APPENDICES

Appendix 1. ARMS PCR condition and primer sequence for *CEP290* c.2991+1655A>G mutation screening.

CEP290 **gene Primer**	**Sequence**	**Product**	**PCR Mix**	**PCR Conditions**
c.2991+1655A>G WT_F	ACCGCACCTGGCCCCAGTT GTAATTGTGGA	136 bp	0 mM $MgCl_2$	ARMS WT: 60^{o}C
c.2991+1655A>G LCA_F	ACCGCACCTGGCCCCAGTT GTAATTGTGGG			ARMS LCA: Touch Down
c.2991+1655A>G WT_ R	AGTAAGGAGGATGTAAGA CTGGAGATAGAG			

Standard PCR used in this condition is universal PCR condition with annealing temperature (Tann): 60^{o}C for the wild-type (WT) reaction. For LCA reaction containing *CEP290* intronic mutation, touch down PCR was used.

Appendix 2. ARMS PCR condition and primer sequence for *CRB1* p.P1305L mutation screening.

CRB1 **gene Primer**	**Sequence**	**Product**	**PCR Mix**	**PCR Condition**
CRB1_p.P1305L ARMS_WT_F	TTCACAACCAATGTATTCAACAG	192 bp	2,5 mM $MgCl_2$	Standard WT: 58^{o}C
CRB1_p.P1305L ARMS_WT_R	CAGACCTCCATTGACACACG			
CRB1_p.P1305L_ ARMS_MUT_F	ATGAGTGTGCCTCTGATCT	113 bp		Standard MUT: 58^{o}C
CRB1_p.P1305L_ ARMS_MUT_R	TCATACGCAAAATGAGGTAAG			

Appendix 3. PCR condition of *CRB1* gene.

***CRB1* gene Primer**	**Sequence**	**Product**	**PCR Mix**	**PCR Conditions**
Exon9_1F	AATGATCATTACTATTAATAACGG	419 bp	2,0 mM $MgCl_2$	59^{o}C
Exon9_1R	GTGTTTCGTTGTCCACTTCC			
Exon9_2F	TTGCAGTCAGTGAATGATGG	423 bp	1,5 mM $MgCl_2$	58^{o}C
Exon9_2R	GGGACAGGAGCAATGATAAG			
Exon9_3F	CATGGAGGAAACTGTGAAGACA	449 bp	2,0 mM $MgCl_2$	59^{o}C
Exon9_3R	TGACCAAATTGTGACAGAAGC			
Exon7_1F	TCCATCCCTTCTGTCTTTTG	390 bp	2,0 mM $MgCl_2$	59^{o}C
Exon7_1R	TCCTAGGTTTTGTGAAGACTGA			
Exon7_2F	GCAATGCTGACTCCAAACTC	475 bp	1,5 mM $MgCl_2$	58^{o}C
Exon7_2F	TGGTGGGTCAGTAACATCATC			

Appendix 4. PCR condition of *AIPL1* gene.

***AIPL1* gene Primer**	**Sequence**	**Product**	**PCR Mix**	**PCR Conditions**
Exon6_F	TGCCTCTGAGGCTGGGAAGG	500 bp	1,5 mM $MgCl_2$	58^{o}C
Exon6_R	CACGATCCTGGTCAATCGAACC			
Exon1_F	TCTCAGCCGCCTAAGTGTCTTCC	436 bp	3,0 mM $MgCl_2$	Touch Down 63-58^{o}C
Exon1_R	TCCCTTTCTTCTCACTCAACATTTAGG			
Exon2_F	CGGGCCTTGAACAGTGTGTCTAG	222 bp	3,0 mM $MgCl_2$	Touch Down 63-58^{o}C
Exon2_R	CGCACCAGAACTCGGCCAC			
Exon3_F	GCCTGGCACACAGTTAACCACAG	389 bp	3,0 mM $MgCl_2$	Touch Down 63-58 ^{o}C
Exon3_R	GTCCCTCTCCAGTGCTGGCAC			
Exon4_5_F	AGGGAGATGTGCCACAGGGTC	603 bp	3,0 mM $MgCl_2$	Touch Down 63-58 ^{o}C
Exon4_5_R	TGGCAGGTGTCTCCGTGGC			

Appendix 5. PCR condition of *GUCY2D* gene.

***GUCY2D* gene Primer**	**Sequence**	**Product**	**PCR Mix**	**PCR Conditions**
Exon12_F	AGAGGCAGCCTTTGTGTTC	281 bp	1,25 mM $MgCl_2$	Touch Down PCR 68-54^{o}C
Exon12_R	AGCTGTCTCAGGTTGCTGAC			

Appendix 6. PCR condition for functional study

Gene	Sequence	Condition
CEP290	TGCTAAGTACAGGGACATCTTGC	Standard PCR + Q Solution
	AGACTCCACTTGTTCTTTTAAGGAG	
ACTB	ACTGGGACGACATGGAGAAG	Standard PCR + Q Solution
	TCTCAGCTGTGGTGGTGAAG	

Primer used in *CEP290* gene encompassed exon 26 and exon 27, which is targeting exon X caused by the c.2991+1655A>G mutation. *ACTB* was used as to normalize samples.

Appendix 7. Protocol of RNA Isolation using NucleoSpin RNA II isolation kit (with modification).

No.	Step	
1	**Homogenize sample** Up to 5 x 10^6 eukaryotic cultured cells can be collected by centrifugation and lysed by addition of Buffer RA1 directly.	 Disrupt sample
2	**Lyse cells** Add 350 µL Buffer RA1 and 3.5 µL β-mercaptoethanol (β-ME) to the cell pellet or to ground tissue and vortex vigorously.	 + 350 µL Buffer RA1
3	**Filtrate lysate** Reduce viscosity and clear the lysate by filtration through NucleoSpin® Filter (violet ring): Place NucleoSpin® Filterin a Collection Tube (2 mL), apply the mixture, and centrifuge for 1 min at 11.000 x g / 9000 rpm.	  9.000 rpm, 1 min
4	**Adjust RNA binding conditions** Discard the NucleoSpin® Filter and add 350 µL ethanol (70%) to the homo genyzied lysate and mix by pipetting up and down (5 times).	 350 µL 70% ethanol Mix
5	**Bind RNA** For each preparation take one NucleoSpin® RNA Column (light blue ring), placed in a Collection Tube. Pipette lysate up and down 2-3 times and load the lysate to the column. Centrifuge for 30 s at 11.000 x g / 9.000 rpm. Place the column in a new Collection Tube (2 mL).	  Load lysate 9.000 rpm, 30 s

6 Desalt silica membrane

Add 350 µL MDB (Membrane Desalting Buffer) and centrifuge at 11.000 x g / 9.000 rpm for 1 min to dry the membrane.

+ 350 µL MDB

9.000 rpm,

1 min

7 Digest DNA

Prepare DNase reaction mixture in a sterile 1.5 mL microcentrifuge tube: For each isolation, add 10 µL reconstituted rDNase to 90 µL Reaction Buffer for rDNase. Mix by flicking the tube.

Apply 95 µL DNase reaction mixture directly onto the center of the silica membrane of the column. Incubate at room temperature for 30 min.

+ 95 µL rDNase reaction mixture

RT, 30 min

8 Wash and dry silica membrane

1st wash

Add 200 µL Buffer RAW2 to the NucleoSpin® RNA Column. Centrifuge for 30 s at 11.000 x g / 9.000 rpm. Place the column into a new Collection Tube (2mL).

+ 200 µL RAW2

9.000 rpm,

30 s

2nd wash

Add 600 µL Buffer RA3 to the NucleoSpin® RNA Column. Centrifuge for 30 s at 11.000 x g / 9.000 rpm. Discard flow-through and place the column back into the Collection Tube (2 mL).

+ 600 µL RA3

9.000 rpm,

30 s

	3rd wash Add 250 µL Buffer RA3 to the NucleoSpin® RNA Column. Centrifuge for 2 min at 11.000 x g / 9.000 rpm to dry the membrane completely. Place the column into a nuclease-free Collection Tube (1,5 mL, supplied).	  + 250 µL RA3 9.000 rpm, 2 min
9	**Elute RNA** Elute the RNA in 50 µL RNase-free H_2O (supplied) and centrifuge at 11.000 x g / 9.000 rpm for 1 min.	  + 50 µL RNase-free H_2O 9.000 rpm, 1 min