Detection and Characterization of Mosaicism in Autosomal Dominant Polycystic Kidney Disease (ADPKD)

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Supplemental Methods

LR-NGS

Patient DNA was diluted to 50ng/μl and all exonic regions, and including some introns, of *PKD1/PKD2* were amplified using ten optimized LR-PCR products. Details on primer sequences and PCR conditions can be found in Supplemental Table 1. Each LR-PCR product was analyzed on a 0.5% agarose gel to assure successful specific amplification and subsequently quantified using the Qubit dsDNA BR Assay kit (Invitrogen). All ten amplicons of each patient were pooled equimolarly at 30fM, with the exception of amplicons PKD1_5UTR-1 and PKD2-5UTR-1, which were enriched 5-fold due to their GC richness and short amplicon size, both reducing NGS library prep efficiency.

Samples were submitted to the Mayo Clinic Genomics Core for NGS library preparation and sequencing. In short, a total of 500ng of equimolarly pooled LR-PCR products per patient were sheered to ~150-200bp fragments using the Covaris E220 system and prepped for Illumina HiSeq2500 rapid 101bp-PE sequencing using the NEBNext DNA Library Prep kit (NEB). A total of 37 indexed samples were pooled per HiSeq2500 lane to achieve an expected average read depth of 5000x high quality reads (GQ>20) per base pair (actual: 4940x+/-2117x; GQ: 92+/-27).

Supplemental Figures

Figure S1. Analysis of two false positive "mosaic" PKD1 families. (**A**) Pedigree of family M796. (**B**) SS shows only a low level of the variant allele in the mother (II-2), including in a kidney tissue sample (II-2 (T)), and the daughter (III-1). (**C**) Analysis by tNGS shows approximately equal read counts for the normal and variant allele. (**D**) Pedigree of family P1317. (**E**) Analysis by tNGS in the mother (II-1) shows that only 7.9% of reads had the duplicated allele, suggesting mosaicism. (**F**) However, SS shows a similar sequence profile in the mother and affected daughter (III-2), with approximate equal peak height between the normal and variant alleles.

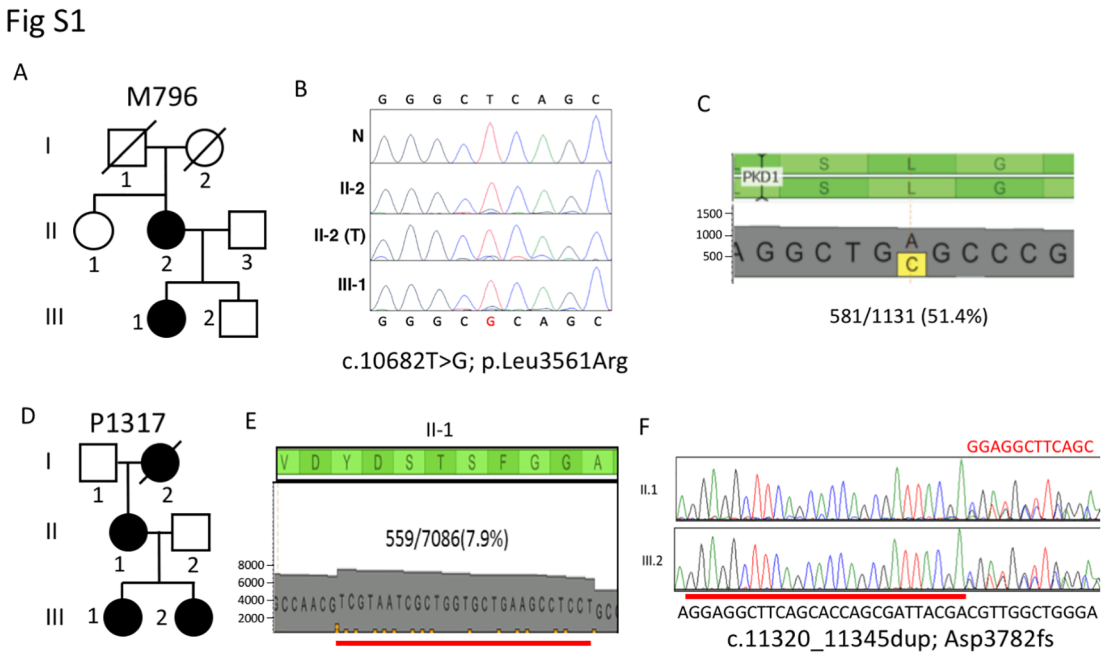
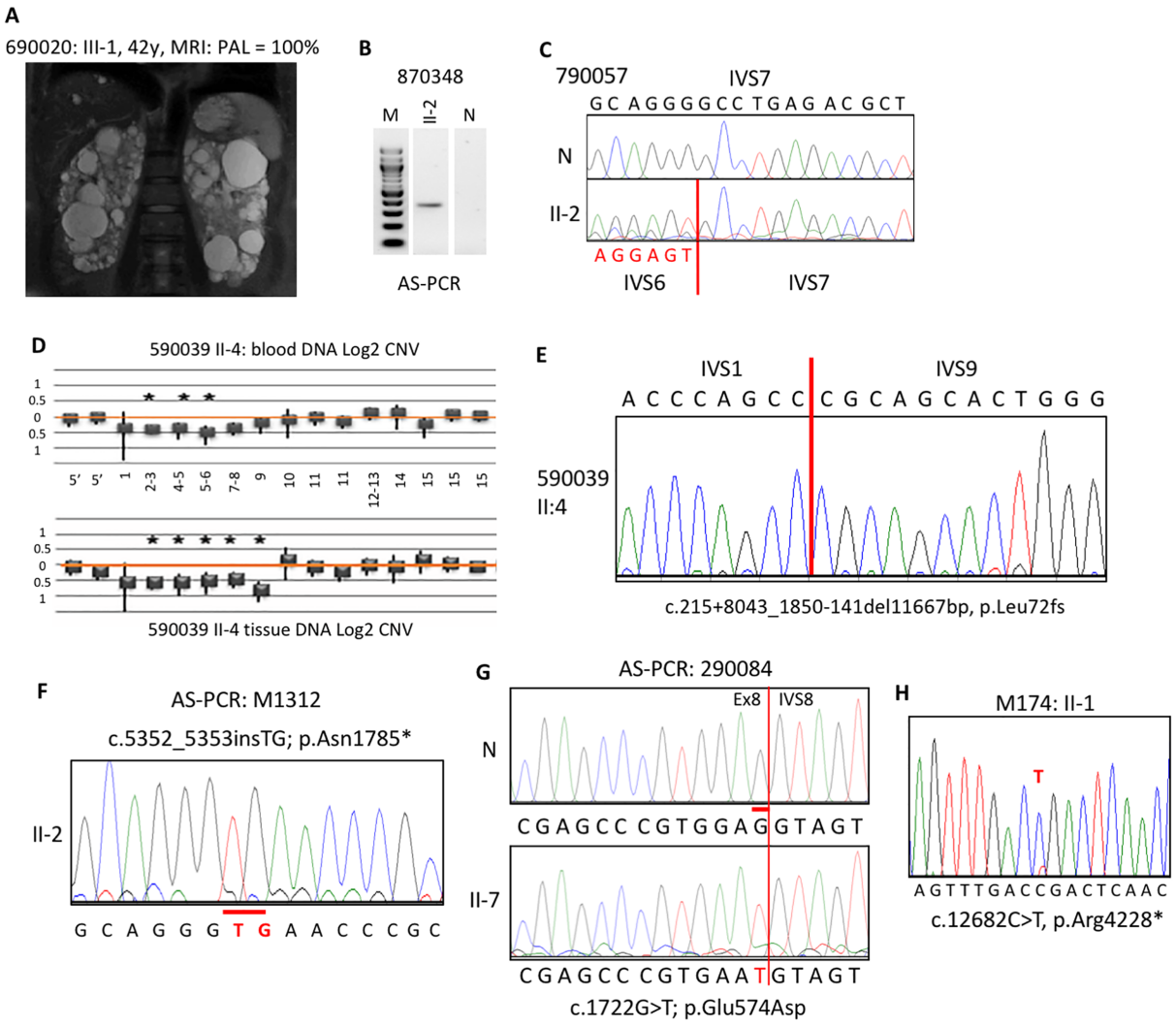


Figure S2. Details of mosaic positive PKD1 families. (**A**) MRI of 690020 III-1 with the fully penetrant mutation showing moderate PKD. (**B**) AS-PCR showing amplification in the mosaic patient, 870348, II-2, but not the normal (N) control. (**C**) SS (reverse strand shown) of the breakpoint fragment from IVS6 to IVS7 in 790057 II-2, compared to normal IVS7 sequence. (**D**) Log2 CNV analysis from the tNGS of 590039 II-4 blood and tissue derived DNA showing reduced signal from exons 2-9, consistent with being mosaic for a deletion. * indicates significantly reduced signal ($p<0.05$). (**E**) SS of the IVS1-IVS9 breakpoint fragment in 590039 II-4. (**F**) SS of the AS-PCR product in M1312 II-2 showing the TG insertion. (**G**) SS of AS-PCR product from 290084 II-7 compared to normal sequence showing substitution of the last nucleotide of exon 8. This substitution, c.1722G>T, is predicted to result in loss of the IVS8 donor site (BDGP: Splice Site Predictions 0.56 to <0.01). The percentage of the observed versus the expected level of the PAL is indicated next to the radiological image.

Fig S2


A
690020: III-1, 42y, MRI: PAL = 100%
B
870348
M
II-2
N
AS-PCR
C
790057
IVS7
G C A G G G G C C T G A G A C G C T
N
II-2
A G G A G T
IVS6
IVS7
D
590039 II-4: blood DNA Log2 CNV
1
0.5
0
0.5
1
5'
5'
1
2-3
4-5
5-6
7-8
9
10
11
11
12-13
14
15
15
15
590039 II-4 tissue DNA Log2 CNV
E
IVS1
IVS9
A C C C A G C C C G C A G C A C T G G G
590039
II:4
c.215+8043_1850-141del11667bp, p.Leu72fs
F
AS-PCR: M1312
c.5352_5353insTG; p.Asn1785*
II-2
G C A G G G T G A A C C C G C
G
AS-PCR: 290084
Ex8
IVS8
N
C G A G C C C G T G G A G G T A G T
II-7
C G A G C C C G T G A A T G T A G T
c.1722G>T; p.Glu574Asp
H
M174: II-1
T
A G T T T G A C C G A C T C A A C
c.12682C>T, p.Arg4228*

Supplemental Tables

Table S1. Genes included on the 137 gene panel

gene	chromosome
AHI1	chr6
ALG8	chr11
ALG9	chr11
ANKS6	chr9
ARL13B	chr3
ARL6	chr3
ATF6B	chr6
ATP2A2	chr12
ATXN10	chr22
B9D1	chr17
B9D2	chr19
BBIP1/BBS18	chr10
BBS1	chr11
BBS10	chr12
BBS12	chr4
BBS2	chr16
BBS4	chr15
BBS5	chr2
BBS7	chr4
BICC1	chr10
CALR	chr19
CANX	chr5
CC2D2A	chr4
CEP290	chr12
CEP41	chr7
CKAP4	chr12
COL4A1	chr13
COL4A2	chr13
COL4A4	chr2
COL4A5	chrX
CSPP1	chr8
DDOST	chr1
DNAJB11	chr3
DNAJB9	chr7
DNAJC1	chr10
DNAJC10	chr2
DNAJC3	chr13
DYNC2H1	chr11
DZIP1L	chr3
EDEM1	chr3
EDEM2	chr20
EDEM3	chr1
EFCAB7	chr1
EIF2AK3	chr2
ERLEC1	chr2
ERO1A	chr14
ERO1LB	chr1
FLCN	chr17
FLNC	chr7
GANAB	chr11
GATA3	chr10
GLA	chrX
GLIS2	chr16
HIVEP1	chr6
HNF1B	chr17
HSP90B1	chr12
HSPA5	chr9
HYOU1	chr11
IFT122	chr3
IFT140	chr16
IFT172	chr2
IFT27/BBS19	chr22
IFT43	chr14
IFT80	chr3
INPP5E	chr9
INVS	chr9
IQCB1	chr3
KIF7	chr15
LRP2	chr2
LRP5	chr11
LZTFL1	chr3
MAPKBP1	chr15
MKKS	chr20
MKS1	chr17
MLEC	chr12
MOGS	chr2
MSTN	chr2
MUC1	chr1
NEK1	chr4
NEK8	chr17
NOTCH2	chr1
NPHP1	chr2
NPHP10/BB1(	chr1
NPHP3	chr3
NPHP4	chr1
NRIP1	chr21
OFD1	chrX
OS9	chr12
PDIA3	chr15
PKD1	chr16
PKD2	chr4
PKHD1	chr6
PMM2	chr16
PRKAR1A	chr17
PRKCSH	chr19
PTHB1	chr7
REN	chr1
RPGRIP1L	chr16
RPN1	chr3
RPN2	chr20
SEC13	chr3
SEC24B	chr4
SEC24C	chr10
SEC31A	chr4
SEC61A1	chr3
SEC61A2	chr10
SEC61B	chr9
SEC61G	chr7
SEC62	chr3
SEC63	chr6
SIL1	chr5
SPIN4	chrX
STT3A	chr11
STT3B	chr3
TCTN1	chr12
TCTN2	chr12
TMEM138	chr11
TMEM216	chr11
TMEM231	chr16
TMEM237	chr2
TMEM260	chr14
TMEM67	chr8
TRIM21	chr11
TRIM32	chr9
TSC1	chr9
TSC2	chr16
TTC21B	chr2
TTC8	chr14
UGGT1	chr2
UGGT2	chr13
UMOD	chr16
VHL	chr3
WDR19	chr4
WDR34	chr9
WDR35	chr2
XPNPEP3	chr22
ZNF423	chr16

Table S2: Mean read counts for the exonic regions of *PKD1* and *PKD2* by the 65 and 137 gene panel

Gene	Exons	Covered Region (GRCh37p.13)	65 Gene Panel Mean DP	137 Gene Panel Mean DP
PKD2	**Overall**		**474.83**	**779.45**
PKD2	5'UTR-Exon 1	chr4:88926749-88929530	70.56	449.89
PKD2	Exon 2	chr4:88940560-88940773	705.08	834.74
PKD2	Exon 3	chr4:88957322-88957555	562.12	964.30
PKD2	Exon 4	chr4:88959353-88959703	877.89	1089.71
PKD2	Exon 5	chr4:88964335-88964659	791.16	887.50
PKD2	Exon 6	chr4:88967744-88968072	715.65	775.61
PKD2	Exon 7	chr4:88973093-88973360	592.46	813.24
PKD2	Exon 8	chr4:88977188-88977469	663.49	900.37
PKD2	Exon 9	chr4:88979085-88979305	567.68	810.83
PKD2	Exon 10	chr4:88983008-88983206	530.35	863.93
PKD2	Exon 11	chr4:88986476-88986697	620.27	896.70
PKD2	Exon 12	chr4:88986864-88987081	752.25	1012.79
PKD2	Exon 13	chr4:88989000-88989263	836.50	1072.94
PKD2	Exon 14	chr4:88995914-88996161	808.74	898.91
PKD2	Exon 15-3'UTR	chr4:88996560-88998981	616.91	972.69
PKD1	**Overall**		**839.24**	**618.89**
PKD1	5'UTR- Exon 1	chr16:2185426-2187949	8.86	343.90
PKD1	Exon 2	chr16:2169258-2169429	1460.35	1038.55
PKD1	Exon 3	chr16:2169065-2169236	764.28	783.20
PKD1	Exon 4	chr16:2168627-2168896	1256.79	1076.63
PKD1	Exon 5	chr16:2167742-2168513	1546.18	755.89
PKD1	Exon 6	chr16:2167440-2167723	1250.75	782.75
PKD1	Exon 7	chr16:2166784-2167104	915.91	468.73
PKD1	Exon 8	chr16:2166480-2166695	854.62	327.74
PKD1	Exon 9	chr16:2165943-2166169	1036.80	585.10
PKD1	Exon 10	chr16:2165329-2165676	1099.17	454.81
PKD1	Exon 11	chr16:2164121-2164976	917.00	527.45
PKD1	Exon 12	chr16:2163112-2163343	791.74	521.77
PKD1	Exon 13	chr16:2162739-2163014	1036.37	831.14
PKD1	Exon 14	chr16:2162291-2162524	891.99	553.96
PKD1	Exon 15	chr16:2158203-2161922	991.00	707.27
PKD1	Exon 16	chr16:2157834-2158083	971.60	769.85
PKD1	Exon 17	chr16:2156756-2156999	773.23	566.16
PKD1	Exons 18-20	chr16:2155816-2156728	1039.77	746.21
PKD1	Exon 21	chr16:2155273-2155525	927.83	564.96
PKD1	Exon 22	chr16:2154449-2154693	1167.33	821.50
PKD1	Exon 23	chr16:2153217-2153946	940.95	542.39
PKD1	Exon 24	chr16:2152765-2153021	853.86	590.50
PKD1	Exon 25	chr16:2152332-2152684	1040.17	931.82
PKD1	Exon 26	chr16:2152012-2152307	1112.61	819.86
PKD1	Exons 27-30	chr16:2149595-2150617	1003.20	590.99
PKD1	Exons 31-32	chr16:2147679-2148035	1012.95	750.22
PKD1	Exons 33-34	chr16:2147099-2147554	959.05	757.81
PKD1	Exons 35-37	chr16:2143495-2144261	929.89	624.54
PKD1	Exon 38	chr16:2142905-2143144	857.67	762.70
PKD1	Exon 39	chr16:2142431-2142643	887.87	908.92
PKD1	Exon 40	chr16:2141998-2142239	918.45	658.07
PKD1	Exon 41	chr16:2141732-2141957	958.96	736.30
PKD1	Exon 42	chr16:2141374-2141648	181.48	54.23
PKD1	Exons 43-3'UTR	chr16:2138661-2141225	687.13	570.26

Table S3. Details of the *PKD1* pathogenic variants detected in the 20 mosaic families

Designation		Variant type	Mutation Strength Group (MSG)	Pub.	gnomAD	ACMG class	ACMG evidence
Nucleotide	Amino acid						
c.74_75delGCinsT	p.(Gly25fs)	Frameshift	1	N	0	Path	PVS1, PM2, PM6, PP4
c.215+8043_1850-141del11.7kb	p.(Leu72fs)	Large deletion	1	N	0	Path	PVS1, PM2, PM6, PP4
c.844_845dupGG	p.(Pro283fs)	Frameshift	1	N	0	Path	PVS1, PM2, PM6, PP4
c.935_937delinsA	p.(Ala312fs)	Frameshift	1	N	0	Path	PVS1, PM2, PP1, PM6, PP4
c.1386-34_1606+26del282bp	p.(Ser463fs)	Frameshift	1	N	0	Path	PVS1, PM2, PM6, PP4
c.1722G>T	p.(Glu574?)	Splicing	2	N	0	L. Path	PM2, PP3, PM6, PP4
c.2548_2557del	p.(Asp850fs)	Frameshift	1	N	0	Path	PVS1, PM2, PP1, PM6, PP4
c.3685delG	p.(Val1229fs)	Frameshift	1	N	0	Path	PVS1, PM2, PM6, PP4
c.4520G>A	p.(Trp1507*)	Nonsense	1	1x[S1]	0	Path	PVS1, PM2, PM6, PP4
c.5352_5353insTG	p.(Asn1785*)	Frameshift	1	N	0	Path	PVS1, PM2, PM6, PP4
c.8426C>T	p.(Pro2809Leu)	Missense	2	1x[S1]	0	L. Path	PM2, PS1, PM6, PP4
c.8970_8972del	p.(Tyr2991del)	Inframe indel	1	N	0	L. Path	PM2, PP3, PP1, PM6, PP4
c.9185_9201+7del24	p.(Val3062fs)	Frameshift	1	N	0	Path	PVS1, PM2, PM6, PP4
c.10373_10386del14	p.(Pro3458fs)	Frameshift	1	N	0	Path	PVS1, PM2, PS2, PP4
c.10922dupC	p.(Arg3642fs)	Frameshift	1	N	0	Path	PVS1, PM2, PM6, PP4
c.11157-2A>G	p.(Arg3719?)	Splicing	1	N	0	Path	PVS1, PM2, PS1, PM6, PP4
c.11379delG	p.(Thr3794fs)	Frameshift	1	8x[S1]	0	Path	PVS1, PS4, PM6, PP4
c.11654_11683del	p.(Val3885_Ser3894del)	Inframe indel	2	N	0	L. Path	PM4, PM2, PP1, PM6, PP4
c.12440_12443dup	p.(Phe4149fs)	Frameshift	1	N	0	Path	PVS1, PM2, PP1, PM6, PP4
c.12682C>T	p.(Arg4228*)	Nonsense	1	11x[S1]	1/244928	path	PVS1, PS4, PM6, PP4

MSG1, truncating, 2 and 3, predicted strongly and weakly penetrant nontruncating, respectively; Pub., Prior description in publication; frequency in the gnomAD database of "normal individuals"; ACMG Class, Pathogenic classification based on the American College of Medical Genetics guidelines for interpretation of sequence variants: Path, pathogenic, L. Path, likely pathogenic; ACMG evidence, evidence supporting the interpretation of sequence variant classification. The evidence is classed as: PVS, pathogenic very strong; PS, pathogenic strong, PM, pathogenic moderate; PP, pathogenic supportive (see Richards et. al.[S2] for details)

S1. Autosomal Dominant Polycystic Kidney Disease Mutation Database (https://pkdb.mayo.edu/), 2019

S2. Richards S, Aziz N, Bale S*, et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; **17:** 405-424.

Table S4. Details of primers used for amplification of *PKD1* and *PKD2* for the LR-NGS protocol

Primer Name	Sequence (red nt denotes mismatch with pseudogenes)	Pair amplicon length	Amplicon Start (hg19)	Amplicon End (hg19)
NGS_PKD1_5UTR-1_F	CGCAGCCTTACCATCCACCT	2277nt	chr16:2185030	Chr16:2187307
NGS_PKD1_5UTR-1_R	TCATCGCCCCTTCCTAAGCA			
NGS_PKD1_2s-12m_NH2_F	CTCTGTCTACTCACCTCCGCATC	8576nt	chr16:2163055	chr16:2171630
NGS_PKD1_2s-12m_NH2_R	AGCGTCCTCGGGCAGCATGAAG			
NGS_PKD1_13s-21s_NH2_F	TGAGTGGAGGGAGGGACGCtAAT	7901nt	chr16:2155151	chr16:2163040
NGS_PKD1_13s-21s_NH2_R	GACAGAACGGCTGAGGCTACTG			
NGS_PKD1_22m-34s_NH2_F	GGGGGTCCAGTCAAGTGGG	7776nt	chr16:2146917	chr16:2154774
NGS_PKD1_22m-34s_NH2_R	CCTACAAAGCCCCATGAGCC			
NGS_PKD1_35-3UTR_NH2_F	ACGCCTGACTTCGGTGCCTGGAGTG	5891 nt	Chr16:2138530	chr16:2144420
NGS_PKD1_35-3UTR_NH2_R	AGCCGAGCCCACACCTGGCTATGAG			
NGS_PKD2_5UTR-1_NH2_F	CTTCAGCTTCAGAAGTTCCTTGGATGAC	4112nt	Chr4:88926589	chr4:88930700
NGS_PKD2_5UTR-1_NH2_R	ATTCTCCCAAAGAGTCAAGTCCTAAGCG			
NGS_PKD2_2_F	AAGAATCATGGCTGTTCCTTAGTGCC	2855nt	chr4:88938934	chr4:88941789
NGS_PKD2_2_R	AAACTGCTTCCTCATCTGTTCTCGTG			
NGS_PKD2_3-5_F	GAGAGGTGGTGGAAGATGAAATCGAG	8082nt	chr4:88957044	chr4:88965126
NGS_PKD2_3-5_R	CCAGGATCTGTGGAAGGAACTTTACG			
NGS_PKD2_5-7_F	TTGGCTGTAATTTTCTCTGGGGAC	9951nt	chr4:88964001	chr4:88973952
NGS_PKD2_5-7_R	TGCTCCTTCCCTCTCTTTCTCTAAG			
NGS_PKD2_8-10_NH2_F	CACTAACTCCCAATTCCCCCAGTCC	6461 nt	chr4:88976965	chr4:88983425
NGS_PKD2_8-10_NH2_R	TGAACCTTCAGCTCAGCTCTTAAAGTGTC			
NGS_PKD2_11-13_NH2_F	GCAATCCAGCCAGGTGACAGGGTAAGAC	4710nt	chr4:88985270	chr4:88989979
NGS_PKD2_11-13_NH2_R	CGTGAATACCACATCTAGGCACATCTAAG			
NGS_PKD2_14-3UTR_F	CTTTTGTAACAAGAGTGGGAAAGCCG	5109nt	chr4:88994370	Chr4:88999479
NGS_PKD2_14-3UTR_R	ATCTACCAGTGTCTGCATTTCTTTGCCT			

Table S5. Primers and conditions for AS-PCR

Primer ID	5'---3' sequence	Tm (^{o}C)	Product size (bp)
290084_F	CCGCACGAGCCCGTGAAT	70	114
290084_R	CCCCAAGTTTTTTGGCGAGACCCAC		
M1312_INSTG _F	ACTTGGTCACCATGACGGCAGGGTGA	70	627
M1312_Ex15(5)_R	GGGTTGGCCCCGCCGACCTGC		
870348DEL10_F	CTTGGTGCTCCAGGTGCCAA	60	311
870348DEL10_R	ACTAGGACTCCCTGCAGTACAC		
M174_F	CTGCTCACCCAGTTTGACT	60	336
M174_R	AACCTACGTGCAGCCATTCT		
390010_DEL30_F	ACCAGCCAGGAGCCCACCCTCGCT	72	259
390010_ DEL30_R	AGCGAGAGGCCCGCGCTGAGGGC		