grcf

Genetic Resources Core Facility
Research Services Catalog
2014
grcf.jhmi.edu

- **bioprocessing**
- **biorepository**
- **bioshipping**
- **cell line testing**
- **digital PCR**
- **dna isolation & handling**
- **genotyping**
- **methylation**
- **products**
- **rna services**
- **sequencing**

GRCF Mission

To provide high quality, cost effective research services and products to investigators throughout the Johns Hopkins Scientific Community.

JOHNS HOPKINS
SCHOOL *of* MEDICINE

To view the Core Store Catalog flip this document.

The Genetic Resources Core Facility

Research Clinic

Patient Samples
- Blood Processing
- Cell line expansion and QC
- Liquid Nitrogen Repository

DNA Processing
- DNA isolation from cells or blood
- Storage • Plating • QC

Genotyping
- Linkage and Whole Genome Arrays
- Custom SNP Panels
- Methylation Arrays

Next Generation Sequencing
- Whole exome and custom capture panels for targeted sequencing.
- RNA Seq & Chip Seq
- Whole Genome Sequencing
- Interpretation

Validation
- Gene-specific PCR
- Taqman genotyping
- Sanger sequencing
- Pyrosequencing

Defining research
one base pair at a time.

For more information visit: grcf.jhmi.edu

grcf
genetic resources core facility

1989-2014

Proudly serving the
Johns Hopkins Research
Community for 25 years.

Dear Researchers,

2014 marks the 25th Anniversary of the Genetic Resources Core Facility. We would like to take this opportunity to thank our customers for the privilege of serving you. We value your business and look forward to serving you in the future. We would also like to invite you to join in our 25th Anniversary Celebration during the 2014 GRCF Core Symposium, April 29th, Turner Concourse, 10 am – 2 pm. Symposium details will be posted as they are finalized on our website, http://grcf.jhmi.edu.

The Genetic Resources Core Facility (GRCF) is a Johns Hopkins service center providing research expertise, products, and services for the study of the human genome. At the leading edge of technology, the GRCF provides sophisticated tools and equipment oftentimes not available in the individual research lab.

The GRCF includes the Core Store and several research services that have been conveniently organized into those related to blood processing & live cells (Biorepository & Cell Center) and to DNA services. These units work together to streamline services such as sample collection, cell line establishment, cell line authentication, cryogenic storage, DNA and RNA isolation, oligo and gene synthesis, methylation testing, both Sanger and next-gen sequencing as well as genotyping from handfuls to millions of variants. The GRCF can also help with project design, grant language, and custom assay development for DNA based studies.

We encourage investigators planning a study to meet with us to discuss your objectives. In many instances we can propose a range of possible approaches and may be able to point you to particular funding opportunities. We are also happy to write letters of support for grant applications that can demonstrate to reviewers that the resources for your study are available at the university.

Finally, thank you for your support of the GRCF. As you may know, we do not receive institutional funding and depend on you, our colleagues, for the support that keeps our services available. We welcome your suggestions for improvements and look forward to being an asset to the Hopkins community for many years to come.

Sincerely,

The Faculty and Staff of the
Genetic Resources Core Facility

THE GENETIC RESOURCES CORE FACILITY

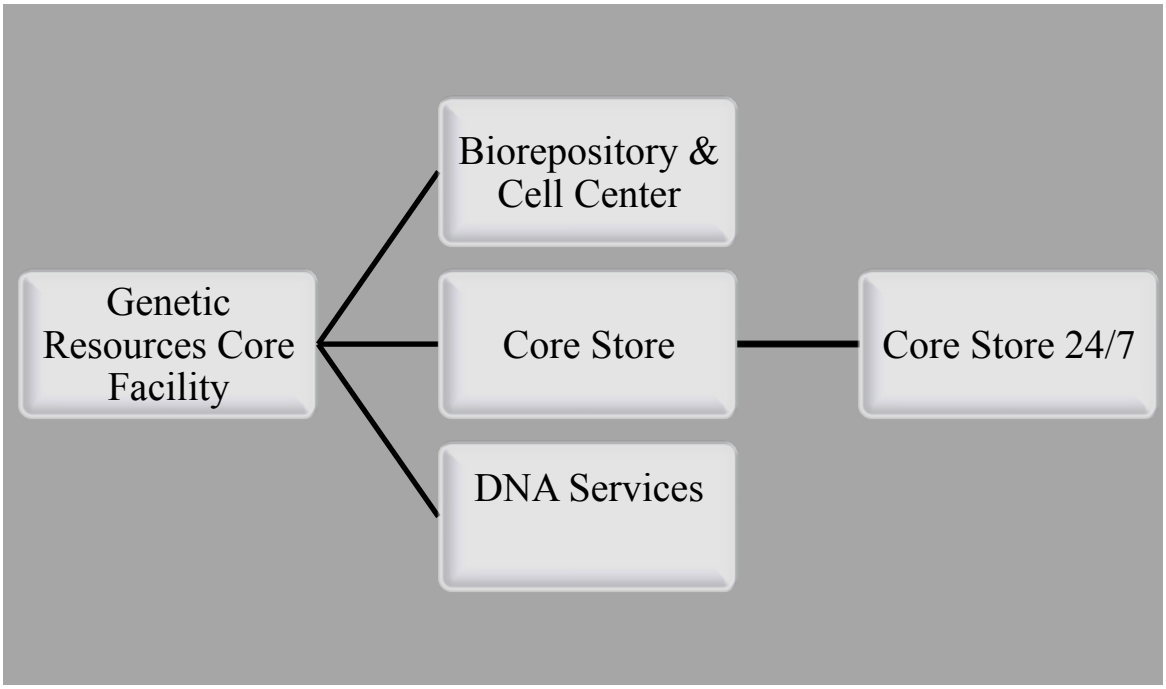
The Genetic Resources Core Facility is a JHU service center including the Core Store, Biorepository & Cell Center and the DNA Services. Collectively, these groups produce a number of products and services to aid researchers performing studies in molecular biology and genetics. It is our mission to provide high quality, cost effective research services and products to investigators throughout the Johns Hopkins Scientific Community.

Biorepository & Cell Center facilitates basic scientific research by providing expertise and service in all mammalian cell culture and long-term cryogenic storage of biospecimens. In 2013 the GRCF Biorepository & Cell Center proudly received the international recognition of CAP (the College of American Pathologist) Accreditation. This voluntary program demands that a laboratory go well beyond acceptable quality and regulatory compliance measures to help achieve the highest standards of excellence in our services. For more information go to http://grcf.jhmi.edu/biorepository-cell-center/

Core Store provides one-stop shopping for more than 150,000 products from 16 of the leading life science companies. In addition to its product offering the store charges no shipping and handling fees and has free delivery to three campuses East Baltimore, Bayview and Homewood. There is also convenient 24/7 access to several hundred products via the Core Store 24/7 at these locations Blalock 1026, CRB 1 B02A and the Asthma and Allergy Building 1st floor. For more information go to http://grcf.jhmi.edu/core-store/

The DNA Services group works together to provide solutions for all of your DNA and RNA needs. We handle basic needs like DNA isolation, plating and storage, "traditional" core services like Sanger sequencing, PCR support and genotyping, and the more complex needs presented by the constantly changing field of next generation sequencing. For more information on these services please go to http://grcf.jhmi.edu

GRCF Organizational Chart



Cross reference table of product/service to the GRCF Division

Product	Core Research Service Division
Dry Ice	Biorepository & Cell Center
Gene Synthesis	DNA Services
Oligonucleotides	DNA Services
Modified Primers and Specialty Probes	DNA Services
QIAGEN GeneGlobe Assays	DNA Services
RNA Oligonucleotides and siRNAs	DNA Services
Taqman Assays	DNA Services
Biorepository	
Onsite Banking of Biospecimens	Biorepository & Cell Center
DNA Storage	Biorepository & Cell Center
Bioprocessing - Cell Lines	
Cell Line Profiling by STR Analysis (Cell Line Authentication)	DNA Services
Cell Line Extablishment (LCL)	
Mycoplasma Detection	DNA Services
Mycoplasma and STR Profiling Analysis	DNA Services
Cell Line Establishment and Cell Line Expansion	Biorepository & Cell Center
Mammalian Cell Line Expansion	Biorepository & Cell Center
Skin Biopsy Processing & Primary Fibroblast Line Establishment	Biorepository & Cell Center
Bioprocessing – Blood Processing	
Blood separation w/isolation, aliquoting, and cryopreservation of sera, plasma or whole blood	Biorepository & Cell Center
Blood separation w/isolation and cryopreservation of viable lymphocytes	Biorepository & Cell Center
Blood separation w/ isolation and transformation of viable lymphocytes (LCL establishment)	Biorepository & Cell Center
DNA & RNA Purification	DNA Services
Bioshipping	
Establishment of cell lines for distribution	Biorepository & Cell Center
Dry Ice	Biorepository & Cell Center
Shipping materials	Biorepository & Cell Center
DNA Handling	
Bisulfate Conversion	DNA Services
DNA Purification	DNA Services
Sample Plating	DNA Services
Whole Genome Amplification	DNA Services
Genotyping	
Specialized Content Products	DNA Services
Custom Genotyping	DNA Services
Genome-Wide Association	DNA Services
Methylation	
Analysis via Genotyping	DNA Services
Analysis via High Throughput Sequencing	DNA Services
Analysis via Pyrosequencing	DNA Services
RNA Services	
Digital PCR	DNA Services
RNA Oligonucleotides, si/shRNA	DNA Services
RNA Purification	DNA Services
Sequencing	
High Throughput Sequencing	DNA Services

Medium Throughput Sequencing	DNA Services
Whole Exome/Targeted Sequencing	DNA Services
Whole Genome Sequencing	DNA Services
Pyrosequencing	DNA Services
Sanger Sequencing	DNA Services

Research Service Divisions Quick Reference

Core Research Service Division	Location	E-Mail	Phone #
Biorepository & Cell Center	Blalock 1001A	biorepository@jhmi.edu bioprocessing@jhmi.edu bioshipping@jhmi.edu	410-614-5201
Core Store	Blalock 1026	jhucorestore@jhmi.edu	410-614-1647
Core Store 24/7	Blalock 1026, CRB I – B02A, Asthma & Allergy 1A.C4	hillia1@jhmi.edu	410-502-3959
DNA Services	Blalock 1004	customorders@jhmi.edu	410-955-2836

Notifications:

Core Research Products/Services are for research purposes only.

Pricing of products and services are subject to change. For the most current pricing please visit our website at http://grcf.jhmi.edu

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Bioprocessing

The GRCF Cell Center specializes in mammalian cell culture propagation, primary cell establishment, lymphocyte isolation and LCL establishment. Our center has processed more than 25,000 blood specimens and serves approximately 300 investigators both from Johns Hopkins as well as other institutions. The GRCF Cell Center continues to be a leader in the Epstein-Barr virus (EBV) lymphocyte transformation. EBV transformation success rates average 98% from frozen lymphocytes and 99% from fresh lymphocytes.

The GRCF Cell Center is open Monday through Thursday from 7:30 am to 4:30 pm and Friday 7:30 am to 4:00 pm and is located on-site at the Johns Hopkins East Baltimore campus for convenient specimen deposit or retrieval. Blood specimens delivered before 2 pm will be processed the same day. All pricing can be determined at: http://grcf.jhmi.edu/place-your-order/

The GRCF Cell Center is always looking to improve our services. If there are services you would like to see us offer in the future or any concerns or comments you have for our current services we invite you to contact Melissa Olson, Ph.D. (molson14@jhmi.edu).

Blood Processing

GRCF Biorepository & Cell Center can help you with your blood processing needs. We offer blood separation, aliquoting and / or cryopreservation of whole blood, lymphocytes, sera and plasma as well as LCL establishment.

Instructions for Blood Collection can be found at: http://grcf.jhmi.edu/biorepository-cell-center/bioprocessing/blood-processing/

Isolation and cryopreservation of viable lymphocytes from whole blood

Service includes sterile lymphocyte isolation by ficoll gradient, cell count, viability determination, cryopreservation of 1 ml aliquot whole blood, cryopreservation of 1-4 aliquots of isolated lymphocytes @ 5 x 10^6 cells, and electronic sample tracking. Cryopreserved lymphocytes are suitable for future transformation. Same-day service is available if whole blood is received by 2pm.

Establishment of cell lines from isolated lymphocytes or whole blood (LCLs)

Service includes B-cell transformation from whole blood isolated blood lymphocytes, culture expansion, and cryopreservation. Specifically includes: lymphocyte isolation by ficoll separation, cell count, viability determination, cryopreservation of whole blood aliquot (1 ml), 1-4 aliquots of lymphocytes @ 5 x 106 cells, and 4 aliquots of (transformed) lymphoblasts @ 10^6 cells and electronic sample tracking. Allow 8-10 weeks for completion

Establishment of cell lines from frozen lymphocytes

Service includes B-cell transformation from previously cryopreserved blood lymphocytes, culture expansion, and cryopreservation. Specifically includes: Thaw of cryopreserved lymphocytes, cell count, viability determination, 1-4 aliquots of freshly cryopreserved lymphocytes @ 5 x 10^6 cells, and 4 aliquots of (transformed) lymphoblasts @ 5 x 10^6 cells and electronic sample tracking. Allow 8-10 weeks for completion.

Isolation, aliquoting, and cryopreservation of sera and plasma

Service includes isolation and cryopreservation of sera and plasma aliquots (0.5-1ml) with electronic sample tracking. Cryopreserved sera and plasma proteins are intact for future analysis. Same-day service is available if whole blood is received by 2pm.

Aliquoting and cryopreservation of whole blood

Service includes the aliquoting fresh whole blood in 0.5-1ml volumes and cryopreserving.

Cell Culture

The GRCF Biorepository & Cell Center is able to help you with your cell culture needs. We are a mycoplasma-free facility offering primary fibroblast isolation and establishment, cell culture expansion, cryopreservation and cell line distribution.

Cell Expansion / Preparation of Cell Line Stocks

Cultured cells from your lab can be tested for mycoplasma, expanded, aliquoted, and cryopreserved in freezing media (5 x 10^6/vial).

Preparation of cells for DNA or RNA extraction

Eukaryotic cells from the your lab or from ATCC can be expanded and pelleted for nucleic acid isolation (1 x 10^8 cells). DNA or RNA extraction is available through the GRCF's DNA Services, http://grcf.jhmi.edu/dna-services/

Establishment of fibroblast cultures from Tissue Biopsies

Service includes fibroblast isolation and establishment in culture, cryopreservation of four (4) aliquots (5 x 10^6 cells per vial) and electronic sample tracking. Submit 5 X 2mm biopsy and allow 8-10 weeks for completion.

Establishment of cell lines from frozen lymphocytes

Service includes B-cell transformation from previously cryopreserved blood lymphocytes, culture expansion, and cryopreservation. Specifically includes: Thaw of cryopreserved lymphocytes, cell count, viability determination, 1-4 aliquots of freshly cryopreserved lymphocytes @ 5 x 10^6 cells, and 4 aliquots of (transformed) lymphoblasts @ 5 x 10^6 cells and electronic sample tracking. Allow 8-10 weeks for completion.

Cell Line Distribution

Service includes cryopreserved cell line stock and maintenance (5 x 10^6 cells per vial), arrangements and shipment (domestic or international) to collaborators of your choosing.

RNA Isolation

The GRCF offers RNA isolation from PAXgene blood collection tubes, tissues (fresh or RNAlater preserved) and cultured cells. RNA is suitable for many downstream applications including cDNA synthesis, qPCR, Northern blots and library construction.

Quantitative analysis is by Nanodrop.

Qualitative analysis is available by Advanced Analytical Fragment Analyzer CE analysis. The system gives results similar and comparable to an Agilent BioAnalyzer. Advanced Analytical's PROSize software determines a RQN (RNA Quality Number) on a scale from 1 to 10.

Please contact Laura Kasch at 410-614-3830 or lkasch@jhmi.edu for additional information and ordering instructions.

Pricing

Source	Sample Size	Yield	Charge (without CE analysis)
Blood	PAXgene collection tube	> 3µg (total RNA)	$25
Tissue	0.15mg-1g	up to 6mg RNA	$35
Tissue	20-250mg	up to 1mg RNA	$30
Tissue	<30mg	up to 100µg RNA	$25
Tissue	<5mg	up to 45µg RNA	$20
Cultured Cells	5 x 10E7 to 5 x 10E8 cells	up to 6mg	$30
Cultured Cells	5 x 10E6 to 1 x 10E8 cells	up to 1000µg	$25
Cultured Cells	< 1 x 10E7	up to 35µg	$20
Cultured Cells	<5 x 10E5 cells	up to 17.5µg	$15

Biorepository

The GRCF Biorepository at Johns Hopkins is dedicated to meeting all of your short and long-term cryostorage requirements. Located onsite at the Johns Hopkins East Baltimore campus, the GRCF Biorepository offers a range of frozen storage options for blood, sera, plasma, and viable mammalian cells. In 2013 the GRCF Biorepository was recognized as a CAP accredited facility, offering superior services in cryopreservation, cryostorage, bioshipping and bioprocessing. Further, the Biorepository & Cell Center is an active member and strictly adheres to guidelines set forth by ISBER best biorepository practices.

The GRCF Biorepository is open Monday through Thursday from 7:30 am to 4:30 pm and Friday 7:30 am to 4:00 pm for convenient deposit and retrieval of specimens. A four-hour advance notice is requested for timely sample retrieval. All pricing can be determined at: http://grcf.jhmi.edu/place-your-order/

The GRCF Biorepository at Johns Hopkins maintains secure and continually monitored storage for up to 1 million vials. Services are available for specimens requiring liquid nitrogen vapor phase temperatures (< -170°C) and standard, ultra-low (-80°C) temperatures. Barcoded vial labels are electronically tracked using a relational database compliant with both CAP regulations and 21CFR part 11 and GxP requirements. Temperatures and liquid nitrogen supply on all freezers is continually monitored by a Rees Scientific 24-7 data tracking and alarm system. GRCF Biorepository personnel are always on call to respond in the event of an emergency.

Biorepository Services

The GRCF Cell Center & Biorepository offers the following cryogenic storage services:

- Secure, cryovial storage in vapor phase liquid nitrogen (LN2) - 170 degrees C
- Secure, cryovial storage in subzero temperatures -80 degrees C
- Inventory Monitoring
- On-site cryogenic storage with rapid retrieval upon request
- Upon request, specimen disposal with certificate of discard

For questions please contact us at: biorepository@jhmi.edu

Safeguards

The GRCF Biorepository & Cell Center takes the following measures to ensure the safety of your specimens:

- Replicate aliquots are stored in separate freezers.
- Because the quality and viability of the specimen is directly related to the way in which temperature and alarms are monitored, we use continuously alarmed equipment to protect the integrity of the specimen itself.
- Every piece of storage equipment that houses specimens of the GRCF Cell Center & Biorepository has an internal alarm, an external alarm, and an in-person monitoring plan.
- Storage equipment is routinely validated for temperature consistency, quality monitoring and cold supply.
- Every label is equipped with both barcoded and human readable information. Label integrity is validated under extreme conditions.
- Disaster contingency plans are in place and routinely tested.

- Barcoded specimens and sample tracking are provided using the Freezerworks 4D relational database.
- Every box is equipped with an indicator that serves for positional orientation and frozen status.
- All samples are store in stainless steel boxes and identified with bar-coded labels made of a material that is impervious to mechanical trauma and liquid nitrogen.
- All inventory information is maintained in a computerized relational database.

Bioshipping

The GRCF Biorepository processes more than 500 domestic and international shipments per year for its customers and their collaborators. The service includes shipping arrangements, documentation, dry ice, shipping boxes, contact with courier for pick-up and telephone or e-mail follow-up with recipient upon shipping.

All GRCF Biorepository personnel have been trained in Department of Transportation (DOT) hazardous materials shipping regulations. Investigators are responsible for preparing material transfer agreements (MTA) through the Johns Hopkins Office of Licensing and Technology. The majority of our shipments are sent via FedEx Priority cryopreserved on dry ice.

Pricing

Package Description	Package Destination	Package Dimension (inches)	Dry Ice Weight (pounds)	Total Cost*
Small	Domestic (USA), Canada, Mexico	11x9x7	6	$30.00
Medium	Europe	14x14x14	20	$70.00
Large	Asia, Africa, South America, Australia	24x16x16	60	$160.00
Cryoport	Upon Request	12x12x24	20(LN2)	$800.00

**Cost based on the sale of dry ice at $2.50/lbs., insulated cooler and cardboard box and does not include courier charges.*

The Bioshipping courier of choice is FedEx. If you prefer a different courier, please let us know and we will be happy to work with you to get your biospecimens safely to their destination.

Dry Ice

Is available at Blalock 1017, Monday – Thursday, 7:30 am – 4:30 pm and Fridays 7:30 am - 4:00 pm

- 1/2″ diameter dry ice pellets
- Used primarily for shipping and transport of temperature-sensitive cells and reagents
- Sold in 1-pound quantities or more
- Bring your own container

Dry ice purchase forms are available when you pick up your dry ice or you can download a dry ice purchase form at http://grcf.jhmi.edu/products/dry-ice

For more information please contact us at: bioshipping@jhmi.edu

Cell Line Testing

Cell Line Authentication

In light of the NIH's recommendation that all cell lines be authenticated before publication, the GRCF's DNA Services offers a short tandem repeat (STR) profiling service to customers who wish to confirm the identity of a line by comparison to a known profile or to establish an identity profile for a cell line generated locally.

General recommendations are to authenticate when a new line is established or acquired to determine an identity for the cell line. Cells should be authenticated again before freezing, once every two months that the culture is actively growing, if the performance of the line is not consistent or results are unexpected, and before publication. If using more than one cell line in the lab, all lines should be initially tested to rule out cross contamination.

The GRCF is currently offering two levels of cell line authentication service, Standard Service or Standard Service plus Profile Search. Standard Service includes a signed cell line authentication report for each line submitted that is suitable for proof of cell line authentication which may be required by journals and grant funding bodies, an STR profile table with allele designations for comparison to reference databases, an electropherogram profile, and a table with the raw data for the alleles (size in base pairs, peak height, peak area). Standard Service is suitable for scientists who prefer to do their own profile comparison analysis. The results can be used to check for cross contamination, mix-ups and percent match between lines or to establish the identity of a new cell line. Standard Service plus Profile Search includes the above, plus cross referencing against repositories ATCC, DSMZ, and JCRB databases to check the authenticity of your cell line.

The GRCF is currently offering analysis using Promega's GenePrint 10 System, PowerPlex 16HS, and PowerPlex 18D kits. The kits are only suitable for cell lines of human origin. For a list of STR markers included in the kits please see http://grcf.jhmi.edu/biorepository-cell-center/bioprocessing/cell-line-authentication/.

Ordering

Please place your order through our secure online ordering system at http://jhu.genesifter.net.

Delivery of Samples

JH Customers: Drop samples off in Blalock 1004 with the GeneSifter order number, Monday – Friday from 9 AM – 4:00 PM.

External Customers: Send the samples with the GeneSifter order number to:

Laura Kasch
Fragment Analysis Facility
Johns Hopkins University
2760 Lighthouse Point East, Suite 201
Baltimore, MD 21224
Phone (443) 287-7948

Email the courier tracking number and GeneSifter order number to FAF@jhmi.edu. Samples must be received by 11AM Tuesday for inclusion in our weekly processing schedule (see turnaround time).

Sample preparation

The FAF accepts purified DNA (please provide a minimum of 100ng of DNA), cell pellets (dried or frozen, between 100,000 and 5 million, preferred) or frozen cell aliquots (Cryopreservation tube) for analysis. A DNA isolation charge applies when submitting cell pellets or frozen cell aliquots. We can also extract DNA from other sources – please inquire.

What you will receive

Standard Service: You will receive a signed cell line authentication report that includes a STR profile table of your cell line with data suitable for comparison to reference databases, an electropherogram (graphic profile), and a table with the raw data for the alleles (size in base pairs, peak height, peak area). The report is suitable for proof of cell line authentication that may be required by journals and grant funding bodies.

Standard Service Plus Profile Search: You will receive a signed cell line authentication report that includes a STR profile table of your cell line with data suitable for comparison to reference databases, an electropherogram (graphic profile), and a table with the raw data for the alleles (size in base pairs, peak height, peak area) plus a database search of repositories ATCC, DSMZ, and JCRB for a known cell line reference profile for comparison.

Turnaround time

Samples submitted for STR profiling are run once a week on Tuesday, with results emailed usually on Monday of the following week. Johns Hopkins customers should drop off samples in Blalock 1004 by 4PM on Monday or hand deliver to the FAF laboratory in Canton by 11AM Tuesday for inclusion in that week's processing schedule. External customers should ship samples to the lab to be received by 11AM Tuesday for inclusion in that week's processing schedule. Samples received after 11AM Tuesday through Friday of any given week will be held and processed the following week.

Pricing

DNA isolation (required for submission of cell pellets): $25 per sample

Standard Service

- GenePrint 10: $85/sample
- PowerPlex 16HS: $125/sample
- PowerPlex 18D: $140/sample

Standard Service Plus Profile Search

- GenePrint 10: $127/sample
- PowerPlex 16HS: $169/sample
- PowerPlex 18D: $182/sample

Mycoplasma and Cell Line Authentication (STR profiling) from the same cell pellet

- GenePrint 10: $131/sample (Standard Service)
- GenePrint 10: $173/sample (Standard Service plus Profile Search)
- PowerPlex 16HS: $171/sample (Standard Service)
- PowerPlex 16HS: $213/sample (Standard Service plus Profile Search)
- PowerPlex 18D: $186/sample (Standard Service)
- PowerPlex 18D: $228/sample (Standard Service plus Profile Search)

Payment

John Hopkins investigators: IO number or credit card (VISA or MasterCard)External U.S. customers: P.O. or credit card (VISA or MasterCard)External foreign customers: P.O., check or credit card (VISA or MasterCard)

*Listed pricing is for Johns Hopkins investigators; all other customers will have a 16% administrative fee added. International customers using a credit card will have an additional $25 processing fee added in addition to the administrative fee.

Mycoplasma Detection

Tissue cell cultures can become contaminated by mycoplasma species without you knowing, which can be a serious problem. Mycoplasma can cause alterations in cell growth and metabolism, or induce morphological changes or chromosomal abnormalities. Potential sources of contamination are laboratory personnel, contaminated products used for cell culture, or exposure to contaminated cultures.

The FAF offers mycoplasma testing using a PCR based MycoDtect kit from Greiner Bio-One. A fluorescent primer pair is used to amplify the conserved 16S – 23S intergenic spacer region of ribosomal RNA. Amplified fragments are hybridized to a MycoDtect DNA-array for detection of all mycoplasma species using a universal probe while simultaneously identifying nine of the most common mycoplasma species with species-specific probes. Species identification can help to identify and treat the source of contamination.

Screening for mycoplasma contamination is recommended on a regular basis, and before cell banking and cryopreservation.

Ordering

Please place your order through our secure online ordering system at http://jhu.genesifter.net.

Delivery of samples

JH Customers: Drop samples off in Blalock 1004 with the GeneSifter order number, Monday – Friday from 9 AM – 4:00 PM.

External Customers: Send the samples with the GeneSifter order number to:

Laura Kasch
Fragment Analysis Facility
Johns Hopkins University
2760 Lighthouse Point East, Suite 201
Baltimore, MD 21224
Phone (443) 287-7948

Samples should be sent for delivery Monday through Friday only.

Sample Preparation

Samples may be submitted from growing culture or as frozen ampoules. We accept pelleted cells, supernatant or frozen stocks. The FAF will accept BSL-1 and BSL-2 samples for testing. If submitting samples for mycoplasma testing and cell line authentication follow the below growing culture cell pellet instructions.

Instructions for growing cultures: Passage cells in antibiotic-free media twice. Do not change the medium or dilute with fresh medium during the last 72 hours before testing. Cells should be 50-80% confluent.

Note: Samples grown in antibiotics or given fresh medium prior to testing may have artificially low mycoplasma levels if present or may give a false negative result.

Cell Pellets: Remove all but approximately 5 to 7 ml of medium in a T25 flask or 8 to 10ml in a T75 flask. Use a cell scraper to dissociated cells from the substrate. Transfer the cell suspension to a 15 ml conical tube for submission. Centrifuge to pellet cells then remove supernatant.

Supernatant: Remove 5ml – 10ml supernatant from flask to a 15ml conical tube.

Frozen stock: Ampoule may be delivered at room temperature.

Pricing

- Mycoplasma testing only: $60/sample

Mycoplasma AND cell line authentication (STR profiling) from the same cell pellet:

- GenePrint 10: $131/sample (Standard Service)
- GenePrint 10: $173/sample (Standard Service plus Profile Search)
- PowerPlex 16 HS: $171/sample (Standard Service)
- PowerPlex 16 HS: $213/sample (Standard Service plus Profile Search)
- PowerPlex 18D: $186/sample (Standard Service)
- PowerPlex 18D: $228/sample (Standard Service plus Profile Search)

Payment

- John Hopkins investigators: IO number or credit card (VISA or MasterCard)
- External U.S. customers: P.O. or credit card (VISA or MasterCard)
- External foreign customers: P.O., check or credit card (VISA or MasterCard)

*Listed pricing is for Johns Hopkins investigators; all other customers will have a 16% administrative fee added. International customers using a credit card will have an additional $25 processing fee added in addition to the administrative fee.

Please see our website, http://grcf.jhmi.edu/grcf-services/cell-line-testing/, for additional information about the testing procedure and sensitivity.

Digital PCR

Digital PCR is a new approach to nucleic acid detection and quantification. It offers a different method for absolute quantification and rare allele detection relative to conventional real-time quantitative PCR. Digital PCR works by partitioning a sample into many individual real-time PCR reactions; some portion of these reactions contain the target molecule (positive) while others do not (negative). Following PCR, the fraction of negative reactions is used to generate an absolute count of the number of target molecules in the sample, without reference to standards or endogenous controls.

At the GRCF, we offer digital PCR using the Life Technologies QuantStudio 3D platform. This technology partitions samples on a chip instead of in droplets, but results in the same type of data.

Digital PCR is an exciting new technology, but it requires careful thought and planning to answer particular questions. Because of this, consultation is required prior to starting an experiment. Please email customorders@jhmi.edu or call 410-955-2836 to make an appointment.

Pricing

$25/chip*

*Life Tech recommends using two chips on each sample to help average aliquoting variation

DNA Isolation & Handling

The GRCF offers a variety of services for researchers working with DNA. These services include:

- Bisulfite Conversion
- DNA Isolation
- Sample Plating
- Whole Genome Amplification (WGA)

For more information, see the individual topic or contact Laura Kasch at 410-614-3830 or lkasch@jhmi.edu.

Bisulfate Conversion

Bisulfite conversion of isolated DNA is offered using Zymo Research Corporation EZ DNA Methylation Kit or Qiagen EpiTect Bisulfite Kits. Converted DNA is suitable for PCR, endonuclease digestion, sequencing, microarrays, and Illumina Infinium and Golden Gate Methylation Assays.

Illumina Methylation Studies

Illumina recommends the following kits for bisulfite conversion. They do not recommend using any other kits.

- EZ DNA Methylation Kit for 50 DNA reactions (Zymo Research, catalog # D5001)
- EZ DNA Methylation Kit for 200 DNA reactions (Zymo Research, catalog # D5002)
- EZ-96 DNA Methylation Kit for 2×96 DNA conversion reactions (deep-well Zymo-Spin I-96 Filter Plate) (Zymo Research, catalog # D5004)

Pyromark Q24

Either type of bisulfite conversion kit may be used for the Pyromark system, though Qiagen guarantees their assays if their conversion kit is used.

Pricing and Ordering

Pricing is sample number dependent. Please contact Laura Kasch at 410-614-3830 or lkasch@jhmi.edu for pricing and ordering instructions.

DNA Isolation

The GRCF offers genomic DNA isolation from whole blood, buffy coat, packed cells, cultured cells, blood spot cards, FFPE samples, buccal swabs/brushes, mouthwash, and Oragene saliva collection kits.

DNA is suitable for archiving, PCR, Southern blotting, library construction and other applications requiring high molecular weight DNA.

DNA is supplied with A260 and A260/A280 values determined by Nanodrop analysis. Sample concentration can be adjusted per customer specifications. DNA aliquoting is also available.

Qualitative analysis is available by agarose gel electrophoresis or Advanced Analytical Fragment Analyzer CE analysis.

Storage of blood on Whatman FTA Classic Cards for archiving is available.

Pricing

Prices range from $25 to $45 per sample. Volume pricing is available for large study projects or batched samples.

Please contact Laura Kasch at 410-614-3830 or lkasch@jhmi.edu, for pricing.

Ordering

Please place your order through our secure online ordering system at http://jhu.genesifter.net.

Delivery of samples

JH Customers: Drop samples off in Blalock 1004 with the GeneSifter order number, Monday – Friday from 9 AM – 4:00 PM.

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Baltimore, MD 21224
Phone (443) 287-7948

Samples should be sent for delivery Monday through Friday only.

Sample Plating

Sample plating includes organization of samples, diluting DNA to a specified concentration, and aliquoting samples into 96-well plates. Samples must be supplied with an optical density (OD) measurement and an electronic inventory of samples. Pricing is per 96-well plate.

If samples lack an OD we can determine the concentration of your samples by Nanodrop or pico green analysis for an additional fee.

Pricing and Ordering

$225 per 96-well plate

Please contact Laura Kasch at 410-614-3830 or lkasch@jhmi.edu for ordering instructions

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Whole Genome Amplification

Whole genome amplification (WGA) of extracted DNA is carried out using a REPLI-g kit (Qiagen). The kit uses Multiple Displacement Amplification technology to produce highly uniform amplification across the genome. The genome is isothermally amplified utilizing a DNA polymerase with $3' - 5'$ exonuclease proofreading activity capable of replicating up to 100 kb without disassociating from the DNA template. Typical yields from the REPLI-g kit are 40ug DNA with a product range of 2 to 100 kb and an average of greater than10 kb. Poor quality genomic DNA may result in synthesis failure, reduced call rates in downstream applications, or loss of loci or alleles in the amplification product.

WGA products are supplied with PicoGreen quantification values.

Pricing

Pricing is sample number dependent. Please contact Laura Kasch at 410-614-3830 or lkasch@jhmi.edu for pricing.

Ordering

Please place your order through our secure online ordering system at http://jhu.genesifter.net.

Genotyping

The Genetics Resources Core Facility offers several different methods of genotyping. We feature high throughput genotyping on the Illumina platform, medium throughput genotyping on the Life Technologies Open Array platform with TaqMan® assays, and lower throughput genotyping via individual TaqMan®, Qiagen Pyrosequencing assays or STR/VNTR analysis. Most platforms offer specialized content for humans and mice, but we can work with you to genotype other organisms as well. The GRCF also offers STR profiling for Cell Line Authentication.

Access to our Illumina technology is available fee-for-service through the GRCF or for free with an application through the CIDR mechanism. To apply to CIDR, you must have grant support through one of the thirteen member NIH institutes. CIDR also requires a written application, the details of which are available on the web site: http://www.cidr.jhmi.edu.

Custom Genotyping

Individual Variants

For customers interested in individual variants, the GRCF offers custom genotyping to analyze short tandem repeats (STR), variable number tandem repeats (VNTR) and single nucleotide polymorphisms (SNP). SNP detection can be done using various methods:

- TaqMan analysis with custom or predesigned kits (Life Technologies) on an Applied Biosystems 7900HT Real Time PCR System
- Sanger sequencing of PCR products on an Applied Biosystems 3730XL DNA Analyzer
- Pyrosequencing of biotinlyated PCR products on the Qiagen Pyromark Q24, using custom or predesigned kits

In addition to the kits offered by Life Technologies and Qiagen, we have developed assays for some common polymorphisms.

Gene	Gene Symbol	Description
Alpha2C adrenergic receptor	ADRA2C	12bp in frame deletion Del322-325
Angiotensin I-converting enzyme	ACE	insertion/deletion polymorphism
Apolipoprotein E	APOE	112T>C and 158C>T (E2, E3, E4 alleles)
Asporin	ASPN	aspartic acid (D) repeat GAT
Dopamine Receptor D4	DRD4	48bp VNTR
Dopamine Transporter	DAT/SLC6A3	40bp VNTR in 3'-untranslated region
Monoamine oxidase A gene	MAOA	30bp VNTR in promoter
Serotonin transporter	5'-HTTLPR	insertion/deletion (Long/Short allele)
Serotonin transporter	5'-HTTLPR	rs#25531, A>G SNP

Pricing

Pricing is sample number and project specific for individual variant projects. Please contact Laura Kasch at 410-614-3830 or lkasch@jhmi.edu for consultation and a quote.

Self Service Genotyping

If you wish to do your own Taqman genotyping, but don't have the equipment to read the results, you may rent time on our self-service equipment: $15/hour to thermocycle on the machine, $10 for an endpoint read.

Medium Throughput Options

Golden-Gate chemistry from Illumina

This is Illumina's original genotyping chemistry. More information on the chemistry can be found on Illumina's website. It can be used to assay SNPs panels of 96, 384, 768, 1536 or multiples of 1536. SNPs should be selected based on their likelihood to work in with the chemistry. Illumina provides a score that can be used to assess this. To select SNPs efficiently, you should submit your entire list for scoring prior to narrowing it down. For more information on this process, contact Roxann Ashworth: rashwor2@jhmi.edu [410-614-0702].

Pricing

Costs for genotyping vary by panel size. Large numbers of SNPs on large numbers of samples may be cheaper with Infinium chemistry.

Number of Samples	96 SNPs	384 SNPs	768 or 1536 SNPs	3072 SNPs
1 plate (90 samples)	$7,500*	$17,500*	N/A	N/A
2 - 5 plates	$5,000*	$9,500*	N/A	N/A
6 - 9 plates	$4,100*	$5,800*	$6,500**	$8,000**
10 - 20 plates	$3,800*	$5,000*	$6,500**	$8,000**
≥ 21 plates	$3,800*	$5,000*	$5,500**	$7,000**

Taqman OpenArray Chemistry from Life Technologies

The OpenArray technology spots off the shelf or custom Taqman assays into 33 through-holes on arrays, which allows many assays to be tested on up to 144 samples at once. Chips can assay 16, 32, 64, 128 or 256 SNPs at once.

Pricing

A minimum of 10 chips must be ordered, but for 16 or 32 SNPs, these arrays are often very cost effective, even if they won't all be used. Pricing in table is for the minimum number of chips and assumes that all chips will be used. Actual prices will be adjusted based on the number of chips used.

Chip Format	Number of Samples/Chip	Minimum # of Chips (Samples)	Price	Total cost
16 SNPs	144	10 (1440)	Chips: $5,000 Other costs: $1,275	$6,275
32 SNPs	96	10 (960)	Chips: $5,000 Other Costs: $1,245	$6,245
64 SNPs	48	20 (960)	Chips: $10,000 Other costs: $2,325	$12,325
128 SNPs	24	40 (960)	Chips: $20,000 Other costs $4,650	$24,650
192 SNPs	16	60 (960)	Chips: $30,000 Other costs: $6,975	$36,975
256 SNPs	12	80 (960)	Chips: $40,000 Other costs: $9,300	$49,300

High Throughput Options

iSelect Infinium BeadChips: The Infinium technology is the same technology as used for the Genome Wide Association Chips. These custom panels can be created using between 3,000 and 1,000,000 SNPs, for any species with sequence information. The minimum number of samples for this to be cost-effective is 1,100. Custom content can also be added to any of Illumina's GWAS chips, often at a lower cost than a completely custom chip.

Pricing

Pricing on this product is based on increments of both SNP numbers and sample numbers. Please contact Roxann Ashworth [rashwor2@jhmi.edu; 410-614-0702] for detailed information on pricing and SNP selection.

Note: When selecting SNPs for this platform, one should select as many SNPs as possible within the appropriate increment, as Illumina estimates up to 20% of the selected SNPs will fail the manufacturing process and will not be placed on the custom chip. The SNP number calculations are further complicated by the fact that there are two different forms of the Infinium chemistry, one of which requires two bead types, the other of which requires only one. Bead types need to be counted when the final number of SNPs in selected. This information is provided by Illumina when the SNP selection files are returned.

Genome Wide Association

Cytogenetics

Human CytoSNP Chip: The HumanCytoSNP-12 BeadChip is designed to scan the whole genome in an efficient, high-throughput analysis of genetic and structural variations that are most relevant to human disease. Resolution is better than with FISH or CGH. The chip can be used to detect duplications, deletions, amplifications, copy-neutral LOH and mosaicism. The panel contains 200,000 "best of the best" SNPs with high tagging power, and includes dense coverage around the 250 genomic regions commonly screened in cytogenetics laboratories.

Pricing*

Number of Samples	Standard Content
49 or 94	$225
140-564	$215
610-1880	$205
>1881	$195

Human Core Product Line

Human Core BeadChip: The HumanCore BeadChip contains about 240,000 highly informative genome-wide tag SNPs found across diverse world populations and an additional 20,000 high-value markers. It is ideal for obtaining baseline sample datasets for downstream applications: common variant, mtDNA, ancestry, sex confirmation, loss of variant, indel, linkage and CNV detection. It can be ordered with Exome content or customized with up to 200,000 SNPs for an additional charge.

Pricing*

Number of Samples	Standard content	Standard + Exome Content
93	$125/sample	$145/sample
94 - 9,999	$110/sample	$132/sample
>10,000	$105/sample	$127/sample

Human Omni Product Line

HumanOmniExpress BeadChip: The HumanOmniExpress genotypes 700,000+ SNPs, gleaned from all three HapMap phases. It is primarily targeted for Caucasian and Asian populations, with genomic coverage of 0.91 at r2 > 0.8 in these populations. It also supports CNV applications, with a 4.0 kb mean distance between CNVs across the genome. Illumina now has developed a method for running this chip with DNA restored from FFPE and other highly degraded samples. This service is available through our facility, for an additional charge. The content of the Exome chip or up to 200,000 SNPs may be added to this chip for an additional charge. The OmniExpress + Exome chip may be customized with up to 30,000 additional SNPs.

Pricing*

Number of Samples	Standard Content	Standard Content + Exome	Standard Content + FFPE restore
93	$285/sample	$305/sample	$410/sample
94 - 9999	$255/sample	$280/sample	$370/sample
>10,000	$245/sample	$265/sample	$355/sample

HumanOmni2.5: This high-density chip has 2.45 million SNPs. It offers a comprehensive set of both common and rare SNP content from the 1000 genome project. It is designed to analyze diverse world populations. Mean SNP spacing is 1.18 kb, and 1.2 million of the SNPs are within 10 Kb of a RefSeq gene. Almost 50,000 of the SNPs are nsSNPs. The content of the Exome chip or up to 200,000 SNPs may be added to this chip for an additional charge. Custom SNPs cannot be added to the Omni2.5+Exome chip.

Pricing*

Number of Samples	Standard Content	Standard Content + Exome
47 or 93	$435/sample	$455/sample
94 - 9,999	$385/sample	$405/sample
>10,000	$370/sample	$390/sample

HumanOmni5: The Omni5 chip is designed to supplement data created from the Omni2.5 and bring it to the equivalent of the Omni5 chip. Leverages tagSNPs selected from the HapMap and 1000 Genomes Projects, targeting genetic variation down to 1% MAF. The chip contains 4.3 million SNPs, and up to 500,000 custom markers can be added to them. The content of the Exome chip or up to 500,000 SNPs may be added to this chip for an additional charge. Up to 200,000 SNPs may be added to the Omni5+Exome chip.

Pricing*

Number of Samples	Standard Content	Standard Content + Exome
47 or 93	$580/sample	$600/sample
94 - 9,999	$520/sample	$540/sample
>10,0000	$500/sample	$520/sample

*Pricing Details

For GWAS projects with more than 94 samples, the pricing includes:

- Our SNP barcode test panel (96 SNPs used to barcode the sample and verify the GWAS dataset released matches the sample performance – can also be used to verify 3 main ethnic groups if desired).
- Identification of problems and replacement of problem samples before running the sample on the GWAS arrays.
- Two HapMap controls per 96 well plate and 1 blind duplicate every other plate.
- Repetition of any sample that has less than a 96.5% call rate for an Illumin array, We attempt the GWAS assay only twice.

Mouse Genotyping

Linkage

There are two Mouse Linkage Panels available from Illumina: the Low Density Linkage and the Medium Density Linkage. Both panels are designed for use to identify quantitative trait loci (QTL) and candidate gene mapping. The SNPs for the panels were chosen from the Welcome-CTC Mouse Strain SNP Genotype Set. A fact sheet comparing both panels is available.

The Low Density Panel consists of 377 loci, optimized for application to N2 & F2 mouse genetic crosses. These can be used for QTL mapping. Information on coverage by chromosome and the expected genotypes in 10 strains are available for download.

The Medium Density Panel consists of 1,449 loci, optimized for various mapping applications including characterization of transgenic, congenic & knockout animals, and genetic mapping in advanced intercross mouse lines. Information on coverage by chromosome and the expected genotypes in 10 strains are available for download.

Pricing

Low Density Panel	Medium Density Panel
$6,300 per plate of 82 samples	$7,800 per plate of 82 samples

Custom

Custom Mouse Genotyping panels can also be created, using the SNPs listed on the Welcome-CTC website.

Pricing

Please inquire for pricing [rashwor2@jhmi.edu; 410-614-0702]. Minimum SNP number for a custom panel is 96 SNPs.

Specialized Content Products

DNA Fingerprinting Panel

A panel of 96 SNPs has been designed by CIDR for Illumina Golden Gate chemistry with enough polymorphic SNPs to uniquely identify samples. All SNPs in this panel are drawn from Illumina's Genome Wide Association products and can be used in several ways: 1) as a record of the original genotypes of a sample that may be stored for long periods of time or frequently distributed to other investigators, to help avoid sample mix-ups; 2) as a pretest for samples of uncertain quality, prior to more extensive genotyping, 3) as a way of insuring that serial samples collected from individuals are indeed from the same individual, 4) for ancestry identification (all SNPs are ancestry informative for distinguishing between the three major world populations).

Pricing

With Genotypes Returned	Without Genotypes Returned
$45/sample	$25/sample

Fingerprinting by STR Analysis/Cell Line Authentication

The GRCF offers a short tandem repeat (STR) profiling service to customers who wish to confirm the identity of a line by comparison to a known profile or to establish an identity profile for a new cell line generated locally. For further information see Cell Line Authentication or our website at http://grcf.jhmi.edu/grcf-services/cell-line-testing/.

Human Exome Chip

This Illumina Infinium chemistry array was developed by a consortium with the goal of enabling an intermediate experiment between current genotyping arrays, which focus on relatively common variants, and exome sequencing of very large numbers of samples, which will enable examination of coding variants, down to singletons. The array aims to include coding variants seen several times in existing sequence datasets. Information from ~12,000 sequenced genomes and exomes was assembled and catalogued for each variant that potentially affects protein structure, the total number of times it was seen and the total number of datasets that included the variant. The chip assays around 250,000 SNPs.

Pricing

Number of Samples	Standard content
93 - 9,999	$76/sample
>10,000	$69/sample

Human Whole Genome Linkage Panel

This product has been discontinued by Illumina. We recommend using the Human Core chip or the Human Exome Chip in its place.

Taqman OpenArray PGx Panel

This panel is derived from the PharmaADME Core Marker Set and assays 158 different polymorphisms in genes encoding drug metabolism enzymes and their associated transport proteins. Each chip tests 16 samples.

Pricing

- 1 chip: $777
- 2 chips: $1449
- 3 chips: $2121
- 4 chips: $2793
- Discounts available for projects of 11 or more chips.

Methylation

The GRCF provides the capability of analyzing DNA methylation through several different technologies. Large numbers of samples can be analyzed via genotyping using the Illumina 450K Methylation BeadChip or via custom Illumina products. Smaller scale projects and quantitative analysis can be carried out via Pyrosequencing. We are also able to provide bisulfite conversion of samples prior to use with one of these technologies.

Analysis via Genotyping

Genome Wide

450K Infinium Methylation BeadChip: The 450K Methylation chip is not reliant on Me-DIP (Methylated DNA immunoprecipitation). It covers 99% of all RefSeq genes, including promoter, 5&prime and 3&prime regions and those without CpG islands. Additional content includes 96% of all CpG islands as well as CpG shores, CpG sites outside of islands, non-CpG methylated sites identified in human stem cells, differentially methylated sited from tumor vs. normal (multiple forms of cancers) and across several tissue types, CpG islands outside of coding regions, miRNA promoter regions, and disease-associated regions. 90% of the HumanMethylation 27 chip is included. A list of all CpG sites is available for download. Bisulfite conversion is included in pricing for projects with more than one full plate. Four PI supplied controls are assayed at no additional charge.

Pricing

Number of Samples	Standard Content	Standard Content + FFPE restore
92	$525/sample	$665/sample
140-564	$482/sample	$610/sample
610-1880	$400/sample	$518/sample
>1881	$380/sample	$480/sample

Custom

Custom Methylation Products: Custom Methylation panels may be created. Panels must be for 384 CpG sites. To create a custom panel, you may submit for evaluation using GeneIDs, Gene symbol, RefSeq mRNA accession numbers or GI numbers, chromosomal region or sequences.

Pricing

Please inquire: rashwor2@Jhmi.edu; 410-614-0702.

Analysis via Pyrosequencing

Specific regions of methylation can be assayed via sequencing on the Pyromark Q24 system from Qiagen. This is not high throughput/next generation sequencing. Bisulfite converted DNA is PCR amplified with a biotinylated primer pair. The non-biotinylated strand is sequenced in the Pyromark system. Custom regions can be amplified, or one of the 60,000 human and rodent predesigned kits can be ordered from Qiagen. Primers ordered through us receive a slight discount and free shipping. Both primer design and analysis software are available for use by customers. Call 5-2836 or email us at customorders@jhmi.edu to arrange to borrow the disk.

This method is ideal for obtaining quantitation of methylation at specific CpGs.

For more information, contact Roxann Ashworth [rashwor2@jhmi.edu; 410-614-0702]

PCR support and bisulfate conversion is available for Pyrosequencing projects

Service includes bisulfate conversion of DNA samples, PCR of samples, amplification confirmation by agarose gel electrophoresis and submission for sequencing. Please contact Laura Kasch at 410-614-3830 or lkasch@jhmi.edu] for ordering instructions.

Pricing

Pyrosequencing

- Individual tubes: $6.50/sample.
- Full plates: $5.50/sample.
- Projects of 1,000 samples or more at once: $5.00/sample.

PCR Support: $6.50/sample. (Discounts available for large projects. Please inquire with Laura Kasch [lkasch@jhmi.edu; 410-614-3830]

RNA Services

cDNA Synthesis & RT-PCR

cDNA Synthesis for MicroRNA Profiling and Custom Gene Expression Taqman Arrays

The GRCF will prepare cDNA from customer supplied total RNA according to Life Technologies quick reference guide for Taqman MicroRNA profiling or by recommended procedures for the Gene Expression Array analysis for use on the QuantStudio 12K Flex Real-Time PCR System. The GRCF also has RNA isolation services available if you require start to finish processing.

RNA Requirements

- RNA should be free of PCR inhibitors
- Has a A260/230 ratio between 2.0 and 2.4
- Has a A260/280 ratio between 1.8 and 2.1
- Has a RNA Integrity Number (RIN) between 6.5 and 10

The recommended amount of total RNA for preparing cDNA for microRNA Profiling is 100ng.

The recommended amount of total RNA for preparing cDNA for Gene Expression Taqman Arrays is 10µl at 200ng/µl (2µg).

Qualitative analysis of your RNA samples is available by Advanced Analytical Fragment Analyzer CE analysis. The system gives results similar and comparable to an Agilent BioAnalyzer. Advanced Analytical's PROSize software determines a RQN (RNA Quality Number) on a scale from 1 to 10.

Pricing

$25 per sample for cDNA synthesis

Ordering

Please contact Laura Kasch [410-614-3830 or lkasch@jhmi.edu] for additional information and ordering.

RT-PCR using Qiagen's PyroMark OneStep RT-PCR Kit or One-Step RT-PCR Kit

The Qiagen OneStep RT-PCR Kits provide highly sensitive and specific reverse transcription of any RNA template by using a blend of Sensiscript and Omniscript Reverse Transcriptases, followed by highly specific and reliable amplification of cDNA using HotStarTaq DNA Polymerase. The entire process takes place in one tube reducing the risk of contamination. The RT-PCR product generated from the OneStep RT-PCR Kits can be used for Pyrosequencing applications such as genotyping and allele-specific gene expression analysis.

Pricing and Ordering

Pricing is project specific. Please contact Laura Kasch at 410-614-3830 or lkasch@jhmi.edu for additional information and ordering.

Custom Gene Expression Arrays

Custom Gene Expression Arrays may be ordered and run through our facility using Life Technologies' OpenArray chips. These chips are ideal for investigators who wish to look at multiple assays on medium to large numbers of samples.

The technology spots Taqman assays onto chips, which are then run in our QuantStudio 12K flex machine. Each chip can assay between 12 and 96 samples at once, depending on the number of assays on the chip. Chips can be formatted with 18, 56, 112, 168 or 224 assays. There is a minimum order of 10 chips, but smaller projects are often cost effective, even when excess chips must be ordered.

Pricing

Pricing varies by SNP and sample number. Assays that require custom designs are more expensive. Examples using minimum numbers, assuming inventoried assays, are given below, but please contact us at customorders@jhmi.edu for detailed information [410-614-0702]. Please note that if you would run your samples in triplicate in a standard gene expression assay, you should do so with these assays as well. Sample numbers given below *do not* take that into consideration.

Chip Format	Number of Samples/Chip	Total Samples	Price	Total cost
18 Assays*	48	480	Chips: $7,000 Other costs: $930	$7,930
56 Assays	48	480	Chips: $8,000 Other Costs: $930	$8,930
112 Assays	24	240	Chips: $9,000 Other costs: $930	$9,930
168 Assays	16	160	Chips: $10,000 Other costs $930	$10,930
224 Assay	12	120	Chips: $11,000 Other costs: $930	$11,930

*18 assay-formatted chips are plated in triplicate, so each sample is used once, but run 3 times.

Fixed Content Gene Expression Arrays

Control Panels

Taqman HS Endogenous Control Panel: This panel targets 56 constitutively expressed human genes. Designed to function as a quality control panel for testing sample preparation methods prior to using custom OpenArray Gene Expression plates. Each chip tests 48 samples.

Pricing

- 1 chip (48 samples): $480
- 2 chips: $830
- 3 chips: $1,180
- 4 chips: $1,530
- Discounts available for projects of 11 or more chips

Specific Pathway Panels

Taqman Human Cancer Panel: This panel targets genes related to DNA repair, angiogenesis, cell adhesion, and ECM, as well as genes involved in the cell cycle and apoptosis, and many of the genes encoding kinases and transcription factors that have been found to be differentially expressed in early cancer and metastatic disease. The 624 genes have been validated for pluripotency. Twenty-four endogenous controls are included for normalization. Each chip tests 4 samples.

Taqman Human Inflammation Panel: This panel covers 586 genes that have been studied as targets for a range of inflammatory disease, plus 21 endogenous control genes. Each chip tests 4 samples.

Taqman Mouse Inflammation Panel: This panel covers 632 genes that have been studied as targets for a range of inflammatory diseases, plus 16 endogenous control genes. Each chip tests 4 samples.

Taqman Human Kinome Panel: This panel targets 772 well-defined genes for human kinases and provides 56 endogenous control genes for normalization. Genes for the panel were chosen based on primary literature and publications. Each chip tests 3 samples (or one sample in triplicate).

Taqman OpenArray Human Signal Transduction Panel: This panel contains 573 signal transduction-related genes plus 24 endogenous controls for normalization. The JAK-STAT, NF κ B, Akt, GPCR, cAMP, and MAP kinase pathways are well represented. Each chip tests 4 samples.

Taqman Stem Cell Panel: This panel targets 609 genes validated for pluripotency, plus 22 endogenous controls for normalization. They use a well-defined set of validated gene expression markers to characterize human embryonic stem (ES) cell identity and assess phenotypic variations between embryonic stem cell isolates. The gene content of this OpenArray® plate resulted directly from the work of the I.S.C.I consortium and characterization of human embryonic stem cell lines by the International Stem Cell Initiative. Each chip tests 4 samples.

Pricing

- 1 chip*: $760
- 2 chips: $1,390
- 3 chips: $2,020
- 4 chips: $2,650
- Discounts available for projects of 11 or more of the same chip.

*Sample number varies by pathway. See descriptions above for specifics.

MicroRNA profiling

Taqman MicroRNA Profiling (Human and Rodent)

We feature Taqman MicroRNA profiling using the QuantStudio 12K Flex Real-Time PCR System. This system allows you to look up to 758 unique assays per sample, quickly and efficiently with only 200ng of total RNA needed for a full miRNA profile. This new, high throughput technology allows us to analyze dozens of samples in a day, generating thousands of data points, at reasonable prices, using highly sensitive and highly specific Taqman assays. Human and rodent chips are available. We can generate the microRNA specific cDNAs from your total RNA sample for an additional charge.

Pricing

Number of Samples	Standard service (human)	Standard Service w/ cDNA synthesis	Standard service (rodent)	Standard Service w/ cDNA synthesis
3*	$875	$950	$670	$745
6	$1,635	$1,785	$1,240	$1,390
9	$2,395	$2,620	$1,810	$2,035
12	$3,155	$3,455	$2,380	$2,680
15	$4,030	$4,405	$3,050	$3,425
18	$4,790	$5,240	$3,620	$4,070
21	$5,550	$6,075	$4,190	$4,715
24	$6,310			

For pricing for projects of more than 75 samples, or if you wish to do the preamplification yourself, contact the GRCF at 410-955-2836 or customorders@jhmi.edu.

*Samples must be run in multiples of 3 (or in triplicate).

cDNA synthesis for MicroRNA Profiling is available. Please contact the GRCF at 410-955-2836 or customorders@jhmi.edu.

Real Time PCR Equipment

Self-Service Real Time PCR

Two Life Technologies QuantStudio 12K Flex machines and a Taqman 7900 are available for use by appointment, in Blalock 1005. All machines accommodate 96 & 384 well plates as well as Taqman Low Density array (TLDA) microfluidic cards. The 12K Flex machines can run in fast mode, as well, reducing your run time, if your assay has been optimized for fast mode. This equipment may be used for SNP genotyping, gene expression, CNV analysis and microRNA assays.

Investigators may reserve the machines by the hour, for assays recording real-time metrics or in 15-minute time increments for end-point reads. All reactions should be set up by the investigators in their own labs and the plate brought at the appropriate step in the process for loading on the machine. The specialized centrifuge and staking equipment necessary for loading the microfluidic cards is available for use in our lab. At the completion of the run, investigators should return and retrieve their data on a flash drive. A typical gene expression run requires a 3-hour reservation to ensure that you have enough time to fill out run details and retrieve data. A fast run typically requires a 1.5 hour reservation

Reservations are scheduled on a first come, first served basis by calling 5-2836, emailing customorders@jhmi.edu or stopping by Blalock 1004. Reservations are available from 7 AM through 4 PM each day. The 4 PM slot runs overnight. The final time slot of the day is for experienced users only. Please provide your PI name, phone number, budget number and the required block type at the time of reservation.

Pricing

- $15 per hour
- $10 for a 15 minute end-point read

RNA Isolation

The GRCF offers RNA isolation from PAXgene blood collection tubes, tissues (fresh or RNAlater preserved) and cultured cells. RNA is suitable for many downstream applications including cDNA synthesis, qPCR, Northern blots and library construction.

Quantitative analysis is by Nanodrop.

Qualitative analysis is available by Advanced Analytical Fragment Analyzer CE analysis. The system gives results similar and comparable to a Agilent BioAnalyzer. Advanced Analytical's PROSize software determines a RQN (RNA Quality Number) on a scale from 1 to 10.

Please contact Laura Kasch [410-614-3830; lkasch@jhmi.edu] for additional information and ordering.

Pricing

Source	Sample Size	Yield	Charge (without CE analysis
Blood	PAXgene collection tube	> 3µg (total RNA)	$25
Tissue	0.15mg-1g	up to 6mg RNA	$35
Tissue	20-250mg	up to 1mg RNA	$30
Tissue	<30mg	up to 100µg RNA	$25
Tissue	<5mg	up to 45µg RNA	$20
Cultured Cells	5 x 10E7 to 5 x 10E8 cells	up to 6mg	$30
Cultured Cells	5 x 10E6 to 1 x 10E8 cells	up to 1000µg	$25
Cultured Cells	< 1 x 10E7	up to 35µg	$20
Cultured Cells	<5 x 10E5 cells	up to 17.5µg	$15

RNAseq

The GRCF offers library prep, sequencing, and analysis of RNAseq data. Due to the nature of these experiments, it is beneficial to discuss sequencing strategy prior to sample submission. We offer free consultation. Please contact David Mohr (dwmohr@jhmi.edu) to discuss your project in detail.

We offer simple, per lane pricing, which allows the investigator control over how many reads required per sample. Please see ENCODE's best practices for current guidelines on RNASeq experimental design: http://encodeproject.org/ENCODE/protocols/dataStandards/RNA_standards_v1_2011_May.pdf

Sample Requirements

Please provide us with high quality total RNA (RIN >8.5). We request >500ng for standard prep, but can work with less if required. We strongly encourage you to QC your RNA prior to submission, as high quality starting material is the key to success. If you cannot QC your sample prior to submission, we can do so for you at a cost of $20/sample.

Sequencing Cost

Sequencing costs vary based on read length and depth per sample required. You can multiplex samples in a single lane to save money. Please see our High Throughput Sequencing page for information on per lane pricing and data yield: http://grcf.jhmi.edu/hts

Analysis

We will happily assist you with analysis free of charge, provided you consider it a collaborative effort. RNASeq is a field that is constantly changing. We have the resources and expertise to run analysis workflows, but will need your input for pipeline design.

Sequencing

High Throughput Sequencing

At the GRCF High Throughput Sequencing Center, our goal is to provide the research community at Johns Hopkins University with access to 'next generation' sequencing platforms. We currently feature three HiSeq 2500 instruments and two MiSeq instruments, and an Ion Proton (currently in R&D). We do our best to sequence samples in a timely manner, but our first priority is high quality data.

We offer free consultation. Please contact David Mohr [dwmohr@jhmi.edu] to discuss your project in detail.

Per Lane Pricing

Single Reads:

50 cycles: $1,200 per lane
100 cycles: $1,600 per lane
100 cycles: $2,000 per lane

Paired End Reads:

50 cycles (x2): $1,900 per lane
100 cycles (x2): $2,400 per lane
150 cycles (x2): $2,900 per lane

Non-standard read lengths are also available, provided they are submitted in pairs (enough to start a 2500 run). Please inquire about pricing.

Library Prep: $250/sample

Sample Requirements

Completed Libraries: Please submit 10 ul of your sample at 2nM. Samples must be pooled. We will do a QC check via Bioanalyzer to increase the likelihood of quality data, but you should quantitate your sample as accurately as possible. Nanodrop is not reliable. qPCR is by far the most accurate, but intercalating dye methods can be used.

Library Prep: Please provide us with >500ng of high molecular weight DNA/RNA. Please contact us to discuss sample submission.

Data Yield

Our facility features the HiSeq 2500 platform. We almost exclusively run our instruments in Rapid Run Mode, due to the fast run times and low error rates. Yield is dependent upon several factors:

- **Read Length:** the longer the read, the more data.
- **Read Type:** paired end reads yield twice the data as single read.

- **Optimal Cluster density:** it is imperative to accurately quantitate your library to ensure high data yield, as we load picomolar amounts on the sequencer. We do our best to QC libraries before sequencing, but we cannot pool samples for you.
- **High Quality Library:** libraries that contain a high level of adapter dimers will yield significantly less data, as the fragments will hybridize to the flowcell. Similarly, over amplified libraries can negatively impact yield.
- **Uniform Base composition:** libraries that have uneven base composition tend to pose problems for the HiSeq analysis software. These issues can be mitigated using several strategies, but the net effect will be lower data yield per lane than a balanced library.

While we regularly achieve greater than 'spec' yields from the HiSeq2500, often matching yields for High Output mode, it is best to be conservative when planning your experiments.

We will also be happy to run your samples in High Output mode, but you will need to submit a full flowcell (i.e. 8 lanes/libraries) at a time.

Data Delivery

Per lane sequencing: Data will be returned in Sanger FASTQ format via our high-speed aspera server

End to end services: alignment files, variant calls, and any intermediate files you wish via aspera server

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Medium Throughput Sequencing

Per Run Pricing

MiSeq reagents are sold by cycle number. They can be run in single/paired end, and/or indexed mode.

50 cycles: $900 per run
300 cycles: $1,000 per run
500 cycles: $1,100 per run
600 cycles: $1,400 per run

Sample Requirements

Please submit 10 μ l of your sample at 2nM. Samples must be pooled. We will do a QC check via Bioanalyzer to increase the likelihood of quality data, but you should quantitate your sample as accurately as possible. Nanodrop is not reliable. qPCR is by far the most accurate, but intercalating dye methods can be used.

Data Delivery

Data will be returned in Sanger FASTQ format via our high-speed aspera server.

PCR Amplification Support Services

Pyrosequencing Projects

PCR amplification support for Pyrosequencing methylation studies are available to customers utilizing the GRCF Pyrosequencing service. We will amplify your samples using a customer supplied Qiagen predesigned or custom kit. Service includes PCR of samples, amplification confirmation by agarose gel electrophoresis and submission for Pyrosequencing.

Sanger Sequencing Projects

For customers interested in custom Sanger sequencing projects we can help you with design of primers, development of PCR amplification conditions, amplification of samples, and sequence analysis.

Pricing

Pricing is sample number dependent. Please contact Laura Kasch [410-614-3830; lkasch@jhmi.edu] for a quote and ordering instructions.

Pyrosequencing

Pyrosequencing is a method of sequencing that relies on light emitted in an enzymatic reaction set in motion by the release of pyrophosphate when a base is added during the sequencing process. The results are quantitative, making the method ideal for sequencing samples of known sequence that may have mutations in proportions as low as 5%, such as tumor samples or mixed viral populations. Test kits exist for APOE, BRAF, KRAS, HFE and MTHFR, but any small region (around 80 bases) can be analyzed with custom primers. Assay design and analysis software is available for use by any Johns Hopkins investigator. This method can be used for characterization of contiguous and multivariable mutations, LOH analysis, unambiguous, fully quantitative genotyping that distinguishes multi-site variations from single nucleotide polymorphisms.

Microbial identification and drug resistance typing is another potential use of this method. It provides rapid and reliable high-throughput screening. Mutations can be accurately identified without use of expensive labels and dyes.

Specific regions of methylation can be assayed via sequencing on the Pyromark Q24 system as well. Bisulfite converted DNA is PCR amplified with a biotinylated primer pair. The non-biotinylated strand is sequenced in the Pyromark system. Custom regions can be amplified, with specific analysis software is available for use by customers. Qiagen also has over 30,000 assays designed for CpG Islands throughout the human genome, which can be ordered through our Geneglobe.com service. Please see the products section of this catalog for more information.

Ordering

To place an order, use the Pyrosequencing order form on the GRCF ordering server: https://jhu.gensifter.net. Samples must be dropped off before 4:30. Results will be available on the JHU ordering server the next business day.

Pricing

- Individual tubes: $6.50 per sample
- Full plate or more of samples: $5.50 per sample
- Project of 1000 samples or more: $5.00 per sample

Sanger Sequencing

Sanger sequencing services are provided using the Applied Biosystems 3730xl DNA Analyzer. These machines utilize a one-capillary cycle sequencing process with refined, four-dye fluorescent labeling methods and a real-time scanning detector. The biochemistry of the one-lane sequencing is similar to Sanger-based methods.

The GRCF ordering server (https://jhu.genesifter.net) automatically processes data after electrophoresis is complete and provides quality assessment for each sample. Data is available for download, viewing, and editing. The service provides standard sequencing primers for several vectors. Custom primers should be provided by the user and premixed with the template. Primers can be ordered through our facility using one of our portals as well. See the products section of this catalog for more information.

Turnaround Time

Samples dropped of in the Blalock building by 4 PM will be ready by 10 AM the following business day. Samples dropped off after 4 PM will be held for processing the next day. Sam day sequencing service is available at a higher fee per sample, with a minimum of 5 samples. For same day sequencing, samples must be ordered on the JHU ordering server and physically present in our facility by 9 AM and the data will released by 5 PM. Samples should be dropped off in our remote locations by 11 AM. Data release will depend on the pick up schedule of the day concerned. On normal days, samples are picked up in the Asthma and Allergy Building by 11:30 and in CRB 1 B02A by 3:00 PM, but pickups may vary depending on staffing.

Pricing

- Single pre-mixed template & primer: $6
- 95 samples in a 96 well plate: $5.50 per sample.
- BAC protocol reactions (used on BACs, other large templates or very GC rich templates): $23
- Same day service: $19 per sample, minimum of 5 samples

Whole Exome/Targeted Sequencing

Exome Sequencing

In conjunction with the Center for Inherited Disease Research, we have developed an extensive, fully automated, production-scale pipeline for processing of both whole exome and custom capture samples. We currently use Agilent's SureSelect Target Enrichment platform.

Lower cost subsidized whole exome and custom targeted sequencing services are available through the NIH CIDR Program: http://www.cidr.jhmi.edu

Pricing

$1350/exome

Please inquire about custom capture, as pricing changes depending on capture size and sample volume. Capture sizes can range from 500Kb and up.

Sample Requirements

- 2.5μg of high molecular weight DNA at 50ng/μl
- DNA should be sent in 1xTE, pH8.0(1mM Tris, 1mM EDTA). Please avoid using water.
- DNA source: blood, cell line, or saliva. Other sources can be used with caveats.

Service Overview

- DNA QC
- Sample pre-testing using a high density SNP array
- Automated Library Prep
- Automated Capture
- Sequencing to a minimum completeness level of 90% coverage at 20X or greater
- Target enrichment report, including capture specificity and completeness
- Quality metrics, including mapping statistics, library fragment size, hybridization and selection metrics, mapping stats, GC bias, and basecall quality distributions
- Sensitivity/Specificity to SNP array data
- Concordance with array data
- Annotated SNP/indel list for targeted regions (dbsnp, SNP type, refseq genes, etc.)

Data Quality

We are committed to providing the highest quality exome and custom capture data available. A team of dedicated scientists review all data we produce. Our current analysis team consists of at least 4 scientists, 3 laboratory managers, 3 statisticians, and 3 bioinformaticians.

We sequence to a completeness metric rather than mean depth as the former gives a much better indication of how many genomic positions are 'callable'. Depth is a poor metric for assessing the quality of exome data. Capture efficiency, library duplication levels, and library complexity are a few examples of factors that can determine how well your exome is covered.

Data quality is monitored and evaluated using a robust alignment and variant calling workflow implemented via CIDRSeqSuite, our in-house pipeline.

Our pipeline is based on open source tools including bwa, Picard, and the GATK2. We can provide detailed information upon request.

Data Delivery

Data will be returned via our high-speed aspera server. Our typical release includes the following:

- Annotated variant lists
- SNPS/indels in VCF format
- BAM alignment files
- QC report
- BED files for regions targeted
- Genotyping files
- Analysis Pipeline details

Whole Genome Sequencing

The GRCF High Throughput Sequencing Center has teamed up with the Illumina Genome Network to offer complete **human** genome sequencing to the Johns Hopkins University.

We are happy to provide in-house whole genome sequencing, but our prices cannot match those of the Illumina Genome Network (it costs us more to buy the reagents than Illumina charges for a genome).

For more information please contact the GRCF.

Products

Core Store

The Core Store is a division of the Genetic Resources Core Facility of the Johns Hopkins University School of Medicine. In operation since 1989, the Core Store is a non-profit resource that offers appreciable savings and fast delivery of a wide variety of research products. The Core Store provides one-stop shopping, saving researchers both time and money. In addition to its product offering, the Store charges no shipping and handling fees and has free delivery to three JHU campuses.

Store Hours

Monday – Friday 9:30am – 5:00pm (Closed from 1:00pm – 2:00pm daily)

The 24/7 Vending Center, http://grcf.jhmi.edu/core-store/core-store-247/, is always open with locations in: Blalock 1026, CRB I-B02A, & the Asthma & Allergy Center 1A.C4

Contact Information

1026 Blalock Building
600 N. Wolfe Street
Baltimore, MD 21287
Phone: (410) 614-1647
FAX: (410) 614-9752
Email: jhucorestore@jhmi.edu
Website: grcf.jhmi.edu/core-store/

Dry Ice

Is available at Blalock 1017, Monday – Thursday, 7:30 am – 4:30 pm and Fridays 7:30 am - 4:00 pm

- 1/2" diameter dry ice pellets
- Used primarily for shipping and transport of temperature-sensitive cells and reagents
- Sold in 1-pound quantities or more
- Bring your own container

Dry ice purchase forms are available when you pick up your dry ice or you can download a dry ice purchase form at http://grcf.jhmi.edu/products/dry-ice.

For more information please contact us at: bioshipping@jhmi.edu

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Gene Synthesis

DNA synthesis technology now allows entire genes to be synthesized. This can be helpful for difficult to clone sequences or for the construction of specific mutations. Custom genes are delivered in standard vectors, but may be ordered in custom vectors for an additional charge. Minimum yield is 2 µg of plasmid.

Genes may be ordered from Bioneer, Eurofins/Operon and IDT through the Johns Hopkins University portals:

Bioneer: http://us.bioneer.com/jhu/
Eurofins/Operon: http://operon.com/custom/jhu_grcf
IDT: http://www.idtdna.com/JohnsHopkins/

All companies adhere to the International Gene Synthesis Consortium's protocols and screen the sequences to identify regulated and potentially dangerous pathogen sequences.

Pricing

Bioneer	Price		Eurofins/ Operon	Price		IDT	Price
Up to 3 kb	$0.39/base (Min $180)		**Mini-genes <400 bp**	$140.00 (flat fee)		**Mini-genes <400 bp**	$198.00 (flat fee)
>3 kb	Please inquire for pricing		**Up to 3 kb**	$0.32/base		**Genes (401-1500 bp)**	$0.50/base
						Genes (>1500 bp)	Please inquire for pricing

Oligonucleotides

The DNA Analysis Facility offers the synthesis of oligonucleotides through several vendors, including Bioneer, Eurofins/Operon, IDT and Sigma-Genosys. Ordering through the Johns Hopkins University portal lets you take advantage of lower pricing and free shipping. Oligos can be delivered to your lab by the Core Store for free or picked up in our office in Blalock 1004.

You can access the Johns Hopkins specific portals by clicking the appropriate button.

Bioneer: http://us.bioneer.com/jhu/
Eurofins/Operon: http://operon.com/custom/jhu_grcf
IDT: http://www.idtdna.com/JohnsHopkins/
Sigma-Aldrich: http://www.sigmaaldrich.com/configurator/servlet/DesignCenter

Please note that you must create a new username and password for each company, even if you have been ordering from their standard web site. If you don't, you will not receive the discounted pricing below, and you will be charged shipping. Specialized quotes from sales representatives cannot be ordered through these portals.

Comparison charts of pricing, size limitations and guaranteed minimum yields are below to help you select a company. Prices listed below are for standard, unmodified oligos, with standard turn around times (48 hours), delivered in lyophilized in tubes. Each company also offers "same day" service, which means that oligos are synthesized and shipped the same day. Same day orders are not eligible for free shipping. See each company's portal for pricing.

Oligo orders must be placed by 2 p.m. for standard shipping times to apply.

Company	Synthesis Scale						
	10 nmole*	25 nmole*	50 nmole*	100 nmole*	200 nmole*	250 nmole*	1 µmol**
Bioneer	N/A	$0.20	$0.34	N/A	$0.65	N/A	$1.24
Eurofins/Operon	$0.14	$0.15	$0.25	N/A	$0.58	N/A	$0.96
IDT	N/A	$0.15	N/A	$0.36	N/A	$0.58	$0.96
Sigma-Genosys	N/A	$0.15	$0.23	N/A	$0.53	N/A	$1.00

*See table below for size limitations

**Larger quantities available from all companies, please call 410-955-2836 for pricing.

Size limitations

Company	Oligo Lengths						
	10 nmole	25 nmole	50 nmole	100 nmole	200 nmole*	250 nmole*	1 µmol*
Bioneer	N/A	15-60mer	10-75mer	N/A	5-110mer	N/A	5-130mer
Eurofins/Operon	15-60mer	15-60mer	5-90mer	N/A	5-125mer	N/A	5-125mer
IDT	N/A	15-60mer	N/A	10-90mer	N/A	5-100mer	5-100mer
Sigma-Genosys	N/A	10-120mer	10-120mer	N/A	10-120mer	N/A	10-130mer

*Oligos between 111-130 bases cannot be purified and yield is not guaranteed

Guaranteed Minimum Yield

Company	Minimum Yield (OD/µg)					
	25 nmole	50 nmole	100 nmole	200 nmole	250 nmole	1 µmol
Bioneer*	2.0	4.0	N/A	8.0	N/A	30.0
Eurofins/Operon	3.0	4.0	N/A	11.0	N/A	36.0
IDT	3.0	N/A	6.0	N/A	15.0	45.0
Sigma-Genosys	3.0	5.0	N/A	12.0	N/A	40.0

*All Bioneer oligos are RP1 purified, standard

Long Oligos

Bioneer and IDT offer long oligos up to 200 bp in length. Bioneer's Extendamers can be 130-200 bases long. IDT's Ultramers can be 60-200 bases long. Extendamers and Ultramers can be PAGE purified for an additional fee. Purification will lower yield.

Pricing

Company	Synthesis Yield		Purification
	3-4 nmole	20 nmole	HPLC
Bioneer*	$0.95/base*	N/A	$75.00
IDT	$0.65/base	$1.34	$94.50

RNA Oligonucleotides

Custom RNA Oligos

Custom RNA oligos can be ordered from all of our portals. They may be purified via HPLC or PAGE for an additional fee. Some modifications are available, see the individual portals for details. Bioneer sells their oligos by total yield. See separate chart for pricing. Bioneer RNA can only be purified by HPLC for an additional fee. RNA oligos usually arrive six business days after ordering, purified oligos take longer. For more information, see the company portals, below.

Bioneer: http://us.bioneer.com/jhu/
Eurofins/Operon: http://operon.com/custom/jhu_grcf
IDT: http://www.idtdna.com/JohnsHopkins/
Sigma-Aldrich: http://www.sigmaaldrich.com/configurator/servlet/DesignCenter

Pricing

Company	RNA base				
	50 nmole	100 nmole	200 nmole	250 nmole	1 µmole
Eurofins/Operon	$3.13	N/A	$5.00	N/A	$15.00
IDT	N/A	$5.69	N/A	$7.44	$17.75
Sigma	$2.82	N/A	$3.62	N/A	$15.75

Bioneer RNA oligos	Price per base			
RNA length	**10 nmole***	**20 nmole**	**50 nmole**	**100 nmole**
<30 bases	$3.00	$5.00	$8.00	$13.00
31-35 bases	$4.50	$7.50	$12.00	$19.50
2'O-me Bases	$8.16	$11.56	$16.95	$23.80
HPLC purification	$40.00	$50.00	$60.00	$70.00

*Guaranteed Yield, up to 30-mer, including overhang

QIAGEN Geneglobe Assays

All assays found on QIAGEN's Geneglobe site (http://qiagen.com/geneglobe) can be ordered through the DNA Analysis Facility.

Products include assays for:

- Gene silencing (siRNAs in several different formats)
- Gene expression analysis (RT-PCR primer sets)
- miRNA (detection primers, mimics, inhibitors, precursor assays, custom assay designs)
- Expression of proteins
- Next gen sequencing primers
- DNA methylation (including Pyrosequencing assays)

Order through the GRCF to receive a discount and free shipping on all orders. To order:

1. Go to QIAGEN's site
2. Fill up your shopping cart
3. Save the cart
4. e-mail it to yourself and customorders@jhmi.edu with the following information:
    a. PI name
    b. budget (IO#) number
    c. contact phone number.

For more information email us at (customorders@jhmi.edu) or call 410-955-2836.

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si/shRNA

Custom siRNA

Custom siRNAs can be used in cell culture experiments to silence genes. They can be ordered Simplex or Duplex, from Bioneer, IDT and Sigma. An optional overhang can be included. siRNAs must be between 19 and 30 bases in length. Pricing is a flat fee, Purification via HPLC or PAGE is extra. siRNAs typically arrive 7 business days after ordering. Modifications, including 2'O-me bases and Locked Nucleic acids are available.

Place your order through our portals:

Bioneer: http://us.bioneer.com/jhu/
IDT: http://www.idtdna.com/JohnsHopkins/
Sigma-Aldrich: http://www.sigmaaldrich.com/configurator/servlet/DesignCenter

Pricing

siRNA, price/base	IDT		Sigma		
	2 nmole	10 nmole	2 OD	5 OD	10 OD
Unlabeled Simplex	N/A	N/A	$68.43	$110.29	$132.83
Unlabeled Duplex	$81.00	$125.00	$120.75	$201.25	$241.50

Bioneer siRNA oligos	Price per base				
	1 nmole*	5 nmole	10 nmole	50 nmole	100 nmole
Unlabeled Duplex (RP1)	$50.00	$95.00	$210.00	$410.00	$440.00
Unlabeled Duplex (HPLC)	$90.00	$170.00	$330.00	$440.00	$570.00

* Guaranteed Yield, up to 30-mer, including overhang

Predesigned siRNA/shRNA

Bioneer, Dharmacon, IDT, Life Technologies, Qiagen and Sigma offer many predesigned siRNA assays. Sigma also offers shRNA. Please see their portals or the specific company pages on the menu bar for details.

Order now

Bioneer: http://us.bioneer.com/jhu/
Eurofins/Operon: http://operon.com/custom/jhu_grcf
IDT: http://www.idtdna.com/JohnsHopkins/
Life Technologies; Use the ordering tool on our site: http://grcf.jhmi.edu/products/taqman-assays/
Qiagen: Find assays on http://qiagen.com/geneglobe, save cart, email to customorders@jhmi.edu
Sigma-Aldrich: http://www.sigmaaldrich.com/configurator/servlet/DesignCenter

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Taqman Assays

Life Technologies custom products are now available through the GRCF. By ordering through us, you will receive small discount and free shipping on all orders! Orders can be delivered to you lab via the Core Store delivery mechanism or picked up in our office in Blalock 1004. Products available include:

- Taqman Gene Expression Assays, Plates and Arrays
- TaqMan MicroRNA Assays
- SNP Genotyping, CNV, siRNA, and mutation detection assays
- siRNAs

To place your order, use the tool from Life Technologies on our site. Once your cart is full, submit the order, providing the requested information. The tool will email your order to our facility. We will place the order, email you a confirmation and deliver the assays when they arrive.

The tool will email us a shopping cart and we will place your order. You will receive an email when the order has been placed.