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research services

Genetic Resources Core Facility

2019

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- bioprocessing
- biorepository
- bioshipping
- cell line testing
- digital PCR
- dna services
- genotyping
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- sequencing
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GRCF MISSION

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

A Unit of Johns Hopkins Genomics

JOHNS HOPKINS
SCHOOL of MEDICINE

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genetic resources core facility

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A unit of the Johns Hopkins Genomics

Dear Researchers,

We would like to take this opportunity to thank you for the privilege of serving you. The Genetic Resources Core Facility (GRCF) is a university service center providing genetic, genomic and cellular expertise, products, and technical services in clinical and basic science research. The GRCF works to stay at the leading edge of technology, by providing sophisticated tools and equipment oftentimes not available in the individual research lab. As a service center, we value and depend on our interactions with the Johns Hopkins research community and look forward to another year of stimulating research.

We encourage investigators planning a study to meet with us to discuss objectives. We have a broad range of expertise and in many instances, can propose an array of possible approaches to your research question. We are also happy to write letters of support for grant applications, demonstrating to reviewers that the resources for your study are available at the university.

We are excited to offer in 2019 through our acclaimed GRCF High-Throughput Sequencing facility, sequencing on the NovaSeq 6000. This system represents the most powerful, scalable, high-throughput Illumina sequencing platform available to date. Other recently added services to the GRCF portfolio include: Oxford Nanopore sequencing, offering real-time, rapid, scalable, long read direct-DNA sequencing, single cell genomics utilizing the 10x Genomics Chromium platform for high throughput isolation, and the ability to order custom CRISPRs direct from vendors through our oligo ordering portal. In addition to utilizing these and the many services described in this catalog, we encourage you to explore our website for other service options, changes and contact information for our network of expert advisors.

Finally, thank you for your continued support of the GRCF. We appreciate your business and the confidence you have placed in us over the years. 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 Johns Hopkins community for many years to come.

Sincerely,

The Faculty and Staff of the

Genetic Resources Core Facility

THE GENETIC RESOURCES CORE FACILITY

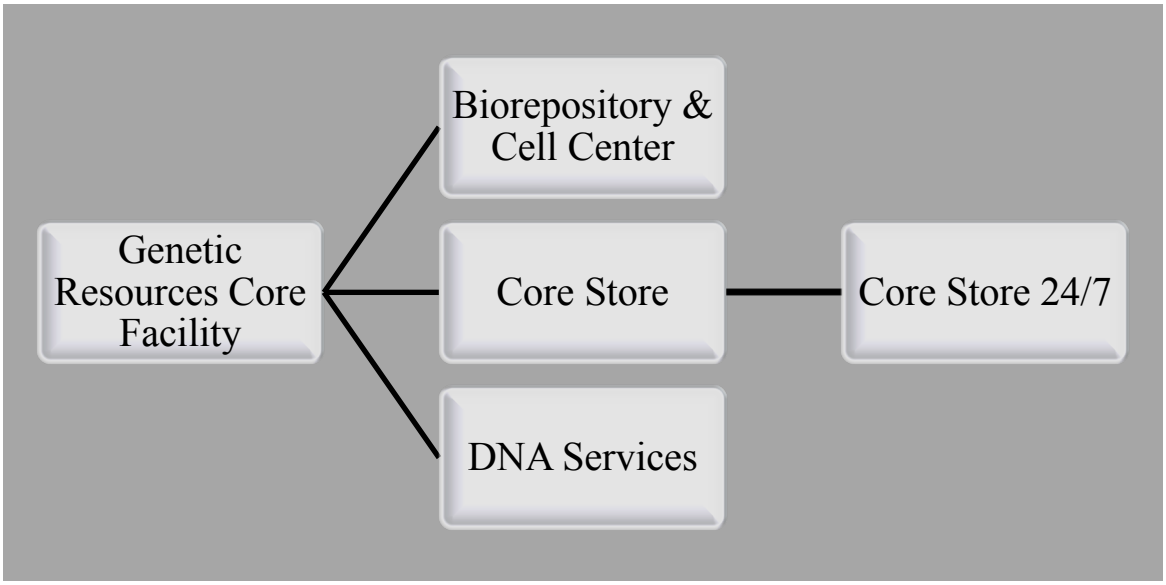
The Genetic Resources Core Facility is a JHU service center including the Core Store, Biorepository & Cell Center and the DNA Services. Collectively, these groups produce a number of products and services to aid researchers performing studies in molecular biology and genetics. It is our mission to provide 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, single cell genomics, clinical trial support and long-term cryogenic storage of biospecimens. The GRCF Biorepository & Cell Center proudly maintains the international quality and regulatory recognition of CAP (the College of American Pathologist) Accreditation. To help further support leading edge research at Johns Hopkins University, the GRCF has worked to develop a single cell genomics facility. Through the joint effort of the GRCF Biorepository & Cell Center and GRCF DNA Services we are able to offer a one-stop single cell isolation (DNA or RNA), sequencing and analysis service. For more information go to http://grcf.jhmi.edu/biorepository-cell-center/

Core Store provides one-stop shopping for more than 350,000 products from 17 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



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	lhillia1@jhmi.edu	410-502-3959
DNA Services	Blalock 1004	customorders@jhmi.edu	410-955-2836
Johns Hopkins Genomics	1812 Ashland Avenue, Suite 200 Baltimore, MD 21205	jhgenomics@jhmi.edu	410-614-8100

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

Bioprocessing

The GRCF Biorepository & Cell Center specializes in CAP qualified clinical processing support services, mammalian cell culture propagation, primary cell establishment, blood isolation products, LCL establishment and the cryopreservation of biological material. Our center has processed more than 30,000 blood specimens and serves approximately 400 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 with EBV transformation success rates at 99%.

The GRCF is excited to now offer a wide and growing range of genomics applications for single cells, including RNA-seq, gene expression profiling by qPCR and DNA amplification for whole-genome or targeted (exome or PCR-based analysis) through two workflows: 10x Genomics Chromium platform (similar to Drop Seq) and Fluidigm's C1 Single Cell Auto-Prep. Single cell genomics through the GRCF is a joint effort between the GRCF Biorepository & Cell Center and GRCF DNA services center to allow for a one-stop single cell isolation, sequencing and analysis service.

The GRCF Biorepository & Cell Center is open Monday through Friday from 8:30 am to 5:00 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. Blood dropped off after hours may be placed in the lock boxes outside the GRCF Biorepository & Cell Center door for processing the next morning. All Single Cell Genomic processing efforts must be pre-arranged with the center at least 72 hours before initiation.

In 2013 the GRCF Biorepository & Cell Center was one of the first in the nation to be recognized as a CAP accredited service facility, offering superior support in cryopreservation, cryostorage, bioshipping and bioprocessing . We are always looking to improve our services to better fit the needs of the Johns Hopkins community. If there are services you would like to see us offer in the future or want to discuss your project requirements or any concerns or comments you have for our current services we invite you to contact Melissa Olson, Ph.D. (mvolson@jhu.edu) or Kakali Sarkar, Ph. D. (ksarkar4@jhmi.edu) or bioprocessing@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 @ 5 X10^6 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: Thawing of cryopreserved lymphocytes, cell count, viability determination, ,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 of 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, cell culture maintenance, 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 fresh or frozen lymphocytes

Service includes B-cell transformation from previously cryopreserved blood lymphocytes or freshly isolated blood lymphocytes, culture expansion, and cryopreservation. Specifically includes: 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 culture maintenance for specific projects

Service includes expanding cells from your lab or ATCC according to your long-term project need and providing you with live cell flasks for your experiment. throughout your project. Cells will also be tested for mycoplasma and cryopreserved @ 5 X 10^6 cells /vial as your back up.

Single Cell Genomics

The GRCF is happy to now provide a single cell genomics service. For all project needs please arrange to discuss study design and implementation with the GRCF Biorepository & Cell Center prior to initiating the experiment.

10x Genomics Platform

Drop Seq technology as presented through the 10x Genomics Chromium Platform. This system offers a high throughput molecular barcoding and analysis suite that delivers cell-by-cell 3'end counting of mRNA transcripts for many tens of thousands of cells per run—similar to the popular DropSeq technology. This technology supports a broad range of applications, including cancer-cell transcriptomics and cell-type identification and discovery. Because the platform works with short read sequencers, it integrates easily into the existing GRCF RNAseq workflow allowing for an end to end service service including 10x data analysis. The GRCF cost for sequencing will vary based on read length.

Further Information for Single Cell Genomics can be found at: https://grcf.jhmi.edu/biorepository-cell-center/single-cell-genomics-2/

Contact us @ 410-614-5201 or bioprocessing@jhmi.edu

If you are looking for DNA Isolation, see p. 16

If you are looking for RNA isolation, see p. 29

For Cell Line Authentication, see p. 11

For Mycoplasma Testing see p. 13

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 and ambient storage options for DNA, RNA, whole blood, sera, plasma, blood spot cards 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 Friday from 8:30 am to 5 pm for convenient deposit and retrieval of specimens. A four-hour advance notice is requested for timely sample retrieval. For more information on these services please visit our website: https://grcf.jhmi.edu/biorepository-cell-center or email us at biorepository@jhmi.edu.

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
- Secure cryovial storage in subzero temperatures -20 degrees C
- Secure cryo-box storage in vapor phase liquid nitrogen (LN2) – 170 degrees C
- Secure cryo-box stoage in subzero temperatures -80 degrees C
- Secure cryo-box stoage in subzero temperatures -20 degrees C
- Secure ambient blood spot card storage
- Inventory Monitoring and specimen tracking
- On-site cryogenic storage with same day specimen retrieval
- Upon request, specimen disposal with certificate of discard
- Upon request, vial relabeling with thermal-printed, cryo-resistant labels

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.
- Specimen storage temperatures are continually monitored with 24 hour dedicated on-call staff responders.
- 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 specimen tracking are provided using a relational database.
- Every box is equipped with an indicator that serves for positional orientation and frozen status.
- Frozen indicators located among specimens routinely validate integrity of the specimen storage box itself.
- All specimens are stored in stainless steel boxes and identified with bar-coded labels made of a material that is impervious to mechanical trauma and liquid nitrogen.
- All specimen information is maintained in a searchable database that is backed up twice daily.

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. For shipping questions, please email us at http://bioshipping@jhmi.edu.

Pricing

Package Description	Package Destination	Package Dimension (inches)	Dry Ice Weight (pounds)	Total Cost*
Small	Domestic (USA), Canada, Mexico	11x9x7	6	$35.00
Medium	Europe	14x14x14	20	$75.00
Large	Asia, Africa, South America, Australia	24x16x16	60	$160.00

**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

Self-service and available at Blalock 1017, Monday – Friday, 8:30 am – 5:00 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 purchases are made using CrossLab. Please make sure you are authorized to purchase items with your budget number in CrossLab prior to coming to make your purchase. If you need help setting up your CrossLab account, detailed instructions can be found on the Core-in-a-Box website:
http://www.hopkinsmedicine.org/research/resources/synergy/core-in-a-box/finder/ilabs-support.html

Cell Line Testing

Cell Line Authentication

In light of the NIH's recommendation https://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-017.htm 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 new human 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.

The GRCF offers 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 and DSMZ databases to check the authenticity of your cell line.

This analysis is offered using Promega's GenePrint 10 System 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 8:00 AM – 4:30 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 should be sent for delivery Monday through Friday only.

Sample preparation

The GRCF accepts purified DNA (please provide >10µl at >10ng/µl of DNA), cell pellets (dried or frozen, between 100,000 and 5 million, preferred) or frozen cell aliquots (Cryopreservation tube) for analysis. A cell pellet is required for combined authentication and mycoplasma testing. 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, a graphic profile (electropherogram), and a table with the raw data for the alleles with base pair size, peak height, and peak area. The signed report provides a STR profile for the cell line submitted, it does not automatically authenticate the cell line. Authentication is determined by comparison to the STR profile of the original donor tissue or repository cell stock. The STR profile is used to verify the cell line is human, evaluate profile consistency between provisionally related cell isolates, detect cross-contamination with another human cell line (intra-species contamination), and can be used to compare to profile databases, which we strongly recommend. Additional information is provided with links to profile databases and information about interpreting your cell line results. The report is suitable for proof of cell line authentication that may be required by journals and grant funding bodies, though you will need to check the profile of your line against published profiles. If preferred, the GRCF provides databases searches with our Plus Profile Search service.

Standard Service Plus Profile Search: You will receive a signed cell line authentication report that includes a STR profile table of your cell line, an graphic profile (electropherogram), and a table with the raw data for the alleles with base pair size, peak height, and peak area, plus a database search summary of repositories ATCC and DSMZ. The report is suitable for proof of cell line authentication that may be required by journals and grant funding bodies

Turnaround time

Please see our website at https://grcf.jhmi.edu/biorepository-cell-center/bioprocessing/cell-line-authentication/ for our up-to-date schedule for processing and data delivery.

Pricing

DNA isolation (required for submission of cell pellets): $20 per sample

Standard Service

- GenePrint 10: $100/sample
- PowerPlex 18D: $140/sample

Standard Service Plus Profile Search

- GenePrint 10: $142/sample
- PowerPlex 18D: $182/sample

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

- GenePrint 10: $145/sample (Standard Service)
- GenePrint 10: $180/sample (Standard Service plus Profile Search)
- PowerPlex 18D: $179/sample (Standard Service)
- PowerPlex 18D: $214/sample (Standard Service plus Profile Search)

Payment

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

International customers using a credit card will have an additional $25 processing fee added.

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 8:00 AM – 4:30 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 should be submitted from growing culture or as frozen ampoules. We accept pelleted cells, supernatant or frozen stocks. The GRCF accepts 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: We recommend passage of 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. 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: Use a cell scraper to dissociated cells from the substrate. Do not treat with trypsin or other enzymatic agents for dissociation. Collect 5 to 7 ml of medium in a T25 flask or 8 to 10 ml in a T75 flask. Centrifuge to pellet cells, then remove supernatant.

Supernatant: Remove 5 ml – 10 ml supernatant from flask to a 15ml conical tube. Parrafin the top if shipping.

Frozen stock: Ampoule may be delivered at room temperature.

Turnaround time

Please see our website at https://grcf.jhmi.edu/biorepository-cell-center/bioprocessing/mycoplasma-detection/ for our up-to-date schedule for processing and data delivery

Pricing

Mycoplasma testing only: $70/sample

Mycoplasma AND Cell Line Authentication (STR profiling): A cell pellet is required for combined service, extracted DNA is not suitable.

- GenePrint 10: $145/sample (Standard Service)
- GenePrint 10: $180/sample (Standard Service plus Profile Search)
- PowerPlex 18D: $179/sample (Standard Service)
- PowerPlex 18D: $214/sample (Standard Service plus Profile Search)

Payment

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

International customers using a credit card will have an additional $25 processing fee added.

Please see our website at 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 ThermoFisher's 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

$26/chip*

*ThermoFisher 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 topics.

Bisulfite 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.

- 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 Q48 Methylation Studies

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

Pricing and Ordering

EpiTect Bisulfite conversion: $15/sample. EZ DNA Methylation conversion 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, PBMCs, packed cells, cultured cells, blood spot cards, FFPE samples, buccal swabs/brushes, mouthwash, Oragene saliva collection kits, and tissue.

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

DNA is returned 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 using a Fragment Analyzer (Advanced Analytical Technologies, Inc.) is available. See our website at https://grcf.jhmi.edu/dna-services/rna-services/nucleic-acid-qualitative-analysis/ for more information. Storage of blood on Whatman FTA Classic Cards for archiving is available.

Pricing

Prices range from $25 to $47 per sample. Volume pricing is available for large study projects.

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 labelled with your GeneSifter tracking number, Monday – Friday from 8:00AM – 4:30 PM.

External Customers: Send the samples labelled with the GeneSifter tracking 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 Plating

Sample plating includes organization of samples, diluting DNA to specified concentration, and aliquoting samples into 96-well plates. Samples must be supplied with concentration and electronic inventory.

If samples lack a concentration, Nanodrop or pico green analysis is available for an additional fee.

Pricing and Ordering

$250 per 96-well plate. Includes standard PCR plate. Matrix plates are available for an additional fee. Matrix plates are provided to CIDR project customers at no additional charge.

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

Whole Genome Amplification

Whole genome amplification (WGA) of extracted DNA is carried out using REPLI-g kits from Qiagen. The kits use 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. 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.

We accept genomic DNA, fresh or frozen whole blood, packed cells, blood spot cards, cultured cells and FFPE. A variety of REPLI-g kits are available. See Qiagen's selection guide. Concentration is determined by pico green analysis using a Spectra Max Gemini XS fluorometer. Concentrations will be returned in an Excel document.

Pricing

Pricing is based on number of samples and kit selected. Please contact Laura Kasch 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 ten 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), single nucleotide polymorphisms (SNP), and insertion/deletions.

We have developed numerous assays for VNTRs, insertion/deletion polymorphisms, STRs and SNPs. Below is a partial list.

Gene	Gene Symbol	Description
Apolipoprotein E	APOE	112T>C and 158C>T (E2, E3, E4 alleles)
Dopamine Receptor D4	DRD4	48bp VNTR
Dopamine Transporter	DAT/SLC6A3	40bp VNTR in 3'-untranslated region
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 a quote.

Medium Throughput Custom Genotyping

Taqman OpenArray Chemistry from Life Technologies

The OpenArray technology spots off the shelf or custom Taqman assays into 33nl through-holes on arrays, which allows many assays to be tested on up to 144 samples at once. Chips can assay 12, 26, 60, 120 or 240 SNPs at once.

Pricing

A minimum of 10 chips must be ordered, but for 12 or 26 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.

PLINK output and detailed metrics (error rates, unexpected duplicate analysis, etc) are available for this service for an additional $500.

Chip Format	Number of Samples/Chip	Minimum # of Chips (Samples)	Price	Total cost
12 SNPs	144	10 (1440)	Chips: $5,728 Other costs: $2100	$8,102
26 SNPs	96	10 (960)	Chips: $5,728 Other Costs: $2,000	$8,002
60 SNPs	48	20 (960)	Chips: $11,456 Other costs: $2,700	$14,594
120 SNPs	24	40 (960)	Chips: $22,912 Other costs $5,400	$29,188
180 SNPs	16	60 (960)	Chips: $34,368 Other costs: $8,100	$43,782
240 SNPs	12	80 (960)	Chips: $45,824 Other costs: $10,800	$58,376

High Throughput Custom Genotyping

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.

Genome Wide Association

Cytogenetics

Human CytoSNP12 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.

Human CytoSNP850 Chip: The HumanCytoSNP-850 BeadChip features nearly 850,000 SNPs that have been chosen with input from the international cytogenetics community and peer-reviewed literature to optimize used for constitutional and cancer studies. Coverage is enriched for dosage sensitive genes and designed to pick up challenging mosaics.

Pricing*

Number of Samples	CytoSNP12	CytoSNP850
49 or 94	$305/sample	$365/sample
140-558	$280/sample	$330/sample
>558	Please inquire	Please Inquire
FFPE restore	Add $100/sample	Add $100/sample

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	$120/sample	$125/sample
94 - 5000	$110/sample	$115/sample
>5001	Please inquire	Please inquire

Global Screening Array

Human Global Screening Array: This array combines multi-ethnic genome-wide content, curated clinical research variants, and quality control (QC) markers for precision medicine research. The 640,000 markers were selected for high imputation accuracy at minor allele frequencies of >1% across all 26 1000 Genomes Project populations. The clinical research content includes variants with established disease associations, relevant pharmacogenomics markers, and curated exonic content based on ClinVar, NHGRI, PharmGKB, and ExAC databases. Quality control content enables sample identification and tracking for large-scale genomics and screening applications. Up to 50,000 custom SNPs may be added to the array for an additional charge.

Pricing*

Number of Samples	Standard Content
93	$120/sample
94 - 5000	$110sample
>5001	Please inquire

Human Multi-Ethnic Global Array (MEGA) Product Line

Human Multi-Ethnic Global Array: Consortium partners developed content for this multi-ethnic global genotyping array using tagging strategies with the power to perform more effective association studies in diverse populations. The novel algorithm selects population-specific and transethnic tag SNPs that maximize imputation accuracy, as imputation has become a standard practice in the interpretation of genotyping data and allows for more accurate statistical inference of genotypes not directly genotyped.

Human Multi-Ethnic EUR/EAS/SAS BeadChip: The Infinium Multi-Ethnic EUR/EAS/SAS chip provides a multi-purpose, multi-ethnic genotyping array focused on European, East Asian, and South Asian populations.

Human Multi-Ethnic AMR/AFR BeadChip: The Infinium Multi-Ethnic AMR/AFR chip provides a multi-purpose, multi-ethnic genotyping array focused on Hispanic and African American populations.

With > 1.4 million expertly selected markers, either of these arrays enable identification of genetic associations with common and rare traits, providing insight to epidemiologists, health care researchers, population geneticists, and genomic researchers.

Pricing*

Number of Samples	Standard Content	Regional only content (either array)
93	$205/sample	$195/sample
94 - 5000	$185/sample	$175/sample
>5001	Please inquire	Please inquire

Human Omni Product Line

HumanOmni Express, 2.5 and 5M chips are still available as products, but are used less frequently due to the informatic improvements made when selecting SNPs for the newer arrays. If you have a project that requires one of them, please inquire for pricing.

***Pricing Details**

For GWAS projects with more than 94 samples, our full service pricing includes:

- The Illumina QC panel (15,000 SNPs used to barcode the sample and verify the GWAS dataset released matches the sample performance).
- Identification of problems and replacement of problem samples before running 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 Illumina array. We attempt the GWAS assay only twice.
- Lower levels of service available at reduced costs on some products. Please see web-site for details (http://grcf.jhmi.edu/dna-services/genotyping/genome-wide-association/).

Non-Human Genotyping

Illumina has products available for several different species, including bovine, canine, ovine, porcine and maize. Additionally, custom panels maybe created for genotyping species with DNA on any of our genotyping platforms.

Mouse Genotyping

The GRCF offers mouse genotyping service from tail snips or ear punches to determine wild-type, heterozygous and homozygous alleles of genetically modified mice. Other tissues can be utilized, please inquire.

The GRCF will optimize PCR conditions with your primers for end-point PCR agarose gel electrophoresis and analysis. An internal control primer set can be included to exclude PCR failure or problems with DNA quality.

We use a KAPA Mouse Genotyping Kit for DNA isolation and PCR.

Pricing: $10 per sample per primer set (DNA extraction and genotyping)

Mouse GigaMUGA Linkage Array

This product is the latest in consortium developed mouse linkage arrays.

Pricing

Number of Samples	Standard content
84 – 2000	$185/sample
>2001	Please inquire

Targeted Content Products

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/.

Fingerprinting by Taqman Barcode Panel

This OpenArray panel consists of 64 markers, including 3 Y-chromosome markers that allow for unique identification of individuals, though it may not be sensitive enough to distinguish siblings from each other. Markers were selected to have high Minor Allele Frequency (MAF) in multiple ethnic groups, so this chip cannot be used for distinguishing between populations. It is useful as an internal control for GWAS studies and next-gen sequencing projects. Each chip runs 48 samples.

Pricing

- 1 chip: $1,175
- 2 chips: $1,960
- 3 chips: $2,750
- 4 chips: $3,530

Discounts available for larger projects. Please email Roxann Ashworth (rashwor2@jhmi.edu) for pricing.

Fingerprinting or Human Linkage by Illumina QC Array

The Illumina QC array contains 15,949 markers which are useful for biobanking, fingerprinting, ethnic ancestry determination and QC. The array contains all of the SNPs which were on the former human linkage array, so it can also be used for linkage studies. It also included ADME content.

Pricing

Number of Samples	Standard content
No minimum or maximum	$45/sample

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	$116/sample
93 – 5000	$105/sample
>5001	Please inquire

Specialized Consortium Arrays

We are happy to work with you to genotype the consortium or commercial version of any of Illumina's specialized arrays. These offerings are constantly changing and pricing and rules for access vary by array. Please contact us if you are interested in running any of the current offerings, which include (but are not limited to): the Onco-Array, the Psych Array, and the H3Africa Array.

Pricing

Please contact Roxann Ashworth (410-614-0702; rashwor2@jhmi.edu) for pricing. Please include the name of the array of interest and approximate sample numbers in your email, as pricing is dependent on the number of samples. Minimum sample number is at least 92, but some arrays may have higher minimums.

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. Price includes data cleaning and returned SNP calls.

Pricing

- 1 chip: $1,120
- 2 chips: $1,850
- 3 chips: $2,570
- 4 chips: $3,300
- Discounts available for projects of 11 or more chips. Please email Roxann Ashworth (rashwor2@jhmi.edu) for pricing.

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. Two Life Technologies QuantStudio 12K Flex instruments are available for use by appointment, in Blalock 1003. The instruments accommodate 96 & 384 well plates as well as Taqman Low Density array (TLDA) microfluidic cards. Both 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 using iLab. Detailed instructions for making the reservations and setting up your iLab account are available on our website: https://grcf.jhmi.edu/dna-services/rna-services/real-time-pcr-equipment/. 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.

Pricing:

- $20 per hour; $10 for a 15 minute end-point read.

Methylation

Genome Wide Analysis

EPIC (850K) Infinium Methylation BeadChip: Illumina's new 850K Methylation chip is not reliant on Me-DIP (Methylated DNA immunoprecipitation). In addition to 90% of the content of the former 450K chip, this chip covers CpG sites outside of CpG islands, non-CpG methylated sites, tumor/normal differentially methylated sites, FANTOM5 enhancers, ENCODE open chromatin & enhancers, DNase hypersensitive sites, and miRNA promoter regions. A list of all CpG sites is available for download. Bisulfite conversion is included in pricing for projects with more than one full plate. Fully methylated and non-methylated controls are assayed at no additional charge.

Pricing

Number of Samples	Standard Content	Standard Content + FFPE restore
46 or less	$500/sample	$600/sample
multiples of 47	$420/sample	$520/sample

Targeted Analysis

Analysis via Pyrosequencing

Specific regions of methylation can be assayed via sequencing on the Pyromark Q48 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 30,000 human and rodent predesigned kits can be ordered from Qiagen. Primers ordered through us receive a slight discount and free shipping. Primer design software is available for use by customers

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

Pricing

Number of Samples	Advanced Reactions
<47 samples	$10.25/sample
Samples in multiples of 47	$10.00/sample

PCR support and bisulfite conversion is available for Pyrosequencing projects
Service includes bisulfite 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:

Pricing is sample number dependent. Please inquire with Laura Kasch [lkasch@jhmi.edu; 410-614-3830]

Bisulfite Conversion

Bisulfite conversion services are available. Please see p. 16 for details

RNA Services

cDNA Synthesis for MicroRNA Profiling and Gene Expression Taqman Arrays

The GRCF offers cDNA synthesis to support customers requesting Taqman OpenArray MicroRNA Profiling Analysis or custom and fixed content Gene Expression Array analysis on the QuantStudio 12K Flex Real-Time PCR System. cDNA synthesis is carried out following recommended protocols using customer supplied total RNA or RNA isolated by GRCF DNA Services for 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) or RNA Quality Number (RQN) 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 RNA samples is available by Advanced Analytical Fragment Analyzer CE analysis. The system determines a RQN (RNA Quality Number) of total RNA on a scale from 1 to 10 that correlates to current industry practices (RIN).

Pricing

cDNA synthesis for custom and fixed content Gene Expression Array analysis using Superscript VILO (Life Technologies part # 4453650: $25 per sample

cDNA synthesis for MicroRNA profiling using High Capacity cDNA Reverse Transcription Kit (Life Technologies part # 4368813): $27 per sample for A and B pools

Ordering

Please contact Laura Kasch [410-614-3830] for 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 48 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 number of gene expression assays and sample numbers. Chips that require custom design assays 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]. 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: $7,657 Other costs: $1,260	$8,917
56 Assays	48	480	Chips: $7,845.50 Other costs: $1,260	$9,105.50
112 Assays	24	240	Chips: $10,136.50 Other costs: $1,260	$12,137.50
168 Assays	16	160	Chips: $10,877.50 Other costs $1,260	$12,137.50
224 Assay	12	120	Chips: $11,970 Other costs: $1,260	$13,230

*18 assay-formatted chips are plated in triplicate, so each sample is used once, but run 3 times.
**Pricing is for chips of entirely inventoried assays. Chips made with some or all custom assays will be about $4,000 more expensive.

Fixed Content Gene Expression Arrays

ThermoFisher/Life Technologies makes several different fixed content array panels for use on the OpenArray system. These chips can be ordered one at a time, and typically contain assays for specific pathways along with several endogenous controls.

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 (Discounts available for projects of 11 or more chips.**)**

1 chip (48 samples)	2 chips	3 chips	4 chips
$710	$1,200	$1,690	$2,180

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*	2 chips	3 chips	4 chips**
$920	$1,620	$2,320	$3,020

*Sample number varies by pathway. See descriptions above for specifics.
** Discounts available for projects of 11 or more of the same chip. Contact us at customorders@jhmi.edu for more information.

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. Please contact the GRCF at 410-955-2836 or customorders@jhmi.edu.

Pricing*

Number of Samples	Standard service
3*	$860
6	$1,620
9	$2,380
12	$3,140

For pricing for projects of more than 300 samples, or if you wish to do the preamplification yourself, please contact the GRCF at 410-955-2836 or customorders@jhmi.edu. cDNA synthesis is also available. Please contact us for pricing.

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

Real Time PCR Equipment (Self Service)

Two Life Technologies QuantStudio 12K Flex instruments are available for use by appointment, in Blalock 1005. The instruments accommodate 96 & 384 well plates as well as Taqman Low Density array (TLDA) microfluidic cards. Both 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. Reservations are scheduled on a first come, first served basis using iLab. Detailed instructions for making the reservations and setting up your iLab account are available on our website: https://grcf.jhmi.edu/dna-services/rna-services/real-time-pcr-equipment/. 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.

Pricing

- $20 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.

Qualitative analysis using a Fragment Analyzer (Advanced Analytical Technologies, Inc.) is available. See our website at https://grcf.jhmi.edu/dna-services/rna-services/nucleic-acid-qualitative-analysis/ for more information. $14 per sample. Discounts for full runs (10 samples) or full plates (92 samples). The system gives results similar and comparable to an Agilent BioAnalyzer. Advanced Analytical's PROSize software determines an RQN (RNA Quality Number) on a scale from 1-10. Nanodrop analysis is also available.

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

Pricing

Source	Sample Size	Yield	Charge
Blood	PAXgene collection tube	> 3µg (total RNA)	$30 (RNeasy Plus Kit)
Tissue	<30mg	up to 100µg total RNA	$32 (RNeasy Plus Kit)
Tissue	< 30mg	up to 100µg total RNA plus miRNA and small RNA	$40 (miRNeasy Kit)
Cultured Cells	< 1 x 10E7 cells	up to 35µg	$27 (RNeasy Plus Kit)
Cultured Cells	< 1 x 10E7 cells	total RNA plus miRNA and small RNA	$35 (miRNeasy Kit)
Plasma/Serum	Up to 240ul	total RNA	$35 (RNeasy Plus Kit)

JH Customers: Drop off on dry ice in Blalock 1004, Monday-Friday from 9 am – 11 am

RNAseq

The GRCF offers QC, library prep and sequencing of RNA. 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 and per flowcell 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:

https://www.encodeproject.org/about/experiment-guidelines/

Sample Requirements

Please provide us with high quality total RNA (RIN >8.5 if possible, but lower values will still work). 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 collaborate with the JHU Computational Biology Consulitng Core for RNASeq analysis. Please visit their website for pricing information: http://ccb.jhu.edu/cbcc/index.shtml.

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 a NovaSeq6000, two HiSeq 2500 instruments and two MiSeq instruments. We do our best to sequence samples in a timely manner, but our priority is producing high quality data.

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

NEW: NovaSeq6000

Illumina's NovaSeq6000 System uses patterned flowcell technology, exclusion amplification, and improved 2 color sequencing chemistry to deliver massive increases in throughput, all while lowering run costs. NovaSeq runs are fixed, but include additional cycles for dual indexed libraries. A cycle is the equivalent of basepair. For example, a 100 cycle kit can be run as paired end 50bp.

S1 Flowcell: ~3.2 billion paired reads, ~1.6 billion single reads

- 100 cycle: $6700
- 200 cycle: $8700
- 300 cycle: $9900

S2 Flowcell: ~6.6 billion paired reads, ~3.3 billion single reads

- 100 cycle: $11,300
- 200 cycle: $15,000
- 300 cycle: $17,100

S4 Flowcell: ~20 billion paired reads, ~10 billion single reads

- 300 cycle: $33,000

COMING SOON: SP Flowcell, ~1.6 billion paired reads, ~800 million single reads.

HiSeq2500 RAPID FLOWCELL PRICING

If you submit a single pool for sequencing on a single Rapid Flowcell (ie. 2 lanes), you can take advantage of lower pricing and fast turnaround times. All barcodes must be unique, and all samples must be in a single pool.

Per Flowcell Pricing

Single Read Flowcell: ~300 million reads

- 50 bp: $2000 per flowcell
- 75 bp: $2400 per flowcell
- 100 bp: $2800 per flowcell
- 150 bp: $3600 per flowcell

Paired End Flowcell: ~600 million reads

- 50 bp (x2): $3400 per flowcell
- 75 bp (x2): $4000 per flowcell
- 100 bp (x2): $4400 per flowcell
- 150 bp (x2): $5400 per flowcell

Per Lane Pricing

Single Reads:

- **50 cycles:** $1,200 per lane
- **100 cycles:** $1,600 per lane
- **150 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.

Sample Requirements

Completed Libraries: Please submit a minimum of 10 ul of your sample at 2nM. Libraries must be pooled as you would like them sequenced. We will do a QC check via Bioanalyzer to confirm library concentration and quality, 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 and pricing.

Data Yield

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.

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

Whole Genome: pipeline data returned via aspera server

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 10µl of your sample at 2nM. Samples must be pooled.

Data Delivery

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

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 Q48 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.genesifter.net/gsle/mainPage. Samples must be dropped off before 4:30. Results will be available on the JHU ordering server within two business days.

Pricing

Number of Samples	Advanced Reactions
<47 samples	$10.25/sample
Samples in multiples of 47	$10.00/sample

Sanger Sequencing

Sanger sequencing services are provided using the Applied Biosystems 3730xl DNA Analyzer. These instruments 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 off 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. Samples should be in our remote locations by the following times: Asthma and Allergy Building at Bayview by 11:00 AM, in Croft Hall and UTL on the Homewood campus by noon, and in CRB I B02A by 3:00 PM, but pickup times may vary depending on staffing. Data release will depend on the pickup schedule of the day concerned.

Same 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 be released by 5 PM.

Pricing

- Single pre-mixed template & primer: $6.25
- 95 samples in a 96-well plate: $6.00 per sample.
- BAC protocol reactions (used on BACs, other large templates or very GC rich templates): $22
- Same day service: $25 per sample, minimum of 5 samples

PCR Amplification Support Services

Pyrosequencing Projects

PCR amplification support for Pyrosequencing methylation studies includes PCR amplification using customer supplied Qiagen predesigned or custom kits, amplification confirmation by agarose gel electrophoresis and submission for Pyrosequencing. Please contact GRCF DNA Services customorders@jhmi.edu or call 410-955-2836 to initiate a custom project.

Sample Requirements

Pyrosequencing (methylation analysis): 40 ng at 5 ng/ul to 10 ng/µl of bisulfite converted DNA per amplicon. If submitting genomic DNA for bisulfite conversion, we recommend converting a minimum of 100ng.

Pyrosequencing (SNP analysis, mutation detection, sequencing): 40 ng at 5 ng/µl to 10 ng/µl per amplicon.

Sanger Sequencing Projects

PCR support is available for Sanger sequencing projects. Service includes primer design, development of PCR amplification conditions, amplification of samples, amplification confirmation by agarose gel electrophoresis, and submission of samples for Sanger sequencing. Please contact GRCF DNA Services customorders@jhmi.edu or call 410-955-2836 to initiate a custom project.

Sample Requirements: 60ng at 10-20ng/µl per amplicon

Pricing

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

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 Twist Biosciences for capture probes.

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

Pricing

Please enquire. Pricing is highly dependent on number of

Our defalut metric is >90% targeted at 20x. If project needs require a different depth metric, sequencing can be adjusted accordingly.

Custom capture is also available. Capture sizes can range from 500Kb and up.

Sample Requirements

- 200ng-2.5ug 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 now offers whole genome sequencing on the NovaSeq 6000.

Human Whole Genome

Human genome sequencing at 30x average coverage, from short-insert paired-end reads using a single library.

Sample Requirements	Input DNA amount: 1ug high molecular weight genomic DNA for PCR free (preferred), 500ng for PCR based methods
Deliverables	▪ Genomic coverage at a minimum of 30x ▪ GATK best practice called sequence variants: (SNPS, CNV's, small indel) ▪ multisample calling (if applicable) ▪ Annotation of SNPs and indels in VCF format (dbSNP, gene, exon, transcript) ▪ Concordance with whole-genome genotyping arrays ▪ Raw data (reads and quality scores) and analyzed data in industry-standard format (archival BAM)
Pricing	$1500/genome, highly dependent upon sample number

Linked Read Whole Genome

10X linked read whole genome sequencing at 30x average coverage, from 10X GemCode library, short-insert paired-end reads.

Sample Requirements	Input DNA amount: 1.2ng high molecular weight genomic DNA (>50Kb minimum)
Deliverables	▪ Genomic coverage at a minimum of 30x ▪ Whole Genome Phasing and SV calling via 10X Longranger Pipeline ▪ SNP/short indel calls using GATK/Freebayes ▪ Concordance with whole-genome genotyping arrays ▪ Annotation of SNPs and indels in VCF format (dbSNP, gene, exon, transcript) ▪ Raw data (reads and quality scores) and analyzed data in industry-standard format (archival BAM). ▪ Loupe browser file
Pricing	$3000/genome, highly dependent upon sample number

We also offer de novo whole genome assembly using 10X linked reads. Please inquire if interested, as pricing depends on genome size and complexity.

Single Cell Genomics

The GRCF offers a wide and growing range of genomics applications for single cells, including RNA-seq, gene expression profiling by qPCR and DNA amplification for whole-genome or targeted (exome or PCR-based analysis) through two workflows: **10x Genomics Chromium** platform (similar to Drop Seq) and **Fluidigm's C1** Single Cell Auto-Prep.

10x Genomics Chromium

> Drop Seq technology as presented through the **10x Genomics Chromium Platform** offers a high throughput molecular barcoding and analysis suite that delivers cell-by-cell 3'end counting of mRNA transcripts for thousands of cells per run. This technology supports a broad range of applications, including cancer-cell transcriptomics and cell-type identification and discovery. Because the platform works with short read sequencers, it integrates easily into the existing GRCF RNAseq workflow allowing for an end to end service.

Single cell genomics through the GRCF is a joint effort between the GRCF Biorepository & Cell Center and GRCF DNA services center to allow for a one-stop single cell isolation, sequencing and analysis service. All single cell genomic service requests must be prearranged to ensure a smooth capture.

Contact us today (bioprocessing@jhmi.edu or 410-614-5201) for more information or to schedule for your experiment!

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 9:00am – 5:00pm (Closed from 1:00pm – 1:30pm 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
Email: jhucorestore@jhmi.edu
Website: grcf.jhmi.edu/core-store/

CRISPRs

CRISPRs have transformed the world of genome editing, opening up great potential and exciting opportunities for understanding the relationships between genes and phenotypes. At the GRCF, we have partnered with three innovative companies to provide you a broad range of products to assist you in every aspect of this rapidly moving field. Dharmacon and IDT both provide online tools for design assistance. MilliporeSigma, through our CRISPR Core Partnership provides detailed, in-person assistance. For more information on various products and types of assistance offered by each company, see their specific pages on our web-site: https://grcf.jhmi.edu/products/crisprs/

Pricing:

Pricing is dependent on product and company selected. See the information for the individual companies in the menu on the left.

Ordering*:

Dharmacon: Order through our portal: https://dharmacon.gelifesciences.com/johns-hopkins-university-grcf/

IDT: Order through our portal: https://www.idtdna.com/johnshopkins

MilliporeSigma: If you design your own CRISPRS, you can order them using our iLab interface: https://johnshopkins.corefacilities.org/service_center/3774/?tab=services

If you need assistance with your designs, you can reach out to our MilliporeSigma representatives (Michelle Laird: michelle.laird@sial.com and Zulfiquer Hossain (mohammad.hossain@sial.com) or use the ordering instructions on our web-site (https://grcf.jhmi.edu/products/crisprs/), complete the pre-designed or custom order form and email it to customorders@jhmi.edu, any day of the week.

**Ordering lentivirus vectors from any of these vendors requires that investigators have a current recombinant DNA and infectious agent registration for this material from the Johns Hopkins Biosafety Office, http://www.hopkinsmedicine.org/hse/biosafety/*

Genes and Gene Fragments

Synthetic Genes

DNA synthesis technology now allows entire genes to be synthesized and inserted into a vector, ready for cloning and other applications. This can be especially 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 typically take between 8 and 25 days to ship, depending on the length and difficulty of the sequence.

Genes may be ordered from IDT through the Johns Hopkins University portal:
http://www.idtdna.com/JohnsHopkins/

IDT adheres to the International Gene Synthesis Consortium's protocols and screens the sequences to identify regulated and potentially dangerous pathogen sequences.

Pricing:

Size	Price
Mini-genes <500 bp	$198.00 (flat fee)
Genes (501-1500 bp)	$0.45/base
Genes (1500 - 3000 bp)	$0.40/base
Genes (3001-5000 bp)	$0.40/base
Genes (5001+ bp)	$0.54/base

gBlocks® Gene Fragments

gBlocks® Gene Fragments are double-stranded, sequence-verified genomic blocks that ship in only a few working days for affordable and easy gene construction or modification, applications such as antibody research and CRISPR-mediated genome editing, use as qPCR standards, and more.

gBlocks® Gene Fragments Libraries are pools of gBlocks Gene Fragments that contain up to 18 consecutive variable bases (N or K) for recombinant antibody generation or protein engineering.

gBlocks® may be ordered from IDT through the Johns Hopkins University portal:
http://www.idtdna.com/JohnsHopkins/

IDT adheres to the International Gene Synthesis Consortium's protocols and screens the sequences to identify regulated and potentially dangerous pathogen sequences.

Pricing

Size	Price
125-250 bp	$89
251-500 bp	$89
501-750 bp	$129
751-1000 bp	$149
Larger sizes also available	See web-site

Oligonucleotides

The DNA Analysis Facility offers the synthesis of oligonucleotides through several vendors, including IDT and MilliporeSigma. 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.

IDT: http://www.idtdna.com/JohnsHopkins/
MilliporeSigma: 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, delivered in lyophilized in tubes. All MilliporeSigma oligos ordered before 1 PM eastern time are shipped as "same day" oligos, arriving within 24 hours of ordering, at no extra charge. IDT ships their standard oligos to arrive within 48 hours of ordering. They offer "same day" oligos with higher pricing then listed below and charge shipping. See each company's portal for pricing.

Oligo orders must be placed by 1 p.m. for standard shipping times to apply.

Pricing

Company	Synthesis Scale					
	25 nmole*	50 nmole*	100 nmole*	200 nmole*	250 nmole*	1 µmol**
IDT	$0.15	N/A	$0.36	N/A	$0.58	$0.96
MilliporeSigma	$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					
	25 nmole	50 nmole	100 nmole	200 nmole	250 nmole	1 µmol
IDT	15-60mer	N/A	10-90mer	N/A	5-100mer	5-100mer
MilliporeSigma	2-60mer	2-120mer	N/A	2-120mer	N/A	2-120mer

Guaranteed Minimum Yield

Company	Minimum Yield (OD/µg)					
	25 nmole	50 nmole	100 nmole	200 nmole	250 nmole	1 µmol
IDT	3.0	N/A	6.0	N/A	15.0	45.0
MilliporeSigma	3.0	5.0	N/A	12.0	N/A	40.0

Long Oligos

IDT offer long oligos up to 200 bp in length. Ultramers can be PAGE purified for an additional fee. Purification will lower yield.

MilliporeSigma offers long oligo synthesis up to 180 bp. Any sequence >120 bp is reviewed by a scientist for synthesis feasibility. To maintain sequence purification >95% and minimize truncation, the minimum scale for long oligos is 0.2 umol and PAGE purification. Please email (customorders@jhmi.edu) your requests to us, and we will have MilliporeSigma provide a custom quote.

Pricing

Company	Synthesis Yield		Purification
	3-4 nmole	20 nmole	HPLC
IDT	$0.65/base	$1.34/base	$94.50
MilliporeSigma	Ask for quote	Ask for quote	Included in quote

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. RNA oligos usually arrive six business days after ordering, purified oligos take longer. For more information, see the company portals, below.

IDT: http://www.idtdna.com/JohnsHopkins/

MilliporeSigma: http://www.sigmaaldrich.com/configurator/servlet/DesignCenter

Pricing

Company	RNA base				
	50 nmole	100 nmole	200 nmole	250 nmole	1 µmole
IDT	N/A	$5.69	N/A	$7.44	$17.75
MilliporeSigma	$2.82	N/A	$3.62	N/A	$15.75

*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.

RNA Interference (RNAi, si/shRNA)

Custom siRNA

Custom siRNAs can be used in cell culture experiments to silence genes. They can be ordered Simplex or Duplex, from IDT and MilliporeSigma. 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:

Dharmacon: https://dharmacon.gelifesciences.com/johns-hopkins-university-grcf/

IDT: http://www.idtdna.com/JohnsHopkins/

MilliporeSigma: http://www.sigmaaldrich.com/configurator/servlet/DesignCenter

Pricing

siRNA, price/base	IDT		MilliporeSigma		
	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

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

Predesigned siRNA/shRNA

Dharmacon, IDT, Life Technologies, Qiagen and MilliporeSigma 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:

Dharmacon: https://dharmacon.gelifesciences.com/johns-hopkins-university-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 and PrimeTime Assays

PrimeTime qPCR Assays

PrimeTime qPCR assays come in two formats: Probe based assays and primer only assays.

- Probe based assays may be ordered with a variety of dye and quencher combinations, in three different scales: 100, 500 or 2500 reactions. Predesigned human, mouse and rat assays are available, and IDT also provides tools to help with the design of custom assays.
- Primer only assays are ideal for using with intercalating dye experiments. Each order of these primers is enough for 500 reactions.

Molecular Beacon assays (highly specific, dual labeled, hairpin based probes) are also available with a variety of dye/quencher combinations.

Order now: http://www.idtdna.com/JohnsHopkins/

Surveyor Mutation Assays

Designed to detect mutations in DNA derived from a variety of organisms including bacteria, fungi, plants, and animals, Surveyor® Mutation Detection Kits allow analysis by:

- Standard gel electrophoresis
- WAVE® and WAVE HS Systems

Surveyor Nuclease is provided as a standardized, quality-controlled enzyme formulation in a ready-to-use kit that can be used without further batch-to-batch optimization. All components other than the thermostable DNA polymerase and PCR products are provided. Reaction buffers and positive controls included in the kit allow users to set up and monitor the assays to obtain reproducible results. In-depth Surveyor Mutation Detection Kit User Guides provide detailed instructions and troubleshooting.

Two types of Surveyor Mutation Detection Kits are available:

- Surveyor Mutation Detection Kit for Standard Gel Electrophoresis—S25 (25 rxn), S100 (100 rxn), S1000 (1000 rxn)
- Surveyor Mutation Detection Kit for WAVE® and WAVE HS Systems—W25 (25 rxn), W100 (100 rxn), W1000 (1000 rxn)

Order now: http://www.idtdna.com/JohnsHopkins/

Taqman Assays

ThermoFisher/Life Technologies custom products are 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.