

DNA testing services

DNA testing services	Positions	Genes	List of Genes	DNA required
1. Thalassemia testing	76	2	beta-globin gene, delta-globin gene	550 ng
2. Hereditary hearing loss testing	198	6	Connexin 26, Connexin 30, Connexin 31, prestin, pendrin, mitochondrial DNA	1,75 µg
3. DNA repair testing	100	58	ATM, BRCA1, BRCA2, CDKN1a, CDKN2A, etc.	5,5 µg
4. Cystic fibrosis testing	254	1	CFTR	1,75 µg
5. Ashkenazi Jewish diseases testing*	77	22	HEX A, BLM, ASPA, SMPD1, IKBKAP, DYT1, MEFV, MCOLN1, FANCC, F11, G6PC, BCKHDB, GJB2, DLD, GDE, SERPINA1, NEB, PCDH15, ABCC8, GBA, CFTR	3 µg
6. Wilson Disease testing	104	1	ATP7B	2 µg
7. Hereditary Breast and Ovarian Cancer testing	88	6	BRCA1, BRCA2, CHEK2, RAD51, NBN, CASP8	3 µg

* For exact list of diseases please contact info@asperbio.com.

HOW TO USE THE DNA TESTING SERVICES AT ASPER

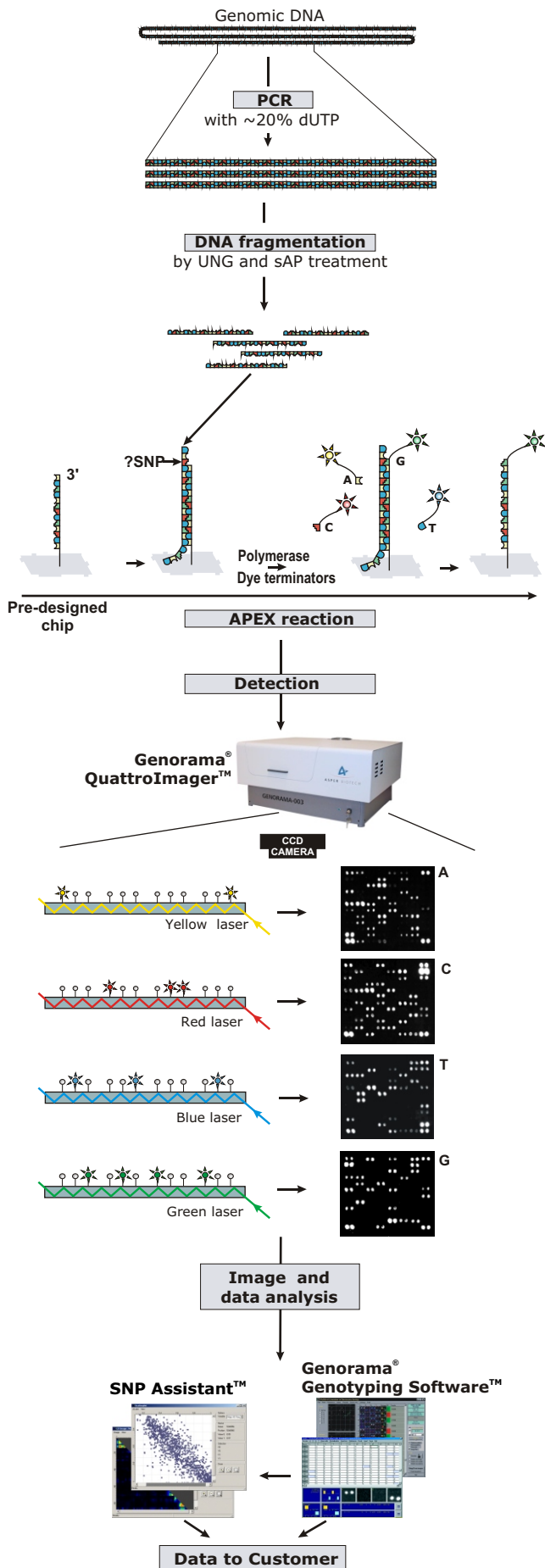
1. Please contact your Asper sales specialist for current pricing information and mutation lists.
2. Prepare the required amount of high-quality DNA.
3. Fill in the DNA sample submission form (downloadable at our homepage) and enclose it in the shipment.
4. Enclose a list of samples included in the package along with information about the DNA concentrations and volumes.
5. Please follow our detailed instructions for secure sample shipment available at our homepage.
6. For speedy and secure delivery use courier services (DHL, UPS, FedEx, etc); alternatively, you can send the samples as a small postal parcel.
7. Notify your Asper sales specialist by email or contact info@asperophthalmics.com or info@asperbio.com.
8. Asper notifies you of the arrival of the samples immediately.
9. Asper delivers the results in an electronic format to the address specified on the sample submission form. A hard copy of the results can be sent upon request for an additional fee.
10. Result confirmation with secondary method can be provided by Asper using verification of the APEX findings by dideoxy sequencing.

REQUIREMENTS FOR SUBMITTING BLOOD SAMPLES

1. Collect 2-4 ml of whole blood to a lavender-cap vacutainer (EDTA)K3. Do not use heparin as an anticoagulant. Make sure the anticoagulant and blood are mixed - do not shake!
2. Blood samples can be preserved at 2-8C, but for no longer than one week. Do not freeze the blood!
3. Labelling on the blood sample and on the submission form should be identical.
4. Blood samples must be packed so that tubes do not get damaged during shipment.
5. For special requirements for sending Guthrie cards and saliva samples contact your Asper sales specialist.

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1. Stargardt disease, cone-rod dystrophy testing	519	1	ABCA4	3 µg
2. Leber congenital amaurosis testing	495	12	AIPL1, CRB1, CRX, GUCY2D, LRAT, TULP1, MERTK, RPE65, RPGRIP1, CEP290, RDH12, LCA5	5 µg
3. Usher syndrome testing	614	9	CDH23, MYO7A, PCDH15, Harmonin, SANS, Usherin, VLGR1, USH3A, Whirlin	6 µg
4. Bardet Biedl syndrome testing	308	14	BBS1, BBS2, BBS3, BBS4, BBS5, BBS6, BBS7, BBS8, BBS9, BBS10, BBS12, PHF6, ALMS1, GNAS1	4 µg
5. Autosomal recessive retinitis pigmentosa testing	585	18	CERKL, CNGA1, CNGB1, MERTK, PDE6A, PDE6B, PNR, RDH12, RGR, RLBP1, SAG, TULP1, CRB, RPE65, USH2A, USH3A, LRAT, PROM1	6 µg
6. Autosomal dominant retinitis pigmentosa testing	370	15	Ca4, FSCN2, IMPDH1, NRL, PRPF3, PRPF31, PRPF8, RDS, RHO, ROM1, RP1, RP9, CRX, TOPORS, PNR	2,5 µg
7. Autosomal dominant optic atrophy testing	118	1	OPA-1	1,5 µg
8. Corneal dystrophy testing	325	13	COL8A2, TGFBI, VSX1, CHST6, KRT3, KRT12, GSN, TACSTD2, CYP4V2, SOD1, TCF8/ZEB1, SLC4A11, UBIAD1	3,5 µg
9. Congenital stationary night blindness testing	126	9	RHO, PDE6B, GNAT1, CABP4, GRM6, SAG, NYX, CACNA1F, CACNA2D	2,5 µg
10. Vitelliform macular dystrophy testing	138	1	BEST1	1µg



PCR and DNA FRAGMENTATION

Polymerase chain reaction (PCR) is used to amplify regions of genomic DNA which harbor mutations or polymorphisms (SNPs) of interest. PCR primers are selected with the help of Genorama™ Chip Design Software.

A fraction of the dTTP-s is replaced by dUTPs in the PCR mix allowing for later fragmentation with Uracil N-Glycosylase (UNG). The products are pooled, concentrated and purified enzymatically from unincorporated dNTP-s using shrimp Alkaline Phosphatase (sAP). After sAP and UNG treatment the samples are heated to deactivate the enzymes and to cleave the DNA at uracil sites.

Fragmentation of the long PCR products facilitates proper hybridization to the complementary oligonucleotides immobilized onto glass.

APEX REACTION

Imminently before APEX reaction, the fragmented PCR products are denatured and transferred to the previously designed chip (oligo array on glass) in a reaction mixture. The buffered reaction mixture contains single-stranded DNA, thermostable DNA Polymerase and four different terminator nucleotides each tagged with an individual fluorophore.

The template-dependent DNA polymerase reaction is carried out at high temperature to minimize the undesirable secondary structures of oligos, yet still permit efficient hybridization of target DNA to oligos and not to compromise the polymerase activity. After incubation, the unattached and non-covalently bound material is washed away, leading to an excellent signal to noise ratio.

In the oligo array each spot comprises of high copy number of the same immobilized synthetic oligonucleotides to determine one specific nucleotide on genomic DNA. The oligos are selected using Genorama® Chip Design Software and printed to the slide. It is important that the oligonucleotides are immobilized to the coated slides surface via an amino linker their 5' ends, leaving their 3' ends of primers free for the single base primer extension reaction.

DETECTION

The processed slides harbouring an APEX array are imaged in our microarray detector (patent pending) - Genorama® QuattroImager™.

Four lasers (one at a time) are used to excite the different dyes. The four spectrally well separated dyes are excited via total internal reflection of the laser beam in the glass slide, which acts as a light guide. The light emitted by fluorophores in response to excitation is captured by a CCD camera.

Since four different dyes per reaction are used, four different images of emitted light per array will be recorded. Each image corresponds to one individual dye, hence to the pattern of the incorporation of one of the four terminator nucleotides on the array.

IMAGE and DATA ANALYSIS

Imaging is followed by analysis with Genorama® Genotyping Software™ to convert the fluorescence information into sequence data. The signal intensities of respective dye terminator images are first normalized. The intensities of four images at all the oligonucleotide positions are compared and the strongest signal is base called. Image analysis includes quality control steps.

In case of interest, Asper offers also haplotype block detection and SNP data analysis (SNP validation (call rate, allele frequencies, HWE), LD calculation and visualization, Case-control tests and TDT tests).