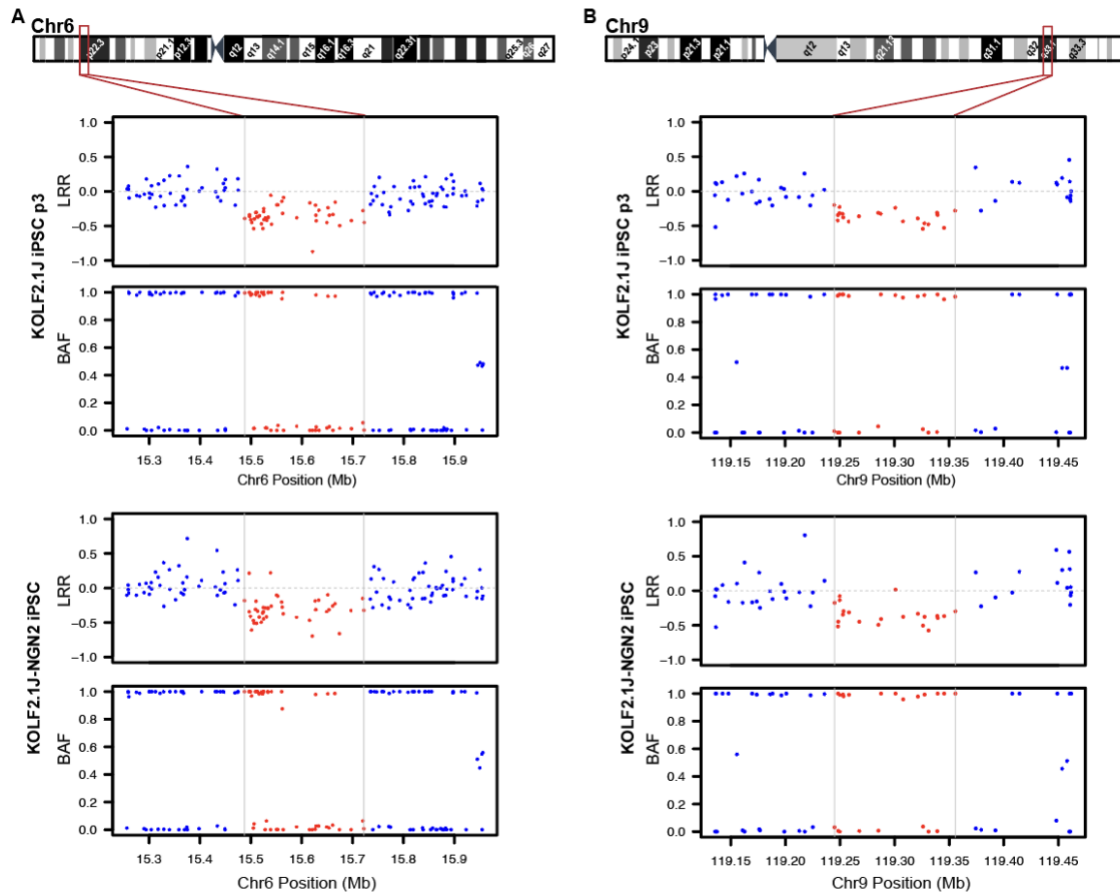


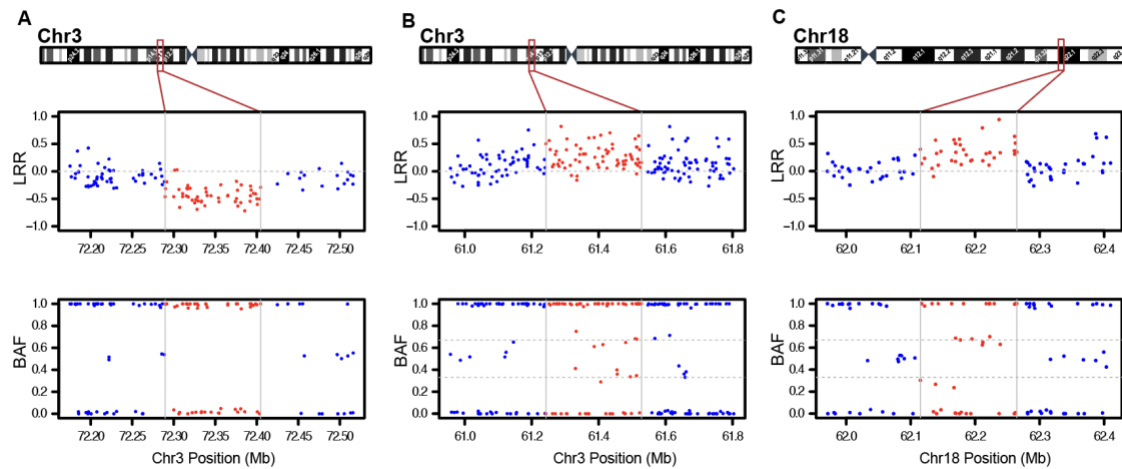
Supplemental Information

Supplemental Figure 1



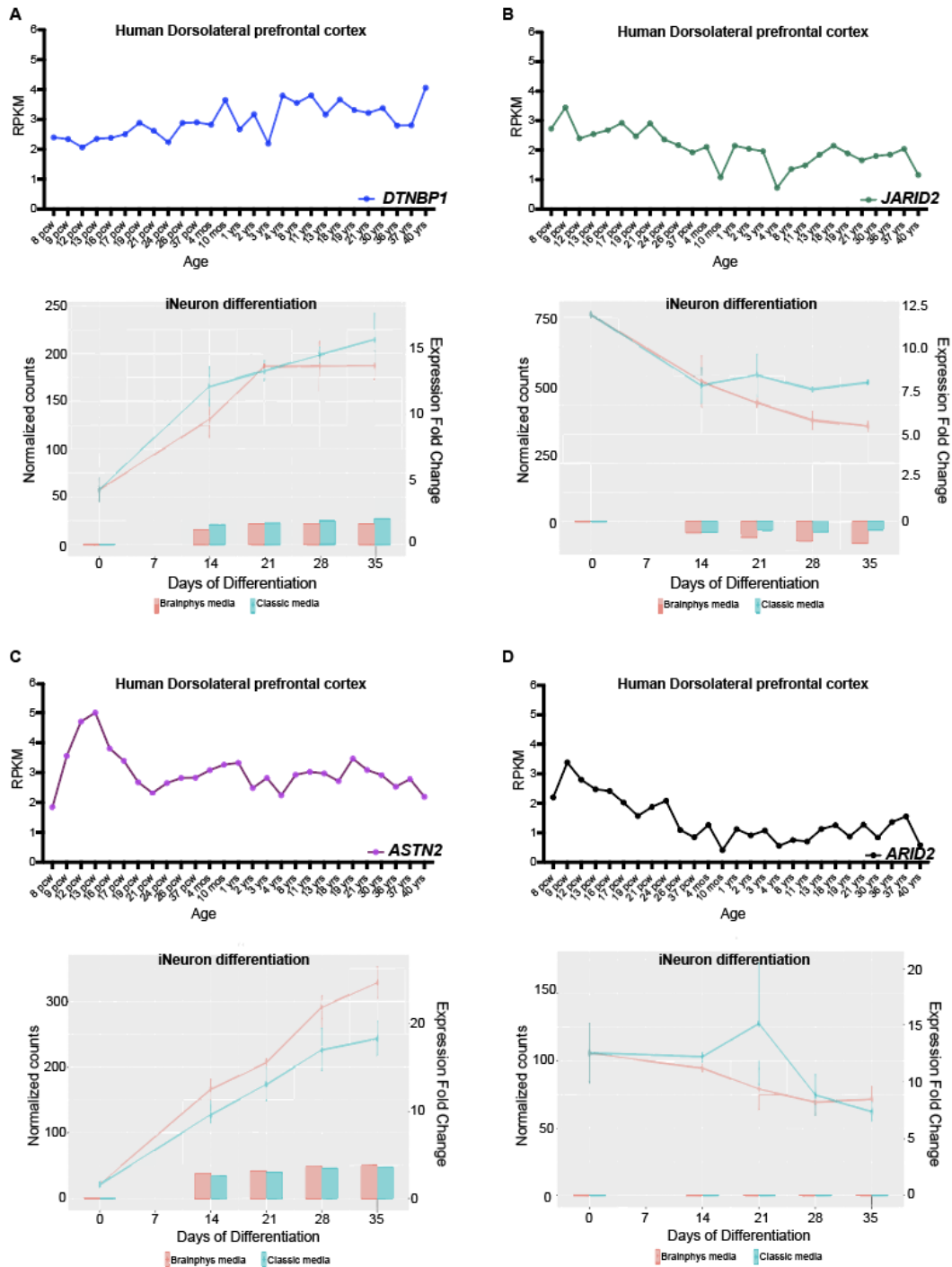
Supplemental Figure 1: High density SNP array uncovers CNVs affecting coding genes in Chr6p22 and Chr9q33 in a stock of KOLF2.1J iPSCs and KOLF2.1J with doxycycline inducible *NGN2* transgene. (A) Chromosome 6 cytoband schematics (top) and Log R Ratio (LRR) and B Allele frequency (BAF) plots (bottom) show reduction of signal intensity and a loss of heterozygosity in 6p22 region of KOLF2.1J iPSCs p3 and KOLF2.1J-NGN2. **(B)** Chromosome 9 cytoband schematics (top) and LRR and BAF plots (bottom) show reduction of signal intensity and a loss of heterozygosity in 9q33 region of KOLF2.1J iPSCs p3 and KOLF2.1J-NGN2.

Supplemental Figure 2



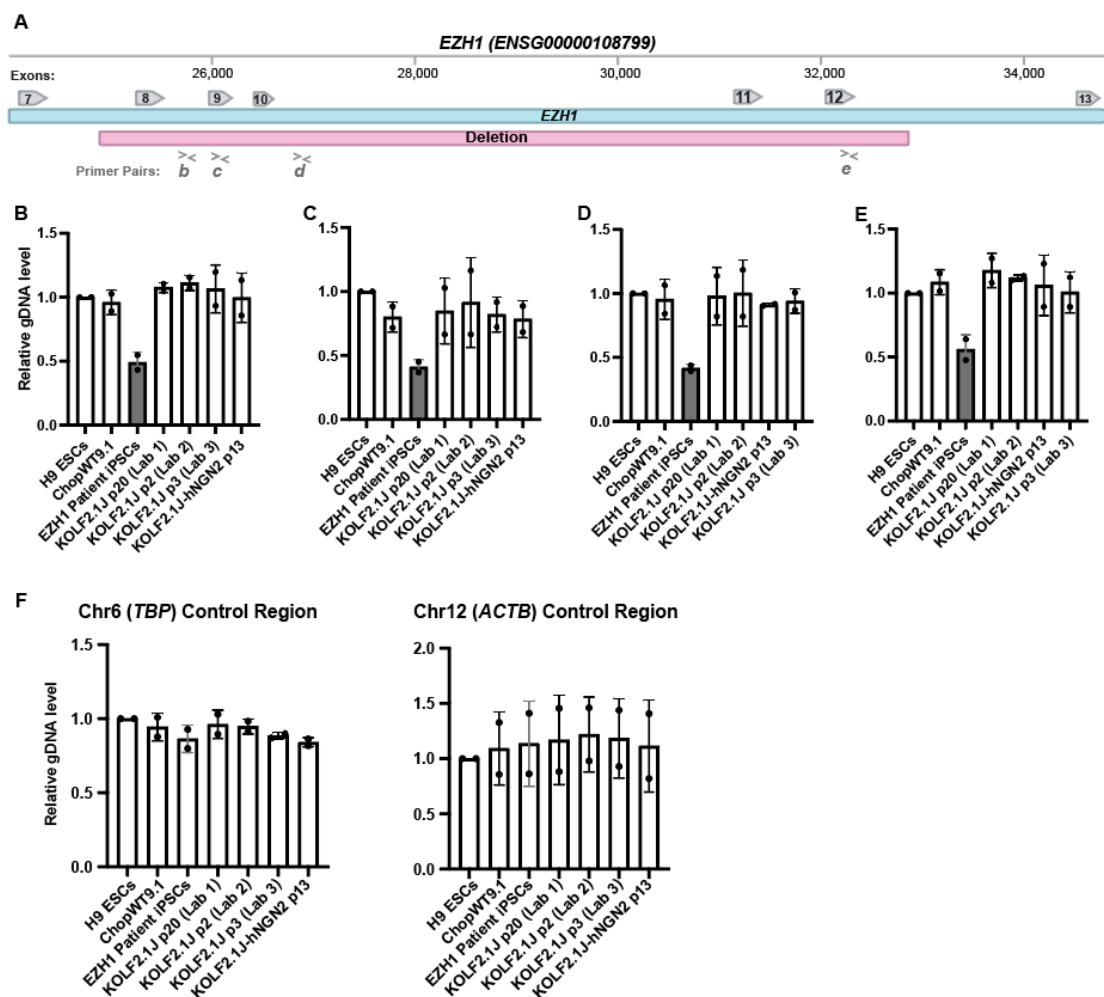
Supplemental Figure 2: CNVs detected by SNP array in Chr3 and Chr18 do not overlap with coding regions. (A-B) Chromosome 3 cytoband schematics (top) and LRR and BAF plots (bottom) showing reduction of signal intensity and a loss of heterozygosity in 3p13 deleted region (A) and increased signal intensity and altered BAF in 3p14 duplicated region (B) of KOLF2.1J iPSCs compared to a control iPSC line. **(C)** Chromosome 18 cytoband schematics (top) and LRR and BAF plots (bottom) showing increased signal intensity and altered BAF in 18q22 duplicated region of KOLF2.1J iPSCs.

Supplemental Figure 3



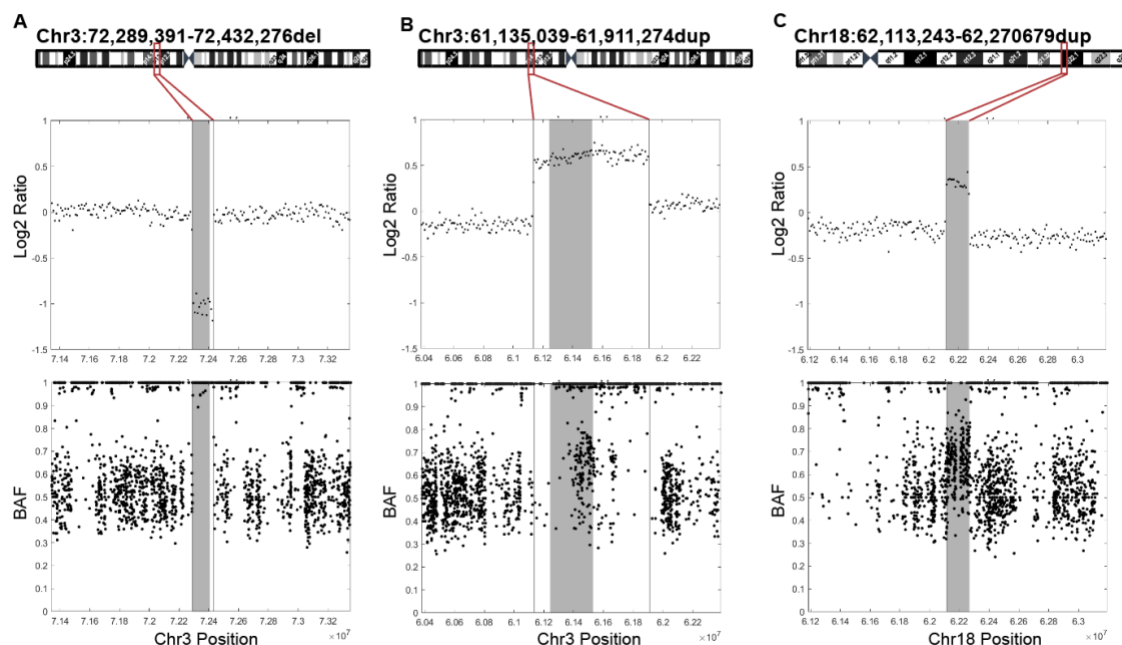
Supplemental Figure 3: Genes affected by Chr6p22 and Chr9q33 are expressed in neurons in the developing and adult brain. (A-D) *DTNBP1* (A), *JARID2* (B), *ASTN2* (C) and *ARID2* (D) expression in developing and adult human prefrontal cortex and in hPSC to iNeuron differentiation. Human prefrontal cortex expression data is retrieved from Brainspan (<https://www.brainspan.org/rnaseq/search/index.html>) and iNeuron differentiation expression plots from the web app created by Connor Ludwig, Kampmann Lab (<https://kampmannlab.ucsf.edu/ineuron-rna-seq>). Pcw; postconception week. Yrs; years.

Supplemental Figure 4



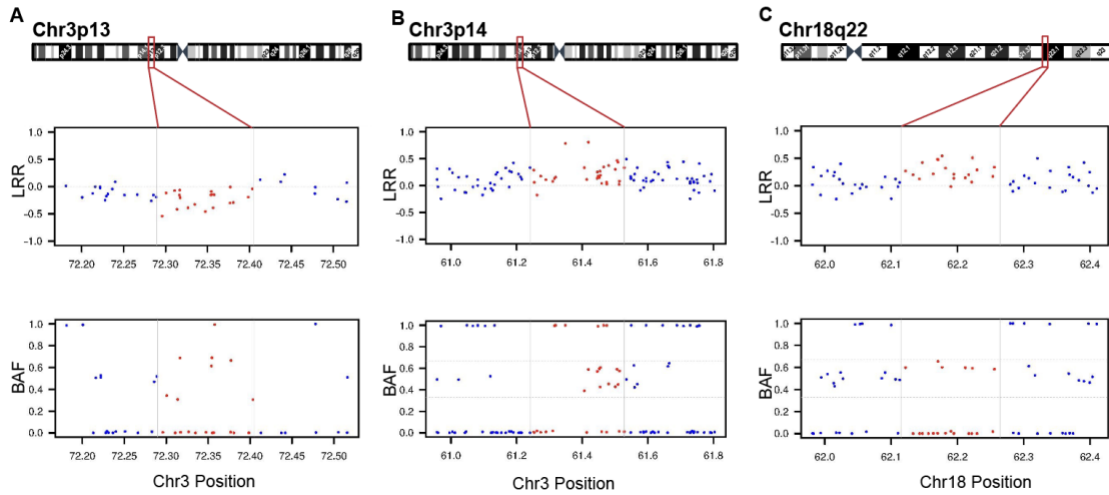
Supplemental Figure 4: gDNA qPCR on an iPSC line with one copy of *EZH1* exon 8-12 deletion demonstrates sensitivity of the technique for detection of hemizygous regions. (A) Schematic representation of *EZH1* exon 7-13. Red line illustrates the region deleted in a *EZH1* patient iPSC line. Arrowheads ($>$, $<$) labeled with b-e indicate position of the primers used for gDNA qPCR. **(B-E)** gDNA qPCR results showing half levels amplification in regions deleted in *EZH1* patient iPSCs compared to control and KOLF2.1Js that are expected to be diploid. **(F)** Randomly selected regions of the genome show similar amplification levels across all the hPSC lines.

Supplemental Figure 5



Supplemental Figure 5: Genome sequencing reanalysis confirms CNVs at Chr3p13, Chr3p14 and Chr18q22. (A-C). Chromosome cytoband schematics with base resolution breakpoints of the CNVs (top) and LRR and BAF plots (bottom) obtained from the KOLF2.1J iPSC genome sequencing reanalysis. LRR plots show reduction of signal intensity and BAF plots show loss of heterozygosity in Chr3p13 (A) and gain of signal intensity and altered BAF in Chr3p14 (B) and Chr18q22 (C) CNV regions compared to diploid up and downstream regions. Shaded area represents the CNV defined by the SNP array and vertical lines the base-resolution breakpoints determined from the genome sequencing data.

Supplemental Figure 6



Supplemental Figure 6: Chr3p13, Chr3p14 and Chr18q22 CNVs in KOLF2.1J were inherited from KOLF2 iPSC line. (A, B) LRR and BAF plots of the KOLF2 iPSC line SNP array deposited in HipSci, show that KOLF2 iPSC line carries two of the Chr3p14 (B) and Chr18q22 (C) CNVs in heterozygosity and is likely mosaic for Chr3p13 (A) deletion.

Supplementary Table 1: Description and sequence of primers used across the study

Primer Pair	Targeted Region (GRCh37/hg19)	Notes	Primer Orientation	Sequence (5'→3')	Product Length (bp)
1	chr6:15372726+15372836	Upstream deleted Chr6 region	Forward	gactacagggcgtgcaccaccac	111
			Reverse	aaggcggacggatcacaggga	
2	chr6:15270358+15270456	Upstream deleted Chr6 region	Forward	tctgtccgtccgtcctccgtc	99
			Reverse	aggaggtggaggctgcagtgag	
3	chr6:15487546+15487631	Within deleted Chr6 region in <i>JARID2</i> : Exon 6	Forward	ggtccagcaggtaacacggg	86
			Reverse	tcgctgtctctccttggcg	
4	chr6:15500050+15500126	Within deleted Chr6 region in <i>JARID2</i>	Forward	gctgtgtcccatccctggct	77
			Reverse	gaaccacctcggcagacacagc	
5	chr6:15514462+15514592	Within deleted Chr6 region in <i>JARID2</i>	Forward	acacactctggtagggacgcc	131
			Reverse	gcactgtgaaagggacgaggc	
6	chr6:15723401+15723550	Within deleted Chr6 region	Forward	acctctcaggctctcacgttccc	150
			Reverse	agcaggagttggaccacagga	
7	chr6:15803765+15803892	Downstream Chr6 deleted region	Forward	tccttcccctcccgcacata	128
			Reverse	ttggttggcgtgccacctgc	
8	chr6:15889438+15889552	Downstream Chr6 deleted region	Forward	gtctgagcgggactggaccctt	115
			Reverse	cgttggcgtccctgtggagac	
9	chr9:119229497+119229617	Upstream deleted Chr9 region	Forward	tgagccagcacagcaaaact	121
			Reverse	gccaagatcgaccactgcact	
10	chr9:119249647+119249723	Within deleted Chr9 region: Exon 20	Forward	ccgtcgggctccagacactga	77
			Reverse	gcagctggtcgaagcctaggag	
11	chr9:119302810+119302918	Within deleted Chr9	Forward	gcttgggtgacagcttggcct	109
			Reverse	tagatggcctgcttggctcct	
12	chr9:119366867+119366974	Within deleted Chr9	Forward	ccatggcccacactgcatgagg	108
			Reverse	agctgagaggatggtggcagca	
13	chr9:119382905+119382984	Downstream deleted Chr9 region	Forward	ccctcccaggagccacaatga	80
			Reverse	ggagtgccaagcctaggggaca	
14	chr17:40870724-40870832	Within <i>EZH1</i> Intron between Exon 8 and 9	Forward	ccaacattgcccaaccagca	109
			Reverse	ggtctccaaggaggatgggt	
15	chr17:40870536-40870611	Within <i>EZH1</i> Exon 9	Forward	agacccaatgcacttcccct	76
			Reverse	tgctcccgtgcacagacttg	
16	chr17:40869846-40869952	Within <i>EZH1</i> Intron between Exon 10 and 11	Forward	ggaacacaagccagctgagcca	107
			Reverse	gagcgttcccacagtaactggg	
17	chr17:40864313-40864437	Within <i>EZH1</i> Exon 12	Forward	tagtggagcaccctcggagcc	125
			Reverse	gcacgttgggtcccagaagc	
18	chr6:170863510+170863606	Chr6 control region within <i>TBP</i>	Forward	cttgccttccgtgcccatag	97
			Reverse	ctcagtgcagtgggtgcttgc	
19	chr7:5596654-5596787	Chr7 control region within <i>ACTB</i>	Forward	tgcttctctgtgcccctcc	134
			Reverse	acgggttctactgtctgccc	
20	chr12:6644756+6644900	Chr12 control region within <i>GAPDH</i> (used for normalization)	Forward	ttcaccgcaaaatggcccctc	145
			Reverse	ccagacacccaatcctccgggt	
21	chr6:15481823+15729819	Chr6 Breakpoint Sanger Seq	Forward	tacctgtcttggctctgtcg	
			Reverse	atctgagccagcatgtagagg	
22	chr9:119240899+119372879	Chr9 Breakpoint Sanger Seq	Forward	ttcttcacctggactagacc	
			Reverse	gcagaatttgcctttgtcg	