Ambry Genetics

Exome Sequencing Data Requisition Form

We offer Exome Sequencing Data, a sequencing-only option for clinicians and scientists looking to capture and sequence the coding regions in the genome, without analysis or interpretation. Whole exome sequencing is performed in our clinical laboratory under CAP- and CLIA-approved processes. This option does not include data analysis or clinical reporting.

EXOME SEQUENCING DATA INCLUDES WHOLE EXOME SEQUENCING AND TWO OPTIONS FOR THE TYPE OF DATA THAT IS PROVIDED:

OPTION	SAMPLE TYPE	TEST CODE	TAT**
1. Exome sequencing and raw data (Fastq, BAM and/or VCF)	DNA or whole blood	9997	4-6 weeks
2. Exome sequencing, filtered variant list, and raw data*	DNA or whole blood	9998	4-6 weeks

* Filtered variant list provided in Microsoft Excel format
** 4-6 week TAT is for projects with 10 samples or fewer

EXOME SEQUENCING ASSAY INFORMATION

Coverage and analytical range: >95% of the bases are expected to have quality scores of Q20 or higher, which translates to an expected base-calling error rate of 1:100, or an expected base-calling accuracy of 99%. >95% of the exome is covered at 20x or higher under current run conditions, sufficient for high quality heterozygous and homozygous variant calling for germline variants. About 92% of characterized Mendelian disease genes are fully covered (100%) at > 20X. Ambry meets the ACMG suggested coverage conditions (Rehm HL, *et al.* Genet Med., 2013). For any given individual ~ 5% of the targeted exome is not sequenced well enough to make a confident call. Each individual may have slightly different coverage yield distributions within the exome.

Methodology: Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's whole blood. Samples are prepared using the IDT hybridization kit. Each DNA sample is sheared, adaptor ligated, PCR-amplified and incubated with the exome baits. Captured DNA is eluted and PCR amplified. Final quantified libraries are seeded onto an Illumina flow cell and sequenced using paired-end, 150 cycle chemistry on the Illumina HiSeq or NextSeq. Initial data processing, base calling, alignments and variant calls are generated by various bioinformatics tools.

Variant filtered lists: Data is annotated with the Ambry Variant Analyzer tool (AVA), including: nucleotide and amino acid conservation, biochemical nature of amino acid substitutions, population frequency, and predicted functional impact. Data analysis is focused on small insertions and deletions, canonical splice site alterations, and non-synonymous alterations. The following sites are used to search for previously described gene mutations and polymorphisms: the Human Gene Mutation Database (HGMD), the Single Nucleotide Polymorphism database (dbSNP), 1000 genomes, HapMap data and online search engines (*e.g.*, PubMed). Variants are then filtered further based on family history and possible inheritance models. In most cases, phase cannot be determined. Exons plus at least 2 bases into the 5′ and 3′ ends of all the introns are analyzed and reported.

Affix blue colored bar code label to TRF below. Affix matching numbered red colored bar code label to specimen. Please do not apply barcode sticker to small DNA vials.

bar code sticker 1 | bar code sticker 2 | bar code sticker 3 | Ambry use only

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Exome Sequencing Data Submission Requirements

Please note that the quality of the specimen received is the primary indicator of the quality of data results you can expect to receive and primary cause of delays in TAT. Please send samples that meet our specimen requirements.

SUBMISSION REQUIREMENTS FOR UNMODIFIED DNA SAMPLES (*I.E.* NO ADAPTERS LIGATED TO DNA)

	DNA QUANTITY	QC SPECS
IDT	≥ 5 µg of genomic DNA in 100-200ul TE (or Qiagen EB)[a]	Qubit fluorometer

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PLEASE INDICATE TEST OPTION:

☐ Exome sequencing and raw data

☐ Exome sequencing, filtered variant list, and raw data

SPECIMEN INFORMATION

	SPECIMEN ID OR PATIENT NAME	DATE OF BIRTH	COLLECTION DATE	FAMILY ID (IF APPLICABLE)	RELATIONSHIP (IF APPLICABLE)	FOR DNA: OD (260/280)
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Please contact the laboratory at CDE@ambrygen.com for projects with more than 10 samples for price and TAT

PLEASE DESCRIBE DETAILS ABOUT THE FAMILY RELATIONSHIPS:

SAMPLE SOURCE:	WHAT METHOD WAS USED FOR EXTRACTION?	COMMENTS:

PLEASE CHECK THE PREFERRED FILE TYPES (DOWNLOADABLE FROM FTP SERVER):

☐ fastq files

☐ BAM files

☐ VCF files

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CONTACT INFORMATION

PURCHASE ORDER NUMBER
Authorizing Personnel (Name)
Phone
Fax
Facility Name and Address (where results should be sent)
Form Completed By
Phone

BILLING INFORMATION

AMBRY QUOTE NUMBER*	
☐ Bill Facility ☐ same as ordering organization	
Accounts Payable Name and Address	
Phone	Fax
☐ Pre-Payment	
Payment Type (click one)	
☐ Check ☐ Mastercard ☐ Discover ☐ Visa ☐ American Express	
Card Number	Expiration Date
Cardholder Name	Amount $
Signature	Date
X	

*The quote number only applies for projects with 10 samples or more

SHIPPING INSTRUCTIONS

Please overnight ship DNA on ice to:
Ambry Genetics
ATTN: Clinical Genomics
15 Argonaut
Aliso Viejo, CA 92656 USA