

CNV detection by Ion semiconductor sequencing

Key findings

- Ion Torrent™ semiconductor sequencing provides simultaneous identification of copy number variants (CNVs), single nucleotide variants (SNVs), and small insertions and deletions (indels) from a research sample using a single integrated workflow
- 34 of 34 assayed CNV regions were detected, using a reference set of 31 samples with known chromosomal aberrations
- Ion Reporter™ Software facilitates user-defined CNV detection, with three sensitivity options so that copy number analysis workflows can be tuned to achieve desired levels of sensitivity and specificity

The missing genome—structural variants of the human genome

Copy number variants (CNVs) represent a class of genomic variation in which large regions (>1 kb) of the genome can be duplicated (gains) or deleted (losses) (Figure 1). With the sequencing of a human reference genome and other human genomes, it was revealed that CNVs are more common than expected, and this form of variation comprises a considerable proportion of the genetic polymorphism found in the human genome [1]. Inherited and *de novo* CNVs of chromosomal regions have been associated with many disease conditions, including cancer and genetic disorders. There can be a variety of phenotypic impacts from CNVs: deletions can unmask recessive mutations; duplications and deletions can alter the copy number of dosage-sensitive genes; deletions can result in position effects that alter the regulation of genes; and selection of duplicated regions containing gain-of-function driver mutations may occur in cancer.

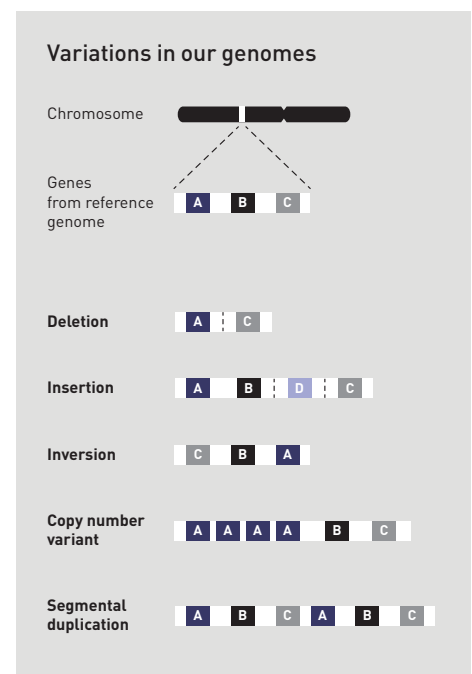


Figure 1. Possible structural variations within the human genome. These variations include deletions, insertions, inversions, copy number variants, and segmental duplications.

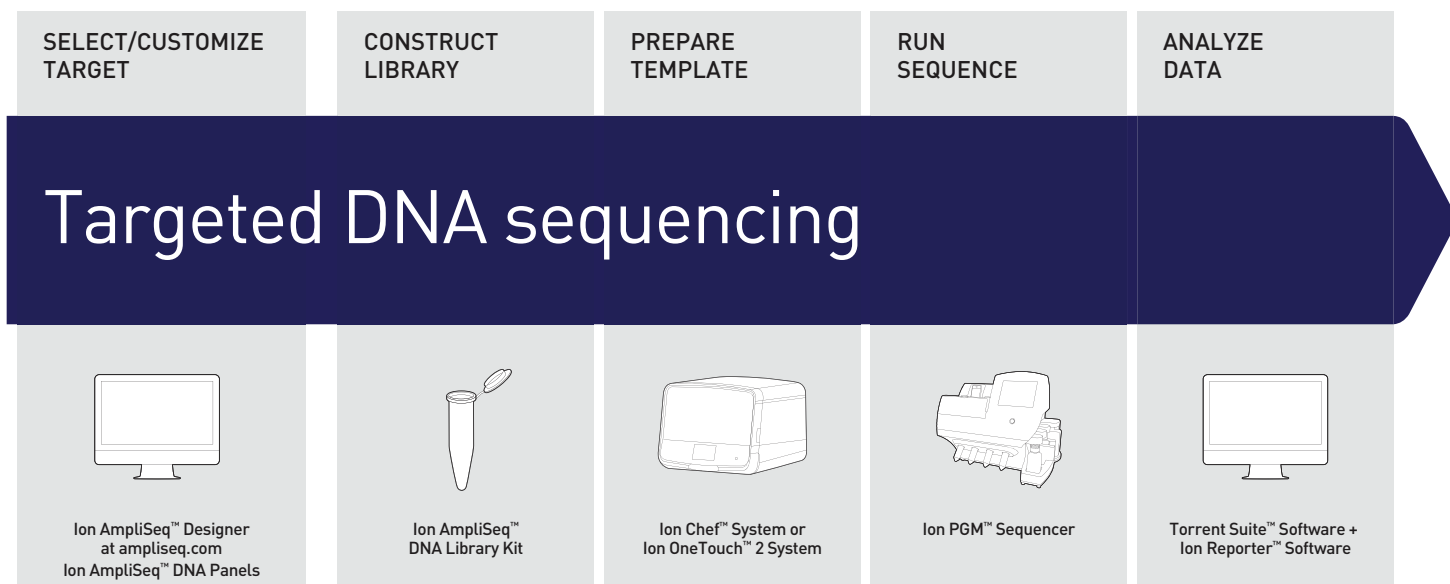


Figure 2. Targeted DNA sequencing workflow. Life Technologies supplies an easy-to-implement, cost-effective, rapid, and scalable targeted DNA sequencing workflow for the Ion PGM™ System that takes <11 hours (3.5-hour library construction, 4-hour template preparation, 2.3-hour sequencing run, and 1-hour data analysis) from library to variant-called results. Following library construction using the Ion AmpliSeq™ Library Kit, template preparation and 200-base sequencing runs are completed in just 2.3 hours for the Ion 314™ Chip and 4.4 hours for the Ion 318™ Chip. Primary data analysis is performed using Torrent Suite™ Software, and SNV, indel, and CNV polymorphisms are determined using Ion Reporter™ Software.

One workflow for the detection of SNVs, indels, and CNVs

Ion AmpliSeq™ technology provides a simple, highly multiplexed, and PCR-based library preparation that enables researchers to rapidly sequence targeted regions of the human genome. For example, the Ion AmpliSeq™ Comprehensive Cancer Panel multiplexes ~16,000 primer pairs in just 4 pools to target >400 genes encoding most of the known oncogenes and tumor suppressors. Ion AmpliSeq™ technology, combined with Ion Torrent™ semiconductor sequencing, delivers a rapid workflow with the power to simultaneously identify single nucleotide variants (SNVs), short insertions and deletions (indels), and CNVs within a research sample using a single, simple workflow (Figure 2). Further, the Ion AmpliSeq™ Designer, a free assay design tool, can be utilized to create custom, ultrahigh-multiplex primer pools to target any region of interest within the genome. In this application note, we describe how researchers can leverage Ion AmpliSeq™

technology and the Ion PGM™ System along with Ion Reporter™ Software to identify CNVs. Using genomic regions targeted by the Ion AmpliSeq™ Comprehensive Cancer Panel, 34 out of 34 previously annotated CNV regions were detected from a reference set of 31 samples using Ion Reporter™ Software.

Results

In a single-blind retrospective study, the performance of the algorithm was assessed on 31 archived samples with known CNVs that were amplified using the Ion AmpliSeq™ Comprehensive Cancer Panel (CCP) primer set and sequenced on the Ion PGM™ instrument (Table 1). Across the sample set, 34 previously annotated CNV regions were assessed based on coverage by amplicons in the ~16,000-amplicon CCP panel (Supplementary information, Table A). Using Ion Reporter™ Software and applying the medium sensitivity setting, the CNV algorithm detected 34 of the 34 CNV segments. Figure 3 illustrates

an example of a previously annotated deletion that was detected in a region of chromosome 11 from sequence read data. This deletion on the short arm of chromosome 11 deletes the *EXT2* gene, seen in Potocki-Shaffer syndrome (Figure 3B).

Additional CNV regions were also identified that were not annotated in the original microarray data. Among these additional detected events were putative false positives with high ploidy counts of ≥ 7 detected on chromosome 14q32 (in a single sample) and on chromosome 16p11.2 (in four samples) that demonstrated low coverage in the control sample but yielded a relatively high coverage ratio between the test samples and the control sample for these regions. Support for CNVs in these regions was low (confidence values of <5.7) in the statistical model, due to the low coverage in the control sample. However, duplications or deletions in regions on chromosomes 14q32 and 16p11.2 are commonly observed in samples analyzed by higher density



Figure 3. Example of a sample (GM22624) with a deletion CNV on chromosome 11. **(A)** The left panel illustrates the visualization of CNVs over the entire genome in the karyotype view. The deletion CNV (red line) was detected in a region of chromosome 11 (selected for focus in the right panel), at the same position as the deletion detected by SNP array. **(B)** The CNV red line in the “sample ploidy (seg)” track was detected in a single gene (*EXT2*), with no evidence of copy number change in amplicons covering flanking genes. CNVs are visualized in Ion Reporter™ Software using a custom version of Integrative Genomics Viewer (IGV), which offers the standard Genome Browser view as well as a CNV track with normalized coverage track for the test and control samples. Note that additional tracks also indicate SNVs, indels, and genes/exons.

Table 1. Confirmation of CNVs by Ion Torrent™ semiconductor sequencing. A reference set of 34 previously annotated CNV regions was assessed using the Ion AmpliSeq™ Comprehensive Cancer Panel across 31 cell line genomes that contain significant chromosomal aberrations. The CDC Genetic Testing Reference Materials Coordination Program (GeT-RM) previously characterized this sample set as reference material. The number and size range of the duplications and deletions as determined by microarray are listed. Note that the detection and size range of CNVs are limited by amplicons spanning the known CNVs. We recommended that at least 10 amplicons cover a region for robust detection of CNVs (see Best Practices sidebar). The performance of the CNV detection algorithm was evaluated using the medium sensitivity setting in Ion Reporter™ Software.

	Microarray		Confirmed by sequence reads	
	Duplications	Deletions	Duplications	Deletions
Number	11	23	11	23
Size range (bp)	2.92 x 10 ⁶ – 1.55 x 10 ⁸	8.33. x 10 ⁵ – 1.55 x 10 ⁸		

microarrays, and may represent common CNVs in some cell lines. A small (4.3 kb) duplication on chromosome 22 (with a ploidy of 3) was also detected in a single sample. Similarly, this duplication had low statistical support with a confidence score of 5.5. It is difficult to classify the above six duplication events as true false positives, due to the use of differing human genome reference annotations (hg18 for the microarray results vs. hg19 for the sequence read data), the use of different normal controls (single control sample for sequence read data vs. a composite normal control for microarray data), and the higher resolution of CNV detection possible using sequence read data.

Detection of CNV events is dependent on position and distribution of amplicons used in the CCP panel. The 11 confirmed duplications detected by sequence reads were spanned by amplicons ranging in size from 51.4 kb to 1.13 Mb (Supplementary information, Table A). The 23 deletions were detected by CCP amplicons ranging in size from 6.69 kb to 1.13 Mb. Using the medium sensitivity setting, detection of the breakpoints for a large 6.11 Mb duplication of chromosome 3 (ploidy of 3) detected by 313 amplicons across the CNV region closely corresponded to the coordinates predicted by the microarray data, whereas recognition of a 4.42 Mb deletion on chromosome 1 was possible with 11 amplicons that spanned only 6,692 bases (Supplementary information, Table A). These results indicate that considerably larger CNVs can be detected with Ion AmpliSeq™ amplicons designed for SNV and indel detection, in this case 409 cancer-related genes encompassing >50% of the Wellcome Trust Sanger Institute Cancer Gene Census.

Conclusion

Ion AmpliSeq™ target selection technology and Ion Reporter™ Software, in combination with the Ion PGM™ System, provide a robust method for the detection of CNVs, SNVs, and indels in a sample in a single, rapid workflow solution for your research. Using a tunable software algorithm that can be user-defined, internal performance

testing demonstrated detection of 34 of 34 CNVs when the Ion AmpliSeq™ CCP primer set was tested on a reference panel of 31 cell line samples that harbor significant chromosomal abnormalities. In conclusion, the Ion AmpliSeq™ targeted sequencing workflow provides a rapid, accurate, and sensitive research solution for CNV detection from sequencing read data.

References

1. Conrad DF, Pinto D, Redon R et al. (2010) Origins and functional impact of copy number variation in the human genome. *Nature* 464: 704–712.

Best practices for CNV detection from Ion AmpliSeq™ sequencing data

Consistent sample preparation and pooling

It is important that the control and experimental Ion AmpliSeq™ libraries be made following the same procedure. Each pool of a particular library should be quantified carefully using the Ion Library Quantitation Kit (Cat. No. 4468802) before pooling libraries.

Choosing a control sample

CNVs will be reported based on their copy number relative to the control sample used. Therefore, it is desirable to choose a control sample with no known CNVs in any region covered by the Ion AmpliSeq™ panel used. If most or all test samples are reported as having a CNV in the same region, one possible cause is that the control sample actually has a CNV in that region.

Filtering by score

By default all candidate CNVs are shown; however, CNVs with higher scores are more likely to be true positives. Users can filter reported CNVs by scores assigned by the software, with scores >10 indicating high-confidence CNVs. However, the exact threshold to use may depend on the panel and the nature of the CNVs under investigation.

Sex chromosomes

Copy number state and confidence scores on X and Y chromosomes will be determined relative to the gender of the control sample. For example, the expected ploidy of X for a male control sample is 1. If the test sample also has 1 copy of X, the confidence score for a CNV will be 0, since there is no evidence of an unexpected ploidy state.

Ion AmpliSeq™ panel design and CNV detection

When using Ion AmpliSeq™ Designer to create an Ion AmpliSeq™ panel that will be used to determine CNVs, consider the following: The algorithm will robustly detect CNVs if <20% of amplicons in a panel are likely to display CNVs. At >20%, the diploid state may not be robustly detected and could result in determination of the wrong copy number. Generally, CNVs can be robustly detected if at least 10 amplicons cover a CNV region. It may be possible to call CNVs with fewer amplicons (especially with the low stringency setting), but there is a risk of increased noise and a CNV covered by <10 amplicons may not be detected.

Limits of CNV detection

If the number of copies of a CNV is >10, this will be reported as a copy number of 10 in the Ion Reporter™ 1.6 release.

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SUPPLEMENTARY INFORMATION

Methods

Targeted DNA sequencing

Cell line gDNA from 31 samples was obtained from The Coriell Institute of Medical Research. These samples are applicable to CNV analysis since the cell line genomes harbor significant chromosomal aberrations and have been previously characterized as part of a 45-sample set used as reference material by the CDC Genetic Testing Reference Materials Coordination Program (GeT-RM). The samples were previously genotyped on the Affymetrix Genome-Wide Human SNP Array 6.0 platform, and G-banding karyotype analysis was performed and, in many instances, fluorescence *in situ* hybridization (FISH) was done. Affymetrix Genome-Wide Human SNP Array 6.0 genotyping data for these samples are available from dbGaP (dbGaP Study Accession: phs000269.v1.p1).

Prior to amplification, the gDNA concentration was determined using the TaqMan® RNase P Detection Reagents Kit following the standard protocol on a ViiA™ 7 Real-Time PCR System. Library construction was performed with the Ion AmpliSeq™ Library Kit Version 2.0 (Cat. No. 4475345) using DNA amplified with the Ion AmpliSeq™ Comprehensive Cancer Panel primer set following

the standard kit protocol (40 ng; 10 ng per primer pool) for each Coriell sample (test). Ion AmpliSeq™ libraries were also constructed from Control DNA CEPH Individual 1347-02 (Cat. No. 403062) and from NA12878 (controls). In all analyses, either of these two samples was used as the control. It is important that both the control and test Ion AmpliSeq™ libraries be constructed following the same procedure and that each pool of a library be quantified carefully using the Ion Library Quantitation Kit (Cat. No 4468802) prior to pooling.

Template preparation and sequencing were performed in separate runs and on separate chips for control and test libraries. Libraries were clonally amplified with the Ion OneTouch™ 200 Template Kit v2 DL and the Ion OneTouch™ System for templating and enrichment prior to chip loading. Sequencing was performed using the Ion PGM™ Sequencing 200 Kit v2 and the Ion 318™ Chip.

Data analysis

CNV detection was performed using an algorithm based on a Hidden Markov Model (HMM). The algorithm uses normalized read coverage across amplicons to predict the copy number or ploidy

states (0, 1, 2, 3, etc.). Prior to copy number state determination, read coverage is corrected for GC bias and compared to a baseline coverage that is constructed from control samples using regions with known ploidy states. When using the paired-sample workflow, the known ploidy state is 2 for autosomes, while customer-specified gender for control samples is required for known ploidy state determination of the sex chromosomes.

CNV region boundary determination

Using the HMM framework, the algorithm utilizes all the coverage information for a sample in a statistical model that determines the likelihood of the coverage for a particular genomic region belonging to an alternate ploidy state. Low read coverage in either the research sample or control sample with significant variation will appropriately lower the support for that genomic region in the statistical model. The HMM framework determines the optimal path through the ploidy states that will maximize the total ploidy state likelihoods when state transition penalties are considered. Transition penalties for regions with no coverage data scale down proportionally with the length of the region.

Tunable CNV detection sensitivity

The state transition penalty value, with scaling factors, allows for a tunable algorithm with large transition penalties, resulting in a smaller number of called CNV regions. Algorithm parameters have been tuned for Ion AmpliSeq™ panel-generated sequencing data using technical sample replicate data as well as samples with known CNVs. There is a choice of three levels of sensitivity: low, medium, or high. High sensitivity enables more permissive CNV detection, detecting smaller CNVs and CNVs with less evidence or in noisy regions, but with a higher probability of detecting

false-positive CNVs. The medium setting is the default setting and is the recommended initial setting for CNV detection in your research.

Confidence and precision quality metrics for CNV prediction output

In addition to predicting copy number regions and the associated ploidy, the algorithm calculates two quality metrics: confidence and precision for each region. Confidence is defined as a log ratio between the called ploidy state likelihood of the region and the expected ploidy state likelihood. Large confidence values (>20) indicate the algorithm is very certain that the ploidy state differs

from the expected. Precision is defined as a log ratio between the likelihood of the assigned ploidy state and a next-closest state. Small precision (<10) denotes the uncertainty in the absolute ploidy value assignment. It is possible that high ploidy states (e.g., copy number state >5) will have low precision and high confidence, indicating that the algorithm is uncertain about the absolute ploidy value, but quite certain about the existence of a ploidy state that is different from the expected (i.e., high confidence that a copy number increase is present, but uncertainty whether the exact copy number is 5 or 6).

Table A. Sample set of 31 samples with a total of 34 CNV regions. The size of duplications (dup) and deletions (del), ploidy, and chromosomal location are indicated as detected by microarray and sequence read data. For each sequence read CNV call, the confidence and precision scores are listed.

Description and Coriell catalog ID	ISCN [hg18]	Microarray [hg18]		Chromosome	Sequence read [hg19]				
		CNV size	Ploidy		Amplicon coverage size across CNV region	No. of amplicons	Ploidy	Confidence	Precision
Potocki-Shaffer Syndrome (GM22624)	46,XX,del(11)(p12p11.2).arr 11p12p11.2(40433344-46031324)x1	5.60 × 10 ⁶ (del)	1	11	1.48 × 10 ⁶ (del)	31	1	33.3	33.3
Chromosome Deletion (GM06936)	46,XX,del(10)(p13).arr 10p15.3p13(94427-12918932)x1	1.28 × 10 ⁷ (del)	1	10	429 × 10 ⁶ (del)	26	1	9.5	9.5
Smith-Magenis Syndrome (GM13476)	46,XX,ish del(17)(p11.2p11.2)(D17S29-).arr 17p11.2(16704280-20336467)x1	363 × 10 ⁶ (del)	1	17	1.45 × 10 ⁴ (del)	24	1	8.7	8.7
Greig Cephalopolysyndactyly Syndrome (GM10925)	46,XY,del(7)(p14p12).arr 7p14.1p11.2(38598541-54681998)x1	1.61 × 10 ⁷ (del)	1	7	1.10 × 10 ⁶ (del)	18	1	15.0	15.0
Chromosome 1p36 Deletion Syndrome (GM22991)	46,XX,ish del(1)(p36.32)(CEB108/T7-,SKI-,D1S3739+).arr 1p36.32(742429-5215341)x1	4.47 × 10 ⁶ (del)	1	1	6.69 × 10 ³ (del)	11	1	10.2	10.2
Chromosome Deletion (GM09216)	46,XY,del(2)(p25.1p23).arr 2p25.1p23.3(10260988-27005382)x1, 4q31.22(145061542-145162384)x1	1.67 × 10 ⁷ (del)	1	2	9.45 × 10 ⁶ (del)	111	1	144.0	144.0
Chromosome Deletion (GM11672)	46,XY,del(10)(q11.2q22.1).arr 10q11.22q22.2(48962457-75120713)x1	2.62 × 10 ⁷ (del)	1	10	3.34 × 10 ⁴ (del) 1.20 × 10 ⁵ (del)	119	1 1	26.5 107.5	26.5 107.5
Gilles De La Tourette Syndrome (GM10989)	46,XY,del(9)(p23).ish del(9)(p23)(ptel 30-,D9Z+,wcp9+).arr 9p24.3p23(36587-11986831)x1	1.20 × 10 ⁷ (del)	1	9	3.71 × 10 ⁶ (del)	129	1	160.6	160.6
Adenosine Deaminase Deficiency With No Immunodeficiency (GM07945)	46,XY,del(20)(q12q13.1).arr 8p23.1(7318699-7847304)x3, 13q12.11(19701062-19932295)x1, 20q11.22q13.12(32961915-44293878)x1	1.13 × 10 ⁷ (del)	1	20	5.50 × 10 ⁶ (del)	168	1	166.9	166.9

Description and Coriell catalog ID	ISCN [hg18]	Microarray [hg18]		Chromosome	Sequence read [hg19]				
		CNV size	Ploidy		Amplicon coverage size across CNV region	No. of amplicons	Ploidy	Confidence	Precision
Chromosome Deletion (GM12606)	47,XY,+del(13)(q21.2).arr 13q11q21.2(17943628-59139422)x3	4.12 × 10 ⁷ (dup)	3	13	2.22 × 10 ⁷ (dup)	175	3	141.9	81.2
Duplicated Chromosome (GM09367)	46,XX,dup(6)(q21q24).ish dup(6)(q21q24)(wcp6+).arr 6q21q24.2(107861056-143105847)x3	3.52 × 10 ⁷ (dup)	3	6	2.93 × 10 ⁷ (dup)	187	3	134.3	96.9
Trichorhinophalangeal Syndrome, Type II (Langer-Giedion Syndrome) (GM09888)	46,XX,del(8)(q23q24.1).arr 1q23.3(159775402-159923110)x3, 8p23.1(7241689-7814569)x3, 8q23. 1q24.12(107189214-119363784) x1,14q22.1(49891851-50724999)x1, 22q11.21(17256416-17420071)x3	1.22 × 10 ⁷ (del)	1	8	5.89 × 10 ⁶ (del)	193	1	250.6	250.6
		8.33 × 10 ⁶ (del)	1	14	9.86 × 10 ⁴ (del)	70	1	83.9	83.9
Trisomy 9 (GM05067)	47,XY,+del(9)(q11)mat.arr 9p24.3p11.2(36587-44806024)x3	4.48 × 10 ⁷ (dup)	3	9	3.20 × 10 ⁷ (dup)	267	3	204.5	81.2
Inverted Duplication Deletion (GM14485)	46,XY,der(8)del(8)(p23.1)dup(8)(p23.1p11.2).ish der(8)del(8)(p23.1)dup(8)(p23.1p11.2)(wcp8+,D8S596-).arr 8p23.3p23.1(160290-7213701)x1, 8p23.1p11.1(12572787-43719525)x3	3.11 × 10 ⁷ (dup)	3	8	1.20 × 10 ⁷ (dup)	284	3	221.6	89.1
Translocated Chromosome (GM06226)	46,XY,der(1)t(1;16)(q44;p12)mat.arr 1q44(245373155-247190999)x1, 16p13.3p12.2(25815-21297471)x3, 16p12.1(21853546-22612021)x3	2.13 × 10 ⁷ (dup)	3	16	1.38 × 10 ⁷ (dup)	285	3	187.8	114.6
Duplicated Chromosome (GM20022)	46,XY,dup(3)(q21q29).ish dup(3)(q21q29)(wcp3+,D3S4560+).arr 3q22.2q29(136044785-197137370)x3	6.11 × 10 ⁷ (dup)	3	3	6.11 × 10 ⁷ (dup)	313	3	284.4	93.3
Chromosome Deletion (GM10985)	46,XX,del(3)(p25).arr 3p26.3p25.3(35333-10305377)x1	1.03 × 10 ⁷ (del)	1	3	7.00 × 10 ⁶ (del)	95	1	122.5	122.5
Chromosome Deletion (GM11213)	46,XX,del(2)(q32.1q33).arr 2q32.1q33.2(186818448-204311174) x1	1.75 × 10 ⁷ (del)	1	2	7.64 × 10 ⁶ (del)	93	1	121.1	121.1
Wolf-Hirschhorn Syndrome (GM22601)	46,XY,del(4)(p15.2).arr 4p16.3p15.2(55665-25591051)x1	2.55 × 10 ⁷ (del)	1	4	1.80 × 10 ⁵ (del)	85	1	44.9	44.9
Aneuploid Chromosome Number - Non-Trisomic (GM01201)	45,XX,-21.arr 21q11.2q22.3(13322592-46921373) x1	3.36 × 10 ⁷ (del)	1	21	1.02 × 10 ⁷ (del)	73	1	40.2	40.2
Chromosome Deletion (GM08331)	46,XY,del(13)(q32q33).arr 8p23.1(7254763-7864765)x3, 13q32.1q33.3(96956971-109061570) x1	1.21 × 10 ⁷ (del)	1	13	2.97 × 10 ⁴ (del)	45	1	56.3	56.3
Derivative Chromosome (GM05966)	46,XY,dup(14)(q22q24).arr 14q22.2q24.3(54038516-75217413) x3	2.12 × 10 ⁷ (dup)	3	14	5.15 × 10 ⁴ (dup)	41	3	8.6	8.6
XXXX Syndrome (GM01416)	48,XXXX.arr(X)x4,(Y)x0	1.55 × 10 ⁸ (dup)	4	X	1.13 × 10 ⁸ (dup)	643	4	3134.9	42.2
Turner Syndrome (GM20027)	45,X.arr(X)x1,(Y)x0	1.55 × 10 ⁸ (del)	1	X	1.13 × 10 ⁸ (del)	643	1	0	735.9
XXY Syndrome; Klinefelter Syndrome (GM17867)	47,XXY.arr(X)x2,(Y)x1	1.55 × 10 ⁸ (dup)	2	X	1.13 × 10 ⁸ (dup)	643	2	1074.3	197.8
Turner Syndrome (GM13019)	46,X,idic(X)(p10)[25]/46,X,del(X)(p10)[16]/45,X[9].arr Xp22.33p11.1(108464-56912309)x1, Xp11.1q28(62260103-153703648) x2~3	5.68 × 10 ⁷ (del)	1	X	1.23 × 10 ⁷ (del)	291	1	0	271.6
		9.14 × 10 ⁷ (dup)	2-3 (mosaic)	X	1.35 × 10 ⁷ (dup)	328	3	402.5	14.0
					7.69 × 10 ⁷		2	362.4	0.6

Description and Coriell catalog ID	ISCN (hg18)	Microarray (hg18)		Chromosome	Sequence read (hg19)				
		CNV size	Ploidy		Amplicon coverage size across CNV region	No. of amplicons	Ploidy	Confidence	Precision
Chromosome Deletion (GM09102)	46,XY,del(11)(q23.3).arr 11q23.3q25(119996189-134449982) x1, 22q11.21(17008946-17386984)x3	1.45 × 10 ⁷ (del)	1	11	3.18 × 10 ⁶ (del)	65	1	48.4	48.4
Chromosome Deletion (GM10800)	46,XY,del(4)(q13.2q22).arr 4q13.2q22.2(70096438-95297116)x1	2.52 × 10 ⁷ (del)	1	4	1.87 × 10 ⁵ (del)	52	1	49.0	49.0
Chromosome Deletion (GM21698)	46,XY,del(6)(q26).ish del(6)(q26) (wcp6+, D62522-).arr 6q26q27(162860228-170761408)x1	7.90 × 10 ⁶ (del)	1	6	4.49 × 10 ⁵ (del)	36	1	29.9	29.9
Tetralogy of Fallot (GM14164)	46,XX,del(13)(q13q32).ish del(13) (q13q32)(RB1-,D13S102+).arr 13q14.2q32.1(46700085-94512977) x1, 22q11.21(17256416-17405213)x3	4.78 × 10 ⁷ (del)	1	13	1.76 × 10 ⁵ (del)	35	1	47.0	47.0
Chromosome Deletion (GM12662)	46,dup(X)(q28),del(Y)(q11.2).ish del(Y)(q11.2)(DXYS129/DXYS153+, SRY+,DYZ3+,DYZ1+,Z43206+).arr Xq28(151659961-154582680)x2, Yq11.223q11.23(22769319-27097245) x0	2.92 × 10 ⁶ (dup)	2	X	1.29 × 10 ⁶ (dup)	34	2	24.2	5.7

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