Free Research-based Sequence Analysis of Genes Associated with Rare Inherited Eye Diseases in Centers of Expertise

(version 2)

At the ERN-EYE meeting in Florence on 12 October 2018, research groups from across Europe volunteered to perform sequence analysis to identify the underlying mutations for one or more inherited eye disease(s). This initiative is important as many new centers cannot afford costly Sanger sequencing or next-generation sequencing-based gene-panel sequencing.

This genotyping can only be done in a research setting, and diagnostic validation for clinical use, e.g. via Sanger sequencing of the identified variant(s) in the proband and/or family members, should be done in a certified diagnostic laboratory afterwards.

The research groups involved will benefit from the sequencing as they can study novel causes or mechanisms of disease. The terms of collaboration will be set by the sending party and the center of expertise. Each group listed below has its own conditions for a collaboration. Written informed consent from the probands is always the responsibility of the physician involved.

If you encounter any problems (e.g. regarding eligibility, turn-around-times, etc.) please send me an e-mail using this address: freeseq@radboudumc.nl

This brochure can also be found at the Retina International website:
http://www.retina-international.org/free-research-based-sequence-analysis-of-genes-associated-with-rare-inherited-eye-diseases-in-centers-of-expertise/

2 June 2019

Prof. Frans P.M. Cremers, Nijmegen, The Netherlands

Phenotype – Genes that are tested (City, Research leader)

- ✓ Achromatopsia – *CNGA3, CNGB3* etc (Tuebingen, S. Kohl)
- ✓ Bardet-Biedl and Alström syndromes (*BBS1-BBS22* and *ALMS1*) (Strasbourg, J. Muller)
- ✓ Blue-cone monochromacy-X-linked - *OPN1LW/OPN1MW* (Tuebingen, S. Kohl)
- ✓ Congenital stationary night blindness – 12 genes (Paris, C. Zeitz)
- ✓ Cornea plana – *KERA* (Prague, P. Liskova)
- ✓ Fuchs endothelial corneal dystrophy - *TCF4* repeats (CTG18.1)(London, A. Davidson)
- ✓ Leber congenital amaurosis / early-onset retinal dystrophy; also syndromic with early onset retinal dystrophy – 70 genes (Paris, J-M. Rozet)
- ✓ North Carolina macular dystrophy – *PRDM13 IRX1* regions (Ghent, E. De Baere)
- ✓ Posterior polymorphous corneal dystrophy – *OVOL2* & *GRHL2* promoters (Prague, P. Liskova)
- ✓ Retinitis pigmentosa (sporadic or autosomal recessive) with one causal variant in *USH2A* after WES or Sanger sequencing of all *USH2A* exons (Nijmegen, S. Roosing)
- ✓ Retinitis pigmentosa (sporadic or autosomal recessive) with one causal variant in *USH2A* after WES or targeted sequencing of all *USH2A* exons (Ghent, E. De Baere)
- ✓ Stargardt disease & ar cone-rod dystrophy- *ABCA4* (Nijmegen, F. Cremers)
- ✓ Stargardt disease with one causal variant in *ABCA4* (Ghent, E. De Baere)
- ✓ Usher syndrome with one causal variant in *USH2A* (Nijmegen, H. Kremer)
- ✓ Usher syndrome type 2 (Nijmegen, H. Kremer)
- ✓ X-linked retinitis pigmentosa – *RPGR ORF15* (Paris; C. Zeitz & I. Audo)

Achromatopsia - *CNGA3, CNGB3* (Tuebingen, Susanne Kohl)

Coverage:	Coding & splice site sequences of ***CNGB3*** Coding & splice site sequences of ***CNGA3***
Sensitivity:	>98% for coding regions
Technology:	Sanger sequencing
Turn-around-time:	2 - 6 months
Period:	1 January 2019 – 31 December 2021
Max. # of samples:	unlimited
Special requirement:	Well defined clinical diagnosis of congenital, non-progressive Achromatopsia (not cone dystrophy!)
Administration costs:	none
Minimal amount DNA:	5 microgram
Contact person:	Susanne Kohl: susanne.kohl@uni-tuebingen.de

Bardet-Biedl and Alström syndromes (Strasbourg, Jean Muller)

Coverage:	Coding and splice sites sequences of all known 22 **BBS-associated genes** and related genes, as well as *ALMS1*. An initial screening will include a fast screening of the recurrent BBS mutations (2 amplicons by Sanger sequencing). WES and WGS on excluded cases if agreed.
Sensitivity:	>98% for coding regions, single nucleotide variation, small indels and structural variations detection.
Technology:	Targeted capture followed by high throughput sequencing, Illumina NextSeq550 (Paired-End sequencing 2x150 bases), Sanger sequencing, qPCR.
Turn-around-time:	12 months
Period:	1 January 2019 – 31 December 2021
Max. # of samples:	100 (for the complete panel), unlimited for the initial screening (recurrent mutations).
Special requirement:	Well-defined clinical diagnosis assessed using the provided clinical form, patient consent.
Administration costs:	none
Minimal amount DNA:	5 microgram
Contact person:	Jean Muller: jean.muller@chru-strasbourg.fr; jeanmuller@unistra.fr

Blue-cone monochromacy, X-linked - *OPN1LW/OPN1MW* (Tuebingen, Susanne Kohl)

Coverage:	Deletions, structural rearrangement, point and splicing mutations in ***OPN1LW/OPN1MW***
Sensitivity:	>95%
Technology:	PCR, RFLP, Sanger sequencing
Turn-around-time:	2 - 6 months
Period:	1 January 2019 – 31 December 2021
Max. # of samples:	unlimited
Special requirement:	Well defined clinical diagnosis of congenital, non-progressive Blue cone monochromatism (not cone dystrophy!)
Administration costs:	none
Minimal amount DNA:	2 microgram (high molecular genomic DNA)
Contact person:	Susanne Kohl: susanne.kohl@uni-tuebingen.de

Congenital stationary night blindness (CSNB) - 12 genes, candidate genes, WES and WGS (Paris, Christina Zeitz)

Coverage:	Coding & splice site sequences of ***CACNA1F, CABP4, CANCA2D4, GNB3RHO, GNAT1, GRM6, GPR179, LRIT3, NYX, PDE6B, TRPM1,*** **candidate genes.** WES and WGS on excluded cases if agreed.
Sensitivity:	>98% for coding regions
Technology:	Sanger sequencing
Turn-around-time:	2 - 6 months for known genes underlying CSNB
Period:	1 January 2019 – 31 December 2021
Max. # of samples:	unlimited
Special requirement:	Well defined clinical diagnosis of CSNB (Riggs, Schubert-Bornschein type sub-classified in complete and incomplete CSNB), pedigree and as much clinical data should be provided. In case of exclusion of known gene defects, C. Zeitz is allowed to screen samples with targeted whole genome, whole exome or whole genome sequencing. If it comes to a publication all partners should be co-authors.
Administration costs:	none
Minimal amount DNA:	5 microgram
Contact person:	Christina Zeitz: christina.zeitz@inserm.fr

Cornea plana - *KERA* (Prague, Petra Liskova)

Coverage:	Coding & splice site sequences of ***KERA***
Sensitivity:	100% for coding regions
Technology:	Sanger sequencing
Turn-around-time:	2 - 4 months
Period:	1 January 2019 – 31 December 2021
Max. # of samples:	200
Administration costs:	none
Minimal amount DNA:	2 microgram (100 ng/ul, 20 ul)
Contact person:	Lubica Dudakova: lubica.dudakova@lf1.cuni.cz

Fuchs endothelial corneal dystrophy (FECD) – *TCF4* (London, Alison E. Davidson)

Coverage:	CTG18.1 microsatellite situated within an intron of ***TCF4***
Sensitivity:	Expansion (≥50 CTG repeats) of a non-coding trinucleotide repeat in *TCF4* confers >76-fold risk for FECD and our functional studies suggest that >31 copies of the repeat are disease-associated (Zarouchlioti *et al.* 2018). The assays used offer high levels of sensitivity and will identify presence and/or absence (including zygosity status) of expansions for all samples; however sizing estimates will not be generated for repeats exceeding 120 copies.
Technology:	The trinucleotide repeat polymorphism (CTG18.1) will genotyped using a combination of short tandem repeat (STR) assay and triplet repeat (TP) primed PCR assay, when required.
Turn-around-time:	2 – 4 months
Period:	1 January 2019 – 31 December 2020
Max. # of samples:	1,000
Administration costs:	≤96 samples free. >96 samples £250 per plate (96 samples)
Minimal amount DNA:	0.5 (preferably 1) microgram
Contact person:	Miss Amanda Sadan: amanda.sadan.18@ucl.ac.uk

Leber congenital amaurosis, non-syndromic; syndromic diseases with early-onset and severe retinal dystrophy as the initial symptom, differential diagnoses (Paris, Jean-Michel Rozet)

Coverage: coding & splice site sequences of 70 genes:

AHI1	*CACNA1F*	*CLUAP1*	*GUCY2D*	*LRIT3*	*PDE6H*	*SPATA7*
AIPL1	*CC2D2A*	*CNGA3*	*IFT140*	*MERTK*	*POC1B*	*TMEM138*
ALMS1	*CCT2*	*CNGB3*	*IMPDH1*	*NMNAT1*	*PRPH2*	*TMEM216*
ARL13B	*CEP104*	*CRB1*	*INPP5E*	*NPHP1*	*RD3*	*TMEM237*
ATF6	*CEP164*	*CRX*	*INVS*	*NPHP3*	*RDH12*	*TRPM1*
C21ORF2	*CEP290*	*CSPP1*	*IQCB1*	*NPHP4*	*RPE65*	*TTC8*
C2ORF71	*CEP290*	*GNAT1*	*KCNJ13*	*NYX*	*RPGRIP1*	*TULP1*
C5ORF42	*CEP41*	*GNAT2*	*KIF7*	*OTX2*	*RPGRIP1L*	*VPS13B*
C8ORF37	*CLN1/PPT1*	*GPR179*	*LCA5*	*PDE6C*	*SDCCAG8*	*WDR19*
CABP4	*CLN3*	*GRM6*	*LRAT*	*PDE6G*	*SLC24A1*	*ZNF423*

Sensitivity:	>98% for coding regions
Technology:	Illumina HiSeq2500 HT (Paired-End sequencing 130x130 bases)
Turn-around-time:	3 - 6 months
Period:	1 march 2019 – 31 December 2021
Max. # of samples:	250 (extendable)
Special requirement:	Diagnosis of LCA unambiguous or highly plausible (copy of all available ophthalmological data required). Affected individual + parental DNA or informative relatives.
Administration costs:	none
Minimal amount DNA:	2 micrograms
Contact person:	Jean-Michel Rozet: jean-michel.rozet@inserm.fr

North Carolina macular dystrophy (Ghent; Elfride De Baere)

Coverage:	Coding and non-coding sequences ***PRDM13*** and ***IRX1***. Complete genome
Sensitivity:	>98% for the coding regions of *PRDM13* and *IRX1*
Technology:	Targeted sequencing and copy number analysis of the reported mutated regions of *PRDM13* and *IRX1*, whole genome sequencing
Turn-around-time:	12 months
Period:	1 January 2019 – 31 December 2021
Max. # of samples:	50 (index cases)
Special requirement:	Informed consent
Administration costs:	None
Minimal amount DNA:	5 microgram
Contact persons:	Ir. Stijn Van De Sompele: kristof.vanschil@ugent.be or Prof. Elfride De Baere: elfride.debaere@ugent.be

Posterior polymorphous corneal dystrophy PPCD1, PPCD4 – *OVOL2, GRHL2* (Prague, Petra Liskova)

Coverage:	promoter region of ***OVOL2*** and ***GRHL2***
Sensitivity:	100% for promotor region
Technology:	Sanger sequencing
Turn-around-time:	2 - 4 months
Period:	1 January 2019 – 31 December 2021
Max. # of samples:	1000
Administration costs:	none
Minimal amount DNA:	2 micrograms (100 ng/ul, 20 ul)
Contact person:	Lubica Dudakova: lubica.dudakova@lf1.cuni.cz

X-linked retinitis pigmentosa - *RPGR-ORF15* (Paris, Christina Zeitz and Isabelle Audo)

Coverage:	***ORF15* of *RPGR***
Sensitivity:	>98% for coding regions
Technology:	Sanger sequencing
Turn-around-time:	2 - 6 months
Period:	1 January 2019 – 31 December 2021
Max. # of samples:	unlimited
Special requirement:	Well defined clinical diagnosis of X-linked RP. Only male cases can be screened. If it comes to a publication all partners should be co-authors.
Administration costs:	none
Minimal amount DNA:	2 microgram
Contact persons:	Christina Zeitz: christina.zeitz@inserm.fr; Isabelle Audo: isabelle.audo@inserm.fr

Retinitis pigmentosa (sporadic or autosomal recessive) with one causal variant in *USH2A* after WES or Sanger sequencing of all *USH2A* exons (Nijmegen; Susanne Roosing)

Coverage:	**Complete genome**
Sensitivity:	>95% for *USH2A*
Technology:	Whole genome sequencing
Turn-around-time:	6 - 12 months
Period:	1 January 2019 – 31 December 2020
Max. # of samples:	50
Special requirement:	Informed consent for genomic sequencing
Administration costs:	none
Minimal amount DNA:	5 microgram
Contact person:	Dr. Susanne Roosing: susanne.roosing@radboudumc.nl or Prof. Frans Cremers: frans.cremers@radboudumc.nl

Retinitis pigmentosa (sporadic or autosomal recessive) with one causal variant in *USH2A* after WES or targeted sequencing of all *USH2A* exons (Ghent; Elfride De Baere)

Coverage:	Coding and non-coding sequences ***USH2A***. Complete genome
Sensitivity:	>98% for *USH2A*
Technology:	Targeted NGS of *USH2A* (coding region), MLPA *USH2A*, whole genome sequencing
Turn-around-time:	12 months
Period:	1 January 2019 – 31 December 2020
Max. # of samples:	35
Special requirement:	Informed consent
Administration costs:	none
Minimal amount DNA:	5 microgram
Contact persons:	Dr. Kristof Van Schil: kristof.vanschil@ugent.be or Prof. Elfride De Baere: elfride.debaere@ugent.be

Stargardt disease & ar cone-rod dystrophy - *ABCA4* (Nijmegen; Frans P.M. Cremers)

Coverage:	Coding and non-coding sequences ***ABCA4*** Coding sequences ***PRPH2***
Sensitivity:	>98% for coding and non-coding sequences of *ABCA4*; 100% of coding sequences of *PRPH2*
Technology:	Single molecule Molecular Inversion Probes and NextSeq 500
Turn-around-time:	6 months
Period:	1 January 2019 – 31 December 2021
Max. # of samples:	1000
Administration costs:	none
Minimal amount DNA:	5 microgram
Contact persons:	Prof. Frans Cremers: frans.cremers@radboudumc.nl

Stargardt disease with one causal variant in *ABCA4* after targeted sequencing of all *ABCA4* exons (Ghent; Elfride De Baere)

Coverage:	Coding and non-coding sequences ***ABCA4***
Sensitivity:	>98% for coding and non-coding sequences of *ABCA4*
Technology:	HaloPlex (or alternative)
Turn-around-time:	12 months
Period:	1 January 2019 – 31 December 2021
Max. # of samples:	Not determined
Special requirement:	Informed consent
Administration costs:	none
Minimal amount DNA:	5 microgram
Contact person:	Dr. Miriam Bauwens Miriam.bauwens@ugent.be and Prof. Elfride De Baere elfride.debaere@ugent.be

Usher syndrome with one causal variant in *USH2A* after WES or Sanger sequencing of all *USH2A* exons (Nijmegen; Hannie Kremer)

Coverage:	**Complete genome**
Sensitivity:	>95% for ***USH2A***
Technology:	Whole genome sequencing
Turn-around-time:	6 - 12 months
Period:	1 January 2019 – 31 December 2020
Max. # of samples:	50
Special requirement:	Informed consent for genomic sequencing
Administration costs:	none
Minimal amount DNA:	5 microgram
Contact person:	Prof. Hannie Kremer: hannie.kremer@radboudumc.nl or Dr. Susanne Roosing: susanne.roosing@radboudumc.nl

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Usher syndrome type 2 (Nijmegen; Hannie Kremer)

Coverage:	***USH2A*** exons and flanking splice sites
Sensitivity:	>95%
Technology:	Molecular inversion probes - NexSeq500
Turn-around-time:	6 - 12 months
Period:	1 January 2019 – 31 December 2020
Max. # of samples:	400
Special requirement:	Unsolved cases after MIPs analysis can proceed to whole genome sequencing if consent of the patient is obtained
Administration costs:	none
Minimal amount DNA:	5 microgram
Contact person:	Ms. Janine Reurink: janine.reurink@radboudumc.nl or Prof. dr. Hannie Kremer: hannie.kremer@radboudumc.nl