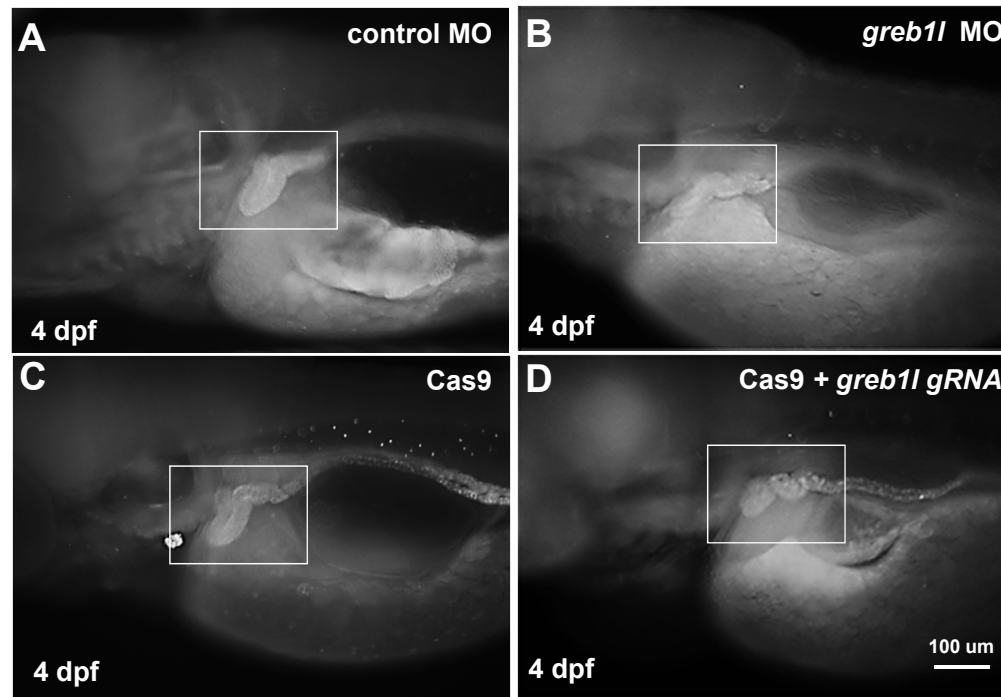**Supplementary Figure S1.** Amino acid sequence comparison between orthologous human and zebrafish GREB1L proteins. Note the strong conservation at the c-termini of the proteins, as well as the conservation at positions affected by the mutations. Circled red L residue = position of the Iowa mutation (recapitulated in two of the mouse mutants), circled blue G residue = position of the Danish frameshift mutation, circled purple W mutation = position of the stop codon mutation in the fish.

```
Hum GREB1L    MGNSYAGQLKSARFEEALHNSIEASLRCSSVVPRPIFSQLYLDPDQHPFSSADVKPKVED    60
              MGNSYAGQLKSARFEEALHNSIEASLR S   P+P+F+QLYL+PDQ+    D+KPK+
Zf Greb1l     MGNSYAGQLKSARFEEALHNSIEASLRSSGGDPQPVFTQLYLEPDQYSGHVEDIKPKM--    58

Hum GREB1L    LDKDLVNRYTQNGSLDFSNNLTV----NEMEDDEDDEEMSDSNSPPIPYSQKPAPEGSCT   116
               DL R     D S ++ V   +  +D DDE+ SD++SPP+PY Q P P+G CT
Zf Greb1l     ---DLSLRS------DPSTHVLVKCHSSNSVEDMDDEDDSDTSSPPLPYLQGPPPDGCCT   109

Hum GREB1L    TDGFCQAGKDLRLVSLCMEQIDIPAGFLLVGAKSPNLPEHILVCAVDKRFLPDDHGKNAL   176
               DGFCQAGKDLRLVS+  E I++PAGF LVGAKSP++PEHILVCAVDKRFLPD++GKNAL
Zf Greb1l     VDGFCQAGKDLRLVSMATESIEVPAGFELVGAKSPSIPEHILVCAVDKRFLPDENGKNAL   169

Hum GREB1L    LGFSGNCIGCGERGFRYFTEFSNHINLKLTTQPKKQKHLKYYLVRSSQGVLSKGPLICWK   236
              LGFSGNC+GCGE+GFRYFTEFSNHINLKL+TQPKKQKHLKYYLV++SQG L KG LICWK
Zf Greb1l     LGFSGNCVGCGEKGFRYFTEFSNHINLKLSTQPKKQKHLKYYLVKNSQGALCKGALICWK   229

Hum GREB1L    ECRSRQSSASCHSIKPSSSVSSTVTPENGTTNGYK-SGFTQTDAA-----NGNSS-----   285
              +C++R  S S K SS SS  + ENG TNG+  S F +D+        +G+SS
Zf Greb1l     DCKTRPFSNSASSSK-PSSSSSLSSKENGDTNGHSPSPFPLSDSPPARMQSGSSSGIFGP   288

Hum GREB1L    -------------HGGK--------------------GSASSSTPAHTGNYSLSPRPSY   311
                           HG K                   G ++  +P T  S +P  Y
Zf Greb1l     QELGFLKPLNTPTHGTKTLPIVPTALRVNGLTNGLSMDGRSTLLSPPRTNPLS-TPSHGY   347

Hum GREB1L    ---ASGDQ--ATMFISGPPKKRHRGWYPGSPLPQPGLVVPVPTVRPLS-RTEPLLSAPVP   365
                 +GD   +T  +GPPKKRHR W+P + +P P  VPVP +RPL+  + PLLS
Zf Greb1l     RTTETGDSPASTAMSTGPPKKRHRSWHPTTLVPIPATAVPVPAIRPLTCSSGPLLSLSNQ   407

Hum GREB1L    Q-TPLTGILQPRPIPAGETVIVPENLLSNSGVRPVILIGYGTLPYFYGNVGDIVVSPLLV   424
              Q  ++G++QP+PI AGETVI+P NLL++ GVRPV+LIG GTLPYF+GNVGD+VVSPLLV
Zf Greb1l     QPASVSGVIQPQPITAGETVIIPDNLLNSYGVRPVLLIGQGTLPYFFGNVGDLVVSPLLV   467

Hum GREB1L    NCYKIPQLENKDLEKLGLTGSQFLSVENMILLTIQYLVRLGPDQVPLREEFEQIMLKAMQ   484
              +CYK +L K L LG++ +Q L+ E MILLT+QYL RLG +Q+PLREEFEQIMLKAM
Zf Greb1l     SCYKGRELNEKTLASLGMSANQLLTTETMILLTLQYLARLGTEQIPLREEFEQIMLKAML   527

Hum GREB1L    EFTLRERALQIGAQCVPVSPGQLPWLARLIASVSQDLVHVVVTQNSLAEGISETLRTLSE   544
                         G   PVSP QLPWLAR+ ASVS  V V+VT  SL EGISE+LR LSE
Zf Greb1l     ----------CGPTGPPVSPAQLPWLARMEASVSGGSVQVLVTHGSLGEGISESLRSLSE   577

Hum GREB1L    M--RHYQRLPDYVVVICASKIRGNEFCVVVLGQHQSRALAESMLTTSEFLKEISYELITG   602
                +  Q LP+YV++IC SK   NEFCV+VLG++QSRALAESML+T+EFLKEISYELITG
Zf Greb1l     TSPQQQQCLPNYVLIICTSKSGANEFCVLVLGKYQSRALAESMLSTNEFLKEISYELITG   637

Hum GREB1L    KVSFLASHFKTTSLGDDLDKLLEKMQQRRGDSVVTPFDGDLNECVSPQEAAAMIPTQNLD   662
              KVS LASHF++TSLGD++DK L + Q++R D VV PF G LE +  QEAA MIP    D
Zf Greb1l     KVSVLASHFQSTSLGDNMDKQLVRYQRKRKDRVVQPFQGHLTEYIHSQEAATMIPESGPD   697

Hum GREB1L    LDNETFHIYQPQLTVARKLLSQVCAIADSGSQSLDLGHFSKVDFIIIVPRSEVLVQQTLQ   722
              L ++ F I+ PQL+VAR LLSQVCAIADSGSQSLDLG F KVDF+I+VP S VLV QT+Q
Zf Greb1l     LLSDDFQIHPPQLSVARSLLSQVCAIADSGSQSLDLGRFCKVDFLILVPPSHVLVHQTVQ   757

Hum GREB1L    RIRQSGVLVDLGLEENGTAHQRAEKYVVRLDNEIQTKFEVFMRRVKQNPYTLFVLVHDNS   782
              RIRQSGVL+DLG+E+    A Q+++KYVVRLD E+ TK E FMR+VKQNPYTLFVL+HDNS
Zf Greb1l     RIRQSGVLIDLGIEDVSLAMQKSDKYVVRLDTEVHTKMEAFMRKVKQNPYTLFVLIHDNS   817

Hum GREB1L    HVELTSVISGSLSHSEPSHGLADRVINCREVLEAFNLLVLQVSSFPYTLQTQQSRISSSN   842
              HV+LTS +SGS+ H E   GLADRV+NC EVLEA NLLVLQVS FP+TLQ++QSRIS+ N
Zf Greb1l     HVDLTSALSGSVCHGE-LQGLADRVVNCPEVLEAINLLVLQVSCFPFTLQSRQSRISTQN   876

Hum GREB1L    EVHWIQLDTGE-DVGCEEKLYFGLSEYSKSLQWGITSPLLRCDETFEKMVNTLLERYPRL   901
              EVHW  + + +    ++ +YFGL +YSKSLQWG+ SP+LRCD+ FE+MV TLLER+P L
Zf Greb1l     EVHWPDTENQQGEASPKDLIYFGLKDYSKSLQWGVASPILRCDDAFERMVKTLLERHPHL   936

Hum GREB1L    HSMVVRCYLLIQQYSEALMALTTMASLRDHSTPETLSIMDDLISSPGKNKSGRGHMLIIR   961
              HSMV+R YLLIQQY+EALMALT    SLRDH TP+TL++++DL S PG++K G GHML++R
Zf Greb1l     HSMVIRSYLLIQQYTEALMALTAAPSLRDHVTPQTLAMVEDLLSVPGRSKHGCGHMLLVR   996

Hum GREB1L    VPSVQLAMLAKERLQEVRDKLGLQYRFEIILGNPATELSVATHFVARLKSWRGNEPEEWI  1021
              VPS+QLA LA+ERL+E RDKLGLQYRF ++LG+PA E+S+  HF ARL++WRG + EEW+
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Zf Greb1l    VPSLQLARLAQERLEEARDKLGLQYRFAVLLGSPAAEISLPVHFCARLRAWRGCKNEEWV      1056

Hum GREB1L   PRTYQDLDGLPCIVILTGKDPLGETFPRSLKYCDLRLIDSSYLTRTALEQEVGLACCYVS      1081
             P TY+DL+GLPCIVILTGKDPLGETFPRSLKYCDLRLIDSSYLTRTALEQEVGLAC YV+
Zf Greb1l    PHTYEDLEGLPCIVILTGKDPLGETFPRSLKYCDLRLIDSSYLTRTALEQEVGLACSYVT      1116

Hum GREB1L   KEVIRGPTVALDLSGKEQERAAVSEN--DSDELLIDLERPQSNSSAVTGTSGSIMENGVS      1139
             + VI    A    + +E   S    + D+L ++LERP SN+SA T TSGS  ENGVS
Zf Greb1l    RRVIPKTKTATSREERPREGERSSGETAEHDDLPMELERPPSNASAATRTSGSTTENGVS      1176

Hum GREB1L   SSSTADKSQKQSLTPSFQSPATSLGLDEGVSASSAGAGAGETLKQECD------------      1187
             SSS DK  QS    P S + + S+              KQECD
Zf Greb1l    SSSILDKPSSQS------DPCGSRTMMDSCSSPV-------RFKQECDSQAPSSSSTSSF      1223

Hum GREB1L   ----SLGPQMASSTTSKPSSSSSGPRTLPWPGQPIRGCRGPQAALPPVVILSKAAYSLLG      1243
                 S   +S   +PS S+ PR          C  Q  P  +LS+AAY+LL
Zf Greb1l    SSASSSSSSSSSPAAQRPSQSTQAPRE----------CNRTQ-VFPRTAVLSRAAYTLLA      1272

Hum GREB1L   SQKSGKLPSSSSLLPHADVAWVSSLRPLLNKDMSSEEQSLYYRQWTLARQHHADYSNQLD      1303
              + G  PSS+SLLPHADV+W S LRP +  +   EQSLYYRQWT ARQHHADY   +
Zf Greb1l    PETLGH-PSSASLLPHADVSWSSPLRPPVPHGLGGAEQSLYYRQWTTARQHHADYEGPVP      1331

Hum GREB1L   PASGTRNFHPRRLLLTGPPQVGKTGSYLQFLRILFRMLIRLLEVDVYDEEEINTDHNESS      1363
                   + HPRRLLL+GPPQVGKTG+YLQFLRILFRMLIRLLEVDVYDEEE+  D + S
Zf Greb1l    ------HPHPRRLLLSGPPQVGKTGAYLQFLRILFRMLIRLLEVDVYDEEELEEDVQDKS      1385

Hum GREB1L   EVSQSEGEPWPDIESFSKMPFDVSVHDPKYSLMSLVYTEKL----AGVKQEVIKESKVEE      1419
             +V  S G  WPD+E  K+ FD+   HD K+   S VY ++    +GVK E +       +
Zf Greb1l    KVPPSSGPQWPDVEDVRKLRFDLCPHDCKFKYSSPVYANRMPKTQSGVKTERLDTEA--D      1443

Hum GREB1L   PRKRETVSIMLTKYAAYNTFHHCEQCRQYMDFTSASQMSDSTLHAFTFSSSMLGEEVQLY      1479
             P KR TVS+ L+ +AA+N FHHCEQC  Y +   A+Q+SD T HAFTF SSMLGEEVQL+
Zf Greb1l    PPKRNTVSVRLSLFAAHNAFHHCEQCHHYSEPIPAAQLSDCTFHAFTFCSSMLGEEVQLH      1503

Hum GREB1L   FIIPKSKESHFVFSKQGKHLESMRLPLVSDKNLNAVKSPIFTPSSGRHEGLLNLFHAME      1539
             FIIPKSKESHFVFS+QG HLESMRLPL+SDK   +KSPIFTP++GR EHGLLN++HAME
Zf Greb1l    FIIPKSKESHFVFSQQGSHLESMRLPLLSDKESGMMKSPIFTPTTGRQEHGLLNIYHAME      1563

Hum GREB1L   GISHLHLLVVKEYEMPLYRKYWPNHIMLVLPGMFNNAGVGAARFLIKELSYHNLELERNR      1599
             G  HLH+LVVK+YEMPLYRKYWPNHI+LVLP MFNN+GVGAARF+IKELSYHNLELERNR
Zf Greb1l    GAEHLHILVVKQYEMPLYRKYWPNHILLVLPAMFNNSGVGAARFMIKELSYHNLELERNR      1623

Hum GREB1L   LEELGIKRQCVWPFIVMMDDSCVLWNIHSVQEPSSQPMEVGVSS-KNVSLKTVLQHIEAT      1658
              EE G+KRQ VWPFIVMMDDSCVLWN      + QP  G +   NVSLK+VLQH+EAT
Zf Greb1l    QEEQGVKRQDVWPFIVMMDDSCVLWN-------AQQPGPDGKTEVMNVSLKSVLQHMEAT      1676

Hum GREB1L   PKIVHYAILGIQKWSSKLTSQSLKAPFSRCHVHDFILLNTDLTQNVQYDFNRYFCEDADF      1718
             PKI   YA+ G++KWSS L+SQ+  +PFSRCH+HD ILLN DLTQNVQYD NR+ CE+ DF
Zf Greb1l    PKISQYAVCGLRKWSSSLSSQAPTSPFSRCHLHDLILLNVDLTQNVQYDLNRFTCEEVDF      1736

Hum GREB1L   NLRTNSSGLLICRFNNFSLMKKHVQVGGQRDFIIKPKIMVSESLAPILPLQYICAPDSEH      1778
             NLR NSSGLL+CRFN FS+MKKH+ +GG +DF+IKPK+M E+  +   QY+CAPDSE
Zf Greb1l    NLRANSSGLLLCRFNQFSIMKKHIPIGGHKDFLIKPKLMRIETPVRVCASQYVCAPDSEQ      1796

Hum GREB1L   TLLAAPAQFLLEKF⌐L⌐QHASYKLFPKAIHNFRSPVLAIDCYLNIGPEVAICYISSRPHSSN      1838
             TLLAAPAQFLLEKF⌐L⌐Q  S++LFP A+ N  +PVL+ID YLN+GPEV +CY+SSRPHS N
Zf Greb1l    TLLAAPAQFLLEKF⌐L⌐QSCSHRLFPLALSNSANPVLSIDSYLNLGPEVQVCYVSSRPHSVN      1856

Hum GREB1L   VNCEGVFFSGLLLYLCDSFVGAD-LKKFKFLK⌐G⌐ATLCVICQDRSSLRQTIVRLELEDF⌐W⌐Q      1897
             V+ +GV FSGLLLYLCDSFV +  LKKF FLK⌐G⌐ATLCVICQDRSSLRQTIVRLELEDF⌐W⌐Q
Zf Greb1l    VDHQGVIFSGLLLYLCDSFVVSSLLKKFNFLK⌐G⌐ATLCVICQDRSSLRQTIVRLELEDF⌐W⌐Q      1916

Hum GREB1L   FRLRDEFQTANSSDDKPLYFLTGRHV   1923
             FRLRDEFQTAN S+D+PLYFLTGRH+
Zf Greb1l    FRLRDEFQTANCSEDRPLYFLTGRHI   1942
```

Supplemental Figure S2. Renal morphology of zebrafish in which *greb1l* expression is inhibited with morpholino or mutated with CRISPR/Cas9. All images are lateral views of zebrafish larvae at 4 days post fertilization, rostral to the left, fixed and processed to reveal anti-Na,K ATPase antibody (a6F) immunoreactivity. Wild type embryos were injected with (A) the "standard control" MO that is complementary to no sequence in the zebrafish genome (Gene Tools), (B) an MO targeting e3/i3 of *greb1l,* (C) Cas9 protein alone, or (D) Cas9 protein plus guide RNA targeting *greb1l* exon 17 (of 34). (A,C) In control embryos, the junction of the proximal convoluted tubule and the neck exhibited the hairpin structure characteristic of wild-type embryos. (B,D) In strongly-affected (B) *greb1l* MO-injected or (D) *greb1l* gRNA injected embryos, the structure of this region was kinked. (E) Table depicting the number of fish observed and the percentage of them that displayed kidney deformities.

E	% abnormal morphology of PCT/Neck junction	Number of embryos (# of experiments)
Control MO	4	45 (3)
greb1l e3i3 MO	85	135 (3)
Cas9	6	33 (3)
Cas9 + *greb1l* gRNA	42	144 (3)

Supplementary Figure S3. Mutagenized alleles recovered from affected CRISPR F0 mouse embryos. (A) Reference genomic sequence is shown at top with recovered mutant alleles shown below for each embryo displaying kidney phenotype. Notably, embryos #1 and 2 shared the same in-frame deletion in addition to the desired KI allele. The number in parentheses indicates the number of clones identified with the specific mutation. (B) Chromatograms of cloned reads corresponding to mutations described in (A).

A

```
          E   K   F   L   Q   H   A   S   Y   K   L   F   P
WT- GAG AAG TTC CTT CAA CAT GCC TCA TAT AAA CTC TTC CCT
```

Greb1l-F0-1

```
          E   K   F   R   Q   H   A   S   Y   K   L   F   P
#1  GAG AAG TTT CGT CAA CAT GCC TCA TAT AAA CTC TTC CCT (4/8)
          E   K   F   L       H   A   S   Y   K   L   F   P
#2  GAG AAG TTC CTT CA- --T GCC TCA TAT AAA CTC TTC CCT (4/8)
```
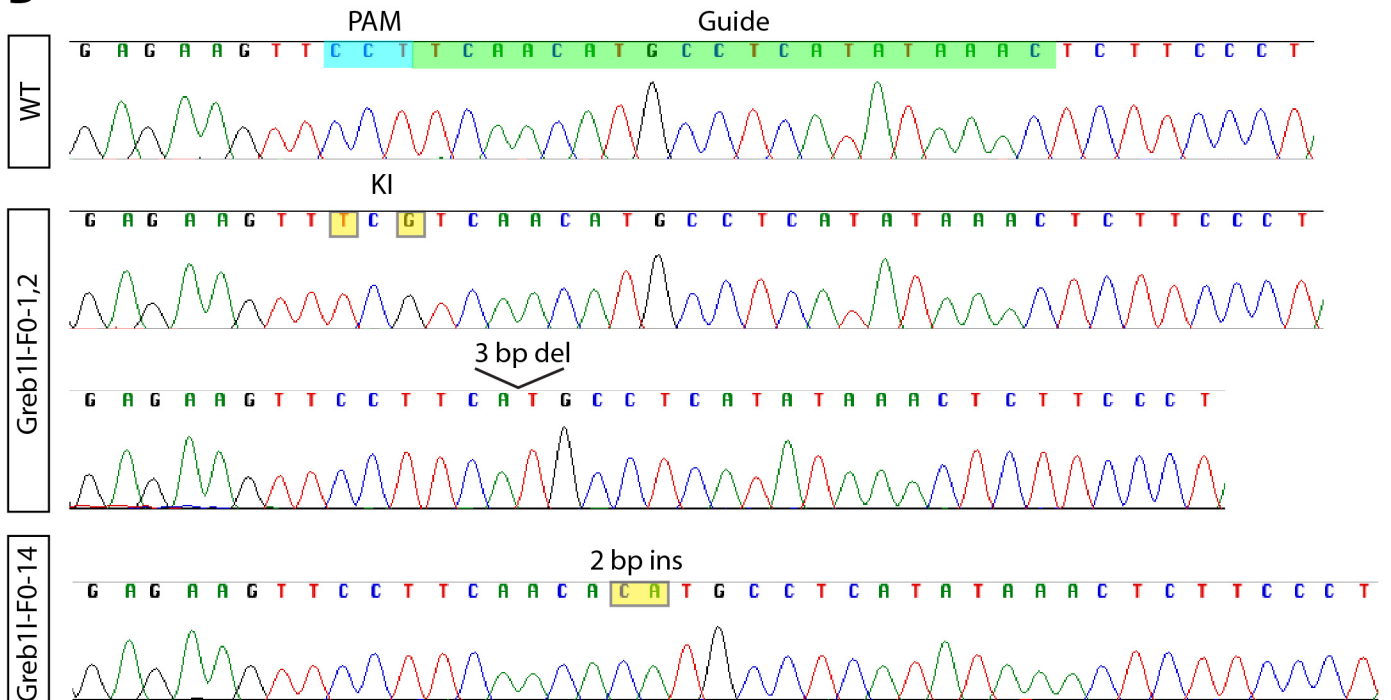
Greb1l-F0-2

```
          E   K   F   R   Q   H   A   S   Y   K   L   F   P
#1  GAG AAG TTT CGT CAA CAT GCC TCA TAT AAA CTC TTC CCT (3/8)
          E   K   F   L       H   A   S   Y   K   L   F   P
#2  GAG AAG TTC CTT CA- --T GCC TCA TAT AAA CTC TTC CCT (5/8)
```

Greb1l-F0-14

```
          E   K   F   L   Q   H   M   P   H   I   N   S   S   R   *
#1  GAG AAG TTC CTT CAA CAc aTG CCT CAT ATA AAC TCT TCC...AGG TAG (3/3)
```

B

Supplementary Table S1. Whole exome mapping statistics for four cases (Iowa family). Across the samples, we achieved an average targeted exome coverage of 171.69X with a mean mapping quality 45.30 for calling high quality variants.

Individual	Total # reads	# mapped reads / % of total reads	# mapped reads in target region / % of total reads	Mean coverage in target region	Mean mapping quality in target region
II.4	138,952,013	121,216,737 / 87.24%	100,480,821 / 72.31%	131.08	44.53
II.6	151,277,545	149,022,896 / 98.51%	123,983,267 / 81.96%	160.78	45.87
III.4	179,371,347	163,104,270 / 90.93%	138,330,046 / 77.12%	177.45	45.07
III.7	201,991,695	198,300,809 / 98.17%	166,643,662 / 82.50%	217.45	45.71
Average	**167,898,150**	**157,911,178 / 93.71%**	**132,359,449 / 78.47%**	**171.69**	**45.30**

Supplementary Table S2. Whole exome mapping statistics for two cases and three unaffected family members (Denmark family). Across the samples, we achieved an average targeted exome coverage of 127X with a mean mapping quality of 62 for calling variants of high quality.

Individual	Total # reads	# mapped reads / % of total reads	# mapped reads in target region / % of total reads	Mean coverage in target region	Mean mapping quality in target region
Fetus	161,010,696	159,630,813 /99.14%	137,922,285 /85.66%	223.7	62.33
Mother	70,125,707	69,581,369 /99.22%	59,504,884 /84.85%	100.4	61.3
Father	69,880,361	69,349,566 /99.24%	59,425,709 /85.03%	98.4	61.56
Maternal Grandmother	64,728,968	64,216,353 /99.2%	54,985,396 /84.94%	92.2	61.44
Maternal Grandfather	85,311,447	84,643,305 /99.21%	72,529,359 /85%	120.5	61.44
Average	**89,484,281**	**99%**	**76,873,527 /85%**	**127**	**62**