from one of those samples (T50) is shown

- Analysis with Partek software
- Deletions on Chromosome 11
- Amplifications on Chromosome 12

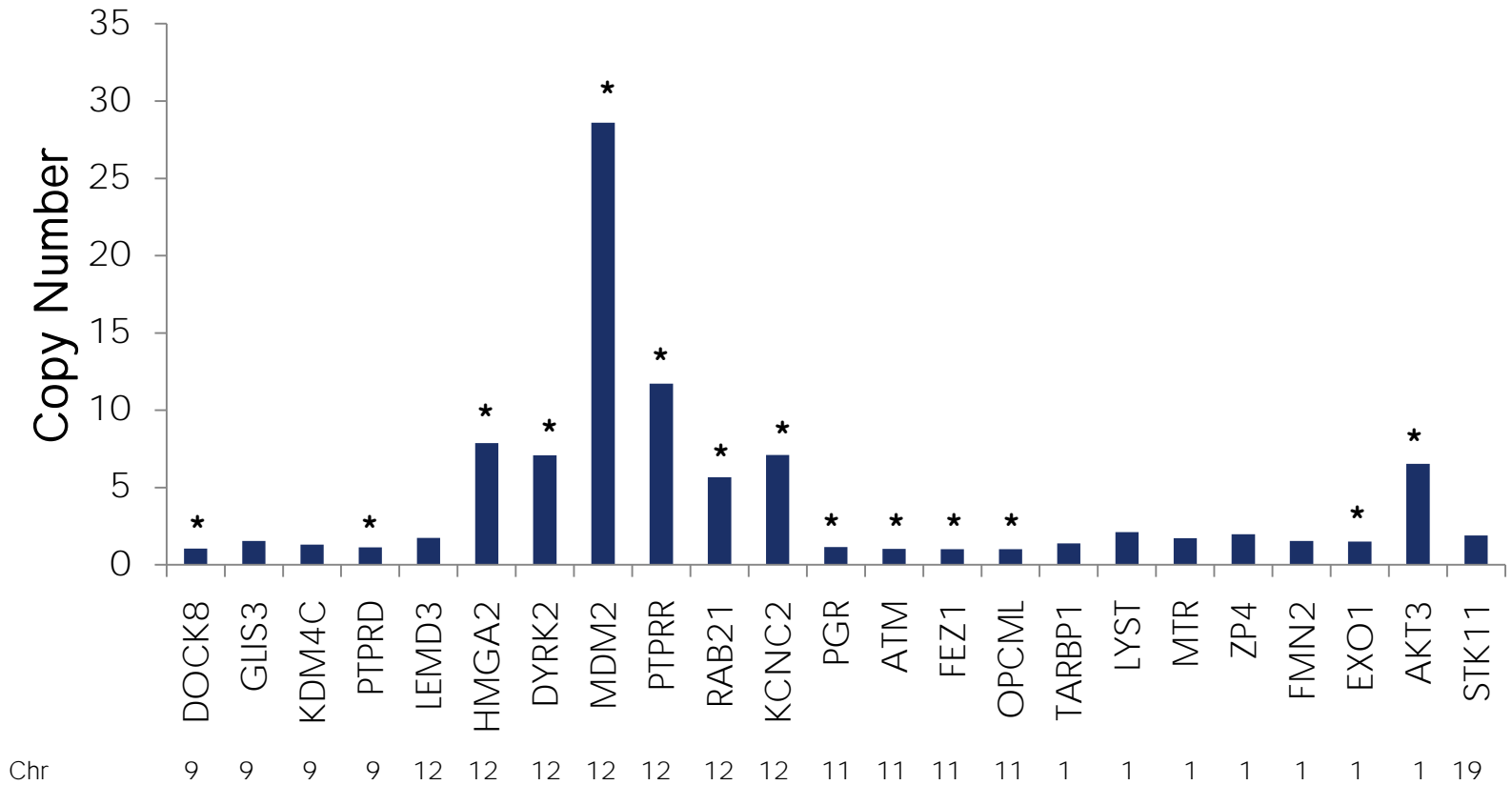
Initial screen yielded a list of 23 genes with copy number changes.

All samples were re-tested using Custom Copy Number PCR Array



Data courtesy of Kara Pascarelli and Dominique Broccoli, Memorial University Medical Center, Savannah, GA, USA; and Lesley Ann Hawthorne, Medical College of Georgia, Georgia Health Sciences University, Augusta, GA, USA)

Copy Number PCR Array Data for Sample T50



aCGH result

qBiomarker result



Data courtesy of Kara Pascarelli and Dominique Broccoli, Memorial University Medical Center, Savannah, GA, USA; and Lesley Ann Hawthorne, Medical College of Georgia, Georgia Health Sciences University, Augusta, GA, USA)



- Overview of Copy Number
- Experimental Strategies
 - Techniques Used
 - Discovery
 - Validation
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- qBiomarker Assay Pipeline
- Disease-focused Copy Number Profiling
 - qBiomarker Copy Number Arrays
- **Data Analysis**
- Ordering Information
- Summary & Questions

qBiomarker Data Analysis version 1.1

Upload Data

Analysis Setup

Analysis

Plots & Charts

Export Data

Experiment Performed Using:

<input type="radio"/> Cataloged Copy Number PCR Array	
<input type="radio"/> Custom Copy Number PCR Array	
<input type="radio"/> Individual Copy Number Assays	

Plate Format: Rotor-Gene Q OtherFile:

* File must be a MS Excel Sheet (in .XLS format, not .XLSX).

Excel Templates for Formatting your Experimental Data:

[Cataloged PCR Array](#)[Custom PCR Array](#)[Single/Multi-Gene Assays](#)

New to Copy Number Analysis? Learn more by:

- [Taking a test run](#)
- [Attending a webinar](#)

Notes:

1. Please note that you must complete all of your work with the PCR Array Data Analysis Web Portal in the same session. Your data is not stored on a server, so all work is lost once the session (or your web browser) is closed. Be sure to export all processed data and results to an Excel file saved on your local computer.
2. Please set your screen resolution to **1024 X 768** or greater, if possible.
3. Turn off any window pop-up blockers. The software will launch separate windows for viewing the plots and charts.

Instructions



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What do you need to measure copy number by qPCR?

DNA Isolation

- [QIAamp DNA Mini Kit \(51304 or 51306\)](#)
- [DNeasy Blood & Tissue Kit \(69504 or 69506\)](#)
- [QIAamp DNA FFPE Tissue Kit \(56404\)](#)

Whole Genome Amplification (optional)

- [REPLI-g](#)

Individual Assays

- [qBiomarker Copy Number PCR Assays](#)
- [qBiomarker Multicopy Reference PCR Assay](#)
- [qBiomarker SYBR Green Mastermix](#) (based on instrument)

Copy Number Profiling

- [qBiomarker Copy Number PCR Arrays](#)
- [Custom qBiomarker Copy Number PCR Arrays](#)
- [qBiomarker SYBR Green Mastermix](#) (based on instrument)

qBiomarker Data Analysis



Use SABiosciences.com

The screenshot shows the SABiosciences.com website interface. At the top left is the QIAGEN logo and the text "SABiosciences A QIAGEN Company". To the right are links for "Live Chat", "QIAGEN Website", "Quick Order", "Online Seminar", "Contact", and "My Account". A search bar contains the text "Enter Search Term" and a "Search" button. Below this is a dark blue navigation bar with the following menu items: "Products and Services", "Catalog", "Support", "Resources", "Order", "About", and "View Cart".

On the left side, there is a sidebar with a "Register for Special Offer" form and a "Research Area" section. The "Research Area" section includes a "Complete Array List" link and a "Browse by Pathway" section with the following categories: Apoptosis, Biomarkers, Cell Cycle, Cytokine & Inflammation, ECM & Adhesion, Neuroscience, Signal Transduction, Stem Cell & Development, and Toxicology & Drug ADME. Below this is a "Browse by Disease" section with categories: Cancer, Cardiovascular Diseases, CNS Disorders, Immune Disorders, Infectious Diseases, and Metabolic Diseases. At the bottom of the sidebar is a "Browse by Epigenetics" link.

The main content area features a large banner for "Mutation identification and validation" with the sub-heading "qBiomarker Somatic Mutation PCR Arrays" and an image of a DNA double helix. Below the banner is a grid of product categories:

- Gene Expression**: PCR Arrays, Mastermix, qPCR Primer Assays, MicroRNA Arrays
- Cell Signaling**: Lentiviral and DNA-based Pathway Reporters
- Epigenetics**: Methylation Analysis, Chromatin IP, MicroRNA, Histone Modification
- RNA Interference**: High potency, guaranteed shRNA plasmids and siRNA
- Gene Regulation**: Transcriptome PCR Arrays identify miRNAs or proteins that regulate gene expression
- Protein Analysis**: Multi-Analyte ELISArray, Single-Analyte ELISArray
- Service Core**: Gene expression Analysis, FFPE, Illumina Genotyping and SNP Analysis
- New! Mutation Analysis**: Pathway-Focused qPCR Analysis
- Induced Pluripotent Stem Cell**: iPS Cell qPCR Arrays, iPS Reprogramming Factors
- New! Copy Number Analysis**: Arrays and Genome-wide Assays for detecting Copy Number Variations and Alterations

On the right side, there is a vertical list of product categories with corresponding images:

- Pathway Maps (<100)**: Download PowerPoint Slides
- Real-time PCR primers**: Genome-wide search
- RNAi**: Genome-wide shRNA and siRNA
- MicroRNA primers**: Reliable and specific qPCR
- Transcription factor search**: Discover which TFs regulate your gene
- DNA methylation primers**: Simple qPCR assays
- Copy Number Assays**: Genome-wide search

Three red arrows are overlaid on the image: one pointing from the "Products and Services" menu item to the "Mutation identification and validation" banner, one pointing from the "New! Copy Number Analysis" category to the "Copy Number Assays" category on the right, and one pointing from the "Copy Number Assays" category to the right edge of the page.



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Summary

From Discovery to Validation

Copy Number Alterations/Variations are important biological changes with ramifications for human health

Experimental Solutions for Copy Number Determination

Discovery experiments

- Arrays, beadchips, etc.

Validation / Pre-Screen / Hypothesis-driven experiments

- qPCR

Better normalization assays yield better data

- Multicopy reference assay vs. single gene

Bench-validated assays at highest resolution

Copy Number Profiling

- Pathway-focused

- Custom designs

