TaqMan Copy Number Assays, Custom Plus TaqMan Copy Number Assays, and Custom TaqMan Copy Number Assays

- Simplest method available to study copy number variation
- Predesigned human and mouse assays for copy number analysis
  - Human
    - Over 1.6 million assays available for genome-wide coverage
    - Genes (exons, introns, and junctions)
    - Known copy number variations (CNVs)
    - Extragenic/nongene regions
  - Mouse
    - Over 180,000 assays available
    - Gene exon coverage
- Assays available for common vector marker and reporter genes
- Applied Biosystems™ Custom Plus TaqMan™ Copy Number Assays for user-defined human and mouse genomic targets
  - Bioinformatics ensures best possible assay design
  - SNPs and repetitive sequence masking
  - Genome specificity checks
- Custom assays for other targets of interest
  - Submit masked target sequences for assay design
  - Submit primer/probe pair sequences for assay synthesis
- Reference assays for unique human and mouse genomic sequences

Human genomes vary from one another at the genetic level. Some genetic variations are large, structural, chromosomal variations, while others occur at the single-nucleotide level.

Easy to use, robust, and accurate—gold standard Applied Biosystems™ TaqMan™ Assays designed for the detection and quantitation of copy number variation targets.

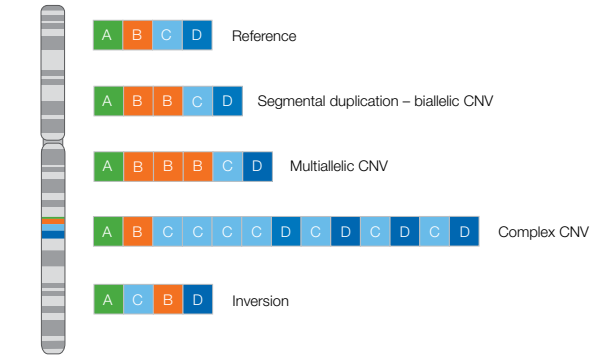

Figure adapted from Estivill et al. (2007) *PLoS Genet.* October; 3(10):e190.

Figure 1. Types of genomic structural changes affecting segments of DNA that lead to different types of variations.

A structural variation that has been described in recent years is copy number variation (CNV). A copy number variation occurs when a DNA segment of 1 kb to several megabases in length is present in variable copy numbers compared to a standard reference genome. There are different types of CNVs, from simple tandem duplications to more complex gains or losses of these sequences at multiple sites throughout the genome (Figure 1). These structural variants are found in all humans as well as other animals and plants.

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A fast and simple method

Genome-wide microarray-based technologies are currently available for CNV analysis, but they are not the optimum platform for target-region or validation needs. Applied Biosystems™ TaqMan™ Copy Number Assays combine TaqMan Assay chemistry with Applied Biosystems™ real-time PCR instruments to form a method for obtaining specific, reproducible, and easy-to-interpret copy number results. This method is fast and simple, and can be completed in hours rather than days. TaqMan Assays are the gold standard for accurate target quantitation, making them ideal for use in microarray follow-up studies. TaqMan Copy Number Assays can also be used to screen specific targets, and the workflow can be automated so that several hundred to thousands of samples can be processed in a single day.

The assays of the TaqMan Copy Number Assays family, including TaqMan Copy Number Assays, Custom Plus TaqMan Copy Number Assays, and Applied Biosystems™ Custom TaqMan™ Copy Number Assays, consist of a FAM™ dye–labeled minor groove binder (MGB) probe and unlabeled PCR primers.

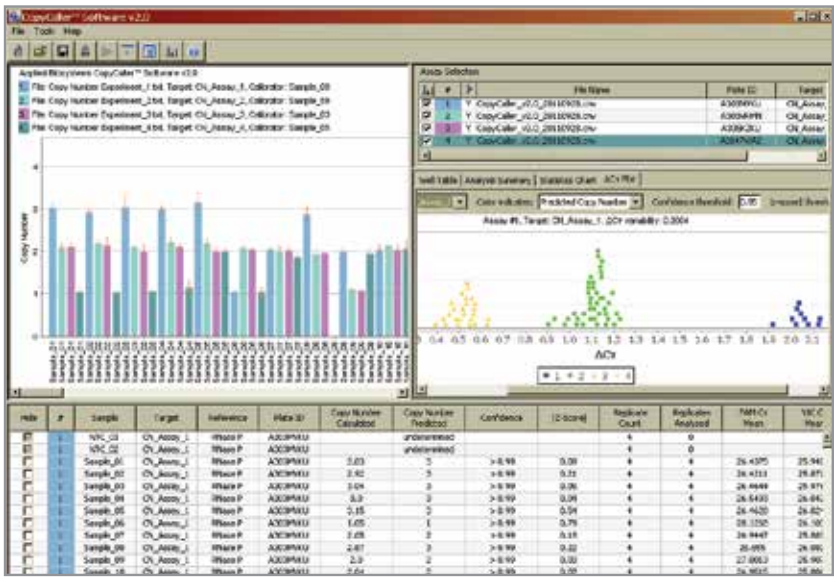

Figure 2. Determining copy number with CopyCaller Software.

TaqMan Copy Number Assays are run simultaneously with a choice of Applied Biosystems™ TaqMan™ Copy Number Reference Assays (VIC™ dye–labeled TAMRA™ probes) in a duplex real-time polymerase chain reaction. The copy number assay detects the target gene or genomic sequence of interest, and the reference assay detects a sequence that is known to be present in two copies in the diploid genome. Relative quantitation analysis is performed with Applied Biosystems™ CopyCaller™ Software (Figure 2) using either a known calibrator sample or no-calibrator sample method.

The simplest workflow

TaqMan Copy Number Assays have the simplest workflow of all currently available CNV analysis methods (Figure 3). The test assay (FAM dye–labeled), the reference assay (VIC dye–labeled), your sample DNA, and Applied Biosystems™ TaqMan™ master mix are combined and then run on an an Applied Biosystems real-time PCR system using the standard Applied Biosystems™ TaqMan™ Genotyping Assay protocol. On average, setup to primary analysis typically takes only 3–4 hours (including a ~2 hour PCR run).

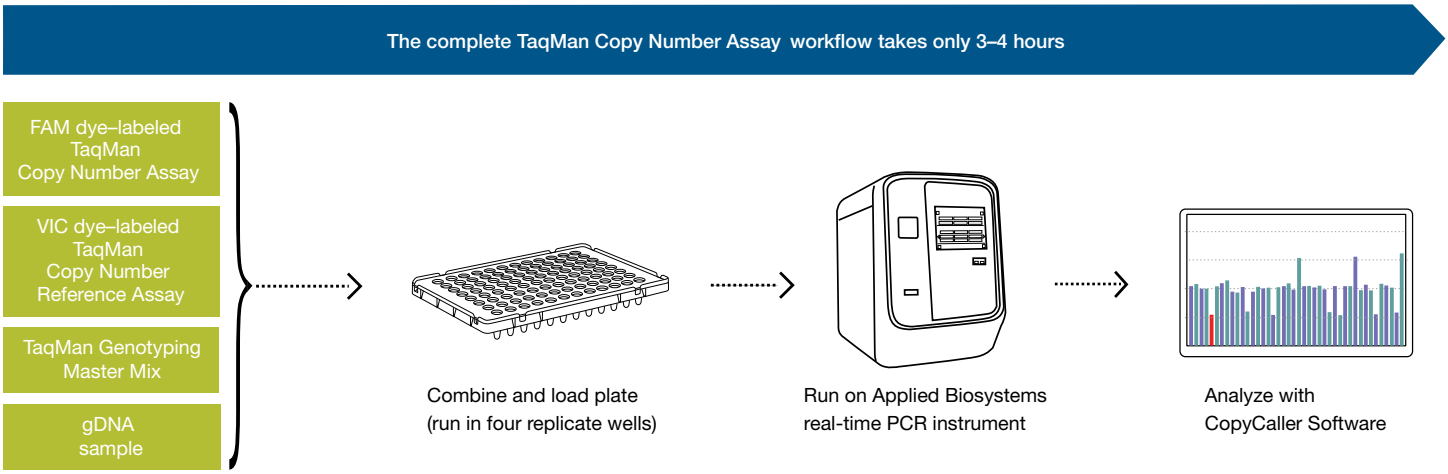

Figure 3. TaqMan Copy Number Assays have a simple workflow, and average setup to primary analysis typically takes only 3–4 hours (including a ~2 hour PCR run). Each copy number quantitation reaction contains four components: a TaqMan Copy Number Assay, a TaqMan Copy Number Reference Assay, TaqMan master mix, and a purified genomic DNA sample, run in four replicate wells.

Choose only what you need

With TaqMan Copy Number Assays, you only choose the assays you need for your project from the large variety of predesigned assays, or create your own Custom Plus or Custom TaqMan Copy Number Assays. Because the assay sets are not fixed, follow-up studies can be easily adjusted as your project develops and changes.

TaqMan Copy Number Assays include predesigned collections for both human and mouse genomes. The human collection includes more than 1.6 million assays targeting known genes, CNV sequences within the Database of Genomic Variants (DGV), and extragenic/nongene regions. For most genes, assays are available for each exon of the gene, where possible, plus assays for intron sequences and junctions. The mouse collection includes more than 180,000 assays targeting gene exons. Predesigned assays for common vector marker and reporter genes are also available for transgenic studies.

Custom Plus TaqMan Copy Number Assays are an optimal solution for studying variation in human and mouse genomic regions of interest for which a predesigned assay is not available. Custom Plus Assays use the same bioinformatics pipeline used to manufacture predesigned TaqMan Copy Number Assays, and can be generated for high-quality genomic targets of interest using the Applied Biosystems™ GeneAssist™ Copy Number Assay Tool. A target range is defined by the user on the genome map, then premasked targets are created and submitted to our proprietary TaqMan Copy Number Assay design pipeline. Benefits include genome quality checks and human/mouse reference assay compatibility checks. Users receive Custom Plus TaqMan Assay annotations that are similar to those for predesigned assays in the assay information files (AIFs); e.g., gene and DGV locations, genomic location, and context sequence. Note that users will not receive their sequence information when they order Custom Plus TaqMan Copy Number Assays.

Custom TaqMan Copy Number Assays are an option for additional targets of interest. Custom assays are designed using Thermo Fisher Scientific assay design algorithms, which are optimized to produce high-performing copy number assays. The GeneAssist Copy Number Assay Tool enables users to submit their own premasked custom target sequences for assay design or primer/probe pair sequences for assay formulation. Custom assay designs do not go through genome quality checks, but can be compared with the human/mouse reference assays for compatibility in duplex reactions. Users will receive custom assay sequences in their AIFs.

TaqMan Copy Number Reference Assays are available to help perform accurate relative quantitation of copy number target sequences. Two reference assays are available for copy number analysis in humans: Applied Biosystems™ TaqMan™ Copy Number Reference Assay RNase P (recommended) and Applied Biosystems™ TaqMan™ Copy Number Reference Assay TERT. This gives users an option in the event that one of the reference assays functions poorly with a sample due to chromosomal aberrations or other issues. Two reference assays are also available for copy number analysis in mice: Applied Biosystems™ TaqMan™ Copy Number Reference Assay, mouse, Tfrc (recommended), and Applied Biosystems™ TaqMan™ Copy Number Reference Assay, mouse, Tert. Note that the reference assays are species-specific. The reference assays are not primer-limited and therefore are highly recommended for copy number analysis.

Web resources

Compilations of reported copy number variable regions can be found at several websites, including:

- Database of Genomic Variants: **dgv.tcag.ca**
- Sanger Institute Copy Number Variation Project: **sanger.ac.uk**
- UCSC Genome Bioinformatics Site: **genome.ucsc.edu**
- Ensembl: **ensembl.org**
- dbVAR: **ncbi.nlm.nih.gov/dbvar**

Powerful data analysis software

CopyCaller Software was developed specifically for TaqMan Copy Number Assay data analysis. This free, easy-to-use software utilizes a graphical interface and quickly calculates the possible copy numbers for a set of samples in a run. It also estimates a confidence value for each copy number call and has outlier removal functionality.

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biosystems

Ordering information

Assay scale	Concentration	Number of reactions		Cat. No.		
Predesigned, Custom Plus, and Custom TaqMan Copy Number Assays						
		384-well, 10 µL	96-well, 20 µL	Predesigned assays	Custom Plus assays	Custom assays
Small	20X	720	360	4400291	4442487	4400294
Medium	20X	1,500	750	4400292	4442520	4400295
Large	60X	5,800	2,900	4400293	4442488	4400296

Assay scale	Concentration	Number of reactions		Cat. No.
TaqMan Copy Number Reference Assays				
Human assays		384-well, 10 µL	96-well, 20 µL	
RNase P, 750 reactions	20X	1,500	750	4403326
RNase P, 3,000 reactions	20X	6,000	3,000	4403328
TERT, 750 reactions	20X	1,500	750	4403316
TERT, 3,000 reactions	20X	6,000	3,000	4403315
Mouse assays		384-well, 10 µL	96-well, 20 µL	
Mouse, Tfrc, 750 reactions	20X	1,500	750	4458366
Mouse, Tfrc, 3,000 reactions	20X	6,000	3,000	4458367
Mouse, Tert, 750 reactions	20X	1,500	750	4458368
Mouse, Tert, 3,000 reactions	20X	6,000	3,000	4458369

Find out more at **thermofisher.com/cnv**.

For more information and full terms of the TaqMan Assays qPCR guarantee, visit **thermofisher.com/taqmanguarantee**

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