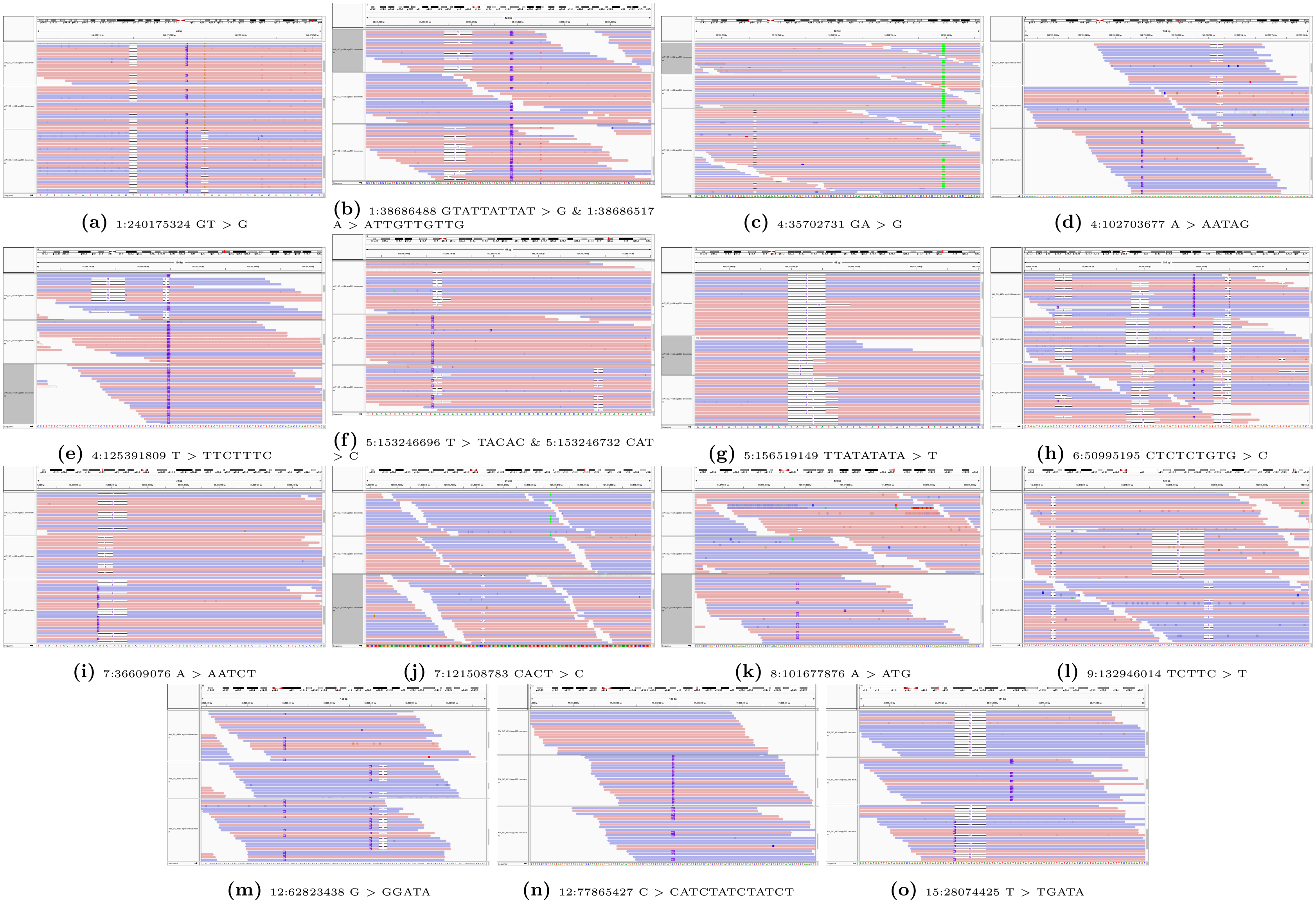
A unified haplotype-based method for accurate and comprehensive variant calling: supplementary material

Supplementary Table 1 Germline benchmarks summary.

Sample	Library	Caller	True-pos-baseline	True-pos-call	False-pos	False-neg	Precision	Sensitivity	F-measure
HG001	Truth	Octopus	3688874	3713175	2486	1987	0.9993	0.9995	0.9994
HG001	Truth	DeepVariant	3687641	3687675	1959	3220	0.9995	0.9991	0.9993
HG001	Truth	GATK4	3686632	3686735	8031	4229	0.9978	0.9989	0.9983
HG001	Truth	Strelka2	3685083	3686196	2287	5778	0.9994	0.9984	0.9989
HG001	Truth	FreeBayes	3650987	3586710	5326	39874	0.9985	0.9892	0.9938
HG001	Truth	Platypus	3502030	3414557	10312	188831	0.9970	0.9488	0.9723
HG001	Consistency	Octopus	3674314	3698104	9629	16547	0.9974	0.9955	0.9965
HG001	Consistency	DeepVariant	3657008	3657066	49097	33853	0.9868	0.9908	0.9888
HG001	Consistency	GATK4	3656111	3656215	52114	34750	0.9859	0.9906	0.9883
HG001	Consistency	Strelka2	3667704	3668747	18828	23157	0.9949	0.9937	0.9943
HG001	Consistency	FreeBayes	3601595	3538355	17834	89266	0.9950	0.9758	0.9853
HG001	Consistency	Platypus	3460120	3373190	17433	230741	0.9949	0.9375	0.9653
HG001	Platinum	Octopus	3680239	3703696	5429	10622	0.9985	0.9971	0.9978
HG001	Platinum	DeepVariant	3669774	3669810	10180	21087	0.9972	0.9943	0.9958
HG001	Platinum	GATK4	3670726	3670826	18685	20135	0.9949	0.9945	0.9947
HG001	Platinum	Strelka2	3668280	3669350	6633	22581	0.9982	0.9939	0.9960
HG001	Platinum	FreeBayes	3597884	3535832	12018	92977	0.9966	0.9748	0.9856
HG001	Platinum	Platypus	3438125	3353648	11285	252736	0.9966	0.9315	0.9630
HG001	10X	Octopus	3506224	3518332	136803	184637	0.9626	0.9500	0.9562
HG001	10X	DeepVariant	3481170	3481225	220935	209691	0.9403	0.9432	0.9418
HG001	10X	GATK4	3480829	3481016	261288	210032	0.9302	0.9431	0.9366
HG001	10X	Strelka2	3260263	3260833	576332	430598	0.8498	0.8833	0.8662
HG001	10X	FreeBayes	3336956	3282617	107384	353905	0.9683	0.9041	0.9351
HG001	10X	Platypus	3213670	3137415	68699	477191	0.9786	0.8707	0.9215
HG002	Truth	Octopus	3509581	3531194	2315	2775	0.9993	0.9992	0.9993
HG002	Truth	DeepVariant	3508465	3508607	1934	3891	0.9994	0.9989	0.9992
HG002	Truth	GATK4	3507537	3507738	7321	4819	0.9979	0.9986	0.9983
HG002	Truth	Strelka2	3504355	3505644	2070	8001	0.9994	0.9977	0.9986
HG002	Truth	FreeBayes	3468607	3404617	5006	43749	0.9985	0.9875	0.9930
HG002	Truth	Platypus	3318750	3235502	10423	193606	0.9968	0.9449	0.9701
HG002	10X	Octopus	3206705	3213519	163573	305651	0.9516	0.9130	0.9319
HG002	10X	DeepVariant	3190100	3190236	299000	322256	0.9143	0.9083	0.9113
HG002	10X	GATK4	3184855	3185054	253559	327501	0.9263	0.9068	0.9164
HG002	10X	Strelka2	2876831	2877241	720157	635525	0.7998	0.8191	0.8093
HG002	10X	FreeBayes	2589977	2549538	29970	922379	0.9884	0.7374	0.8446
HG002	10X	Platypus	2825392	2758545	64893	686964	0.9770	0.8044	0.8824
HG005	GIAB	Octopus	3429673	3445448	2275	2738	0.9993	0.9992	0.9993
HG005	GIAB	DeepVariant	3429961	3430084	1150	2450	0.9997	0.9993	0.9995
HG005	GIAB	GATK4	3427314	3427526	5324	5097	0.9984	0.9985	0.9985
HG005	GIAB	Strelka2	3428363	3429451	1986	4048	0.9994	0.9988	0.9991
HG005	GIAB	FreeBayes	3409962	3349333	3359	22449	0.9990	0.9935	0.9962
HG005	GIAB	Platypus	3292042	3207968	11481	140369	0.9964	0.9591	0.9774
Syndip	Broad	Octopus	3971376	3990730	82342	105228	0.9798	0.9742	0.9770
Syndip	Broad	DeepVariant	3948110	3949749	70922	128552	0.9824	0.9685	0.9754
Syndip	Broad	GATK4	3830761	3834235	132829	245820	0.9665	0.9397	0.9529
Syndip	Broad	Strelka2	3924087	3924538	56453	152633	0.9858	0.9626	0.9741
Syndip	Broad	FreeBayes	3557667	3473034	77584	519066	0.9781	0.8727	0.9224
Syndip	Broad	Platypus	3527485	3435146	52729	549232	0.9849	0.8653	0.9212



(a) 1:240175324 GT > G

(b) 1:38686488 GTATTATTAT > G & 1:38686517 A > ATTGTTGTTG

(c) 4:35702731 GA > G

(d) 4:102703677 A > AATAG

(e) 4:125391809 T > TTCTTTC

(f) 5:153246696 T > TACAC & 5:153246732 CAT > C

(g) 5:156519149 TTATATATA > T

(h) 6:50995195 CTCTCTGTG > C

(i) 7:36609076 A > AATCT

(j) 7:121508783 CACT > C

(k) 8:101677876 A > ATG

(l) 9:132946014 TCTTC > T

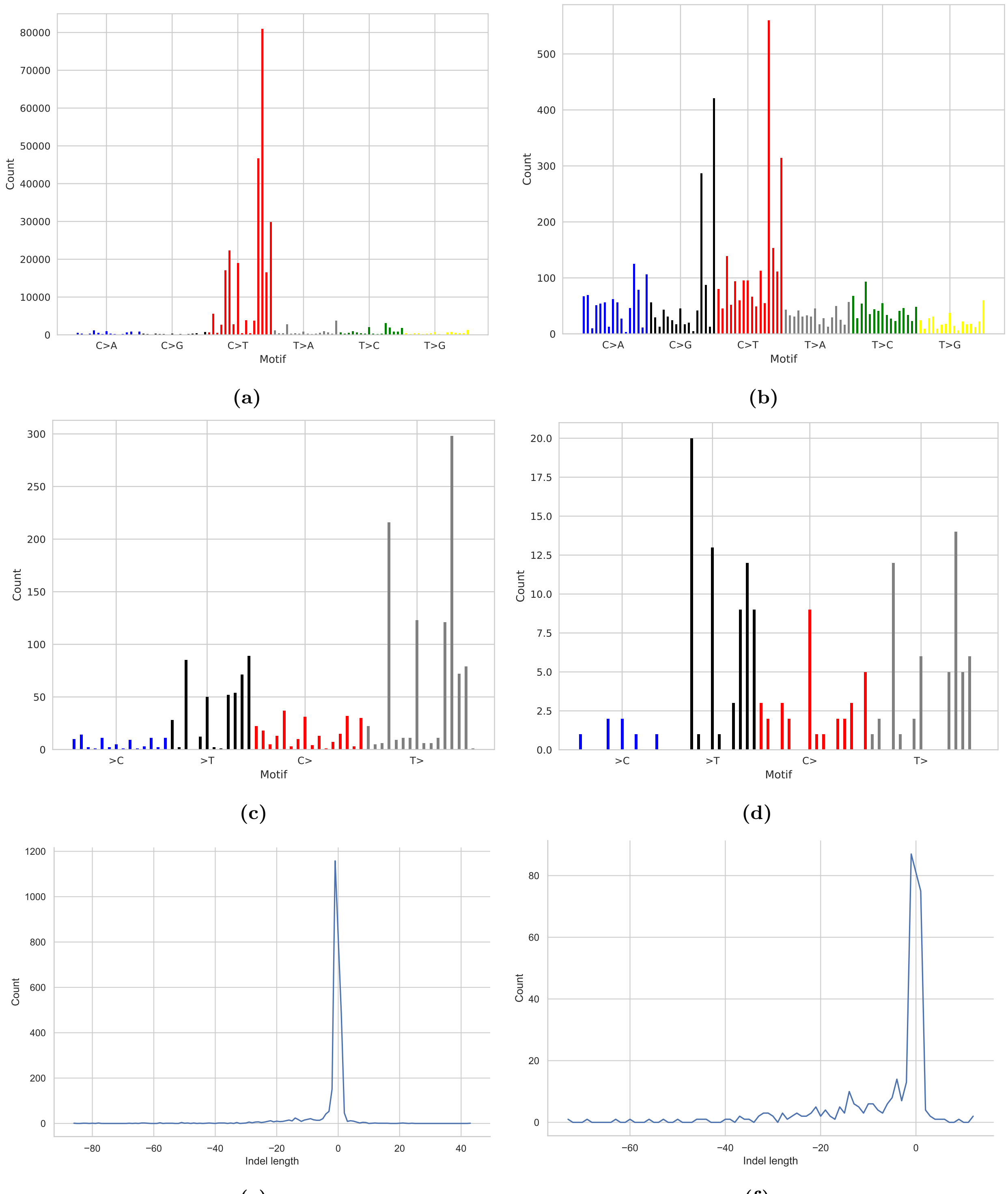
(m) 12:62823438 G > GGATA

(n) 12:77865427 C > CATCTATCTATCT

(o) 15:28074425 T > TGATA

Supplementary Figure 1 Octopus evidence BAM realignments for curated indel *de novo* mutations. Realigned reads are shown for parents (top two panels) and offspring (bottom panel).

.



(a)

(b)

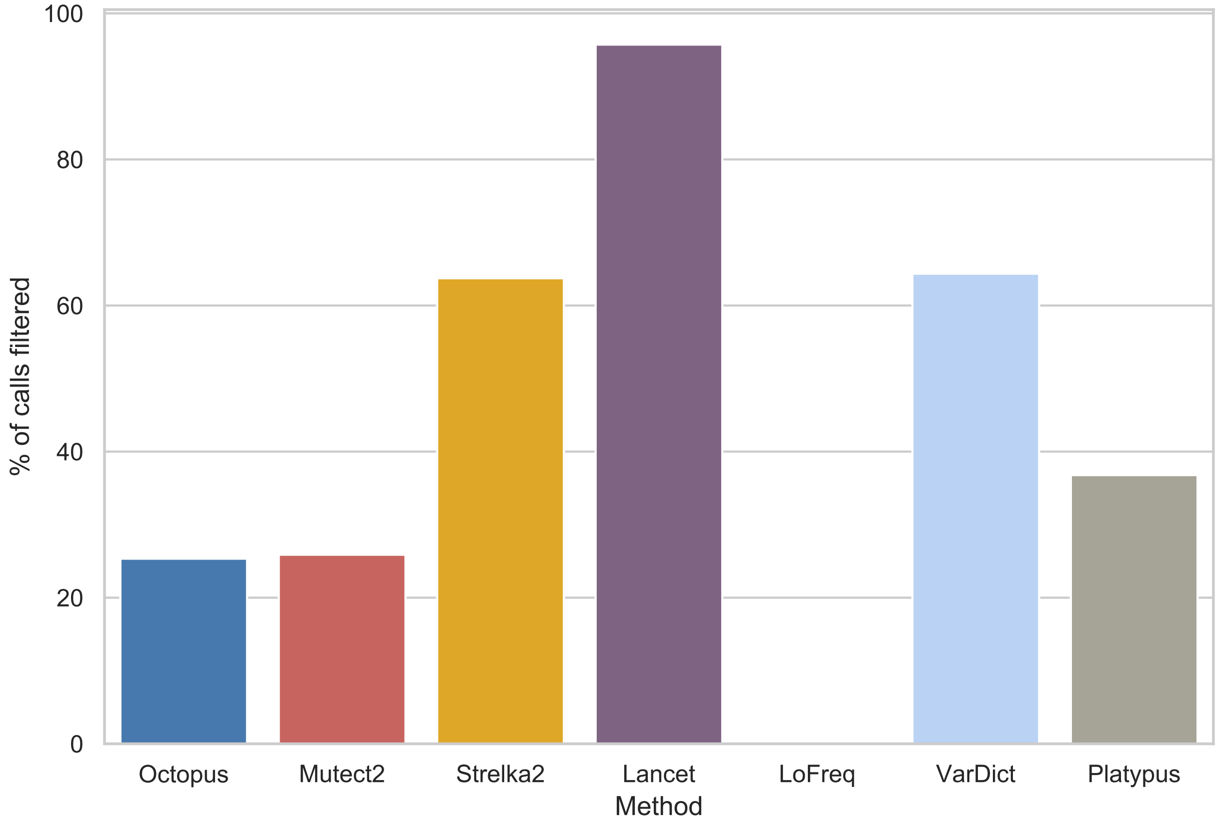
(c)

(d)

(e)

(f)

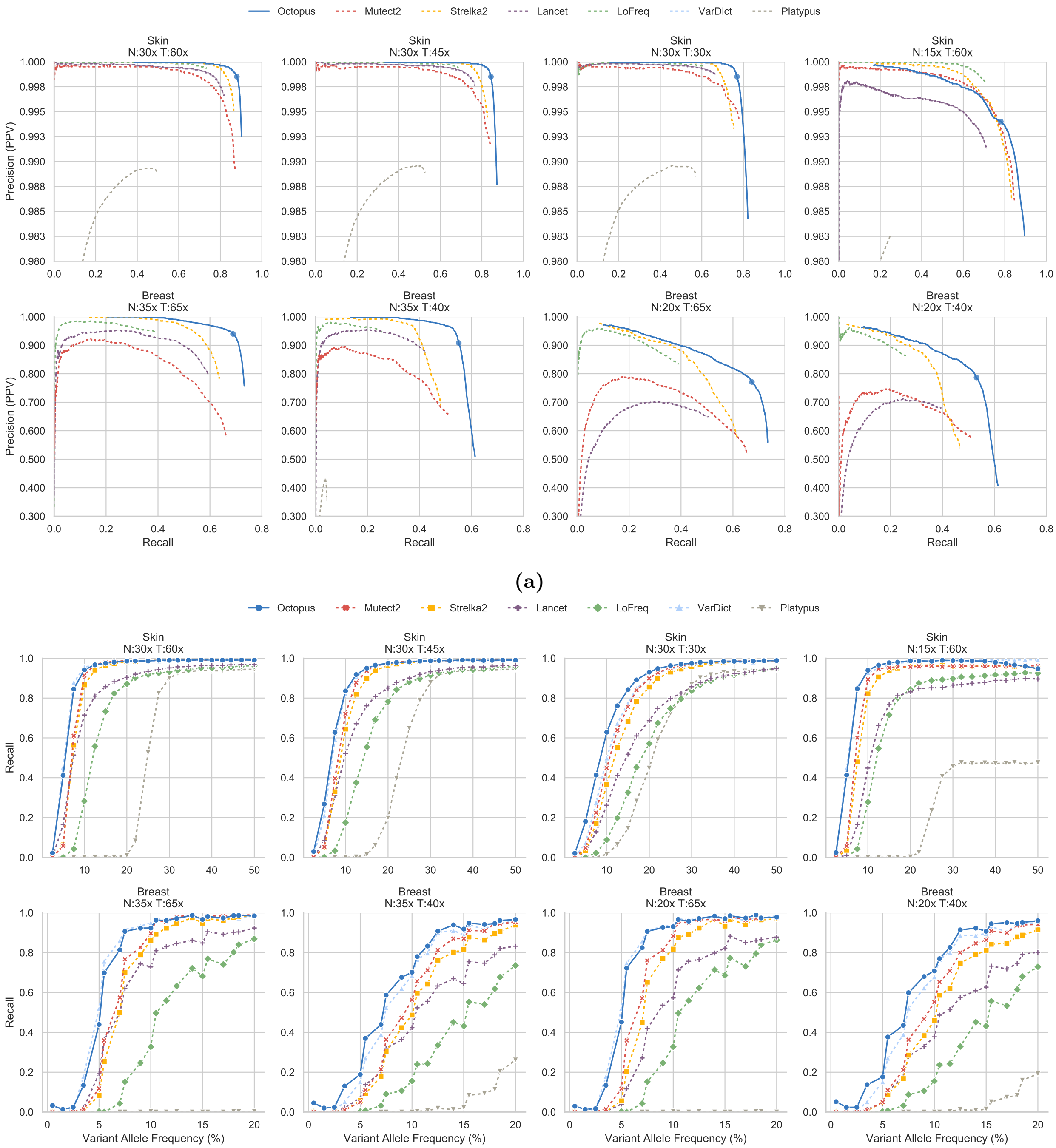
Supplementary Figure 2 Mutation profiles of synthetic tumours. **a**, **b** show the SNV distribution with 1 bp context in the synthetic skin tumour (left) and synthetic breast tumour (right). **c**, **d** show the 1 bp indel distribution with 1 bp context in the synthetic skin tumour (left) and synthetic breast tumour (right). **e**, **f** show the size distribution of indels in the synthetic skin tumour (left) and synthetic breast tumour (right).



Supplementary Figure 3 Proportion of somatic calls filtered for the paired synthetic skin (N:30x T:60x).

Supplementary Table 2 Paired somatic benchmarks summary.

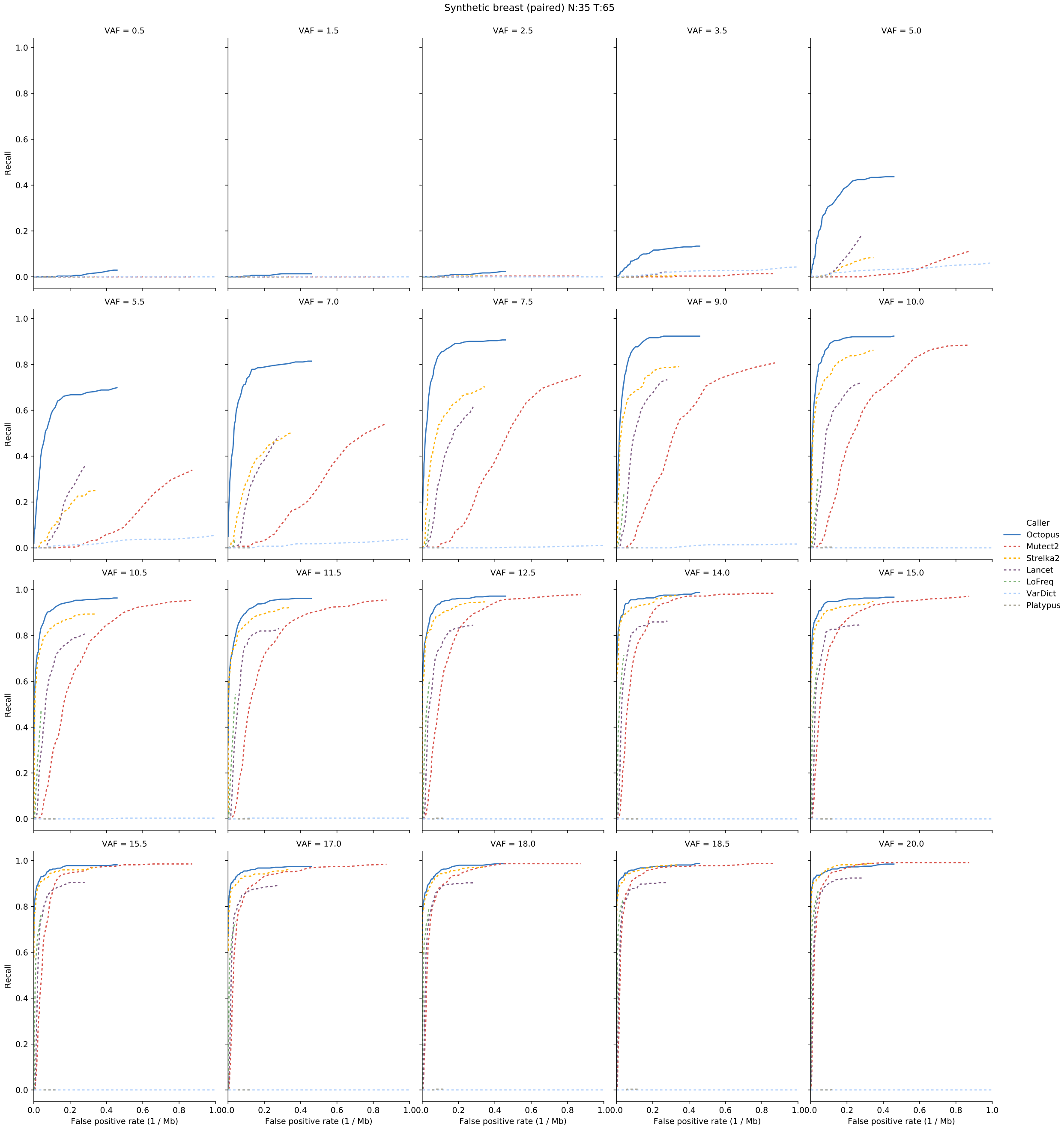
Tumour	Normal depth	Tumour depth	Caller	True-pos-baseline	True-pos-call	False-pos	False-neg	Precision	Sensitivity	F-measure
skin	15	60	Octopus	251710	251720	4467	29586	0.9826	0.8948	0.9366
skin	15	60	Mutect2	238088	238087	3344	43208	0.9861	0.8464	0.9109
skin	15	60	Strelka2	234156	234155	3345	47140	0.9859	0.8324	0.9027
skin	15	60	Lancet	200337	200337	1767	80959	0.9913	0.7122	0.8289
skin	15	60	LoFreq	199181	199181	418	82115	0.9979	0.7081	0.8284
skin	15	60	VarDict	253293	253292	60745	28003	0.8066	0.9005	0.8509
skin	15	60	Platypus	69530	69530	1235	211766	0.9825	0.2472	0.3950
skin	30	30	Octopus	231214	231221	3689	50082	0.9843	0.8220	0.8958
skin	30	30	Mutect2	219304	219304	1322	61992	0.9940	0.7796	0.8739
skin	30	30	Strelka2	212106	212106	1460	69190	0.9932	0.7540	0.8572
skin	30	30	Lancet	186781	186781	245	94515	0.9987	0.6640	0.7977
skin	30	30	LoFreq	170193	170193	71	111103	0.9996	0.6050	0.7538
skin	30	30	VarDict	221108	221108	14820	60188	0.9372	0.7860	0.8550
skin	30	30	Platypus	161007	161007	1876	120289	0.9885	0.5724	0.7250
skin	30	45	Octopus	245479	245489	3067	35817	0.9877	0.8727	0.9266
skin	30	45	Mutect2	236521	236521	1970	44775	0.9917	0.8408	0.9101
skin	30	45	Strelka2	232496	232496	1339	48800	0.9943	0.8265	0.9027
skin	30	45	Lancet	215495	215495	514	65801	0.9976	0.7661	0.8667
skin	30	45	LoFreq	193533	193533	112	87763	0.9994	0.6880	0.8150
skin	30	45	VarDict	242924	242923	24927	38372	0.9069	0.8636	0.8847
skin	30	45	Platypus	148213	148213	1661	133083	0.9889	0.5269	0.6875
skin	30	60	Octopus	253689	253701	1908	27607	0.9925	0.9019	0.9450
skin	30	60	Mutect2	244985	244984	2686	36311	0.9892	0.8709	0.9263
skin	30	60	Strelka2	243142	243141	1200	38154	0.9951	0.8644	0.9251
skin	30	60	Lancet	229919	229919	782	51377	0.9966	0.8174	0.8981
skin	30	60	LoFreq	206142	206142	139	75154	0.9993	0.7328	0.8456
skin	30	60	VarDict	254466	254465	37176	26830	0.8725	0.9046	0.8883
skin	30	60	Platypus	139286	139286	1553	142010	0.9890	0.4952	0.6599
breast	20	40	Octopus	3652	3652	5304	2302	0.4078	0.6134	0.4899
breast	20	40	Mutect2	3041	3041	2230	2913	0.5769	0.5107	0.5418
breast	20	40	Strelka2	2778	2778	2402	3176	0.5363	0.4666	0.4990
breast	20	40	Lancet	2355	2355	1115	3599	0.6787	0.3955	0.4998
breast	20	40	LoFreq	1547	1547	242	4407	0.8647	0.2598	0.3996
breast	20	40	VarDict	3443	3443	30806	2511	0.1005	0.5783	0.1713
breast	20	40	Platypus	186	186	548	5768	0.2534	0.0312	0.0556
breast	20	65	Octopus	4369	4369	3432	1585	0.5601	0.7338	0.6353
breast	20	65	Mutect2	3913	3913	3620	2041	0.5194	0.6572	0.5803
breast	20	65	Strelka2	3664	3664	2746	2290	0.5716	0.6154	0.5927
breast	20	65	Lancet	3009	3009	1633	2945	0.6482	0.5054	0.5680
breast	20	65	LoFreq	2327	2327	467	3627	0.8329	0.3908	0.5320
breast	20	65	VarDict	4406	4406	60024	1548	0.0684	0.7400	0.1252
breast	20	65	Platypus	4	4	517	5950	0.0077	0.0007	0.0012
breast	35	40	Octopus	3656	3656	3529	2298	0.5088	0.6140	0.5565
breast	35	40	Mutect2	3074	3074	1642	2880	0.6518	0.5163	0.5762
breast	35	40	Strelka2	2864	2864	1299	3090	0.6880	0.4810	0.5662
breast	35	40	Lancet	2527	2527	371	3427	0.8720	0.4244	0.5709
breast	35	40	LoFreq	1554	1554	89	4400	0.9458	0.2610	0.4091
breast	35	40	VarDict	3464	3464	20879	2490	0.1423	0.5818	0.2287
breast	35	40	Platypus	254	254	466	5700	0.3528	0.0427	0.0761
breast	35	65	Octopus	4359	4359	1399	1595	0.7570	0.7321	0.7444
breast	35	65	Mutect2	3960	3960	2889	1994	0.5782	0.6651	0.6186
breast	35	65	Strelka2	3785	3785	1062	2169	0.7809	0.6357	0.7009
breast	35	65	Lancet	3529	3529	904	2425	0.7961	0.5927	0.6795
breast	35	65	LoFreq	2337	2337	142	3617	0.9427	0.3925	0.5543
breast	35	65	VarDict	4421	4421	41031	1533	0.0973	0.7425	0.1720
breast	35	65	Platypus	6	6	389	5948	0.0152	0.0010	0.0019



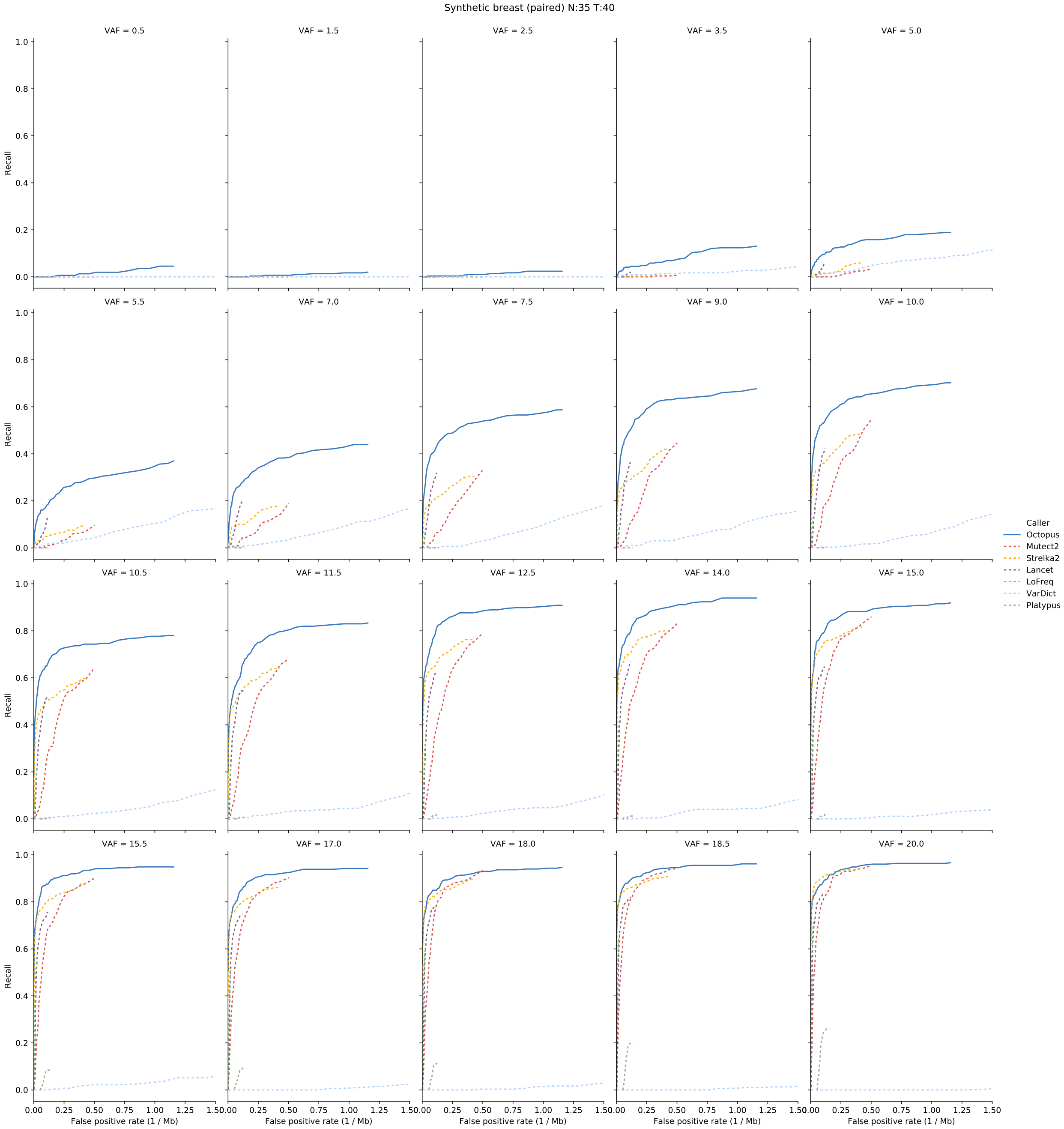
(a)

(b)

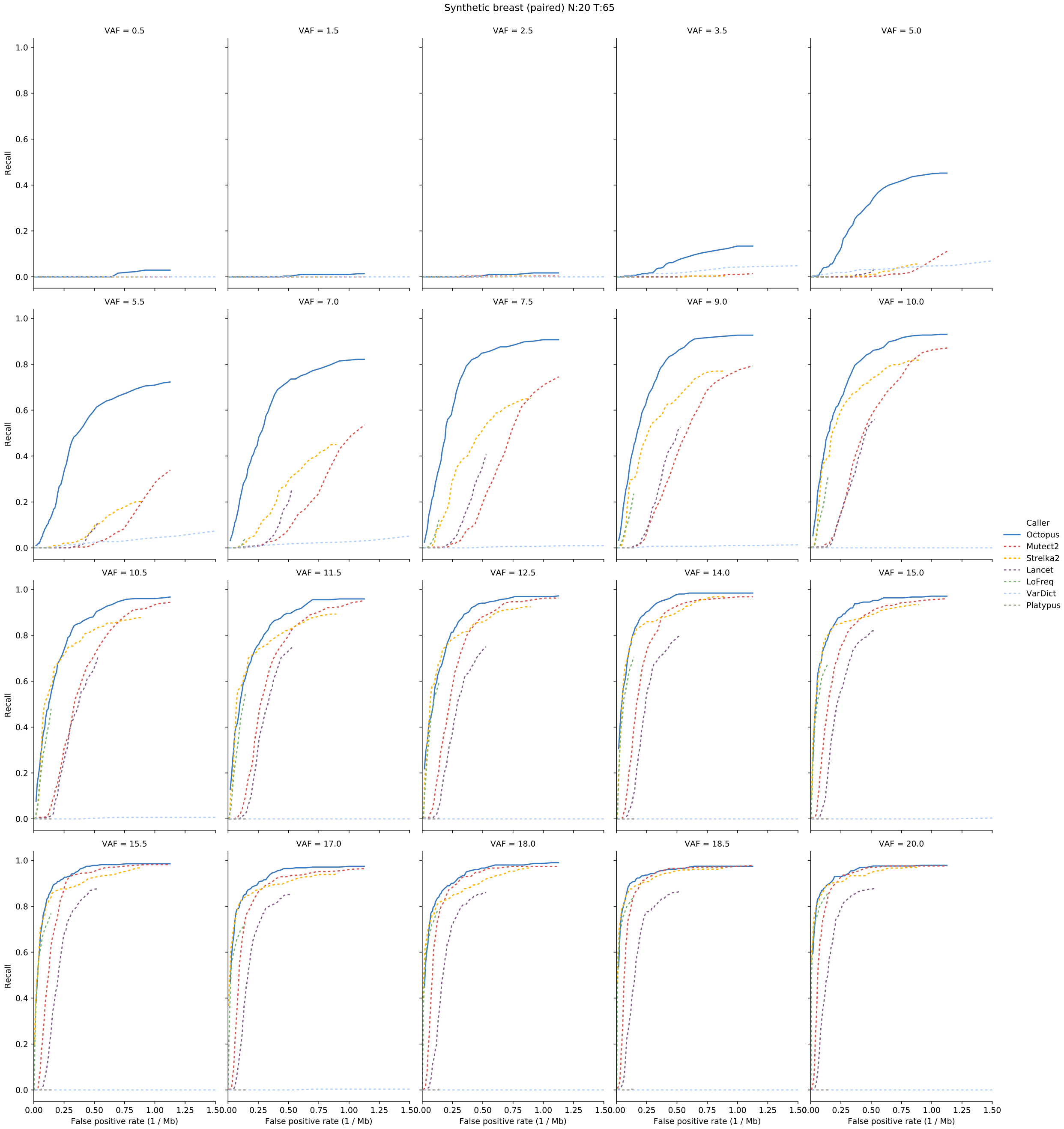
Supplementary Figure 4 Somatic mutation calling accuracy for synthetic skin and breast tumours with a paired normal sample with downsampling applied. **a** Precision-recall curves. Scoring metrics used to generate curves were RFQUAL (Octopus), TLOD (Mutect2), SomaticEVS (Strelka2), QUAL (Lancet), QUAL (LoFreq), SSF (VarDict), and QUAL (Platypus). Only PASS calls are used. VarDict is not visible as it is outside the axis limits due to low precision. Precisions on the two tests are substantially different as the skin set has almost 50 times as many true mutations as the breast set. Dots on the Octopus curve are placed at RFQUAL 7 (3 is used for the entire curve). **b** Recalls for each Variant Allele Frequency (VAF) using PASS variants. Points show true spike-in VAFs. All comparisons to the synthetic tumour truth sets were performed using RTG Tools vcfeval (version 3.9.1).



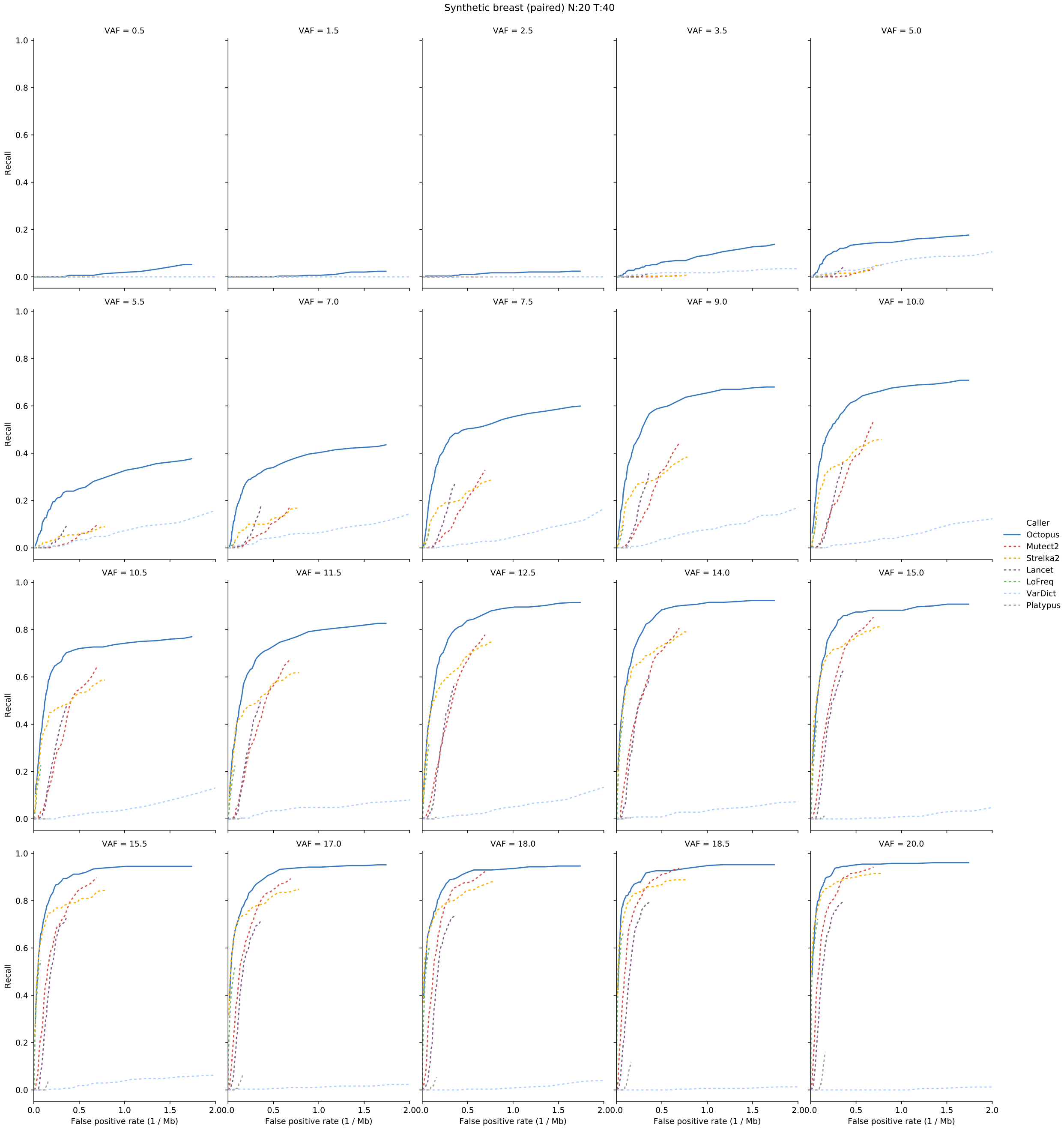
Supplementary Figure 5 Evaluation of VAF sensitivity in paired synthetic breast sample. Scoring metrics used to generate curves were RFQUAL (Octopus), TLOD (Mutect2), SomaticEVS (Strelka2), QUAL (Lancet), QUAL (LoFreq), SSF (VarDict), and QUAL (Platypus). The false positive rate were calculated using all false positive calls at each score threshold. Calls were compared to the truth sets using RTG Tools vcfeval.



Supplementary Figure 6 Evaluation of VAF sensitivity in paired synthetic breast sample with downsampled tumour sample. Scoring metrics used to generate curves were RFQUAL (Octopus), TLOD (Mutect2), SomaticEVS (Strelka2), QUAL (Lancet), QUAL (LoFreq), SSF (VarDict), and QUAL (Platypus). The false positive rate were calculated using all false positive calls at each score threshold. Calls were compared to the truth sets using RTG Tools vcfeval.



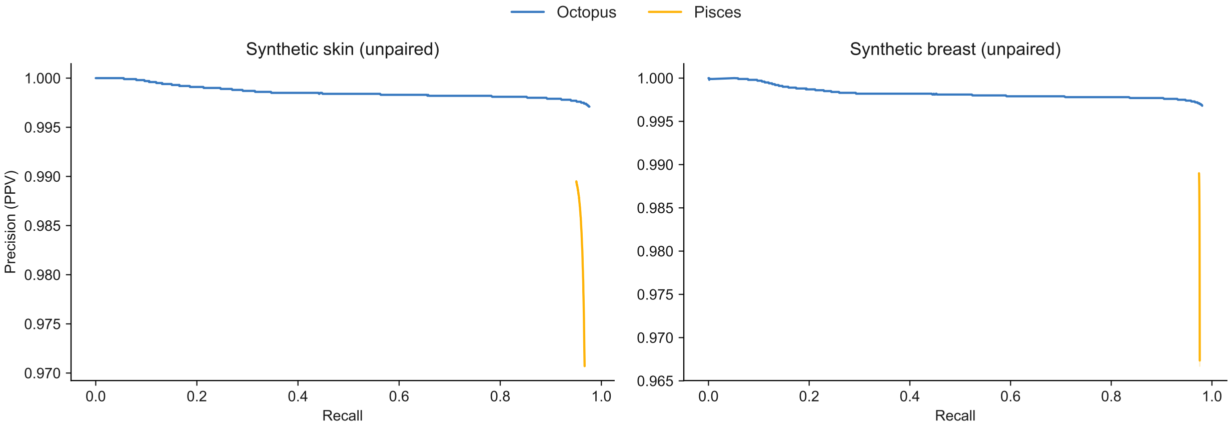
Supplementary Figure 7 Evaluation of VAF sensitivity in paired synthetic breast sample with downsampled normal sample. Scoring metrics used to generate curves were RFQUAL (Octopus), TLOD (Mutect2), SomaticEVS (Strelka2), QUAL (Lancet), QUAL (LoFreq), SSF (VarDict), and QUAL (Platypus). The false positive rate were calculated using all false positive calls at each score threshold. Calls were compared to the truth sets using RTG Tools vcfeval.



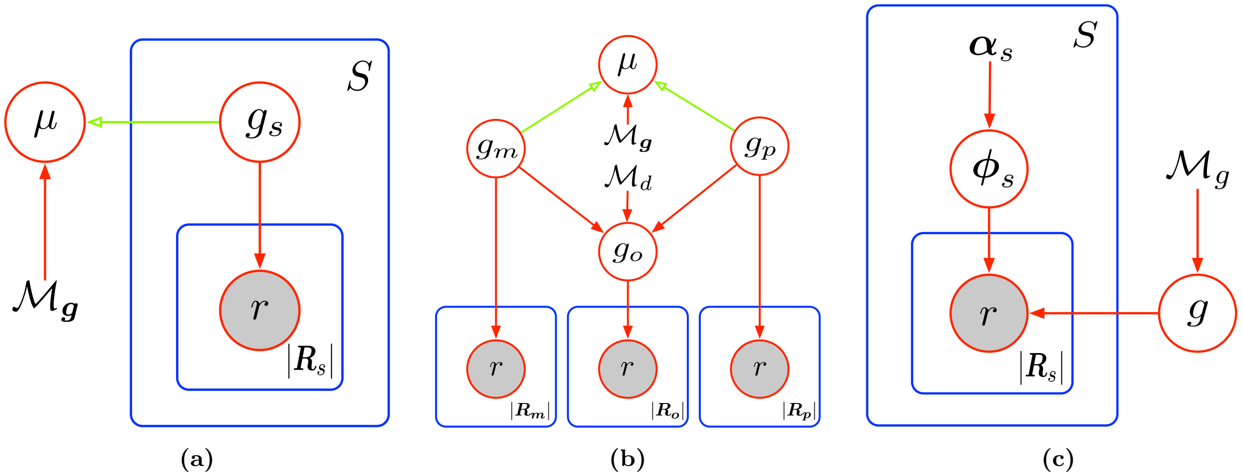
Supplementary Figure 8 Evaluation of VAF sensitivity in paired synthetic breast sample with down-sampled normal and normal samples. Scoring metrics used to generate curves were RFQUAL (Octopus), TLOD (Mutect2), SomaticEVS (Strelka2), QUAL (Lancet), QUAL (LoFreq), SSF (VarDict), and QUAL (Platypus). The false positive rate were calculated using all false positive calls at each score threshold. Calls were compared to the truth sets using RTG Tools vcfeval.

Supplementary Table 3 Unpaired somatic benchmarks summary.

Tumour	Caller	Test	True-pos-baseline	True-pos-call	False-pos	False-neg	Precision	Sensitivity	F-measure
skin	Octopus	combined	3748037	3770554	11129	90523	0.9971	0.9764	0.9866
skin	Octopus	somatic	153332	153332	10740	108579	0.9345	0.5854	0.7199
skin	Pisces	combined	3711460	3718366	112203	127088	0.9707	0.9669	0.9688
skin	Pisces	somatic	65242	65242	71600	196669	0.4768	0.2491	0.3272
breast	Octopus	combined	3624884	3648238	11566	71775	0.9968	0.9806	0.9886
breast	Octopus	somatic	3978	3978	9948	1527	0.2857	0.7226	0.4094
breast	Pisces	combined	3607097	3614212	124363	89555	0.9667	0.9758	0.9712
breast	Pisces	somatic	3115	3115	84648	2390	0.0355	0.5658	0.0668



Supplementary Figure 9 Tumour-only combined germline and somatic precision-recall.



Supplementary Figure 10 Genotype models shown in plate notation. **a** Population. **b** Trio. **c** Subclone. Symbols insides circles are latent variables, observed variables are shaded. Symbols inside boxes are repeated. Symbols not inside a circle are parameters or models. Arrows define conditional relationships, red for stochastic and green for deterministic. μ is used to denote parameters for a joint genotype prior model $\mathcal{M}_g$. Remaining symbols are defined in the the Online Methods.

Supplementary Note 1. Data and software

Software versions

General

- BWA (0.7.17-r1188)
- Samtools (1.7)
- Bcftools (1.7)
- RTG Tools (3.9.1)

Germline and *de novo* analysis

- Octopus (v0.5.2-beta)
- DeepVariant (v0.5.1)
- GATK4 (v4.0.0.0)
- Platypus (0.8.1)
- Strelka2 (v2.9.1)

Somatic analysis

- Octopus (v0.5.1-beta)
- Mutect2 (GATK4 v4.0.0.0)
- Platypus (0.8.1)
- Strelka2 (v2.9.1)
- LoFreq (v2.1.3.1)
- VarDict (1.5.2-java)
- Lancet (v1.0.6)
- Pisces (5.2.7.47)

Data sources

References

We used hs37d5 (`ftp://ftp-trace.ncbi.nih.gov/1000genomes/ftp/technical/reference/phase2_reference_assembly_sequence/hs37d5.fa.gz`) for all analysis.

Germline truth data

Name	Truth VCF	High confidence BED
GIAB HG001	ftp://ftp-trace.ncbi.nlm.nih.gov//giab/ftp/release/NA12878_HG001/NISTv3.3.2/GRCh37/HG001_GRCh37_GIAB_highconf_CG-IllFB-IllGATKHC-Ion-10X-SOLID_CHROM1-X_v.3.3.2_highconf_PGandRTGphasetransfer.vcf.gz	ftp://ftp-trace.ncbi.nlm.nih.gov//giab/ftp/release/NA12878_HG001/NISTv3.3.2/GRCh37/HG001_GRCh37_GIAB_highconf_CG-IllFB-IllGATKHC-Ion-10X-SOLID_CHROM1-X_v.3.3.2_highconf_nosomaticdel.bed
GIAB HG002	ftp://ftp-trace.ncbi.nlm.nih.gov//giab/ftp/release/AshkenazimTrio/HG002_NA24385_son/NISTv3.3.2/GRCh37/HG002_GRCh37_GIAB_highconf_CG-IllFB-IllGATKHC-Ion-10X-SOLID_CHROM1-22_v.3.3.2_highconf_triophased.vcf.gz	ftp://ftp-trace.ncbi.nlm.nih.gov//giab/ftp/release/AshkenazimTrio/HG002_NA24385_son/NISTv3.3.2/GRCh37/HG002_GRCh37_GIAB_highconf_CG-IllFB-IllGATKHC-Ion-10X-SOLID_CHROM1-22_v.3.3.2_highconf_noinconsistent.bed
GIAB HG005	ftp://ftp-trace.ncbi.nlm.nih.gov//giab/ftp/release/ChineseTrio/HG005_NA24631_son/NISTv3.3.2/GRCh37/HG005_GRCh37_highconf_CG-IllFB-IllGATKHC-Ion-SOLID_CHROM1-22_v.3.3.2_highconf.vcf.gz	ftp://ftp-trace.ncbi.nlm.nih.gov//giab/ftp/release/ChineseTrio/HG005_NA24631_son/NISTv3.3.2/GRCh37/HG005_GRCh37_highconf_CG-IllFB-IllGATKHC-Ion-SOLID_CHROM1-22_v.3.3.2_highconf_noMetaSV.bed
CHM1-CHM13	https://github.com/lh3/CHM-eval/releases/download/v0.5/CHM-evalkit-20180222.tar (full.37m.vcf.gz)	https://github.com/lh3/CHM-eval/releases/download/v0.5/CHM-evalkit-20180222.tar (full.37m.bed.gz)

Germline analysis sequencing data

Name	BAM/FASTQ	URL(s)	Notes
Precision FDA Truth (HG001)	FASTQ	https://precision.fda.gov/challenges/truth	Login required to access data
Precision FDA Truth (HG002)	FASTQ	https://precision.fda.gov/challenges/truth	Login required to access data
Precision FDA Consistency (HG001)	FASTQ	https://precision.fda.gov/challenges/truth	Login required to access data
GIAB (HG005)	FASTQ	ftp://ftp-trace.ncbi.nlm.nih.gov//giab/ftp/data/ChineseTrio/HG005_NA24631_son/HG005_NA24631_son_HiSeq_300x/basespace_250bps_fastqs/150430_HG005_Homogeneity_03_FCB-22310288 **and** ftp://ftp-trace.ncbi.nlm.nih.gov//giab/ftp/data/ChineseTrio/HG005_NA24631_son/HG005_NA24631_son_HiSeq_300x/basespace_250bps_fastqs/150506_HG005_Homogeneity_04_FCA-22365346	Fastq files were merged.
10X (HG001)	BAM	ftp://ftp-trace.ncbi.nlm.nih.gov//giab/ftp/data/NA12878/10XGenomics/NA12878_phased_possorted_bam.bam	Mapped to hg19.
10X (HG002)	BAM	ftp://ftp-trace.ncbi.nlm.nih.gov//giab/ftp/data/AshkenazimTrio/HG002_NA24385_son/10XGenomics/NA24385_phased_possorted_bam.bam	Mapped to hg19.
Platinum Genomes (HG001)	FASTQ	https://storage.googleapis.com/genomics-public-data/platinum-genomes/fastq/ERR194147_1.fastq.gz **and** https://storage.googleapis.com/genomics-public-data/platinum-genomes/fastq/ERR194147_2.fastq.gz	.
CHM1-CHM13	BAM	ftp://ftp.sra.ebi.ac.uk/vol1/ERA596/ERA596361/bam/CHM1_CHM13_2.bam	.

Sample	Library	Library prep	Read Length	Coverage	Instrument
HG001	Truth	TruSeq DNA PCR-Free	2x148bp	50x	HiSeq 2500
HG001	Consistency	TruSeq Nano DNA Library Prep kit	2x150bp	40x	HiSeq X Ten
HG001	10X	10X GemCode	2x98bp	34x	HiSeq 2500
HG001	Platinum	TruSeq DNA PCR-Free	2x101bp	50x	HiSeq 2000
HG002	Truth	TruSeq DNA PCR-Free	2x148bp	50x	HiSeq 2500
HG002	10X	10X GemCode	2x98bp	25x	HiSeq 2500
HG005	GIAB	TruSeq DNA PCR-Free	2x250bp	50x	HiSeq 2500
Syndip	Broad	Kapa Biosystems DNA PCR-Free	2x151bp	45x	HiSeq X Ten

De novo analysis sequencing data

Fastq files for each sample in the trio were obtained from the Wellcome Centre for Human Genetics.

Somatic analysis sequencing data

Fastq files were downloaded from `ftp://ftp-trace.ncbi.nlm.nih.gov//giab/ftp/data/NA12878/NIST_NA12878_HG001_HiSeq_300x`.

Germline random forest training data

For the germline analysis, we used three whole-genome replicates of NA12878 (HG001) for training the random forest:

1. High coverage Illumina reads from the 1000G phase 3 project, Aligned reads were downloaded from `ftp.1000genomes.ebi.ac.uk:/vol1/ftp/phase3/data/NA12878/high_coverage_alignment/NA12878.mapped.ILLUMINA.bwa.CEU.high_coverage_pcr_free.20130906.bam`.

2. Low coverage Illumina reads from the 1000G phase 3 project. Aligned reads were downloaded from `ftp.1000genomes.ebi.ac.uk:/vol1/ftp/phase3/data/NA12878/alignment/NA12878.mapped.ILLUMINA.bwa.CEU.low_coverage.20121211.bam`.

3. High coverage Illumina X Ten reads. Raw FASTQ files were downloaded from `https://s3-ap-southeast-2.amazonaws.com/kccg-x10-truseq-nano-v2.5-na12878/NA12878_V2.5_Robot_2_R1.fastq.gz` and `https://s3-ap-southeast-2.amazonaws.com/kccg-x10-truseq-nano-v2.5-na12878/NA12878_V2.5_Robot_2_R2.fastq.gz`.

Resources

1. GATK4 resources were downloaded from `ftp://ftp.broadinstitute.org//bundle/b37`.

2. Mutect2 resources were downloaded from `ftp://ftp.broadinstitute.org//bundle/Mutect2`.

Supplementary Note 3. Command lines

General

Read mapping

We used BWA-MEM with default configuration to map all reads:

```
$ bwa mem -t $nthreads -R "@RG\tID:${read_group}\tSM:${sample}\tLB:${library}\tPU:${platform}" \
      $reference \
      $fastq1 $fastq2 \
      | samtools view -bh | samtools sort -o $bam -
$ samtools index $bam
```

Read pre-processing

For tools that recommended that reads be pre-processed before variant calling, we used GATK4 to mark duplicates and recalibrate base qualities:

```
$ gatk --java-options -Xmx12G MarkDuplicates \
     -I $bam \
     -O $bam_dedup \
     -M $bam_dedup_metrics

$ gatk --java-options -Xmx4G BaseRecalibrator \
    -R $reference \
    -I $bam \
    --known-sites dbsnp_138.b37.vcf.gz \
    --known-sites Mills_and_1000G_gold_standard.indels.b37.vcf.gz \
    --known-sites 1000G_phase1.indels.b37.vcf.gz \
    -O $bam_recal_table
$ gatk --java-options -Xmx4G ApplyBQSR
    -R $reference \
    -I $bam \
    --bqsr-recal-file $bam_recal_table \
    -O $bam_recal
```

Germline analysis

Germline variant calling

DeepVariant

We followed the guidance given on GitHub (https://github.com/google/deepvariant/blob/r0.5/docs/deepvariant-case-study.md).

```
$ BUCKET="gs://deepvariant"
$ BIN_VERSION="0.5.1"
$ MODEL_VERSION="0.5.0"
$ MODEL_CL="182548131"
$ BIN_BUCKET="${BUCKET}/binaries/DeepVariant/${BIN_VERSION}/DeepVariant-${BIN_VERSION}+cl-*"
$ MODEL_BUCKET="${BUCKET}/models/DeepVariant/${MODEL_VERSION}/DeepVariant-inception_v3-${
    MODEL_VERSION}+cl-${MODEL_CL}.data-wgs_standard"
$ BIN_DIR="deepvariant"
$ N_SHARDS=$nthreads
$ EXAMPLES="$bam.tfrecord@${N_SHARDS}.gz"
$ CALL_VARIANTS_OUTPUT="${bam}.cvo.tfrecord.gz"
$ LOG_DIR=log
$ ( time seq 0 $((N_SHARDS-1)) | \
  parallel --halt 2 --joblog "${LOG_DIR}/log" --res "${LOG_DIR}" \
    python "${BIN_DIR}"/make_examples.zip \
      --mode calling \
      --ref "${reference}" \
      --reads "${bam}" \
      --examples "${EXAMPLES}" \
      --task {}
) >"${LOG_DIR}/make_examples.log" 2>&1
$ ( time python "${BIN_DIR}"/call_variants.zip \
    --outfile "${CALL_VARIANTS_OUTPUT}" \
    --examples "${EXAMPLES}" \
    --checkpoint "${MODEL}" \
    --batch_size 32
) >"${LOG_DIR}/call_variants.log" 2>&1
$ ( time python "${BIN_DIR}"/postprocess_variants.zip \
    --ref "${REF}" \
    --infile "${CALL_VARIANTS_OUTPUT}" \
    --outfile "${deepvariant_vcf}"
) >"${LOG_DIR}/postprocess_variants.log" 2>&1
```

FreeBayes

```
$ freebayes \
    -f $reference \
    -b $bam \
    -t $chromosomes \
    -= | bgzip > $freebayes_vcf
$ tabix $freebayes_vcf
```

GATK4

```
$ gatk --java-options -Xmx12G HaplotypeCaller \
    -R $reference \
    -I $bam_dedup_recal \
    -O $gatk_vcf \
    -L $chromosomes
```

Octopus

```
$ octopus \
    -R $reference \
    -I $bam \
    -t $chromosomes \
    -o $octopus_vcf \
    --sequence-error-model $octopus_error_model \
    --forest $octopus_germline_forest \
    --legacy \
    --threads $nthreads
```

`$octopus_error_model` was set to `hiseq` for the Precision FDA Truth tests and then GIAB HG005 test, and `x10` for the other tests. The ’legacy’ VCF that Octopus reports was used for benchmarking as RTG Tools vcfeval has trouble parsing VCF v4.3 that Octopus outputs by default. All evaluation commands are available from `https://github.com/luntergroup/octopus-paper`.

Platypus

```
$ python Platypus.py callVariants \
    --refFile $reference \
    --bamFiles $bam \
    --regions $chromosomes \
    --output $platypus_vcf
$ bgzip $platypus_vcf
$ tabix ${platypus_vcf}.gz
```

Strelka2

```
$ configureStrelkaGermlineWorkflow.py \
    --referenceFasta $reference \
    --bam $bam \
    --callRegions ${chromosomes}.gz \
    --runDir $strelka_tmp
$ ${strelka_tmp}/runWorkflow.py -m local -j $nthreads
$ mv ${strelka_tmp}/results/variants/variants.vcf.gz $strelka_vcf
$ mv ${strelka_tmp}/results/variants/variants.vcf.gz.tbi ${strelka_vcf}.tbi
```

Germline calling evaluation

We used RTG Tools vcfeval to evaluate germline variant calls:

```
$ rtg vcfeval \
    -t $reference_sdf \
    -b $truth_vcf \
    --evaluation-regions $truth_bed \
    --ref-overlap \
    -c $caller_vcf \
    -f $caller_score_metric \
    -o $caller_eval_dir
```

The value for the environment variable `$caller_score_metric` is given in the main text. Note: the two X10 samples were pre-mapped to a version of the reference genome with ”chr” prefix contig names, which we needed to remove these before comparison with RTG Tools.

Trio analysis

Caller commands

DeepVariant

Following the guidance given on GitHub (`https://github.com/google/deepvariant/issues/45`), we called each sample called similarly to the germline analysis but with GVCF output requested:

```
$ ( time seq 0 $((N_SHARDS-1)) | \
  parallel --halt 2 --joblog "${LOG_DIR}/log" --res "${LOG_DIR}" \
    python "${BIN_DIR}"/make_examples.zip \
      --mode calling \
      --ref "${reference}" \
      --reads "${bam}" \
      --examples "${EXAMPLES}" \
      --gvcf "${GVCF_TFRECORDS}" \
      --task {}
) >"${LOG_DIR}/make_examples.log" 2>&1
$ ( time python "${BIN_DIR}"/call_variants.zip \
    --outfile "${CALL_VARIANTS_OUTPUT}" \
    --examples "${EXAMPLES}" \
    --checkpoint "${MODEL}" \
    --batch_size 32
) >"${LOG_DIR}/call_variants.log" 2>&1
$ ( time python "${BIN_DIR}"/postprocess_variants.zip \
    --ref "${REF}" \
    --infile "${CALL_VARIANTS_OUTPUT}" \
    --outfile "${OUTPUT_VCF}" \
    --nonvariant_site_tfrecord_path "${GVCF_TFRECORDS}" \
    --gvcf_outfile "${deepvariant_gvcf}"
) >"${LOG_DIR}/postprocess_variants.log" 2>&1
```

Native DeepVariant GVCF files are not compatible with GATK (used to identify *de novo* calls).

```
$ bioawk -tHc vcf ’{gsub("<\\*>","<NON_REF>",$alt); print}’ \
    $deepvariant_gvcf \
    | bgzip > $deepvariant_gatk_compatible_gvcf
$ tabix $deepvariant_gatk_compatible_gvcf
```

The GATK4 pipeline is then followed, without the VQSR and CalculateGenotypePosteriors steps.

FreeBayes

```
$ freebayes \
    -f $reference \
    -b $offspring_bam_dedup $maternal_bam $paternal_bam \
    -t $chromosomes \
    -= | bgzip > $freebayes_vcf
$ tabix $freebayes_vcf
```

The GATK4 pipeline is then followed, without the VQSR and CalculateGenotypePosteriors steps.

GATK4

```
$ gatk --java-options -Xmx12G HaplotypeCaller \
    -R $reference \
    -I $bam \
    -L $chromosomes \
    -ERC GVCF \
    -O $gatk_gvcf

$ gatk --java-options -Xmx12G CombineGVCFs \
    -R $reference \
    -V $gatk_maternal_gvcf \
    -V $gatk_paternal_gvcf \
    -V $gatk_offspring_gvcf \
    -L $chromosomes \
    -O $gatk_trio_gvcf

$ gatk --java-options -Xmx12G GenotypeGVCFs \
    -R $reference \
    -V $gatk_trio_gvcf \
    -L $chromosomes \
    -D dbsnp_138.b37.vcf.gz \
    -O $gatk_trio_vcf

$ gatk VariantRecalibrator \
    -R $reference \
    -V $gatk_vcf \
    --resource hapmap,known=false,training=true,truth=true,prior=15.0:hapmap_3.3.b37.vcf.gz \
    --resource omni,known=false,training=true,truth=false,prior=12.0:1000G_omni2.5.b37.vcf.gz \
    --resource 1000G,known=false,training=true,truth=false,prior=10.0:1000G_phase1.snps.
        high_confidence.b37.vcf.gz \
    --resource dbsnp,known=true,training=false,truth=false,prior=2.0:dbsnp_138.b37.vcf.gz \
    -an DP -an QD -an FS -an SOR -an MQ -an MQRankSum -an ReadPosRankSum \
    -mode SNP \
    --max-attempts 4 \
    -tranche 99.5 \
    --tranches-file $gatk_snp_tranches \
    -L $chromosomes \
    -O $gatk_snp_recal
$ gatk ApplyVQSR \
    -R $reference \
    -V $gatk_vcf \
    -ts-filter-level 99.5 \
    --tranches-file $gatk_snp_tranches \
    --recal-file $gatk_snp_recal \
    -mode SNP \
    -L $chromosomes \
    -O $gatk_vqsr_snp_vcf
$ gatk VariantRecalibrator \
    -R $reference \
    -V $gatk_vqsr_snp_vcf \
    --resource mills,known=false,training=true,truth=true,prior=12.0:
        Mills_and_1000G_gold_standard.indels.b37.vcf.gz \
    --resource dbsnp,known=true,training=false,truth=false,prior=2.0:dbsnp_138.b37.vcf.gz \
    -an DP -an QD -an FS -an SOR -an MQRankSum -an ReadPosRankSum \
    -mode INDEL \
    --max-attempts 4 \
    --max-gaussians 4 \
    -tranche 99.0 \
    --tranches-file $gatk_indel_tranches \
    -L $chromosomes \
    -O $gatk_indel_recal
$ gatk ApplyVQSR \
    -R $reference \
    -V $gatk_vqsr_snp_vcf \
    -ts-filter-level 99.0 \
    --tranches-file $gatk_indel_tranches \
    --recal-file $gatk_indel_recal \
    -mode INDEL \
    -L $chromosomes \
    -O $gatk_vqsr_vcf

$ gatk CalculateGenotypePosteriors \
    -V $gatk_trio_vqsr_vcf \
    -ped $trio_ped \
    --de-novo-prior 1.3e-8 \
    -O $gatk_trio_vqsr_cgp_vcf

$ java -jar GenomeAnalysisTK.jar -T VariantAnnotator \
    -R $reference \
    -V $gatk_trio_vqsr_cgp_vcf \
    -A PossibleDeNovo \
    -ped $trio_ped \
    -o $gatk_trio_vqsr_cgp_denovo_ann_vcf

$ bcftools view \
    -i 'INFO/hiConfDeNovo="${offspring}"' \
    -Oz -o $gatk_denovo_vcf \
    $gatk_trio_vqsr_cgp_denovo_ann_vcf
```

Octopus

```
$ octopus
    -R $reference \
    -I $offspring_bam $maternal_bam $paternal_bam \
    -t $chromosomes \
    --ped $trio_ped \
    --denovos-only \
    -o $octopus_vcf \
    --threads $nthreads
```

Platypus

```
$ python Platypus.py callVariants \
    --refFile $reference \
    --bamFiles ${offspring_bam},${maternal_bam},${paternal_bam} \
    --regions $chromosomes \
    --output $platypus_vcf
$ bcftools view \
    $platypus_vcf \
    | python findDeNovoMutations.py 11 9 10 10 \
    | bgzip > $platypus_denovo_vcf
$ tabix $platypus_denovo_vcf
```

Strelka2

```
$ configureStrelkaGermlineWorkflow.py \
    --referenceFasta $reference \
    --bam $offspring_bam \
    --bam $maternal_bam \
    --bam $paternal_bam \
    --callRegions $chromosomes.gz \
    --runDir strelka_tmp
$ strelka_tmp/runWorkflow.py -m local -j $nthreads
$ mv strelka_tmp/results/variants/variants.vcf.gz $strelka_vcf
$ mv strelka_tmp/results/variants/variants.vcf.gz.tbi ${strelka_vcf}.tbi
```

We found the Strelka2 output contained haploid genotypes calls, which we needed to remove to identify *de novo* variants:

```
$ bcftools view \
    -a -U -c 1 \
    -e ’GT="0"|GT="1"’ \
    -Oz -o $strelka_fixed_vcf \
    $strelka_vcf
$ tabix $strelka_fixed_vcf
```

We then ran the GATK4 pipeline (without VQSR or CalculateGenotypePosteriors) to find *de novo* variants, which were then filtered:

```
$ bcftools view \
    $strelka2_denovo_vcf \
    | awk -F $"\t" \
        ’BEGIN {OFS = FS} {if (NF>1 && $7 != "PASS" && $9~/:FT:/) $7="FAIL"; print} \
        | bgzip > $strelka2_denovo_filtered_vcf
$ tabix $strelka2_denovo_filtered_vcf
```

De novo calling evaluation

We used RTG Tools vcfeval to evaluate *de novo* calls:

```
$ rtg vcfeval \
    -t $reference_sdf \
    -b $truth_vcf \
    -c $caller_vcf \
    --squash-ploidy \
    --sample $offspring \
    -o $caller_eval_dir
```

All evaluation commands are available from `https://github.com/luntergroup/octopus-paper`.

Paired tumour analysis

Lancet

```
for chrom in ‘seq 1 22‘ X; do
    lancet \
        --ref $reference \
        --normal $normal_bam \
        --tumor $tumour_bam \
        --reg $chrom \
        --num-threads $nthreads \
        | bgzip > ${lancet_tmp_vcf}.chr${chrom}.vcf.gz
        tabix ${lancet_tmp_vcf}.chr${chrom}.vcf.gz
done
$ bcftools concat -Oz -o $lancet_vcf ${lancet_tmp_vcf}.chr1.vcf.gz ${lancet_tmp_vcf}.chr2.vcf.gz
    ${lancet_tmp_vcf}.chr3.vcf.gz ${lancet_tmp_vcf}.chr4.vcf.gz ${lancet_tmp_vcf}.chr5.vcf.gz ${
    lancet_tmp_vcf}.chr6.vcf.gz ${lancet_tmp_vcf}.chr7.vcf.gz ${lancet_tmp_vcf}.chr8.vcf.gz ${
    lancet_tmp_vcf}.chr9.vcf.gz ${lancet_tmp_vcf}.chr10.vcf.gz ${lancet_tmp_vcf}.chr11.vcf.gz ${
    lancet_tmp_vcf}.chr12.vcf.gz ${lancet_tmp_vcf}.chr13.vcf.gz ${lancet_tmp_vcf}.chr14.vcf.gz ${
    lancet_tmp_vcf}.chr15.vcf.gz ${lancet_tmp_vcf}.chr16.vcf.gz ${lancet_tmp_vcf}.chr17.vcf.gz ${
    lancet_tmp_vcf}.chr18.vcf.gz ${lancet_tmp_vcf}.chr19.vcf.gz ${lancet_tmp_vcf}.chr20.vcf.gz ${
    lancet_tmp_vcf}.chr21.vcf.gz ${lancet_tmp_vcf}.chr22.vcf.gz ${lancet_tmp_vcf}.chrX.vcf.gz
$ tabix $lancet_vcf
```

LoFreq

```
$ lofreq somatic \
    -f $reference \
    -n $normal_bam \
    -t $tumour_bam \
    --call-indels \
    -l $chromosomes \
    -d dbsnp_138.b37.vcf.gz \
    --threads $nthreads \
    -o $lofreq_vcf_prefix

$ bcftools concat \
    -a -Oz \
    -o $lofreq_vcf \
    ${lofreq_vcf_prefix}.somatic_final_minus-dbsnp.snvs.vcf.gz \
    ${lofreq_vcf_prefix}.bwa-mem.somatic_final_minus-dbsnp.indels.vcf.gz
$ tabix $lofreq_vcf
```

Mutect2

```
$ gatk --java-options -Xmx12G Mutect2 \
    -R $reference \
    -I $normal_bam \
    -I $tumour_bam \
    -normal $normal_sample \
    -tumor $tumour_sample \
    --germline-resource af-only-gnomad.raw.sites.b37.vcf.gz \
    --af-of-alleles-not-in-resource 0.0000025 \
    --disable-read-filter MateOnSameContigOrNoMappedMateReadFilter \
    -L $chromosomes \
    -O $mutect_raw_vcf
$ gatk GetPileupSummaries \
    -I $tumour_bam \
    -V small_exac_common_3_b37.vcf.gz \
    -O $mutect_pileup_table
$ gatk CalculateContamination \
    -I $mutect_pileup_table \
    -O $mutect_contamination_table
$ gatk FilterMutectCalls \
    -V $mutect_raw_vcf \
    --contamination-table $mutect_contamination_table \
    -O $mutect_vcf
```

Octopus

```
$ octopus \
    -R $reference
    -I $normal_bam $tumour_bam \
    -t $chromosomes \
    -N $normal_sample \
    --forest $octopus_somatic_forest \
    --somatics-only \
    --threads $nthreads \
    -o $octopus_vcf
```

Platypus

```
$ python Platypus.py callVariants \
    --refFile $reference \
    --bamFiles ${normal_bam},${tumour_bam} \
    --regions $chromosomes \
    --output $platypus_vcf
$ python findSomaticMutationsInTumour.py \
    --inputVCF $platypus_vcf \
    --outputVCF $platypus_somatic_vcf \
    --tumourSample $tumour_sample \
    --normalSample $normal_sample \
    --minPosterior 1
$ bgzip $platypus_somatic_vcf
$ tabix ${platypus_somatic_vcf}.gz
```

Strelka2

```
$ configureStrelkaSomaticWorkflow.py \
    --referenceFasta $reference \
    --normalBam $normal_bam \
    --tumorBam $tumour_bam \
    --callRegions $chromosomes.gz \
    --runDir strelka_tmp
$ strelka_tmp/runWorkflow.py -m local -j $nthreads
$ bcftools concat -a \
    -Oz -o $strelka_vcf \
    strelka_tmp/results/variants/somatic.snvs.vcf.gz \
    strelka_tmp/results/variants/somatic.indels.vcf.gz
$ tabix $strelka_vcf
```

VarDict

In addition to VarDict's default filtering, we also applied filtering recommended by bcbio author Brad Chapman (`http://bcb.io/2016/04/04/vardict-filtering/`).

```
$ VarDict \
    -G $reference \
    -f 0.01 \
    -N $tumour_sample \
    -b "${tumour_bam}|${normal_bam}" \
    -z 0 -c 1 -S 2 -E 3 \
    $chromosomes \
    | testsomatic.R \
    | var2vcf_paired.pl \
      -N "${tumour_sample}|${normal_sample}" \
      -f 0.01 \
      | bgzip > $vardict_raw_vcf
$ bcftools view \
    -i ’INFO/STATUS="StrongSomatic"’ \
    -Oz -o $vardict_vcf \
    $vardict_raw_vcf
$ bcftools filter \
    -s "BCBIO" \
    -e ’((FORMAT/AF[0:0] * FORMAT/DP[0:0] < 6) && ((MQ < 55.0 && NM > 1.0) || (MQ < 60.0 && NM >
        2.0) || (FORMAT/DP < 10) || (QUAL < 45)))’ \
    -Oz -o $vardict_filtered_vcf \
    $vardict_vcf
$ tabix $vardict_vcf
```

Paired somatic calling evaluation

We used RTG Tools vcfeval to evaluate somatic calls:

```
$ rtg vcfeval \
    -t $reference_sdf \
    -b $truth_vcf \
    --evaluation-regions $truth_bed \
    -c $caller_vcf \
    --squash-ploidy \
    --sample $tumour_sample \
    -f $caller_score_metric \
    -o $caller_eval_dir
```

The value for the environment variable `$caller_score_metric` is given in the main text. We needed to use some pre-processing steps for Octopus, Strelka2, and LoFreq in order to run vcfeval. In particular, Octopus reports non-diploid genotypes for somatic calls, but vcfeval only supports haploid and diploid genotypes. Strelka2 did not report GT fields, and renamed samples. LoFreq did not report genotype information. All evaluation commands are available from `https://github.com/luntergroup/octopus-paper`.

Tumour-only analysis

Octopus

```
$ octopus \
    -R $reference
    -I $tumour_bam \
    -t $chromosomes \
    -C cancer \
    --forest $octopus_germline_forest \
    --somatic-forest $octopus_somatic_forest \
    --threads $nthreads \
    -o $octopus_vcf
```

Pisces

```
$ dotnet CreateGenomeSizeFile.dll \
    -g $reference_dir \
    -s "Homo sapien (b37)" \
    -o $pisces_reference

$ dotnet /data/apps/Pisces/Pisces.dll \
    -bam $tumour_bam \
    -g $pisces_reference \
    -i $chromosomes \
    -CallMNVs false \
    -gVCF false \
    -RMxNFilter 5,9,0.35 \
    -OutFolder $pisces_somatic_results_dir

$ dotnet Pisces.dll \
    -bam $tumour_bam \
    -g $pisces_reference \
    -i $chromosomes \
    -CallMNVs false \
    -gVCF false \
    -RMxNFilter 5,9,0.35 \
    -ploidy diploid \
    -crushvcf true \
    -OutFolder $pisces_germline_results_dir
```

```
$ dotnet VennVcf.dll \
    -if ${pisces_somatic_chr_vcf},${pisces_germline_chr_vcf} \
    -o $pisces_results_dir
```

Note: the `VennVcf.dll` program did not work with our output, we therefore followed with workout recommended by the Pisces author (https://github.com/Illumina/Pisces/issues/18) by adding ”chr” to the chromosome names in the VCF files.

Unpaired somatic calling evaluation

We used RTG Tools vcfeval to evaluate somatic and germline calls, as for the germline and paired somatic analysis above. All evaluation commands are available from https://github.com/luntergroup/octopus-paper.

Supplementary Note 3. Synthetic tumours

All raw sequencing reads were downloaded from `ftp://ftp-trace.ncbi.nlm.nih.gov//giab/ftp/data/NA12878/NIST_NA12878_HG001_HiSeq_300x`.

We then merged read sets from the same flow cell and lane, in order to create four samples of NA12878 with approximate depths of 30X, 35X, 60X, and 65X. In particular:

- Set1 30X: Sample_U5c
- Set2 35X: Sample_U2a, Sample_U2b, and Sample_U3a
- Set3 60X: Sample_U0a and Sample_U5a
- Set4 65X: Sample_U0b and Sample_U5b