Supplementary Figures

Fig. S1 – Schematic overview of the computational pipeline to identify somatic mutations in NG-TAS data from longitudinal samples.

Fig. S2 - Representative image of the Bioanalyser gel plot. The 8plex PCR products were analysed using Bioanalyser for primer efficiency and quality control.

Fig. S3 - Fragment size distribution according to the Bioanalyser results for cfDNA extracted from the media where NA12878 cells were grown (main peak at around 160-170bp).

Fig. S4 – (A) Percentage of amplicons having more than 100x coverage for 2, 5 and 10 ng of input cfDNA from NA12878 sample. (B) Percentage of reads on target for 2, 5 and 10 ng of input cfDNA from NA12878 sample.

Fig. S5 – Detailed representation of mutations identified in tumour or plasma samples of 21 metastatic breast cancer cases. The colour gradient indicates the VAF as indicated; PT = primary tumour, M = metastasis biopsy, V1…n = plasma.

Fig S1

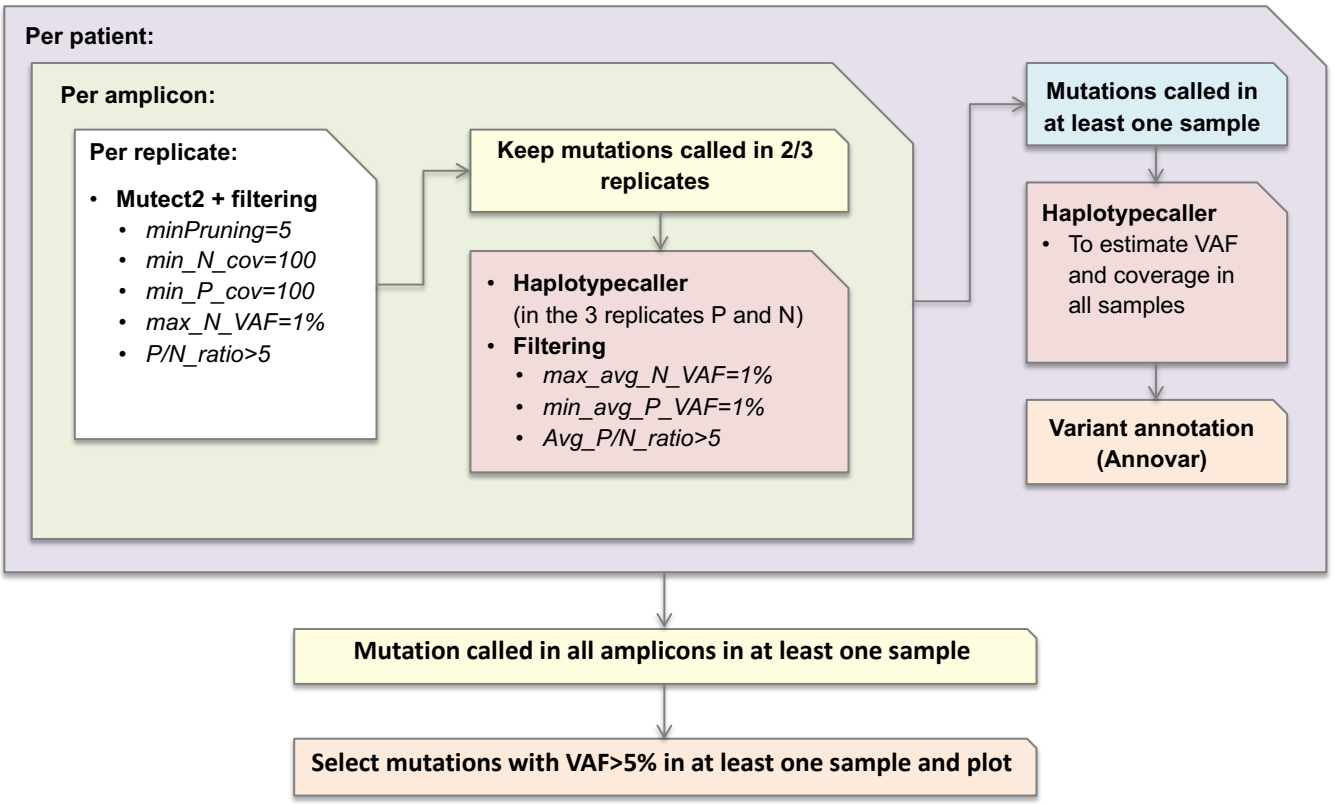

Per patient:
Per amplicon:
Per replicate:
• Mutect2 + filtering
• minPruning=5
• min_N_cov=100
• min_P_cov=100
• max_N_VAF=1%
• P/N_ratio>5
Keep mutations called in 2/3 replicates
• Haplotypecaller (in the 3 replicates P and N)
• Filtering
• max_avg_N_VAF=1%
• min_avg_P_VAF=1%
• Avg_P/N_ratio>5
Mutations called in at least one sample
Haplotypecaller
• To estimate VAF and coverage in all samples
Variant annotation (Annovar)
Mutation called in all amplicons in at least one sample
Select mutations with VAF>5% in at least one sample and plot

Fig S2

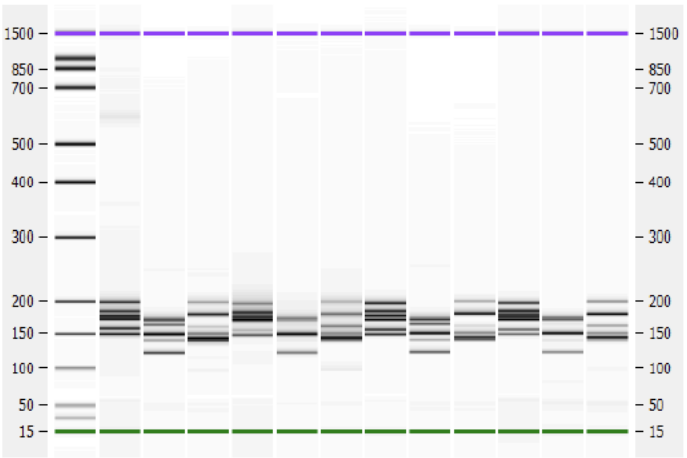

1500
850
700
500
400
300
200
150
100
50
15

Fig S3

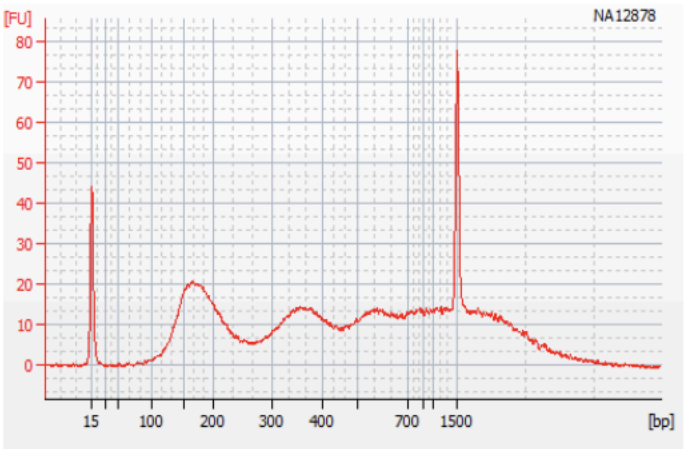
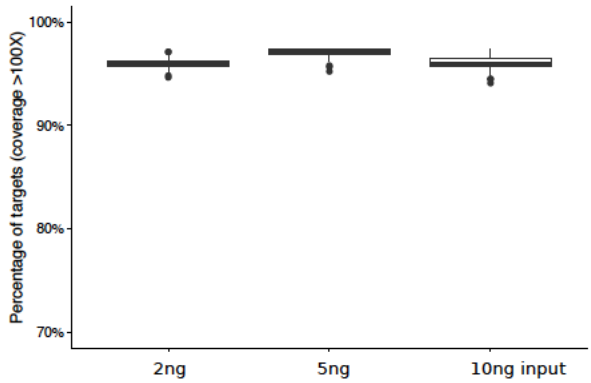

[FU]
NA12878
80
70
60
50
40
30
20
10
0
15
100
200
300
400
700
1500
[bp]

Fig S4

A


Percentage of targets (coverage >100X)
100%
90%
80%
70%
2ng
5ng
10ng input

B

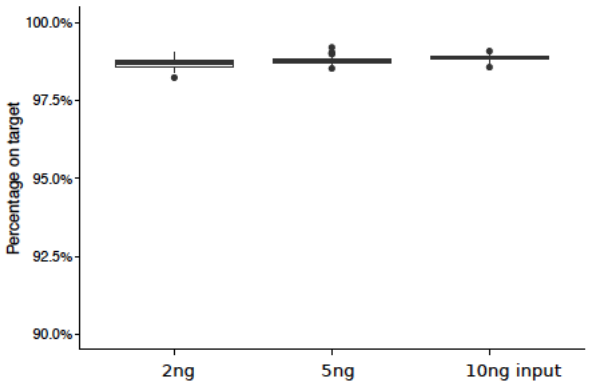
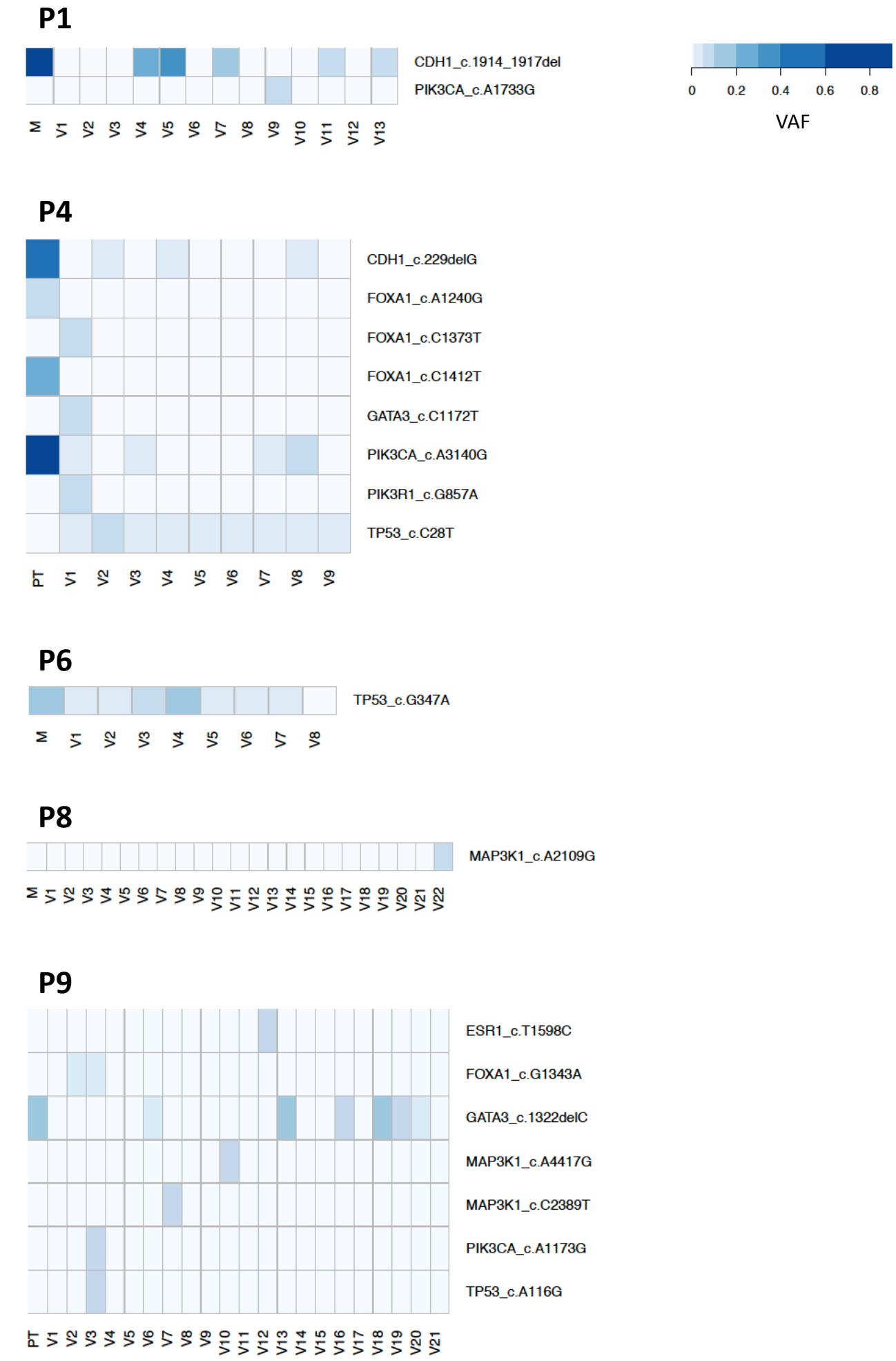
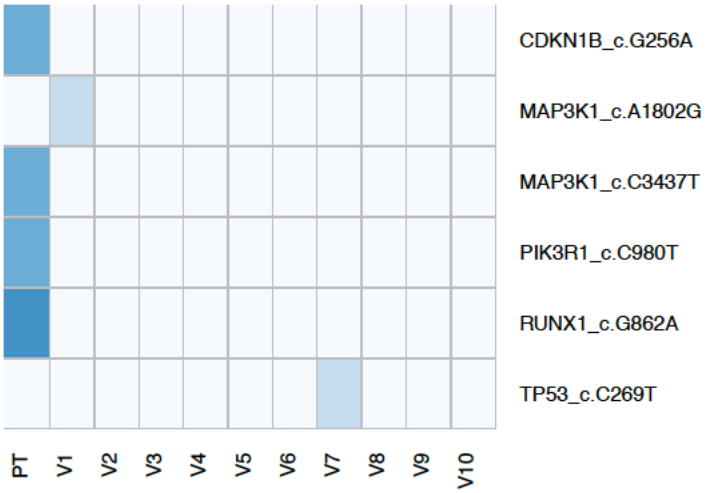

Percentage on target
100.0%
97.5%
95.0%
92.5%
90.0%
2ng
5ng
10ng input

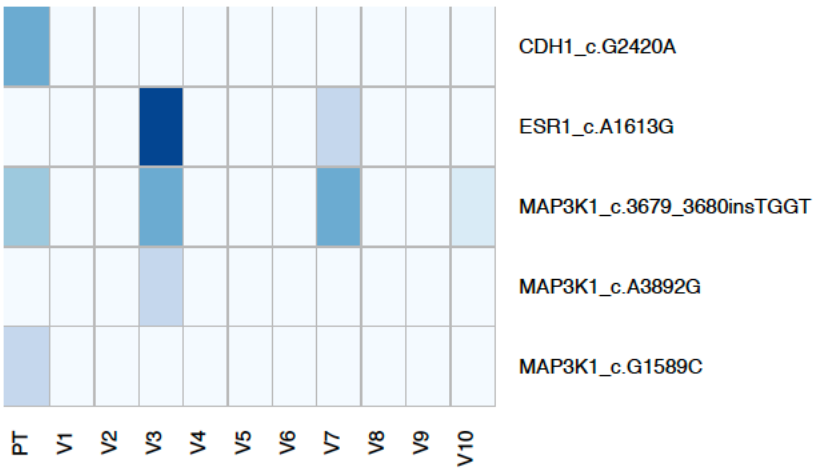
Fig S5


P1
CDH1_c.1914_1917del
PIK3CA_c.A1733G
M V1 V2 V3 V4 V5 V6 V7 V8 V9 V10 V11 V12 V13
0 0.2 0.4 0.6 0.8
VAF
P4
CDH1_c.229delG
FOXA1_c.A1240G
FOXA1_c.C1373T
FOXA1_c.C1412T
GATA3_c.C1172T
PIK3CA_c.A3140G
PIK3R1_c.G857A
TP53_c.C28T
PT V1 V2 V3 V4 V5 V6 V7 V8 V9
P6
TP53_c.G347A
M V1 V2 V3 V4 V5 V6 V7 V8
P8
MAP3K1_c.A2109G
M V1 V2 V3 V4 V5 V6 V7 V8 V9 V10 V11 V12 V13 V14 V15 V16 V17 V18 V19 V20 V21 V22
P9
ESR1_c.T1598C
FOXA1_c.G1343A
GATA3_c.1322delC
MAP3K1_c.A4417G
MAP3K1_c.C2389T
PIK3CA_c.A1173G
TP53_c.A116G
PT V1 V2 V3 V4 V5 V6 V7 V8 V9 V10 V11 V12 V13 V14 V15 V16 V17 V18 V19 V20 V21

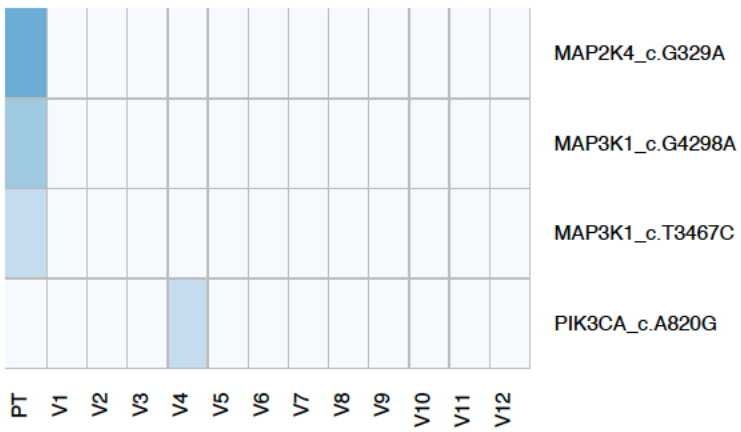
P11


CDKN1B_c.G256A
MAP3K1_c.A1802G
MAP3K1_c.C3437T
PIK3R1_c.C980T
RUNX1_c.G862A
TP53_c.C269T
PT
V1
V2
V3
V4
V5
V6
V7
V8
V9
V10

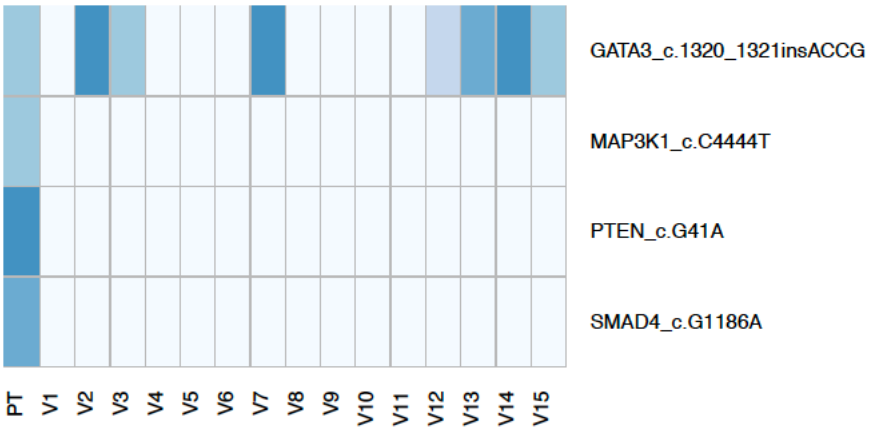
P13


CDH1_c.G2420A
ESR1_c.A1613G
MAP3K1_c.3679_3680insTGGT
MAP3K1_c.A3892G
MAP3K1_c.G1589C
PT
V1
V2
V3
V4
V5
V6
V7
V8
V9
V10

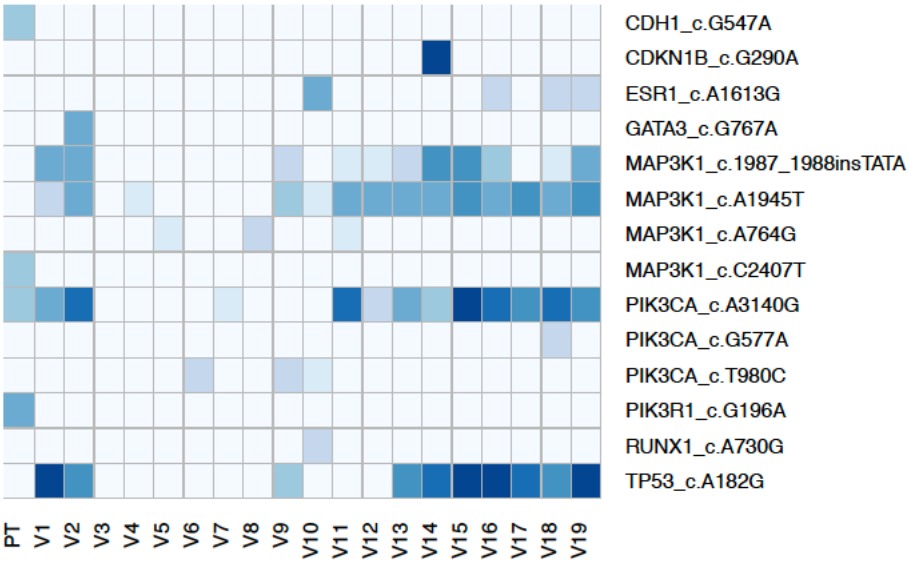
P14


MAP2K4_c.G329A
MAP3K1_c.G4298A
MAP3K1_c.T3467C
PIK3CA_c.A820G
PT
V1
V2
V3
V4
V5
V6
V7
V8
V9
V10
V11
V12

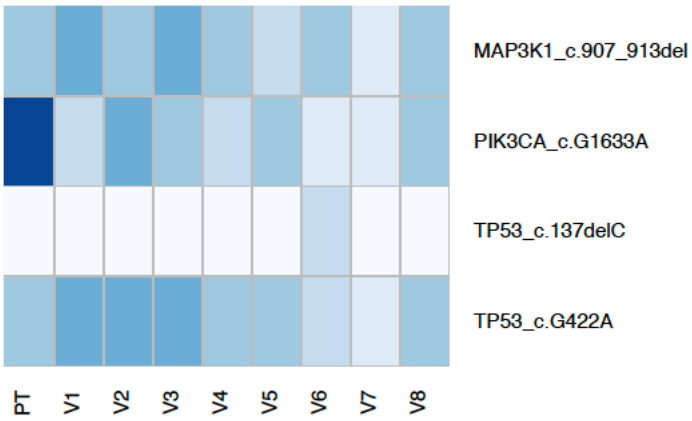
P15


GATA3_c.1320_1321insACCG
MAP3K1_c.C4444T
PTEN_c.G41A
SMAD4_c.G1186A
PT V1 V2 V3 V4 V5 V6 V7 V8 V9 V10 V11 V12 V13 V14 V15

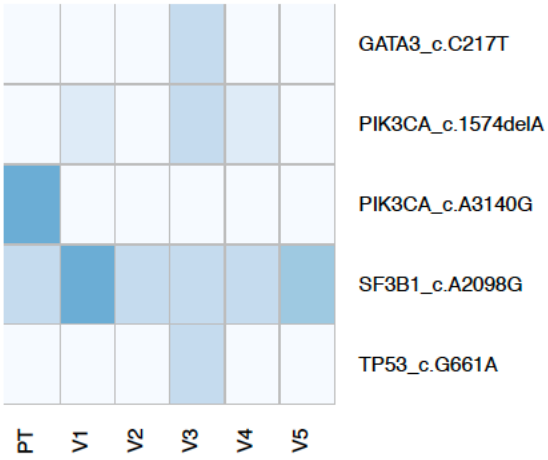
P16


CDH1_c.G547A
CDKN1B_c.G290A
ESR1_c.A1613G
GATA3_c.G767A
MAP3K1_c.1987_1988insTATA
MAP3K1_c.A1945T
MAP3K1_c.A764G
MAP3K1_c.C2407T
PIK3CA_c.A3140G
PIK3CA_c.G577A
PIK3CA_c.T980C
PIK3R1_c.G196A
RUNX1_c.A730G
TP53_c.A182G
PT V1 V2 V3 V4 V5 V6 V7 V8 V9 V10 V11 V12 V13 V14 V15 V16 V17 V18 V19

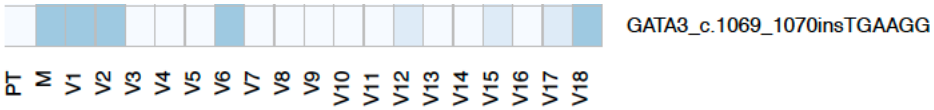
P17


MAP3K1_c.907_913del
PIK3CA_c.G1633A
TP53_c.137delC
TP53_c.G422A
PT V1 V2 V3 V4 V5 V6 V7 V8

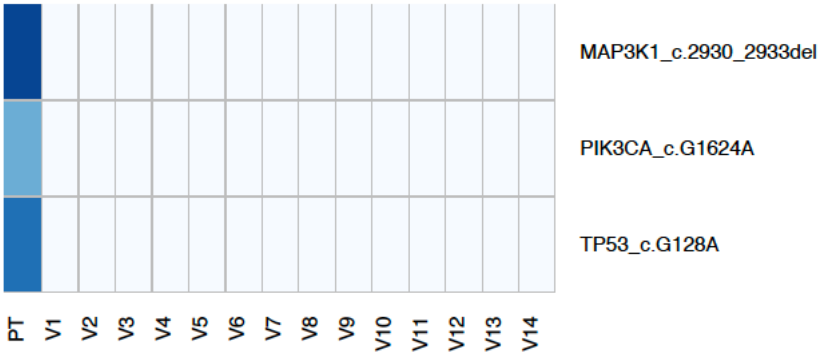
P19



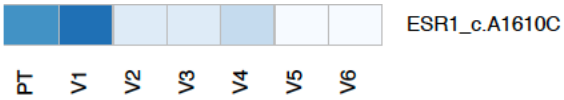
P20



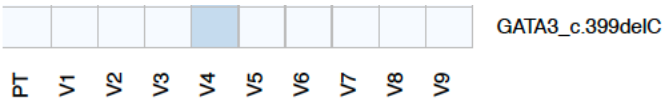
P21



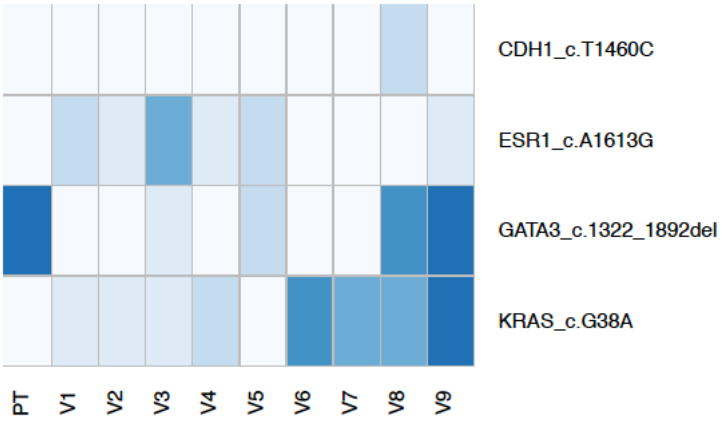
P24



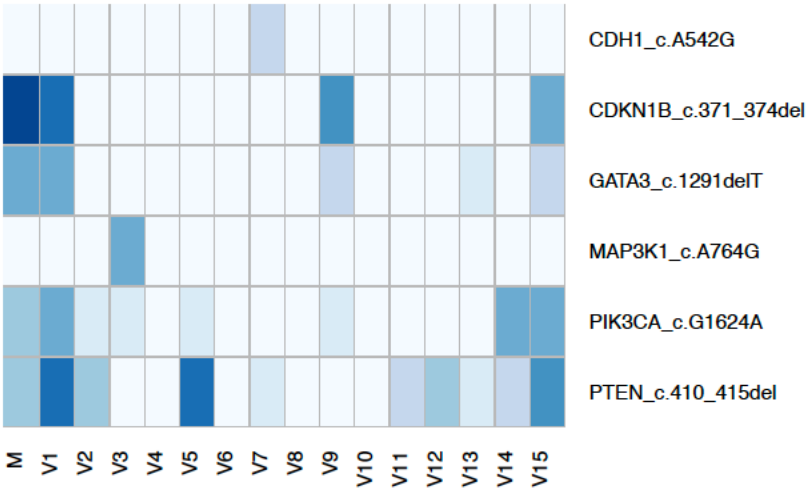
P27



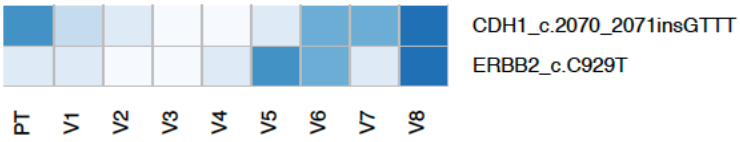
P29



P30



P31



P34

P37

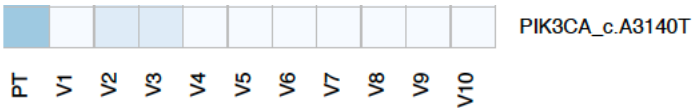


Table S1: The proportion of NA12878 and NA11840 for the generation of the cfDNA dilution series with expected VAF

Expected VAF (%)	NA12878	NA11840
50	100	0
40	80	20
30	60	40
20	40	60
10	20	80
5	10	90
2.5	5	95
1	2	98
0.5	1	99
0.25	0.5	99.5
0.1	0.2	99.8
0	0	100

Table S2: Primers and Probes for PIK3CA and ESR1 hotspot mutations for digital PCR

Gene	Assay	F Primer	R Primer	WT probe (5' VIC/3' MGB)	Mutant probe (5' 6-FAM/3' MGB)
PIK3CA	p.E545K (c.1633 G>A)	GCAATTTCTACACGAGATCC TCTCT	CATTTTAGCACTTACCTGTGA CTCCAT	TGAAATCACTGAGCAGGAG	TGAAATCACTAAGCAGGA
	p.H1047R (c.3140 A>G)	AAGAGGCTTTGGAGTATTTC ATGAA	TGTTTAATTGTGTGGAAGAT CCAATC	CAAATGAATGATGCACATC	TGATGCACGTCATGGT
ESR1	p.D538G (c.1613 A>G)	AGGCATGGAGCATCTGTAC A	TTGGTCCGTCTCCTCCA	TGGTGCCCCTCTATGACCTG	CCCTCTATGGCCTGCTGCT