Supplemental Digital Content including 1 figure and 5 tables.

DEFINITIONS:

Analytical sensitivity is the ability to obtain positive results in concordance with positive results obtained by the reference method.

Analytical specificity is the ability to obtain negative results in concordance with negative results obtained by the reference method. (Or the ability to identify only a specific substance, i.e. sequence variant.)

Validation is confirmation, through the provision of objective evidence, that requirements for a specific intended use or application have been fulfilled (ISO 9000).

Precision is the closeness of agreement between repeated independent test results obtained under stipulated conditions. It is important to demonstrate analytical consistency which is measured by performing intra- and inter-run reproducibility studies. These studies are done to determine if the same results are obtained regardless of the operator, lot of reagent, and other variables. This is a measure of random error and is usually expressed as standard deviation or coefficient of variation.

Supplementary Figure 1. Bioinformatics Pipeline at The Children's Hospital of Philadelphia (CHOP), Philadelphia, PA:

1. The first step of the pipeline is alignment to the hg19v37 1000 Genomes (1000G) reference (ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/technical/reference/human_g1k_v37.fasta.gz) (October 31, 2016) using NovoAlign (Novocraft, Selangor, Malaysia). The output of this step is a sam file (http://samtools.sourceforge.net/SAM1.pdf) (October 31, 2016). Modified parameters include:
    a) –k enables NovoAlign base quality recalibrator
    b) –i PE 240,150 which are respectively the average and the median of the insert sizes used during library prep. These values proved to generate the greatest number of reads aligned (optimal alignment rate) during the validation phase to be the best parameters for the Genomic Diagnostics Laboratory (GDL) MiSeq library prep workflow.
    c) The rest of the input parameters identify the input fastq files and the reference file
2. The sam file from the alignment step is converted to compressed binary format (bam) using samtools
3. Samtools also sorts the bam file and the result is a sorted bam file
4. Duplicate reads are marked and removed using Picard (http://broadinstitute.github.io/picard/) (October 31, 2016)
5. Picard HSmetrics and Genome Analysis Toolkit (GATK) depth of coverage is used to calculate coverage metrics for the regions of interest intervals. The following modified parameters are used for GATK depth of coverage tool:
    a) -ct 1 -ct 10 -ct 30 -ct 40 -ct 50 -ct 60 -ct 80 which specify different depth of coverage levels for calculating coverage statistics
    b) –omitBaseOutput
    c) –omitLocusTable
6. GATK: RealignerTargetCreator (limited to region of interest (ROI)) – modified parameters:
    a) -known Mills_and_1000G_gold_standard.indels.b37.sites.vcf
    b) -known 1000G_phase1.indels.b37.vcf

7. GATK: IndelRealigner (limited to ROI) – modified parameters:
    a) -known Mills_and_1000G_gold_standard.indels.b37.sites.vcf
    b) -known 1000G_phase1.indels.b37.vcf
    c) --maxReadsForRealignment 100000
    d) --maxReadsInMemory 750000
    e) --maxReadsForConsensuses 600

8. GATK: BaseRecalibrator (limited to ROI) – modified parameters:
    a) -knownSites Mills_and_1000G_gold_standard.indels.b37.sites.vcf
    b) -knownSites 1000G_phase1.indels.b37.vcf
    c) -knownSites dbsnp137.vcf

9. GATK: PrintReads (limited to ROI)
10. GATK: UnifiedGenotyper (limited to ROI) – modified parameters:
    a) -stand_call_conf 50.0
    b) -stand_emit_conf 10.0
    c) -glm BOTH
    d) –dt NONE
    e) -A BaseCounts -A AlleleBalan -A IndelType -A GCContent -A NBaseCount -A LowMQ
    f) –dbsnp dbsnp137.vcf
11. Bench Lab Next Generation Sequencing software (Cartagenia, Cambridge, MA) (Functional annotations and predictions) – modified parameters:
    a) –ud 0
    b) –splice 6

Supplementary Table 1. Gene list for targeted panel testing developed at The Children's Hospital of Philadelphia (CHOP), Philadelphia, PA.

Gene Name	Associated Disease or Phenotype	Panel
ALX1	Frontonasal dysplasia 3	Craniofacial
ALX3	Frontonasal dysplasia 1	Craniofacial
ALX4	Frontonasal dysplasia 1, Parietal foramina 2	Craniofacial
EFNB1	Craniofrontonasal syndrome	Craniofacial
FBN1	Weill-Marchesani syndrome 2	Craniofacial
FGFR1	Jackson-Weiss syndrome Pfeiffer syndrome	Craniofacial
FGFR2	Apert syndrome, Beare-Stevenson syndrome, Crouzon syndrome, Jackson-Weiss syndrome, Pfeiffer syndrome	Craniofacial
FGFR3	Crouzon syndrome with acanthosis nigricans, Muenke syndrome	Craniofacial
GLI3	Pallister-Hall syndrome	Craniofacial
IFT122	Cranioectodermal dysplasia 1	Craniofacial
*MSX2**	Parietal foramina with cleidocranial dysplasia	Craniofacial
POR	Antley-Bixler syndrome with genital anomalies and disordered steroidogenesis	Craniofacial
RAB23	Carpenter syndrome 1	Craniofacial
RECQL4	Rothmund-Thomson syndrome	Craniofacial
TGFBR1	Loeys-Dietz syndrome 1	Craniofacial
TGFBR2	Loeys-Dietz syndrome 2	Craniofacial
TWIST1	Saethre-Chotzen syndrome	Craniofacial
ARHGEF9	Epileptic encephalopathy, early infantile, 8	EIEE
ARX	Epileptic encephalopathy, early infantile, 1	EIEE
CDKL5	Epileptic encephalopathy, early infantile, 2	EIEE
GABRD	Epilepsy, generalized, with febrile seizures plus, type 5, susceptibility to	EIEE
GABRG2	Epilepsy, generalized, with febrile seizures plus, type 3, Febrile seizures, familial, 8	EIEE
KCNQ2	Epileptic encephalopathy, early infantile, 7	EIEE
MEF2C	Mental retardation, stereotypic movements, epilepsy, and/or cerebral malformations	EIEE
PCDH19	Epileptic encephalopathy, early infantile, 9	EIEE
PLCB1	Epileptic encephalopathy, early infantile, 12	EIEE
PNKP	Microcephaly, seizures, and developmental delay	EIEE
SCN1A	Epilepsy, generalized, with febrile seizures plus, type 2, Febrile seizures, familial, 3A, Epileptic encephalopathy, early infantile, 6	EIEE
SCN2A	Epileptic encephalopathy, early infantile, 11	EIEE
SLC25A22	Epileptic encephalopathy, early infantile, 3	EIEE
SPTAN1	Epileptic encephalopathy, early infantile, 5	EIEE
STXBP1	Epileptic encephalopathy, early infantile, 4	EIEE
ACTG1	Deafness, autosomal dominant 20	Hearing Loss

Gene Name	Associated Disease or Phenotype	Panel
ADGRV1	Usher syndrome, type 2C	Hearing Loss
ATP6V1B1	Renal tubular acidosis with deafness	Hearing Loss
BCS1L	Bjornstad syndrome	Hearing Loss
BSND	Bartter syndrome, type 4a, Sensorineural deafness with mild renal dysfunction	Hearing Loss
CCDC50	Deafness, autosomal dominant 44	Hearing Loss
CDH23	Deafness, autosomal recessive 12, Usher syndrome, type 1D	Hearing Loss
CEACAM16	Deafness, autosomal dominant 4B	Hearing Loss
CLDN14	Deafness, autosomal recessive 29	Hearing Loss
CLRN1	Usher syndrome, type 3A	Hearing Loss
COCH	Deafness, autosomal dominant 9	Hearing Loss
COL11A1	Marshall syndrome, Stickler syndrome, type II	Hearing Loss
COL11A2	Deafness, autosomal dominant 13, Deafness, autosomal recessive 53, Otospondylomegaepiphyseal dysplasia, Stickler syndrome, type III, Weissenbacher-Zweymuller syndrome	Hearing Loss
COL2A1	Epiphyseal dysplasia, multiple, with myopia and deafness, Stickler syndrome, type I	Hearing Loss
COL4A3	Alport syndrome, autosomal dominant, autosomal recessive	Hearing Loss
COL4A4	Alport syndrome, autosomal recessive	Hearing Loss
COL4A5	Alport syndrome	Hearing Loss
COL9A3	Hearing loss, non-syndromic	Hearing Loss
CRYM	Deafness, autosomal dominant 40	Hearing Loss
DFNA5	Deafness, autosomal dominant 5	Hearing Loss
DFNB59	Deafness, autosomal recessive 59	Hearing Loss
DIABLO	Deafness, autosomal dominant 64	Hearing Loss
DIAPH1	Deafness, autosomal dominant 1	Hearing Loss
DIAPH3	Auditory neuropathy, autosomal dominant, 1	Hearing Loss
DSPP	Deafness, autosomal dominant 39, with dentinogenesis	Hearing Loss
EDN3	Waardenburg syndrome, type 4B	Hearing Loss
EDNRB	Waardenburg syndrome, type 4A, ABCD syndrome	Hearing Loss
ESPN	Deafness, autosomal recessive 36	Hearing Loss
ESRRB	Deafness, autosomal recessive 35	Hearing Loss
EYA1	Branchiootorenal syndrome 1, with or without cataracts, Branchiootic syndrome 1	Hearing Loss
EYA4	Deafness, autosomal dominant 10	Hearing Loss
FOXI1	Deafness, autosomal recessive 4, with enlarged vestibular aqueduct	Hearing Loss
GATA3	Hypoparathyroidism, sensorineural deafness, and renal dysplasia	Hearing Loss
GIPC3	Deafness, autosomal recessive 15	Hearing Loss
GJB2	Deafness, autosomal dominant 3A, Deafness, autosomal recessive 1A	Hearing Loss

Gene Name	Associated Disease or Phenotype	Panel
GJB3	Deafness, autosomal dominant 2B, Deafness, autosomal recessive, Deafness, digenic, GJB2/GJB3	Hearing Loss
GJB6	Deafness, autosomal dominant 3B, Deafness, autosomal recessive 1B, Deafness, digenic, GJB2/GJB6	Hearing Loss
GPSM2	Chudley-McCullough syndrome	Hearing Loss
GRHL2	Deafness, autosomal dominant 28	Hearing Loss
GRXCR1	Deafness, autosomal recessive 25	Hearing Loss
HGF	Deafness, autosomal recessive 39	Hearing Loss
ILDR1	Deafness, autosomal recessive 42	Hearing Loss
KCNE1	Jervell and Lange-Nielsen syndrome 2	Hearing Loss
KCNJ10	Deafness, autosomal recessive 4, with enlarged vestibular aqueduct, SESAME syndrome	Hearing Loss
KCNQ1	Jervell and Lange-Nielsen syndrome	Hearing Loss
KCNQ4	Deafness, autosomal dominant 2A	Hearing Loss
LHFPL5	Deafness, autosomal recessive 67	Hearing Loss
LOXHD1	Deafness, autosomal recessive 77	Hearing Loss
LRTOMT	Deafness, autosomal recessive 63	Hearing Loss
MARVELD2	Deafness, autosomal recessive 49	Hearing Loss
MITF	Tietz albinism-deafness syndrome, Waardenburg syndrome, type 2A	Hearing Loss
MSRB3	Deafness, autosomal recessive 74	Hearing Loss
*MSX2**	Parietal foramina with cleidocranial dysplasia	Hearing Loss
MYH14	Deafness, autosomal dominant 4A	Hearing Loss
MYH9	Deafness, autosomal dominant 17, Epstein syndrome, Fechtner syndrome, Macrothrombocytopenia and progressive sensorineural deafness	Hearing Loss
MYO15A	Deafness, autosomal recessive 3	Hearing Loss
MYO1A	Sensorineural deafness, nonsyndromic	Hearing Loss
MYO1C	Sensorineural hearing loss, bilateral	Hearing Loss
MYO1F	Sensorineural hearing loss, bilateral	Hearing Loss
MYO3A	Deafness, autosomal recessive 30	Hearing Loss
MYO6	Deafness, autosomal dominant 22, Deafness, autosomal recessive 37	Hearing Loss
MYO7A	Deafness, autosomal dominant 11, Deafness, autosomal recessive 2, Usher syndrome, type 1B	Hearing Loss
OTOA	Deafness, autosomal recessive 22	Hearing Loss
OTOF	Deafness, autosomal recessive 9	Hearing Loss
PAX3	Craniofacial-deafness-hand syndrome, Waardenburg syndrome, type 1 and 3	Hearing Loss
PCDH15	Deafness, autosomal recessive 23, Usher syndrome, type 1D/F digenic	Hearing Loss
PDZD7	Usher syndrome, type IIC, GPR98/PDZD7 digenic	Hearing Loss
POU3F4	Deafness, X-linked 2	Hearing Loss
POU4F3	Deafness, autosomal dominant 15	Hearing Loss
PRPS1	Arts syndrome, Charcot-Marie-Tooth disease, X-linked recessive, 5, Deafness, X-linked 1	Hearing Loss

Gene Name	Associated Disease or Phenotype	Panel
PTPRQ	Deafness, autosomal recessive 84A	Hearing Loss
RDX	Deafness, autosomal recessive 24	Hearing Loss
SERPINB6	Deafness, autosomal recessive 91	Hearing Loss
SIX1	Branchiootic syndrome 3, Deafness, autosomal dominant 23	Hearing Loss
SIX5	Branchiootorenal syndrome 2	Hearing Loss
SLC17A8	Deafness, autosomal dominant 25	Hearing Loss
SLC26A4	Deafness, autosomal recessive 4, with enlarged vestibular aqueduct, Pendred syndrome	Hearing Loss
SLC26A5	Deafness, autosomal recessive 61	Hearing Loss
SLC33A1	Congenital cataracts, hearing loss, and neurodegeneration	Hearing Loss
SMPX	Deafness, X-linked 4	Hearing Loss
SNAI2	Waardenburg syndrome, type 2D	Hearing Loss
SOX10	PCWH syndrome, Waardenburg syndrome, type 2E, with or without neurologic involvement, Waardenburg syndrome, type 4C	Hearing Loss
STRC	Deafness, autosomal recessive 16	Hearing Loss
TCOF1	Treacher Collins syndrome 1	Hearing Loss
TECTA	Deafness, autosomal dominant 8/12, Deafness, autosomal recessive 21	Hearing Loss
TIMM8A	Mohr-Tranebjaerg syndrome	Hearing Loss
TJP2	Hearing loss, non-syndromic, autosomal dominant	Hearing Loss
TMC1	Deafness, autosomal dominant 36, Deafness, autosomal recessive 7	Hearing Loss
TMIE	Deafness, autosomal recessive 6	Hearing Loss
TMPRSS3	Deafness, autosomal recessive 8/10	Hearing Loss
TPRN	Deafness, autosomal recessive 79	Hearing Loss
TRIOBP	Deafness, autosomal recessive 28	Hearing Loss
USH1C	Deafness, autosomal recessive 18A, Usher syndrome, type 1C	Hearing Loss
USH1G	Usher syndrome, type 1G	Hearing Loss
USH2A	Usher syndrome, type 2A	Hearing Loss
WFS1	Deafness, autosomal dominant 6/14/38, Wolfram syndrome	Hearing Loss
WHRN	Deafness, autosomal recessive 31, Usher syndrome, type 2D	Hearing Loss
FH	Leiomyomatosis and renal cell cancer	Hereditary Cancer #
FLCN	Birt-Hogg-Dube syndrome	Hereditary Cancer
MAX	Pheochromocytoma, susceptibility to	Hereditary Cancer
MET	Renal cell carcinoma, papillary, 1, familial	Hereditary Cancer
RET	Pheochromocytoma	Hereditary Cancer
SDHAF2	Paragangliomas 2	Hereditary Cancer
SDHB	Paragangliomas 4, Pheochromocytoma	Hereditary Cancer
SDHC	Paragangliomas 3	Hereditary Cancer

Gene Name	Associated Disease or Phenotype	Panel
SDHD	Paraganglioma and gastric stromal sarcoma, Pheochromocytoma	Hereditary Cancer
TMEM127	Pheochromocytoma, susceptibility to	Hereditary Cancer
VHL	Pheochromocytoma, von Hippel-Lindau syndrome	Hereditary Cancer
BRAF	Cardiofaciocutaneous syndrome, LEOPARD syndrome 3, Noonan syndrome 7	RASopathy
CBL	Noonan syndrome-like disorder with or without juvenile myelomonocytic leukemia	RASopathy
HRAS	Costello syndrome	RASopathy
KRAS	Cardiofaciocutaneous syndrome 2, Noonan syndrome 3	RASopathy
MAP2K1	Cardiofaciocutaneous syndrome 3	RASopathy
MAP2K2	Cardiofaciocutaneous syndrome 4	RASopathy
NRAS	Noonan syndrome 6	RASopathy
PTPN11	LEOPARD syndrome 1, Noonan syndrome 1	RASopathy
RAF1	LEOPARD syndrome 2, Noonan syndrome 5	RASopathy
SHOC2	Noonan-like syndrome with loose anagen hair	RASopathy
SOS1	Noonan syndrome 4	RASopathy
SPRED1	Legius syndrome	RASopathy

* In two disorders

^EIEE, Early Infantile Epileptic Encephalopathy

Please note that this is not a comprehensive hereditary cancer panel. The genes selected here were based on specific herediatry cancer syndromes tested in this laboratory

Supplementary Table 2. Gene list for targeted hearing loss panel testing developed at the Laboratory for Molecular Medicine (LMM) at Partners HealthCare Personalized Medicine, Boston, MA.

Gene Name	Associated Disease or Phenotype	Panel
ACTG1	Baraitser-Winter syndrome	Hearing Loss
ATP6V1B1	Distal renal tubular acidosis	Hearing Loss
BSND	Bartter syndrome	Hearing Loss
CACNA1D	Bradycardia and deafness	Hearing Loss
CCDC50	Postlingual, progressive, moderate to profound SNHL*	Hearing Loss
CDH23	Usher syndrome type 1	Hearing Loss
CEACAM16	Postlingual, progressive, moderate SNHL	Hearing Loss
CIB2	Usher syndrome type 1	Hearing Loss
CLDN14	Prelingual, flat SNHL (variable progression)	Hearing Loss
CLPP	Perrault syndrome	Hearing Loss
CLRN1	Usher syndrome type 3	Hearing Loss
COCH	Vestibular impairment	Hearing Loss
COL11A2	Non-ocular Stickler syndrome (STL3), OSMED syndrome	Hearing Loss
DIABLO	Adulthood onset, progressive, mild to moderate, flat SNHL	Hearing Loss
DFNA5	Postlingual, progressive sloping SNHL	Hearing Loss
DFNB31	Usher syndrome type 2	Hearing Loss
DFNB59	Auditory neuropathy	Hearing Loss
DIAPH1	Postlingual, low frequency progressive SNHL	Hearing Loss
EDN3	Waardenburg syndrome type 4	Hearing Loss
EDNRB	Waardenburg syndrome type 4	Hearing Loss
ESPN	Vestibular areflexia, in some	Hearing Loss
ESRRB	Early onset, severe to profound, flat/slightly sloping SNHL	Hearing Loss
EYA1	Branchio-oto-renal syndrome	Hearing Loss
EYA4	Postlingual, progressive, moderate to profound, flat SNHL	Hearing Loss
GIPC3	Prelingual, mild to profound, flat SNHL	Hearing Loss
GJB2	Congenital/childhood onset, mild to profound SNHL	Hearing Loss
GJB6	Congenital/childhood onset, mild to profound SNHL, Hidrotic Ectodermal Dysplasia	Hearing Loss
GPR98	Usher syndrome type 2	Hearing Loss
GPSM2	Chudley-McCullough syndrome	Hearing Loss
GRHL2	Postlingual, progressive, mild to severe SNHL	Hearing Loss
GRXCR1	Congenital, moderate to profound, flat/slightly sloping SNHL	Hearing Loss
HARS	Usher syndrome type 3B	Hearing Loss
HARS2	Perrault syndrome	Hearing Loss
HGF	Prelingual, severe to profound, sloping SNHL	Hearing Loss
HSD17B4	Perrault syndrome, Bi-functional Protein Deficiency	Hearing Loss
ILDR1	Prelingual, moderate to profound, sloping SNHL	Hearing Loss
KARS	Peripheral neuropathy	Hearing Loss
KCNE1	Jervell and Lange-Nielsen syndrome	Hearing Loss
KCNQ1	Jervell and Lange-Nielsen syndrome	Hearing Loss
KCNQ4	Postlingual, progressive, sloping SNHL	Hearing Loss
LARS2	Perrault syndrome	Hearing Loss

Gene Name	Associated Disease or Phenotype	Panel
LHFPL5	Prelingual, severe to profound SNHL	Hearing Loss
LOXHD1	Fuchs corneal dystrophy	Hearing Loss
LRTOMT	Congenital, moderate to profound, flat SNHL	Hearing Loss
MARVELD2	Prelingual, moderate to profound, flat/sloping SNHL	Hearing Loss
MIR96	Vertigo in some	Hearing Loss
MITF	Waardenburg syndrome type 2	Hearing Loss
MSRB3	Prelingual, severe to profound, flat SNHL	Hearing Loss
MTRNR1	Aminoglycoside ototoxicity sensitivity	Hearing Loss
MTTS1	Variable, progressive SNHL	Hearing Loss
MYH14	Peripheral neuropathy	Hearing Loss
MYH9	Macrothrombocytopenia, MYH9-related disorder	Hearing Loss
MYO15A	Congenital, severe to profound, flat SNHL	Hearing Loss
MYO3A	Postlingual, progressive, moderate to severe, sloping SNHL	Hearing Loss
MYO6	Vestibular impairment in some	Hearing Loss
MYO7A	Usher syndrome type 1, Vestibular impairment	Hearing Loss
OTOA	Prelingual, severe to profound, flat SNHL	Hearing Loss
OTOF	Auditory neuropathy	Hearing Loss
OTOG	Vestibular impairment in some	Hearing Loss
OTOGL	Congenital, moderate to moderately severe, sloping SNHL	Hearing Loss
P2RX2	High frequency tinnitus	Hearing Loss
PAX3	Variable Waardenburg syndrome types 1 and 3	Hearing Loss
PCDH15	Usher syndrome type 1	Hearing Loss
POU3F4	Internal auditory canal dilation, perilymphatic gusher	Hearing Loss
POU4F3	Adult onset, progressive, moderate to severe, sloping SNHL	Hearing Loss
PRPS1	Charcot-Marie-Tooth disease, Arts syndrome, and prelingual non-syndromic deafness	Hearing Loss
RDX	Prelingual, severe to profound, flat SNHL	Hearing Loss
SERPINB6	Postlingual, moderate to severe, sloping SNHL	Hearing Loss
SIX1	Branchio-oto-renal syndrome	Hearing Loss
SLC26A4	Pendred syndrome	Hearing Loss
SMPX	Postlingual, progressive, moderate to profound, flat/sloping SNHL	Hearing Loss
SNAI2	Waardenburg syndrome type 2	Hearing Loss
SOX10	Waardenburg syndrome types 2 and 4	Hearing Loss
STRC	Deafness male infertility syndrome (DIS)	Hearing Loss
SYNE4	Pre/postlingual progressive, mild to profound, sloping SNHL	Hearing Loss
TBC1D24	Prelingual, profound, flat SNHL, Epilepsy	Hearing Loss
TECTA	Pre/postlingual, progressive (in some), mild to severe SNHL	Hearing Loss
TIMM8A	Mohr-Tranebjaerg syndrome	Hearing Loss
TMC1	Postlingual, progressive SNHL, Congenital, profound, flat/slightly sloping SNHL	Hearing Loss
TMIE	Congenital, severe to profound, flat SNHL	Hearing Loss
TMPRSS3	Congenital/childhood onset, severe to profound, flat SNHL	Hearing Loss
TPRN	Prelingual, severe to profound, flat/slightly sloping SNHL	Hearing Loss
TRIOBP	Prelingual, severe to profound, flat SNHL	Hearing Loss
TSPEAR	Congenital, profound, flat SNHL	Hearing Loss
USH1C	Usher syndrome type 1	Hearing Loss

Gene Name	Associated Disease or Phenotype	Panel
USH1G	Usher syndrome type 1	Hearing Loss
USH2A	Usher syndrome type 2, Isolated autosomal recessive retinitis pigmentosa	Hearing Loss
WFS1	Wolfram-like disorder, Wolfram syndrome	Hearing Loss

*SNHL, Sensorineural hearing loss

Supplementary Table 3. General quality control metrics tracking for samples.

Sequence metric	Note
Quality Score	Represents the purity of the sequencing read, signal-to-noise ratio. Reads with low quality scores are typically filtered out before base calling
Total number of reads generated	Density of sequencing reads immobilized on a flow cells. A higher cluster density decreases the ability of the instrument to decide independent reads. When the targeted number of reads is exceeded, the background noise is increase and multiple reads receive low purity scores and are filtered out before base calling and subsequent analysis
Percentage of total reads that pass filter	If the percentage of total reads that pass filter is low, it is an indication of suboptimal run
Signal strength	Intensity of the signal of each incorporated base, particularly at the first base and at specified subsequent bases
Phasing	Rate at which nucleotides are incorporated into the read per cycle that are higher/lower that the average of all simultaneous sequencing reactions, creating background noise and incorrect base calling
Base call	Quality (Q) score+ score on a log scale
Base call (all reads)	How well read maps to the reference
Genome Mappabiltiy Score	Probability that any read can be unambiguously mapped to a given position

Supplementary Table 3. General QC metrics tracking for samples (cont.).

Coverage Depth	Number of times each base has been sequenced
Supporting reads	Number of reads that support a variation call, count in each read direction and balance between directions
Allele Frequency	Percentage reads that contain a variant sequence
Duplicate reads	Number of reads with the same start position
Variation score	P-value or Bayes factor
Expected vs observed data yield	Overall data yield is within expected values obtained during validation
Evenness of coverage	Number of reads per base and throughout out the region(s) tested is similar
Depth of coverage	Number of reads per base
Phasing and Prephasing	Rate at which nucleotides are incorporated into sequencing read per cycle that are higher /lower than the average of all simultaneous sequencing reactions, creating background noise and incorrect base calling
Balance of forward and reverse reads (where applicable)	Number of reads in both directions of sequence is similar

Supplementary Table 4. Data used for analytical sensitivity and specificity for detection of single nucleotide variants.

Sample ID	True Negative	False Negative	True Positive	False Positive
1	72	0	76	1*
2	71	0	77	1*
3	69	0	77	2*
4	65	0	82	2*
5	613	0	198	0
6	633	0	182	2*
7	71	0	78	0
8	635	0	179	0
9	21549	0	4	0
10	21548	0	5	0
11	21548	0	5	0
12	21543	0	10	0
13	21547	0	6	0
NA12878	469121	0	264	1*
NA19240	469047	0	339	0
TOTAL	**1048132**	**0**	**1582**	**0**

* A recurring false positive variant in *HRAS* (chr11:534332) and *MAP2K2* (chr19:4102449) was identified. After extensive analysis, it was identified as an artifact most likely as a result of enrichment procedure. Repeating the enrichment assay with same specimen and a new enrichment kit led to improvement in the data with NO false positives.

Supplementary Table 5. Data used for analytical sensitivity and specificity for detection of insertion/deletions (indels).

	Indels (1-5 base pairs)	**False negative**	**True positive**	**False positive**
NA12878	469365	0	10	0
NA19240	469378	0	8	0
TOTAL	**938743**	**0**	**18**	**0**