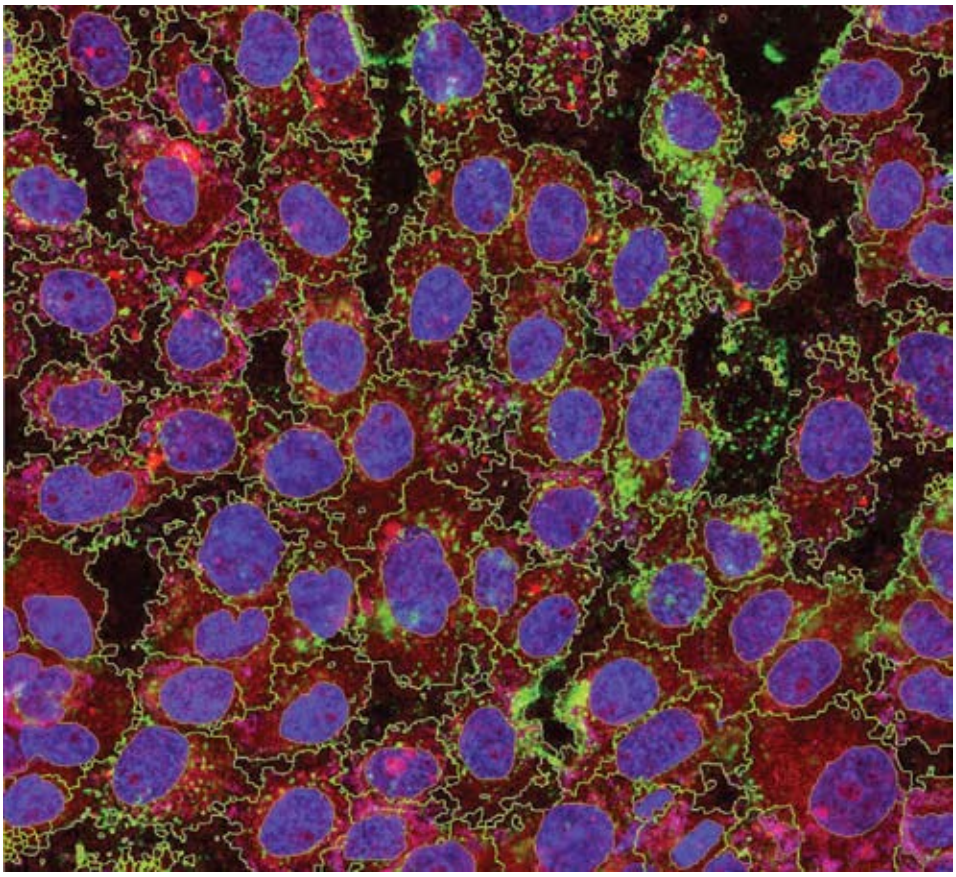
Cell Collections

A Publication of the Coriell Cell Repositories
2007/2008



Using Locational Proteomics to Study Cell Signaling

Confocal assay using green Quantum dot stains to study EGF-receptor internalization. The image is computer processed for nuclei and cytoplasmic boundary detection to study local protein concentrations.

In This Issue:

2006-2007: A Year of Challenge, Opportunity, and Great Promise

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The past year has been a time of continued growth and the promise of exciting new opportunities. It has been a year of optimism and challenge as Coriell welcomes Dr. Michael F. Christman, its new president, who will guide the Institute following the principles of excellence established by Dr. Lewis L. Coriell at the Institute's inception in 1953. Dr. Christman is pursuing research in genetics and genomics through Coriell's initiative in personalized medicine, the foundation of which is the newly established Coriell Genotyping and Microarray Center.

On behalf of the Board of Trustees and Coriell Staff, I am pleased to share several highlights of the year 2006/2007 that exemplify Coriell's ongoing success in scientific research, in the banking and storage of cells, and in the commitment to education.

Fall 2006

- The consulting firm of Schultz and Williams was retained to promote Coriell's Development program, most notably major gifts and endowment giving.
- Two new scientists joined the Coriell Faculty: Margaret Keller, Ph.D., Principal Investigator - New Jersey Stem Cell Resource and Roderick Corriveau, Ph.D., Principal Investigator of NINDS Human Genetic Resource Center / DNA and Cell Line Repository at Coriell.

Winter 2006

- The Governor signed P.L. 2006, Chapter 102 on December 20, 2006, authorizing stem cell research facilities in New Jersey. Coriell was named as one of four partners to operate this $50M laboratory.
- Coriell Institute received an award of $3.1 million from the National Human Genome Research Institute (NHGRI) to establish a sample repository for the international HapMap project. It will be used for the study of human genetic variation.
- Coriell continues its global presence through its licensing agreement with BioRep, Srl, Milan, Italy.

Spring 2007

- Coriell hosted the NJ Spinal Cord Commission Program: "Genetics of Neurodegenerative Disorders."
- Coriell Institute sponsored the 26th Annual Science Fair. Ninety judges and 280 students participated. Forty-two Fair winners competed in the Delaware Valley Science Fair held in Valley Forge, Pennsylvania, and one student was a semi-finalist at the International Science Fair in Albuquerque, New Mexico.
- The New Jersey Technology Council (NJTC) recognized Josefina Nash, Director of Information Systems, as the NJ Chief Information Officer (CIO) of the Year.
- Michael Christman, Ph.D. accepted the position of President and CEO of the Coriell Institute.

Summer 2007

- The National Institute of Neurological Diseases and Stroke (NINDS) extended its contract with Coriell through September 2008.
- The Coriell Board of Trustees approved the establishment of the Genotyping and Microarray Laboratory, to serve as a central resource in Dr. Christman's research program. An investment of $2.4M has been made in Affymetrix Equipment and supplies as well as in recruitment costs as well as $5.0M to refurbish the building and its facilities.
- Norman Gerry, Ph.D. joined Coriell as Director of the Genotyping and Microarray Center.
- Alan Herbert, MBChB, Ph.D. a scientist from Boston University's Department of Genetics and Genomics heads collaboration on the Delaware Valley Personalized Medicine Project and will soon join Coriell's Scientific Staff.
- Scientists and physicians at Coriell Institute and the Cooper Cancer Institute (Director, Dr. Generosa Grana) began planning for collaborative studies of lung cancer.

Fall 2007

- Courtney Sill, Ph.D., joined Coriell as the Director of Communications.

Editorial Staff

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Coriell Institute Welcomes Michael F. Christman, Ph.D.

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Coriell Institute is pleased to welcome Michael F. Christman, Ph.D., as the Institute's new President and CEO. Dr. Michael Christman officially began in his new position on June 1, 2007. Dr. Christman was professor and founding chair of the Department of Genetics and Genomics for Boston University School of Medicine since its inception in 2001. In that position, he recruited primary faculty and led an international team of scientists in one of the first genome-wide scans associated with human genetic variation in disease.

Dr. Christman has also served as Associate Professor, Department of Microbiology, University of Virginia (1997- 2001), Assistant Professor, Department of Radiation Oncology, University of California at San Francisco (1991- 1996), and was a Jane Coffin Childs postdoctoral fellow at Massachusetts Institute of Technology (1986- 1990). His educational credits include a doctorate in biochemistry from the University of California at Berkeley (1985) and a bachelor's degree in chemistry from the University of North Carolina at Chapel Hill (1981). He is a member of the Genetics Society of America and the American Association for the Advancement of Science. He is also a member of the NIH Drug Discovery and Molecular Pharmacology (DMP) Study Section.

Michael Christman's recent research accomplishments are impressive: Principal Investigator on the first dense genome scan for human genetic variants that predispose to disease; Identification of first common obesity-predisposing human genetic variant (Herbert et al., 2006); Initiation of first genome scan of disease genes of an African American cohort, the Howard University Family Study (in progress). Coriell's new president brings with him a program of research that can help point the way to treatments for illnesses as diverse as heart disease, obesity, Alzheimer's disease, and cancer.

"This is an exciting time for Coriell as our exhaustive search for the best possible candidate who can build upon our solid worldwide reputation and take us to a new, higher level of discovery has culminated with the appointment of Dr. Christman," said Peter Driscoll, Board Chairman. "Dr. Christman's scientific experience in leading and supervising human genome-related research combined with his vision will take Coriell to the next level in scientific research initiatives - and beyond."

"Mike" Christman looks forward to leading the Institute to even greater levels of prominence and working in partnership with Trustees and the scientific community to help Coriell contribute more directly to advances in medical research and attain an even greater status as one of the world's most important research institutes. In keeping with Coriell's mission "to discover the causes of cancer and other genetic diseases in order to improve the health of mankind through scientific research, banking of cells and tissues, and education." Michael Christman's vision for Coriell's future includes initiation of a "personalized medicine project" in the Delaware Valley that he hopes will become a model for how genome-informed medicine should work.

The completion of the human genome sequence and the more recent understanding of patterns of human genetic variation have unlocked the door to a new level of knowledge about the causes of human diseases such as cancer, heart disease and Alzheimer's. With the door unlocked, scientists are poised for the first time to open the door and enter a new world of molecular pathways that cause these deadly diseases and to understand how each person's disease course and response to therapy may be different. Personalized medicine will fundamentally change medical practice in the next five to ten years, but major challenges remain regarding how genome-informed medical practice should operate.

The Delaware Valley Personalized Medicine Project is a forward thinking, joint effort involving patient volunteers, physicians, scientists, ethicists and information technology experts whose goal is to better understand the coming impact of genome-informed medical practice and to guide its ethical, legal and responsible implementation. "Trustee Marc Maser suggested the project be called the Delaware Valley Project" to broaden its scope and I think that is an excellent idea," said Christman. The aim is to allow patients to benefit from the advances that genome-informed medical practice will bring, while ensuring that patient privacy is vigorously protected.

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Genotyping and Microarray Center

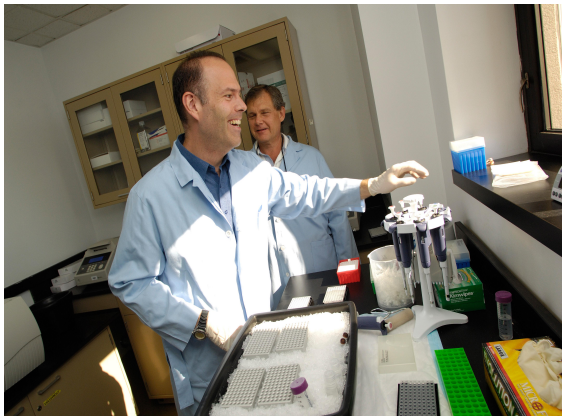
The Genotyping and Microarray Center has instrument capacity to process 800 samples per week. This advanced facility consists of state-of-the-art technology including:

- **3 Affymetrix GeneChip GCS3000 scanners equipped with autoloaders**
- **12 Affymetrix GeneChip FS450 fluidics stations**

The Genotyping and Microarray Center was established in August 2007 and is directed by Dr. Norman Gerry. The mission of the Coriell Institute Microarray Resource is to provide investigators with the tools they need to fulfill their research goals. The widespread use of microarrays has led to a paradigm shift in detecting sequence variations and gene expression on a genomic scale. This is just the tip of the iceberg in terms of possibilities. There are continuing developments in the areas of pathogen detection, mapping of DNA protein binding sites (ChIP on chip), analysis of recombination, and determination of copy number variations. In addition, the fields of protein and tissue/whole cell microarrays are just beginning to mature.

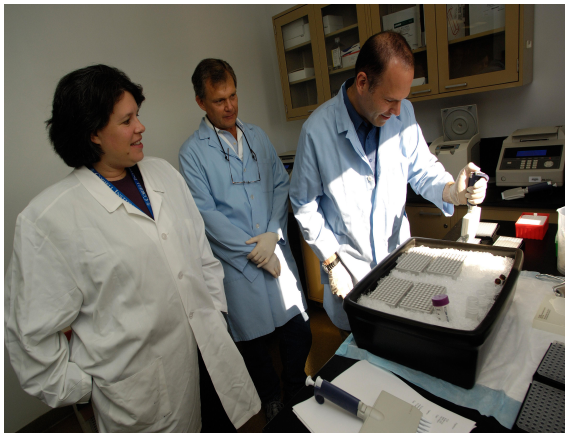
Genotyping and Microarray services fall into two main categories. The first is sample preparation/data analysis for Affymetrix expression and SNP profiling GeneChips. The second is to assist in the design and analysis of arrays for non-model organism expression profiling or unique applications such as ChIP on chip or resequencing. For these experiments, the Resource, along with Affymetrix or other 3rd party vendors will work with investigators to produce an array as well as prepare samples for hybridization.

The Coriell Institute Genotyping and Microarray Center is a high capacity facility, consisting of 12 FS450 Affymetrix fluidics stations and 3 state of the art GCS3000 scanners. Each scanner is equipped with an autoloader. The facility can process up to 3,200 DNA or RNA samples per month. Optional assistance with data interpretation, both statistical and bioinformatics, is available.



Norman Gerry, Ph.D., is a genetics scientist recruited from the Department of Genetics and Genomics, Boston University, to join Dr. Michael Christman in the Personalized Medicine Project. Dr. Norman Gerry, former director of the Boston University Genotyping and Microarray Resource, has established and now directs the Genotyping and Microarray Center at the Coriell Institute facility.

Dr. Gerry has been involved in Microarray and DNA testing for several years and has successfully processed thousands of SNP and gene expression arrays, including all genotyping in the first genome scan of the Framingham Heart Study (Herbert et al. (2006), Science; Herbert et al. (2007) Nature Genetics). Dr. Gerry was a beta-tester for the new Affymetrix 6.0 Genechips (1 million SNPs) as part of the Quality Control procedure. He is the author or co-author of several peer-reviewed scientific publications.

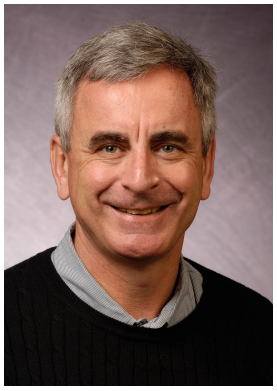


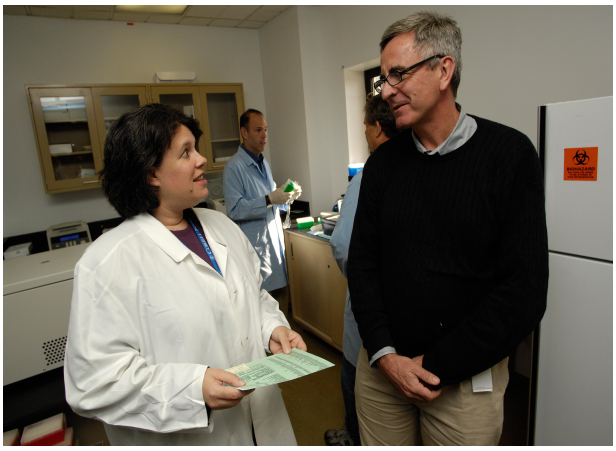
Services provided by the Coriell Institute Genotyping and Microarray Center:

Genotyping and Microarray – Affymetrix GeneChip® Systems

- Genotyping
- Gene Expression Analysis
- CustomSeq® Resequencing Arrays
- Whole Genome Tiling Arrays

Statistical and bioinformatics data interpretation also available.





Alan Herbert, MBChB, Ph.D., was recruited by Dr. Michael Christman from the Department of Genetics and Genomics at Boston University to join the Delaware Valley Personalized Medicine Project. Dr. Alan Herbert, with Dr. Christman and Dr. Gerry, have just completed a whole genome scan of families from a community-based population in Framingham, Massachusetts, that involved genotyping 100,000 single nucleotide polymorphisms per individual and identified a common variant that increases risk of obesity.

Coriell Institute Welcomes Michael F. Christman, Ph.D.

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The study will seek to discover presently unknown genes that elevate a patient's risk of cancer, heart disease and neurological diseases, to understand why patients often respond differently to treatment, and to explore how the resulting information can best be viewed and utilized by patients and their physicians in a secure and user-friendly environment.

Coriell is the largest repository of human cell cultures in the world. It manages thousands of samples and materials for scientific exploration. As Dr. Christman has begun to assemble a team of researchers to continue his program of cutting-edge genomics research, he is establishing collaborations with the Coriell Cell Repositories that provide greatly enhanced characterizations of the collections. Plans are now in place to provide SNP and copy number analyses for 900 cultures in the NIGMS Repository. Four hundred cultures from the ethnicity collection and 500 cultures from the chromosomally abnormal collection will be studied. The ethnic panels are presently available as DNA samples as are many of the chromosomally abnormal samples. This will greatly expand the range of reference materials available to microarray laboratories.

Dr. Christman also plans to work with local and state officials to transform the region into a magnet for high-tech and biotech industries. Genome-informed medicine is a major growth area that involves several important high-tech businesses. There remains a treasure trove of information about disease waiting to be unlocked. Coriell - and Mike Christman - believe they have the key.

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Using Locational Proteomics to Study Cell Signaling

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Simultaneous application of specialized imaging techniques, advanced image analysis and computer simulation can enhance our understanding of receptor and signaling dynamics. We apply these capabilities to the specific application of endocytosis of the EGF receptor and its effect on the MAPK signal transduction pathway to demonstrate the interplay between spatial heterogeneity, diffusion and endocytosis in the signaling process. The activation of epidermal growth factor receptor (EGFr) by ligand or antibody can be of significance in understanding the kinetics of its internalization in various cell lines and hence serve as a discovery tool in cell biology, cancer research, and studies of the biology of aging.

The ability to dynamically monitor EGFr internalization and activation of downstream proteins has been recently improved by the use of appropriately functionalized quantum dots (Q-dots), due to the long lasting fluorescence and brightness of these nanoparticles. However, quantitative information on EGFr kinetics is derived from average properties of many cells in a culture, rather than tracking individual events within one cell. Further, relative positional information on the location of tagged species is gained from traditional staining procedures prone to noise, fading and low contrast. Therefore, we apply confocal microscopy to image cells in cultures followed by a novel multiscale image segmentation to define regional sectors in cells prior to determination of concentration of Q-dots.

We use an imaging systems based on a Leica inverted microscope, which is combined with a confocal real-time spinning disc unit (BD Atto Biosciences). The system is automated with a well plate loading robot and scanning stage (Clemex Corp.). The system belongs to a new class of highly automated microscopes enabling comprehensive cell biological studies (Debernardi et al., 2006). Images are taken with a sensitive CCD camera and stored on a host computer. We deploy computer image analysis to detect the cells, cell boundaries and cell compartments in these images (see figure 1), using a software toolbox (Definies Corp., Munich). The analytical process starts with a generation of several multi-resolution levels of the images to address the low signal-to-noise ratio and fuzzy boundaries inherent in fluorescent images. The resulting objects represent object primitives, which can be combined and related to cellular compartments like nucleus, cytoplasm, membranes and organelles (see Figure 2). We use multiple fluorescence channels jointly to make use of the resulting color information to enhance this process.

Once the compartments are determined, we automatically detect location and concentration of proteins stained with fluorescent-labeled Q-dots within these compartments. To study the kinetics of tagged proteins, such as the migration of proteins involved in signaling pathways, we fix cells at regular time intervals after activation for a multiplexed (multiple proteins), high-resolution imaging. Image captures reveal location and mean concentration of Q-dots in compartments of the cell, and their dynamics as these proteins change location or activation over time. As an example, to monitor changes of the EGF-receptor location in the cytoplasm, first a distance ratio between the cell membrane and the nucleus is used to assign equidistant regions in the cytoplasm. Subsequently, the concentration of proteins in these regions is determined and averaged over multiple cells (Kriete et al., 2006).

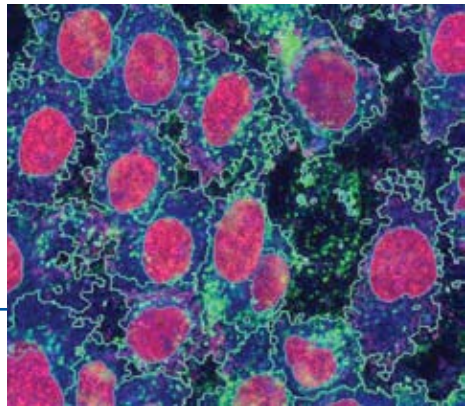

Figure 1) Confocal image processed for cell boundaries. Nuclei are shown in red, cytoplasm in blue, and Quantum dots, bound to the EGF-receptor, in green.

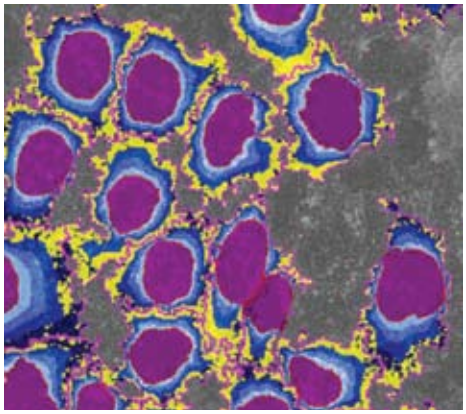

Figure 2) Further image analysis to investigate the progression of the receptor internalization and advection dynamics towards the nucleus. Protein concentrations (yellow) can be measured in dedicated cytoplasmic regions (color coded in shades of blue) at sequential time points.

This process allows quantitative description of the internalization of EGFr and other activated protein species. The capabilities realizable by the simultaneous application of specialized high resolution imaging techniques and functionalized quantum dots in conjunction with advanced image analysis, capable of being carried out in a multiplexed fashion, are a prerequisite for a computational cell modeling of signaling and other key heterogeneous intra-cellular processes (Ghosh et al., 2005). In collaboration with computational groups at Drexel University, these data sets are used to develop spatio-temporal cell models.

Expression Profiling of Aging Cell Strains

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To gain insight into the mechanisms of aging, we are investigating fibroblast cell strains from the NIA cell collection. This includes the application of microarrays to profile changes in mRNA expression. We have applied human whole genome arrays with 55K probes using the Codelink platform (Amersham Biosciences). The Codelink platform is based on single-stranded oligonucleotide probes. RNA from the cell strains was extracted by an established protocol (Trizol reagent). After hybridization, the chips were scanned and the spots identified. The Amersham Biosciences CodeLink Analysis Software gives an integrated optical density (IOD) value for each spot, along with a measurement of an integrated background intensity, which is used for background correction. The sensitivity of these oligo-based arrays is 1:300,000, about 1 copy RNA per cell.

We are using cells from Coriell's aging cell repository, sponsored by the National Institute on Aging (NIA). It is a resource facilitating cellular and molecular research studies on the mechanisms of aging and the degenerative processes associated with it. The cells in this resource have been collected over the past three decades using strict diagnostic criteria and banked at Coriell under the highest quality standards of cell culture. We have propagated several cell lines from different donor ages and starved the cells by a serum free of growth factors.

We applied a 2-fold thresholding and classified the genes using gene ontology tools. Many of the genes differentially expressed (> 2-fold down) between young (around 20 yrs.) and old (around 80 yrs.) donors (see figure 1) were in relevant biological processes, specifically related to metabolism. Genes upregulated involved stress response and DNA damage. We have further investigated the change in expression after GF-activation.

Genes involved in signal transduction, cell cycling, cell proliferation, and intracellular signaling cascade that were upregulated included MAPK10 (mitogen activated protein kinase 10), cyclin e1, rab11b, rab31, rab9a, rab2b, rab38, rab35 (member ras oncogene family), STMN1 (stathmin 1/oncoprotein 18), SNX6 (sorting nexin 6), PIAS1 (protein inhibitor of stat-1), PRKAA2 (protein kinase, amp-activated, alpha 2 catalytic subunit), IFNAR2 (interferon (alpha, beta and omega) receptor 2), and STK24 (serine/threonine kinase 24).

Coriell cell lines have been previously used to investigate steady state by gene expression analysis, and a mitotic disregulation had been reported (Ly et al., 2000). Our investigative approach is complementary and points to further phonotypical shifts in molecular machinery of the cell, even before cellular senescence is reached. The results are supportive of an increased state of stress response, which also changes many dynamic properties like cell signaling.

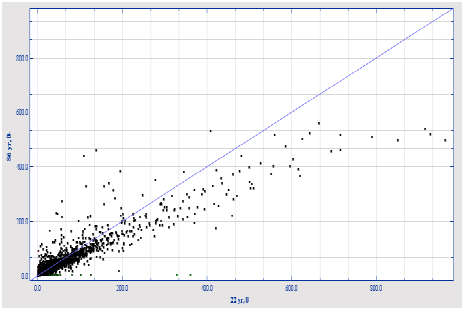

Figure 1. Differential gene expression of young cell strain control (22 year old donor) after 12 hours growth-factor starvation vs. old donor age (86 years). Genes considered in this plot have > 2-fold change. Many genes appear downregulated in old cells, including processes related to metabolism. Upregulated are genes related to stress response and DNA repair.

Highlight on Andres Kriete

Dr. Andres Kriete has a background in systems biology, bioinformatics and bioimaging. Since he received a joint appointment from Drexel University, Philadelphia, and the Coriell Institute, he developed a focus on the biology of aging. At Drexel, he teaches bioimaging, statistical design, pharmacogenomics and systems biology classes at the School of Biomedical Engineering, Science and Health Systems.

Since aging is a systems phenomenon, Dr. Kriete is implementing novel frameworks and computational approaches to study spatio-temporal processes and interactions on multiscales, in part funded by an NIH multiscale modeling initiative. This research provides insights into the sensitivity and robustness of biological organization at different levels, scaling properties, emerging aging phenotypes and pro-oncogenic processes. Trained as a physicist and computationally oriented, he augmented his research with an experimental component and explores new experimental techniques like high-content screening assays and genome wide analyses to study changes in aging cells and tissues. He uses Coriell's aging collections to carry out investigations with human cell lines from longitudinal studies.

Currently, the team of Dr. Kriete has four master and two graduate students, and a research assistant for lab operations. He also supervises a senior design team at Drexel and interns at Coriell. For his research he is cooperating with Drexel's physics and biology departments as well as with Jefferson University. He is involved in developing aging research strategies at the Santa Fe Institute, NM.

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New to the Repositories: Matched Fibroblasts and Differentiated Cells from Skin

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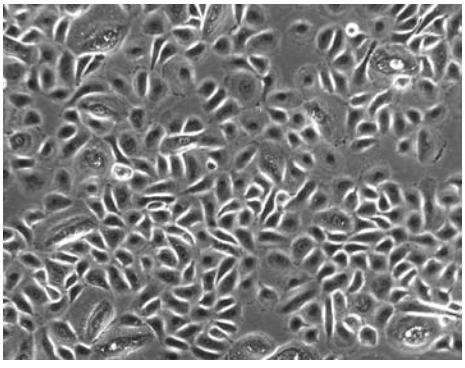
The Cell Repositories at The Coriell Institute for Medical Research hold a variety of differentiated cell lines for research use. As culture methods and media for growing various kinds of differentiated cells have improved, we will develop and offer more unique cultures and new kinds of differentiated cells to the research community. In the near future, we will be introducing SkinTrios to the NIA and NIGMS Repositories: sets of skin cell cultures established from individual donors. These trios will be comprised of dermal fibroblasts, epidermal keratinocytes, and melanocytes, all derived from the same neonatal foreskin (Figure 1). Given the aging population and increases in skin cancers, especially melanomas (Byrd-Miles et al., 2007; Markovic et al., 2007), these cultures will be especially valuable to basic and applied researchers in skin biology and medicine. While Coriell also produces pooled, primary keratinocytes from multiple foreskins, these are not useful for genetic research in the same way that many of the cells from single donors that are distributed by Coriell are used.

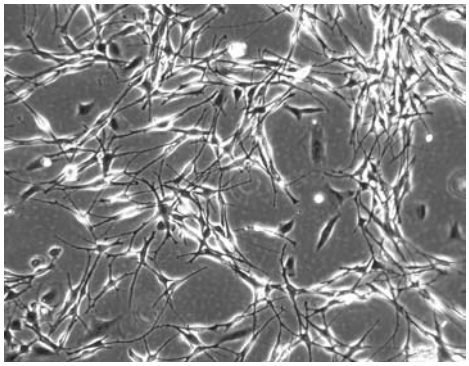
With the completion of the human genome project, researchers are actively investigating human genetic diversity through such programs as the International HapMap Project (http://www.hapmap.org/), and studies on gene expression at the whole genome level are expected to increase dramatically. While some studies have been published comparing gene expression in different cell types (c.f. Ivanova et al., 2002; Ramalho-Santos et al., 2002), or in the same cell type from different individuals (Allegrucci and Young, 2007), it has already become clear that underlying genetic differences between individual donors complicate such comparisons. Furthermore, cultures of the same cell type obtained from different sources may have been established using different methods or media, or grown for varying amounts of time before cryopreservation, all of which could affect the properties of the cultures (Skottman et al., 2006). By enabling researchers to obtain matched sets of different cell types from the same individuals, and making sets from multiple individuals established in the same way available, the new SkinTrios will provide important tools for a variety of studies.

Source and Establishment of SkinTrio Cultures

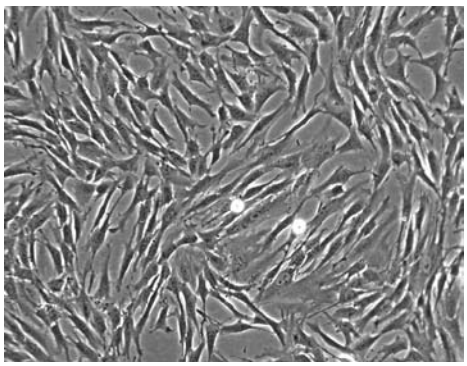
Skin is comprised of two layers, the epidermis and the dermis, separated by a basement membrane zone. The epidermis is a stratified, squamous, keratinized epithelium, an avascular outer layer that is primarily a protective barrier, preventing entrance of microorganisms into the body and retaining water. Keratinocytes are the main cell type in the epidermis, which also contains much smaller numbers of pigment-producing melanocytes, Langerhans cells, and neuroendocrine Merkel cells. The dermis is a vascular inner layer that provides nutrients for the maintenance of the epidermis, and contains cellular components of sensory and autonomic neurons, and for immune



GM21806 keratinocytes



GM21807 Melanocytes



GM21808 Fibroblasts

Figure 1. Photomicrographs of keratinocyte, melanocyte, and fibroblast cultures obtained from a single foreskin. All images of live cells were obtained at the first passage after recovery, using a 10X phase contrast objective.

surveillance and wound healing. The cellular components of the dermis include endothelial cells, fibroblasts, neural cells, monocytes, and macrophages, in an extensive extracellular matrix.

We obtain these cells from neonatal foreskins, as the proliferative capacity of cells, especially keratinocytes, decreases with increasing age of the donor. The foreskins are obtained from "apparently healthy" individuals; the ethnicity is as reported by the hospital personnel and the samples are anonymous. We enzymatically dissociate the epidermis from the dermis, disaggregate the epidermal keratinocytes and melanocytes, and expand the cell populations *in vitro*. Because the growth requirements of keratinocytes and melanocytes are substantially different, one-half of the epidermal cell suspension is plated in medium for keratinocytes, and one-half is plated in medium optimized for growth of melanocytes. The keratinocytes are plated in flasks coated with collagen IV, a key component of the basement membrane, which enhances both attachment and growth of the keratinocytes. We use a new, serum-free medium, CnT-07, developed to target the growth of the epidermal keratinocyte progenitors. These conditions enable expansion of the keratinocytes sufficient to freeze with minimal expansion.

Although far fewer melanocytes than keratinocytes are obtained from the epidermis, the tetradecanoyl phorbol acetate (TPA, also known as phorbol myristate acetate or PMA) component of the melanocyte growth medium rapidly eliminates the keratinocytes, while at the same time stimulating the proliferation of the melanocytes. Because some fibroblasts may also be present, the primary melanocyte cultures are treated with geneticin in medium with 10% FBS for two to three days to eliminate the more rapidly growing fibroblasts (Halaban and Alfano, 1984).

The dermis is processed for the establishment of matched fibroblast cultures by explant in standard medium containing FBS. The explant method has been widely used to isolate fibroblasts from skin for decades (Hentzer and Kobayasi, 1978). Because the fibroblast cultures are established from the dermis, initial keratinocyte and melanocyte contamination is minimal, and the dermal fibroblasts grow so robustly in the 15% FBS used in the primary cultures that cross contamination with other dermal cell types has not been observed. The number of medical applications using autologous fibroblasts in tissue engineering (Lamme et al., 2000), cosmetic plastic surgery (West and Alster, 1998), and gene therapy (Roth et al., 2001) is increasing, and they are key to producing organotypic skin constructs.

All cultures are frozen at the first or second passage to balance the proliferation required to obtain a sufficient number of cells for distribution against the limited lifespan of normal keratinocytes in culture. We test the cultures for bacterial, fungal, and mycoplasma contamination when they are frozen, and again when they are recovered.

Characterization of Cultures

Many differentiated cell types have more limited *in vitro* lifespans than fibroblasts, so it is important to carry out lifespan studies on such cells in order to allow researchers to plan their experiments accordingly. Thus, phenotypic characterization of differentiated cell types both early and late in lifespan is very important for all differentiated cells. This is of particular concern with respect to slow growing melanocyte cell cultures that were not cloned (derived from single cells). In such cases, a few cells of a different type, such as fibroblasts, may persist in a melanocyte cell culture. Due to the rapid growth rate of fibroblasts relative to melanocytes, the proportion of fibroblasts would increase after successive expansions of cryopreserved cells. A similar problem may occur with keratinocyte cultures. Thus, we re-examine such sequentially passaged cultures to ensure that the melanocytes are free of detectable fibroblasts.

While keratinocytes and fibroblasts are readily distinguished from melanocytes and from one another based on morphology (Figure 1), we will also stain these cultures with antibodies to cytokeratins, which stain the epidermal keratinocytes (Yamamoto, 1999), and to the mesodermal marker vimentin, which stains the fibroblasts (Schiller et al., 2001) and melanocytes (Pitz et al., 2002), but not quiescent keratinocytes. In addition to *in vitro* lifespan, characterization involves immunocytochemical staining for specific markers, e.g., a surface marker, gp100 (Sheffield et al., 2002), and a transcription factor, (MiTF) for melanocytes (Busam et al., 2001; Gaggioli et al., 2003), and cytokeratin and vimentin as markers for the epidermal keratinocytes and fibroblasts, respectively (Katagata et al., 1999). Examples of cultures immunostained for expression of melanocyte-specific antigens and vimentin are shown in Figure 2. Neither fibroblasts nor keratinocytes express gp100, and MiTF shows nuclear localization in melanocytes (Busam et al., 2001), whereas, although present in fibroblasts (Zhu et al., 1998; Busam et al., 2001), this transcription factor is localized in the cytoplasm by interaction with 14-3-3 proteins in these and other cell types (Bronisz et al., 2006).

Potential Applications

Because keratinocytes spontaneously differentiate in culture, they have long been used as a model system to study differentiation *in vitro*. They are also used for the study of certain human viruses, particularly Herpes simplex virus (HSV-1) and human papilloma virus (HPV; Chow and Broker, 1997); certain subtypes of the latter have been implicated in the genesis of cervical cancer (Kao and Kao, 1994; DiMaio and Liao, 2006; Jones and Wells, 2006). When grown on permeable substrates, such as collagen gels with dermal fibroblasts, and subsequently raised to the medium-air interface, keratinocytes can produce stratified, organotypic skin cultures (Dekker et al., 2000; Ehrenreich and Ruszczak, 2006). Melanocytes can be incorporated into organotypic skin constructs for various research purposes such as studies of melanocyte growth and pigmentation (Bertaux et al., 1988; Medalie et al., 1998), tumor metastasis (Dekker et al., 2000), and molecular and physiological responses to ultraviolet light (Hachiya et al., 2005).

Besides being useful model systems for basic cell biology and applied research (Poumay and Coquette, 2007), organotypic cultures can also be used to study abnormal development, such as occurs in psoriasis (Fransson, 2000), the effects of various cytokines on cell behavior or gene expression (Fransson et al., 1996; Fransson, 2000), and invasion by pathogens (Bhattacharyya, 1998). Organotypic cultures can be used for studies in a variety of other

continued on page 10

New to the Repositories

continued from page 9

areas including phototoxicity (Augustin et al., 1997a), carcinogenesis (Dekker et al., 2000; Harriger and Hull, 1994) and chemoprevention (Moore et al., 2006), differentiation (Fransson, 2000; Koria and Andreadis, 2006), drug permeability/toxicity testing (Augustin, et al. 1997b; Michel-Buono et al., 1997), cell-cell interactions (Lacroix et al., 1995; Fransson et al., 1996), and virology (Chow and Broker, 1997).

Perhaps the most medically relevant application of cultured keratinocytes is their use as skin grafts for burn patients (O'Connor et al., 1981). This technique has been developing for over 25 years and has proved lifesaving in many cases (Terskikh and Vasiliev, 1999). However, considerable work remains to be done to improve and standardize the cultivation and transplantation techniques. Many researchers still use fetal bovine serum (FBS) or dialyzed FBS to grow keratinocytes, adding cholera toxin and other reagents to block differentiation. While cultured skin can be lifesaving for burn patients in the short-term, the use of FBS is problematic for transplantation, since the foreign proteins can result in graft rejection (Ehrenreich and Ruszczak, 2006). The defined, serum-free medium currently used at Coriell for culture of keratinocytes provides adequate *in vitro* lifespan for experimental purposes. There is also hope that cultured, autologous keratinocytes or dermal fibroblasts will be useful, in combination with gene therapy, in the treatment of inherited blistering skin disorders such as epidermolysis bullosa (Goto et al., 2006; Andreadis, 2007).

Cultures of differentiated cells are widely used to determine the functions of genes that are only expressed in particular tissues. Other applications include studies of chemical and physical agents relevant to development of skin cancers, including melanoma. These cultures will soon be available through the NIA and NIGMS Cell Repositories.

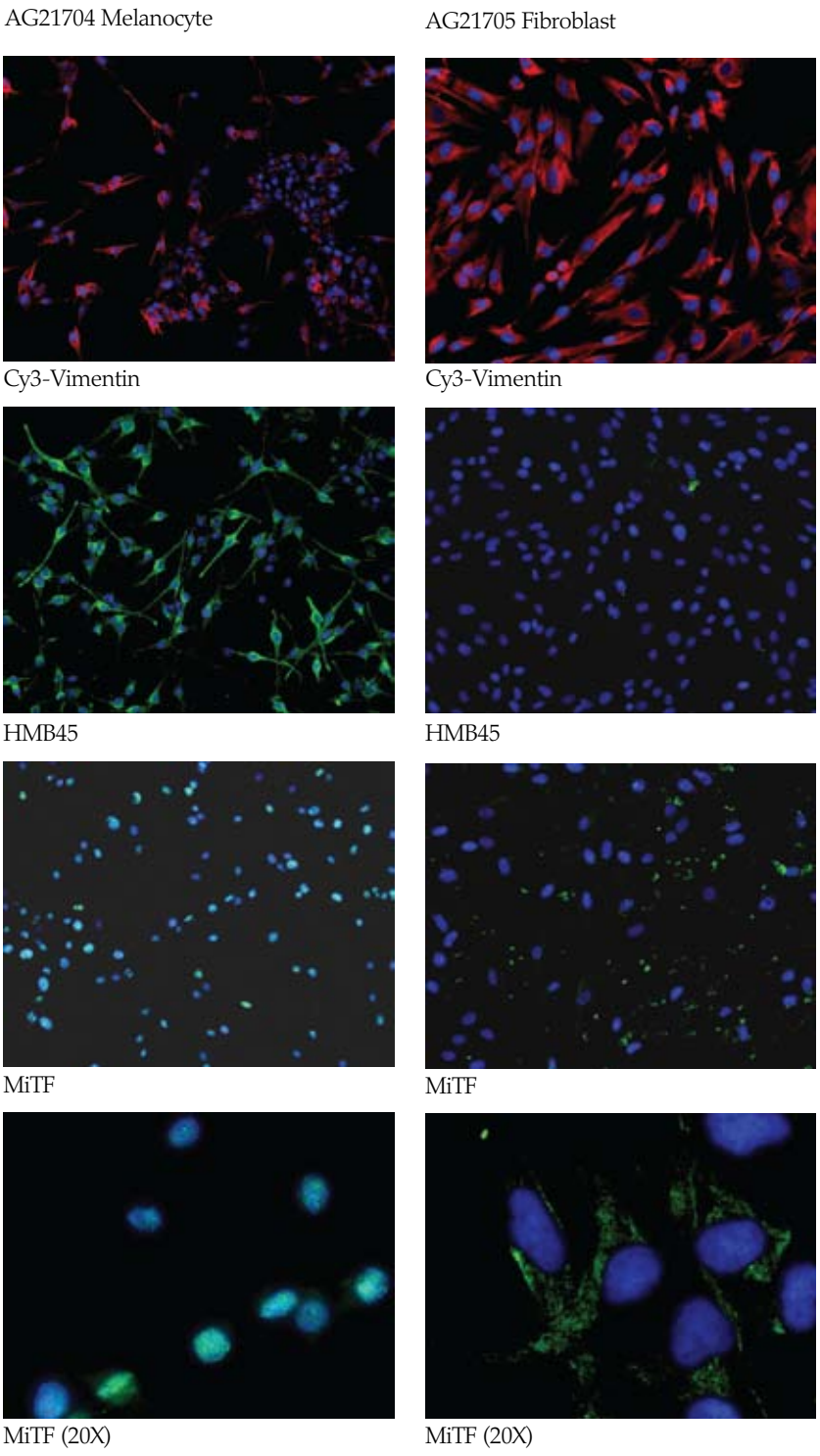


Figure 2. Immunofluorescent images were obtained using a 10X objective except as noted. The anti-vimentin antibody was directly labeled with Cy3. The anti-melanoma antibody (anti-gp100, Clone HMB45) and anti-micropthalmia transcription factor (MiTF, Clone D5) antibodies were detected with AlexaFluor 488-labeled anti-mouse IgG. Nuclei were visualized with DAPI. Note the exclusively nuclear staining of the melanocytes with the anti-MiTF antibody (green to turquoise nuclei), in contrast with the cytoplasmic staining of the fibroblasts (bottom panels).

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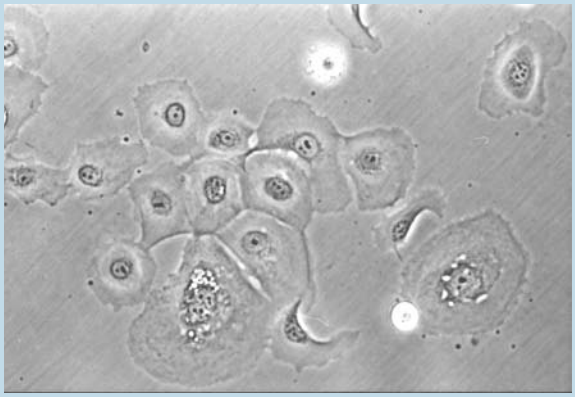
Technical Note



This photomicrograph shows a terminally differentiating clone of a foreskin keratinocyte culture, late in the lifespan. As keratinocyte cultures are repeatedly passaged, more cells enter the terminal differentiation program, although submerged cultures do not form a normal, stratified squamous epithelium as occurs in organotypic cultures. These cells, recognized by their large, flat morphology, can only undergo a few divisions prior to terminal differentiation. With each passage, more of the culture surface will be occupied by such cells, which do not contribute to subsequent culture growth. If a low-passage culture is allowed to remain at confluence, the cells will also begin to terminally differentiate. Therefore, it is essential to carefully examine keratinocyte cultures at each passage to ensure that they are subcultivated prior to complete confluence, and to perform experiments with keratinocytes well before the end of the culture lifespan. When your culture starts to look like this, it's time to order new cells!

A New Resource for the Study of Rare Disorders: Example of Propionic Acidemia

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To develop new resources for The Human Genetic Cell Repository, which is sponsored by the National Institute of General Medical Sciences (NIGMS), we have initiated interactions with voluntary health organizations. These organizations are established by concerned parents who want to be sure that the medical establishment brings all of its skills and resources to bear on the particular disease of concern. The voluntary health organization functions as a place to which patients and their families can go for mutual support, focuses on research on the disease, pushes for drug and treatment development and interacts with governmental agencies for support of the patients. Many of the diseases of concern are extremely rare with only a few hundred to a few thousand patients.

Many voluntary health organizations vigorously promote research into their diseases by providing grants directly to scientists, by educating scientists about the disorders and by helping to make patient materials available to scientists. Coriell has worked with several voluntary health organizations in the past and here we describe an expanded effort to make research materials and information available.

The NIGMS Human Genetic Cell Repository

The NIGMS Repository, established in 1972 at Coriell, has cell lines and DNA samples from more than 500 inherited diseases, many of which are extremely uncommon. These cell lines have been an important resource for the identification of genes in which a mutation or chromosomal defect leads to the disorder. Yet, this is often only the first step. For some inherited disorders, it has been possible to perform a "genotype-phenotype" analysis, connecting the patient outcome with the type of symptoms displayed by the patient with the genotype of the patient. For others this connection is not obvious. One possibility is that the outcome of the disease is due to variants in other genes besides the principal disease causing gene. In order to investigate these possibilities, it is necessary to have excellent longitudinal clinical information in addition to the laboratory analyses. To build these resources we have begun establishing interactions with groups which interact directly with the patients on a continuing basis.

Propionic Acidemia

Propionic acidemia (PA) is a very rare disorder (~1 per 100,000 live births) of the catabolism of essential amino acids valine, threonine, methionine and isoleucine. The disorder is due to a defect in propionyl-CoA carboxylase, a tetrameric enzyme with two subunits. The defect can occur in either subunit (encoded by PCCA or PCCB) and the source of the defect can be detected by complementation or DNA sequencing.

The disorder can present in the first weeks of life as acute onset or later in life as "late onset." PA can be detected through newborn screening using tandem mass spectrometry, however, not all states are screening for PA at this time. Symptoms of the disorder may include: refusal to eat, vomiting, dehydration, lethargy, acidosis, hyperammonemia, seizures, and coma. Those affected by the disorder are prone to: pancreatitis, cardiomyopathy, pancytopenia, optic nerve damage, developmental disabilities, hypotonia, and other types of metabolic instability. Current treatments primarily involve the dietary restriction of offending amino acids and supplementation with L-carnitine.

Propionic Acidemia Foundation

The Propionic Acidemia Foundation (PAF) (http://www.pafoundation.com/) was started in 2001 and is dedicated to identifying improved treatments and a cure for Propionic Acidemia (PA) by funding research and by providing information and support to families and medical professionals. Since 2004, PAF has given almost $160,000 in research grants through the support of thousands of people worldwide.

Coriell and the PAF have initiated a collaboration to provide the NIGMS Human Genetic Cell Repository with samples from subjects and their families with PA and a thorough set of clinical, molecular and laboratory data. We have developed an extensive questionnaire with the expertise of the Medical Advisory Board of the PAF which will be administered for each submission.

A new type of collaboration

As noted above it is critically important, not only to find the gene which is the primary cause of the disorder, but also to understand the genetic and other factors which could lead to an understanding of the way the disease unfolds. To achieve this Coriell and the PAF have developed a protocol whereby subjects who submit samples to the Repository for the preparation of a cell line, will also be recontacted in the future so that the course of the disease can be known to the scientists who obtain the cell lines or DNA from the Human Genetic Cell Repository. To protect the identity of the subjects and their families, Coriell developed a protocol, approved by the Institutional Review Board, where the Propionic Acidemia Foundation would be the only ones who would be able to connect the identity of the subject with the sample in the Repository. The PAF would also undertake to follow up on the patients every few years to create a database on the status of the patients in addition to information about cells derived from the patients. Our goal is to create a new type of resource for the study of rare diseases. We plan to establish similar arrangements for a series of rare diseases.

Development of a Comprehensive CCR Online Catalog

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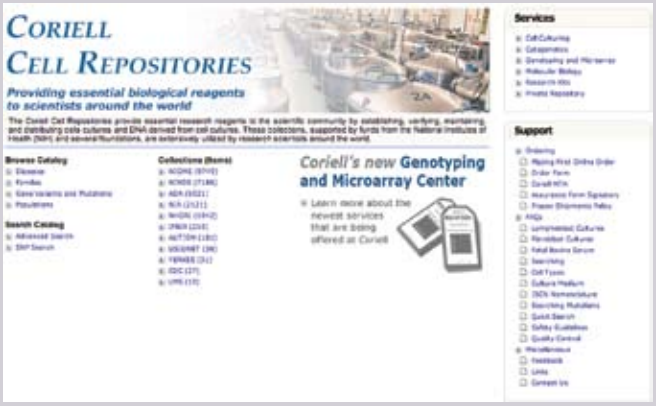


One of the core curatorial responsibilities of the Coriell Cell Repositories is to facillate usage of biomaterials in collection through well-documented descriptions that permit our users to select those most approriate to their studies. Our catalog has evolved from paper catalogs dealing with only individual collections to our present online catalog that enables biomaterials searches across all collections.

Coriell has provided the scientific community with a catalog of biomaterials since 1973. Beginning in 1977, a printed catalog was computer-generated every two years until 1995. The first Coriell electronic catalog was made available to the user community via a telephone dial-up connection in 1993. A second-generation electronic catalog was developed in 1996. This catalog, developed for the World Wide Web through a contract with Johns Hopkins University, incorporated some of the features available in the printed catalog version, such as ideogram images that were unavailable with the first version of the electronic catalog. A third-generation catalog was developed in 1997 at the Coriell Institute. This catalog had search functionality, enhanced navigation tools, and direct links to several external databases such as OMIM, PubMed, dbSNP, Gene Expression Omnibus, and GenBank.

The need for a comprehensive approach evolved as the number of collections at the managed by the CCR grew as shown in table 1.

Organization	Size of Collection*	Date Launched on Web
National Institute of General Medical Sciences	9749	06/97
National Institute on Aging	2113	01/98
American Diabetes Association	5923	02/98
Autism Research Resource at Coriell	182	05/00
Integrated Primate Biomaterial and Information Resource	136	06/03
National Institute of Neurological Diseases and Stroke	6401	11/03
US Immunodeficiency Network	36	10/04
Centers for Disease Control and Prevention	27	10/04
Leiomyosarcoma Foundation	16	04/05
National Human Genome Research Institute	951	10/06
CHDI - Cure Huntington's Disease Initiative	65	06/07

Table 1 summarizes the currently maintained WWW catalogs by Coriell as of June 2007

The missions and consequently the contents of the individual collections can overlap to different degrees. In the past scientists using one collection have initially missed opportunities presented by another collection. In response to this, the Coriell Cell Repositories launched a completely redesigned catalog in June 2007. Coriell Information Systems has not only extended the search for biomaterials across collections but has developed tools that will facilitate both searching and browsing.

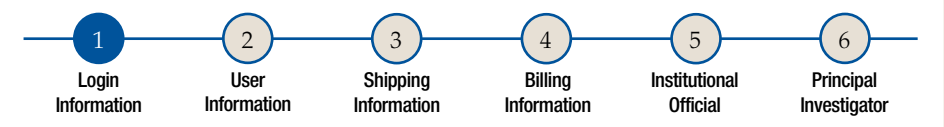
The catalog provides improved navigation and organization throughout the site, to more easily browse the biomaterials in the different collections, as well as across collections. A new search engine, using a Google search appliance, is integrated into the catalog. This new search engine combines the strengths of Google technology, specifically search speed, relevance matching, and keyword indexing, with customized search results.

A new SNP search is available that dynamically queries NCBI's dbSNP database for a gene or SNP of interest. This SNP search returns samples that have SNP data, and their corresponding genotypes.

The new catalog streamlines the ordering process, by offering online ordering, in addition to the option of placing orders via Fax. Once registered for an account, researchers can place orders for biomaterials, save "favorites" lists, and check order history.

New from Coriell Cell Repositories: Online Ordering

Josefina Nash
Director, Information Systems



Here are the steps you need to follow to register for an account and place an order. If you have any problems when following these steps, please contact our customer service by phone (800-752-3805 in USA or 856-757-4848 from other countries) or by e-mail at CCR@coriell.org.

1. Create an Account

Click on the "new user?" link on the login page to go to the first account registration page.

a. Step 1 - Email and Password
The first account registration page asks for your email address and password (Keep in mind that the e-mail address you provide here will be the only e-mail address to which we can send information about subsequent orders). You can check and see if you already have created an account with your e-mail address by clicking on the "Check Username Availability" button. Click on "Next" to proceed to the next account registration page.

b. Step 2 - User Information
The second account registration page asks for your contact information (name, job title, phone number) and mailing address. Fill in the required information and click on "Next" to proceed to the next account registration page.

c. Step 3 - Shipping Information
The third account registration page asks for your shipping address. Click on the "Same as Mailing Information" checkbox to automatically fill in the shipping address if it is the same as your mailing address. The information you provide on this screen should be for the location to which your orders will be shipped. Please ensure that the shipping address is correct to avoid any problems with delivery of your orders.

d. Step 4 - Billing Information
The fourth account registration page asks for your billing address. Click on the "Same as Mailing Information" checkbox to automatically fill in the billing address if it is the same as your mailing address. Fill in the other required and optional fields such as name of billing department, phone number, e-mail, and for the type of organization you are purchasing the biomaterials.

e. Step 5 - Institutional Official Information
The fifth account registration page asks for the contact information of the institutional official that can make legal commitments on behalf of your institution. This individual will need to sign the assurance forms and material transfer agreements that are required before your orders can be shipped. Click on the "Same as Mailing Information" checkbox to automatically fill in the address if it is the same as your mailing address. Fill in the other required and optional fields such as name, phone number, email, job title and job function.

f. Step 6 - Principal Investigator Information
The last account registration page asks for contact information of the principal investigator whose lab will receive the biomaterials that are shipped from Coriell. Any correspondence about orders and shipments will also be sent to the principal investigator selected when the order is placed. Click on the "Same as Mailing Information" checkbox to automatically fill in the address if it is the same as your mailing address. Fill in the other required and optional fields such as department or division, phone number, and e-mail.

g. Post-registration Processes
Once you have completed the registration process you will immediately be sent an e-mail to validate the e-mail address that you entered in the first registration page. Click on the link provided in the e-mail to activate your account. At this point you can save favorites lists and manage your account information.

Coriell customer service must validate your principal investigator, institutional official, and billing information BEFORE any orders can be placed and processed. You can check the status of your account by going to the "My Account" page. Click on the "Manage Account" link to view and edit the information you entered during the account registration process. You will see if your account information has been approved or is pending approval by Coriell customer service.

2. Find the Items you Want

First you will need to browse or search for the items you would like to order. Keyword search boxes are located on every page of the catalog. You will also find links to browse lists and more detailed product-specific searches in the right navigation bar of each catalog collection, as well as on each collection home page. When you find an item that interests you, click the catalog ID of the item to see its product detail page. Here you will find more information about the item, including the products available (such as cell cultures, DNA, plates or panels).

If you do not find what you are looking for in the catalog, you may want to contact our customer service by phone (800-752-3805 in USA or 856-757-4848 from other countries) or by e-mail at CCR@coriell.org.

3. Add the Items to your Shopping Cart

If you want to order an item from Coriell, select the type of product you would like to order from the "Product" dropdown list (if there is more than one option), and then click the "Add to Cart" button on the item's product detail page. Once you have added an item to your Cart, use the cart's top navigation bar or your browser's back button to keep searching or browsing until your cart contains all of the items you want to order. You can access the contents of your Cart at any time by clicking the "View Cart" icon at the top of every page of our Web site.

4. Proceed to Checkout

Take a moment to review all of the items you have placed in your Cart. If you decide that you do not want to purchase a particular item, click the "Remove" button at the beginning of the item's row. You may also update the quantity of any items in your Cart, but please note that certain products are only available in pre-defined quantities. When you are ready to place an order for everything in your Cart, click the "Proceed to Checkout" button. If you are not logged in to your account, you will be taken to the login page where you can either log in or register for an account. If you are logged in, you will be taken to the first page of the order form.

If you are not ready to place the order at this time, you can click on the "Save List for Later" button. You will be asked to supply a name for your saved list so that you can easily reference this Cart in the future.

The instructions below outline each step of our online order form. If at any point you encounter difficulty or receive an error message, please contact our customer service by phone (800-752-3805 in USA or 856-757-4848 from other countries) or by e-mail at CCR@coriell.org.

5. Configure your Delivery Information

Select the principal investigator, institution official, shipping address and billing address that apply to your order from the dropdown lists provided. If your account only has one principal investigator, institutional official, shipping or billing address on file, that information will be be pre-selected for you. Click on the "Next" button to proceed to the next checkout page.

6. Choose a Payment Method

Select the method of payment for your order. The options are bank transfer, check, credit card or purchase order. If purchasing by credit card or purchase order, you will be prompted for additional information at this time.

You may also choose to bill shipping costs directly to your FedEx account at this time. Click on the "Bill your FedEx Account Directly" checkbox and you will be prompted to enter your FedEx account number. Click on the "Next" button to proceed to the next checkout page.

7. Verify your Assurance Form Status

Depending on the items that you are ordering, there may be different assurance forms that are required to be submitted. The assurance forms that are required for your order will be listed on this page, and you will see if your assurance form is current and on file, or if you must submit an assurance form so that your order can be processed.

If you must submit an assurance form for the items that you are ordering, click on the link to download the assurance form, obtain the required signatures from your institutional official and principal investigator, and FAX the form to Coriell customer service. You can proceed with the checkout if you do not have the required assurance form on file, but your order will not be fulfilled until the form is received by Coriell. Click on the "Next" button to proceed to the next checkout page.

8. Enter your Research Intent

Enter information that indicates your research intentions with the biomaterials that are in your order. Select a research intent type from the dropdown list that is provided. Also, briefly provide research aims and general methods to be used with the biomaterial(s). Pasting of research astract, grant or other document is acceptable for this requirement. If you are unable to complete the research intent information, please have the principal investigator use the forms that can be downloaded from this page as an aid in completing the information.

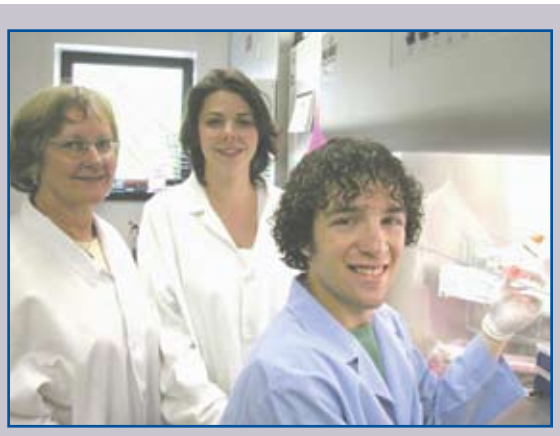
If the information cannot be entered directly at this time, please save this current order by using the "Save List for Later" button below. You will be able to continue with this order at any time by going back to your saved list. Click on the "Next" button to proceed to the next checkout page.

9. Indicate any Secondary Distribution Intentions

Depending on the items that you are ordering, there may be different policies about secondary distribution that are applicable to your order. Some collections prohibit secondary distribution, and others allow limited shared use. If the items in your order cannot be shared or distributed to another party, click on the "I Agree" button next to each collection that is displayed on this page. If the collection that you are ordering items from allows secondary distribution, select your proposed intentions for secondary distribution, if any, from the dropdown list, in addition to clicking on the "I Agree" button. Click on the "Next" button to proceed to the next checkout page.

10. Review and Submit your Order

Review the information that you have entered for accuracy before submitting your order. If you have any special handling requests, enter this information at this time in the input box provided. Click on "Submit" to submit your order to Coriell.



Interleukin-10 Enhances Epstein-Barr Virus Transformation in Human Lymphocytes

Kyle Tretina
Student Intern
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Transformation of human lymphocytes by Epstein-Barr Virus (EBV) has been utilized for decades to produce renewable resources of cells and DNA (Steinberg et al., 2002; Beck et al., 2001; Bernacki, et al., 2003; Bernacki et al., 2005a; Bernacki et al., 2005b). Although the transformation of B-lymphocytes with EBV is extremely efficient, there are a number of samples that do not produce lymphoblastoid cell lines (LCLs) despite repeated attempts at transformation. Using standard protocols of EBV infection, we examined factors related to the successful transformation of human lymphocytes. Samples were collected from three healthy individuals who have been shown to produce LCLs upon exposure to EBV *in vitro* (transformer) and an individual who has repeatedly failed to produce LCLs (non-transformer). Cells from this individual have been observed to die within the first month after the application of the transformation protocol, presumably by apoptosis (unpublished observations).

EBV infection occurs through the interaction of the viral envelope proteins gp350 and gp42 with CD21 and HLA II molecules, respectively (Janz, et al., 2000; Fingeroth et al., 1984; Li et al., 1997;Wang et al., 1998), resulting in the endocytosis of the virus. Once the virus enters the cell, the expression of six EBV nuclear antigens (EBNAs 1-3, and a leader protein, LP) and three latent membrane proteins (LMP 1-3) act cooperatively to induce cell cycle re-entry leading to the outgrowth of LCLs (Bornkamm and Hammerschmidt, 2001). It was therefore hypothesized that the non-transforming sample may express low levels or altogether lack the CD21 receptor necessary for entry of EBV into the host cell.

Previous experiments (Tretina, unpublished) have demonstrated that non-transforming human B-cells possess a statistically equivalent density of CD21 (the EBV receptor) expressed on the cell surface as compared to transforming B-cells (Figure 1A). These results indicate that the lack of LCL outgrowth is not a result of the cell's inability to bind and internalize the virus. In this study, we examined whether the lymphocytes from the non-transforming sample were infected as determined by expression of EBV viral proteins. We confirmed that the levels of expression of EBNA-2, an early EBV protein associated with transformation, is similar in both non-transforming and transforming samples (Figure 1B).

A possible explanation for the failure of an individual's B-cells to transform is the response of T-lymphocytes, to EBV infection. The protocol used for the isolation of leukocytes from whole blood yields a heterogeneous white blood cell population that includes a large number of T-lymphocytes capable of mounting an immune response similar to that observed *in vivo* following primary EBV infection (Schneider and zur Hausen, 1975). Upon infection of the host with EBV, B-lymphocytes produce viral proteins and rapidly proliferate. In response to expression of viral proteins on the surface of the B-lymphocytes, T-lymphocytes attack and kill the infected B-cells to eliminate the viral threat. Following viral clearance, the T-cells down-regulate their response. However, sentinel T-lymphocytes (memory T-cells) are left behind to ensure a faster immune response should the host be re-exposed to the viral threat. These memory T-lymphocytes have the ability to limit the expansion of EBV infected cells and, in some cases, eliminate virus infection *in vitro* (Thorley-Lawson et al., 1977; Gudgeon et al., 2005). Therefore, T-cell

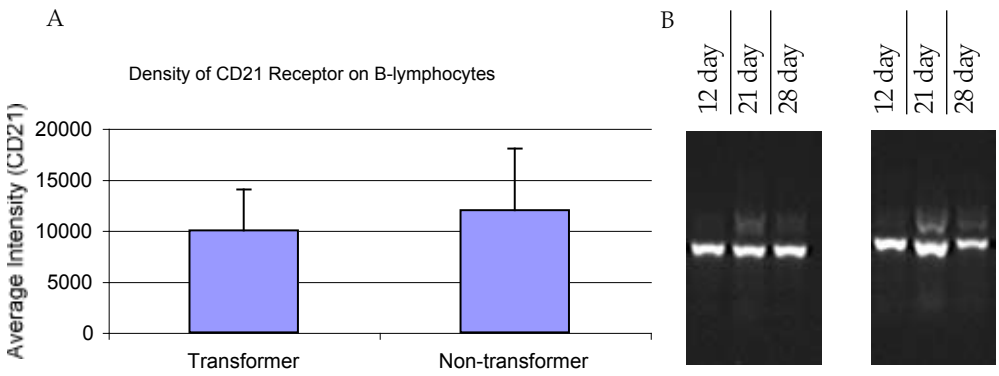

Figure 1: (A) Bar graph representing the average pixel intensity of CD21 receptor on the surface of transforming and non-transforming B-lymphocytes in leukocyte-enriched cultures derived from a peripheral mononuclear blood sample. Cells were stained for CD21 and visualized on Imagestream. :(B) PCR for EBNA-2, a viral protein, from RNA extracted from transforming and non-transforming samples at 12, 21 and 28 days post-infection.

immune response to EBV infection could be a vital factor in the inhibition of the EBV transformation of human B-cells, perhaps by the recognition and activation toward markers of EBV transformation such as LMP-1 (Jurgens et al., 2006). We therefore supplemented the cultures with Cyclosporin A (CsA), a potent T-cell inhibitor at the time of infection with EBV. As seen in figure 2A, T-cell inhibition failed to induce transformation in the non-transforming sample.

Cluster formation and size is a determining factor in the transformation of a sample upon exposure to EBV *in vitro*, identified microscopically as early as 7-10 days following exposure to the virus and macroscopically after 28 days.

EBV has the ability to regulate and induce the expression of pro-apoptotic genes that would affect the course of EBV infection: latent vs. lytic (Prang et al., 1997). An alternate hypothesis to explain the failure of some B-cells to transform is that EBV induces apoptosis by inducing the lytic phase rather than the latent form used to produce LCLs. Cells were infected in the presence and absence of the general caspase inhibitor, Z-Vad to inhibit viral apoptosis and perhaps push the cells to enter the latent phase of the viral infection to produce LCL outgrowth. As seen in figure 2B, the inhibition of Z-Vad did not produce LCL outgrowth in the non-transforming sample.

Although it appears that neither T-cell inhibition nor cellular apoptosis is the cause for non-transformation, an interesting effect was discovered in the non-transforming sample (Figure 2B). Although repeated experimentation has shown that the non-transforming sample does not transform following EBV infection and transformation, the sample succeeded in transformation (Figure 2B). Because the non-transforming sample was the only female in the study, a gender assay was undertaken in order to determine if this sudden outgrowth of these B-cells was due to cross-contamination between samples. The results confirmed that there was no cross-contamination across samples.

Upon further investigation into donor health history, it was noted that at the time of donation the patient had a common cold most likely caused by a human rhinovirus (HRV) infection. HRV infection leads to the systemic release of multiple cytokines such as IL-10, which induces immune responses in the host (Stockl et al., 1999). IL-10 is one of the key factors that regulate inflammatory responses by inhibiting the production of pro-inflammatory cytokines like IL-1 and IL-6. It is interesting to note that EBV encodes a viral IL-10 indicating a role for IL-10 in EBV infection and proliferation (Stuart et al., 1995) and that the presence of IL-10 aids in the outgrowth of LCLs (Burdin et al., 1993; Beatty et al., 1997). We therefore added exogenous IL-10 to the samples three days prior to infection with EBV. At Coriell Institute, a cell line is considered transformed after meeting the criteria of (a) proliferation to 100 million cells and (b) outgrowth following cryopreservation, doubling the cell number within 4 days. We therefore used these criteria to assess for transformation. As seen in figure 3A, the non-transforming sample proliferated at similar levels as compared to the transforming control, reaching a cell density of 100 million cells. Cells were then cryopreserved and thawed 3 weeks later to assess recovery. Following recovery, cells were cultured in either the presence or absence of IL-10 to assess whether the continued proliferation of the cells was dependent upon IL-10 stimulation. It was determined that withdrawal of exogenous IL-10 during recovery did not effect the proliferation of the non-transforming sample, indicating that IL-10 is not necessary for the continued culture of the transformed cell line (Figure 3B). The transformation of the non-transforming sample in the presence of IL-10 has been repeated twice. With this success in mind, the lab is now attempting EBV transformations using the revised protocol of samples from different banks within the repository that have been labeled as non-transformers.

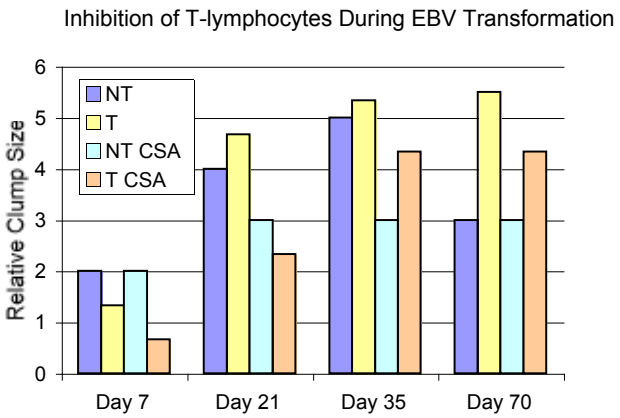

Figure 2: (A) Bar graph representing the average cell cluster size of transforming and non-transforming B-lymphocytes in leukocyte-enriched cultures derived from a peripheral mononuclear blood sample infected with EBV and treated with CSA, a T-cell inhibitor.

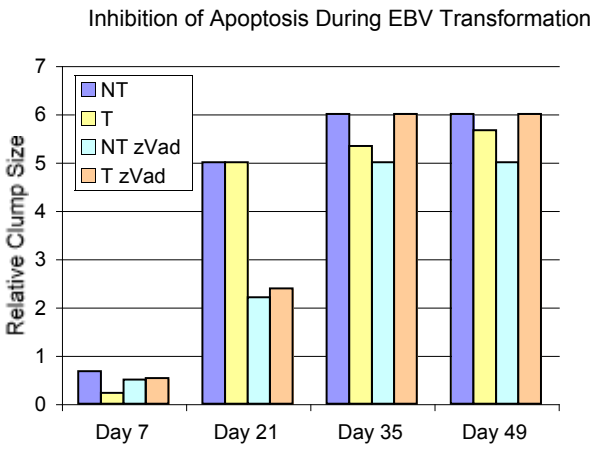

Figure 2: (B) Bar graph representing the average cell cluster size of transforming and non-transforming B-lymphocytes in leukocyte-enriched cultures derived from a peripheral mononuclear blood sample infected with EBV and treated with Z-Vad, an apoptosis inhibitor. The average cell cluster size was determined by visual inspection and rated on a scale of 0 to 6 where 0 represents no clusters and 6 represents large clusters of grapelike cells as observed in transformed cultures.

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Interleukin-10

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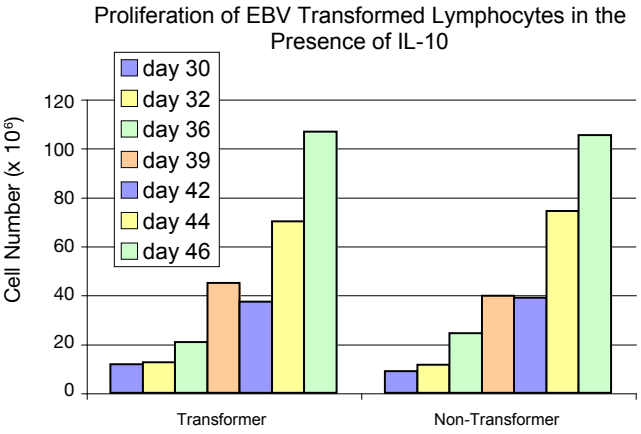
A



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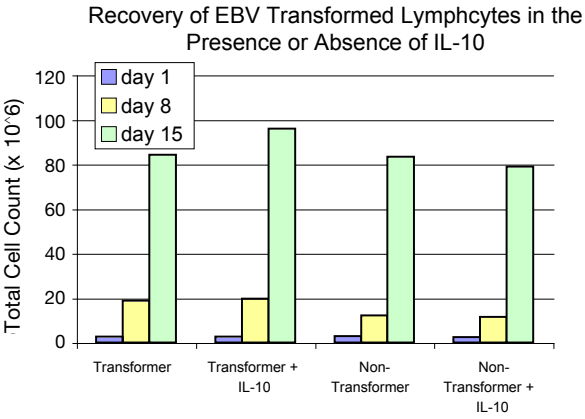

Figure 3: EBV Transformation of Non-transforming Sample. (A) Histogram is representative of cell counts taken over five weeks following infection of cells with EBV in the presence of 10 ng/ml of IL-10. (B) Histogram is representative of the recovery of transformed samples indicated in histogram A. Cells were cultured in the presence or absence of 10 ng/ml of IL-10 and cell counts were taken at 24 hour intervals for two weeks.

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Highlight on Kyle Tretina

This work was done at the Coriell Cell Repository Research Laboratory by Kyle Tretina as part of Coriell's education program. Kyle is a high school student who has participated in research at Coriell for the previous two years. Kyle's research has been presented at multiple science fairs throughout New Jersey, including the 2007 Coriell Science Fair where Kyle took home First Place in his division and Second Place in the entire fair. Kyle went on to the Delaware Valley Regional Fair where he took home second place in Microbiology. Kyle is graduating from high school this summer and has been accepted into Wheaton College where he plans to continue his study of science. We thank him for all of his hard work and wish him luck in college and his future endeavors.

Highlight on Karen Fecenko-Tacka

Karen Fecenko-Tacka, Ph.D. is a Research Associate in the Cell Biology Research Laboratory of the Coriell Cell Repositories. Prior to her promotion and appointment this past year as Research Associate, Dr. Fecenko-Tacka was a Post-Doctoral Fellow at the Institute. She also served as a member of the science faculty that organized and taught the Stem Cell Training Course at Coriell in January of 2006. Dr. Fecenko-Tacka holds a Bachelor of Science degree with a major in Biology and minor in Neuroscience from Kings College, Wilkes-Barre, Pennsylvania. She earned her Doctorate degree in Neuroscience from State University of New York (SUNY) – Upstate Medical Center, Syracuse NY. As guide and mentor to Kyle Tretina during his research at Coriell, Dr. Fecenko-Tacka is pleased with the findings of Kyle's study and hopes to publish his work in a peer-reviewed journal.

Multipotent Mesenchymal Stem Cell Populations Isolated from Human Umbilical Cord Blood

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The laboratory of Stem Cell and Matrix Biology at the Coriell Institute for Medical Research has isolated, propagated and characterized multipotent mesenchymal stem cell (MSC) populations from human umbilical cord blood (Markov et al., 2007). Umbilical cord blood (UCB), a known source of hematopoietic stem cells, is obtained from the New Jersey Cord Blood Bank (NJCBB) housed within the Coriell Institute where stem cell-rich cord blood is anonymously donated for transplantation and research. UCB is collected postnatally from neonates, both male and female, and from a varied ethnic population whose parents have provided informed consent prior to donation. UCB is already a widely accepted source of hematopoietic stem and progenitor cells, and transplants have been performed for a number of malignant diseases (Broxmeyer, 2005).

Mesenchymal stem/stromal cells (MSCs) are fibroblast-like cells that were first described several decades ago and found in bone marrow (BM) as precursors of non-hematopoietic tissue. MSCs are self-renewing multipotent cells that can differentiate into mesoderm-derived tissues, including cartilage, bone, fat and muscle (Baksh et al., 2004). UCB has been increasingly explored as an alternative source to BM for multipotent MSCs (Broxmeyer, 2005).

Phenotypic heterogeneity has been observed among MSC populations, but specific genes associated with this variability have not been defined. To study this question, we analyzed two distinct isogenic MSC populations isolated from umbilical cord blood (UCB1 and UCB2). UCB1 cells are spindle-shaped small cells that give confluence; UCB2 cells are larger in comparison and grow in focal patches. The use of isogenic populations eliminated differences contributed by genetic background. We characterized these UCB-MSCs for cell morphology, growth kinetics, immunophenotype and potential for differentiation. UCB1 displayed faster growth kinetics, higher population doublings (Figure 1) and increased adipogenic lineage differentiation compared to UCB2; however, osteogenic differentiation was stronger for the UCB2 population (Figure 2).

To identify MSC-specific genes and developmental genes associated with observed phenotypic differences, in collaboration with Dr. Kenro Kusumi at the Children's Hospital of Philadelphia (CHOP), we performed expression analysis using Affymetrix microarrays. We compared UCB1, UCB2 and human bone marrow (BM) MSCs, and identified distinct gene expression patterns (Figure 3). Selected clusters were analyzed demonstrating that genes of developmental pathways, markers of early embryonic stages and mesodermal differentiation displayed significant differences among the MSC populations. Overall, the results confirmed the presence of two distinct isogenic UCB-derived cell populations, identified gene profiles useful to distinguish MSC types with different lineage differentiation potentials, and

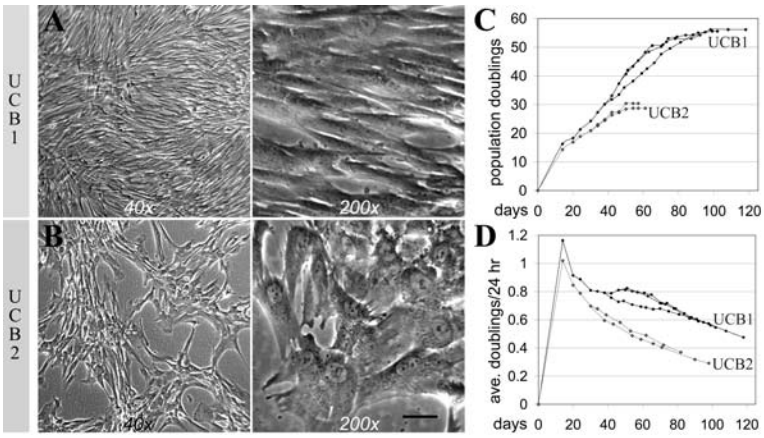

Figure. 1. Identification of distinct umbilical cord blood (UCB) derived mesenchymal stem cell (MSC) populations with different morphology and growth kinetics.

By phase contrast microscopy, UCB1 cells (A) are spindle-shaped, small cells that grow to confluence, while UCB2 cells (B) are larger and grow in focal patches (passage 1 for both cells). This morphology was maintained through all population doublings. The magnifications shown are 40x and 200x (black bar = 15 µm). UCB1 cells doubled more than 50 times in culture and grew for 19 passages, while UCB2 cells doubled less than 30 times for 13 passages (C). Average cell doublings per 24 hours over several passages is shown for all three replicates of UCB1 and two replicates of UCB2 cells (D). (Modified from Markov et al., 2007).

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Multipotent Mesenchymal Stem Cell Populations

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helped to clarify the heterogeneity observed in these cells (Markov et al., 2007). These studies will help to refine gene expression profiles associated with faster growing cells, and may also distinguish UCB-MSC types more suitable for therapeutic use as in vitro models of cell lineage differentiation.

The Stem Cell and Matrix Biology laboratory has also established a novel method for the isolation and identification of different MSC populations using low volume (~34ml) samples. This method has been the basis for the development of new Standard Operating Procedures (SOPs) for the New Jersey Stem Cell Resource (NJSCR) Grant at Coriell. The laboratory has initiated several collaborative projects on both a national and international level, aimed at establishing the basis for a program at Coriell in stem cell biology.

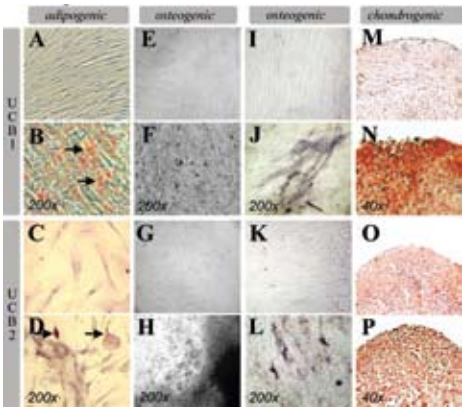

Figure 2. Differentiation capacities of distinct UCB-MSC populations.

UCB1 and UCB2 MSC populations were assayed for adipogenic (A-D), osteogenic (E-L), and chondrogenic (M-P) differentiation. Induced cells were compared to control cells grown in DMEM-high glucose with 10% FBS medium. Cells (A-P) were visualized by phase contrast- microscopy after two and three weeks in culture. Adipogenic induction, assayed by Oil Red O stained lipid droplets, is seen in induced UCB1 (B) and UCB2 (D) cells, but not in control cells (A, C). Osteogenic induction, as assayed by von Kossa staining, is seen as dark regions in treated UCB1 (F) and UCB2 (H) cells, but not in untreated controls (E, G). Osteogenic induction, assayed alternately by alkaline phosphatase, is seen as purple regions of staining in treated UCB1 (J) and UCB2 (L) cells, but not in untreated controls (I, K). Chondrogenic differentiation, detected by COL2A1 antibody staining of pellet cultures, is seen in induced UCB1 (N) and UCB2 (P) cells, but not in control cells (M, O). (Modified from Markov et al., 2007).

Among the different collaborative projects are:

a) In collaboration with Dr. Kusumi at CHOP, Dr. Saitta's laboratory used microarray expression techniques to study gene expression of oscillatory genes in UCB-MSCs while differentiating into musculoskeletal lineages. These studies are complemented by developmental studies of somitogenesis in early murine embryos (William et al., 2007) and supported by funding from the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS).

b) Studying the response of UCB-MSCs in an *in vitro* model of heart tissue damage. The research team will utilize an *in vitro* model of myocardial damage and explore extracellular matrix proteins associated with cardiac fibrosis that often follows the damage suffered from a heart attack. Through a collaborative effort with clinicians and cardiovascular researchers (Drs. Steven Hollenberg and Joseph Parrillo) at Cooper Heart Institute in Camden NJ, the research team will also analyze functional outcomes of their model and whether MSCs can improve the contractility of cardiac cells that have been injured. This study was supported by Heart Day Foundation and a recently awarded grant by the New Jersey Commission on Science and Technology (NJCST).

c) In collaboration with Drs. Parnigotto and Conconi of the University of Padova, Italy, the research team is testing the potential of the UCB-MSCs toward muscle regeneration in model systems. A Ph.D. student from Italy (supported by the University of Padova) has been working in the lab on this project as part of his research thesis during the past year.

d) In collaboration with Dr. Zhang (Thomas Jefferson University, Philadelphia), the Coriell lab is trying to determine if cord blood-derived MSCs are an effective tool for slowing down or even reversing intervertebral degeneration (IVD) using a rabbit organ culture system and degenerating rabbit discs *in vivo*. This work is supported by the Cervical Spine Research Society (CSRS).

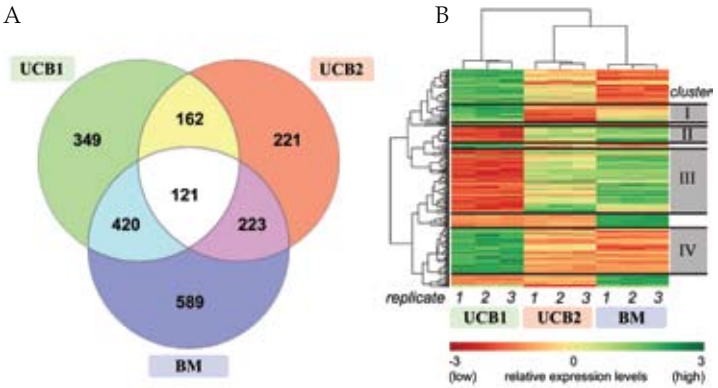

Figure 3. Microarray profile analysis of UCB1, UCB2 and BM-MSCs identifies clusters of distinct gene expression patterns.

(A) A Venn diagram is shown, representing the distribution 2,085 probe sets found to be differentially expressed on the HG-U133A at an FDR of 0.01. (B) Heatmap display of mean-centered and standardized gene expression patterns. The patterns were detected by hierarchical clustering of 290 probe sets differentially expressed between UCB1, UCB2 and BM-MSCs at an FDR of 0.001. (Modified from Markov et al., 2007).

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New Jersey Stem Cell Resource and the Umbilical Cord Blood Stem Cell Laboratory

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The New Jersey Stem Cell Resource (NJSCR) is an important new resource for researchers, offering human neonatal stem cells for use in basic and preclinical research. The Resource was established at Coriell Institute for Medical Research in 2006, with funding from the New Jersey Commission on Science and Technology. The mission of the NJSCR is to salvage umbilical cord blood specimens (UCB) that are donated to a public cord blood bank but do not meet the requirements for cord blood banking. These specimens, that would routinely be discarded, can be used in a variety of ways to meet the needs of a growing stem cell research community. The NJSCR distributes UCB samples without processing and cryopreserves mononuclear cells (MNC), cell subpopulations (CD34-enriched and depleted), and cultured mesenchymal stromal cells (MSC) derived from UCB.

NJSCR obtains units of UCB from the New Jersey Cord Blood Bank, located within Coriell Institute. These units do not meet the NJCBB laboratory's criteria for banking for clinical use. Typically, these UCB are of insufficient volume and/or cell count for use in bone marrow transplant. Donors have consented to the use of their UCB for research purposes if it cannot be used for clinical UCB banking. The NJSCR processes the UCB to meet the needs of the research community. The NJSCR was established to support stem cell research within New Jersey; however, its biomaterials also are available to the broader scientific community. While most NJSCR biomaterials are shipped to customers by overnight courier, local researchers have the option of picking up the UCB at Coriell on the same day that it is obtained.

The NJSCR laboratory staff fractionate UCB to isolate MNC. The yield of MNC from a single unity of CB varies widely, depending on the volume of UCB processed; in general, it ranges from ~100 to ~ 500 million MNC. The laboratory has established an inventory of cryopreserved UCB-MNC which are available for distribution, each vial containing ~ 100 million cells. Representative aliquots of each UCB-MNC preparation are used to assess yield and viability. Researchers can use the MNC as starting material to culture the very rare subpopulation of UCB-MSC or to perform immunomagnetic separation of a particular cell subpopulation, such as CD34+ cells.

The NJSCR has implemented a commercially-available immunomagnetic bead isolation procedure to separate UCB-MNC into CD34-enriched and CD34-depleted fractions. The NJSCR lab staff can assess subpopulation purity by flow cytometry following ISHAGE parameters (Gratama et al., 2003).The CD34-enriched fraction represents a good source of hematopoietic progenitor cells while the CD34-depleted fraction is a good source of stromal cell types, including MSC.

It has been appreciated for some time that bone marrow contains a type of stem cell that can generate new connective tissue, including bone and cartilage (Hirano and Urist, 1981). These cells, referred to as mesenchymal stem cells or mesenchymal stromal cells (MSC) hold much promise for cell-based therapies and the growing field of regenerative medicine. More recently, umbilical cord blood (Erices et al., 2000), adipose tissue (Zuk et al., 2001) and dental pulp (Pierdominico et al., 2005) have been shown to be sources of these multilineage cells. Umbilical cord blood is an attractive source of MSC, as it is easily obtained and does not require an invasive procedure.

The NJSCR has an inventory of cryopreserved MSC obtained from umbilical cord blood (UCB-MSC). The MSC were obtained using a procedure developed in the laboratory of Dr. Biagio Saitta at Coriell (Markov et al., 2007). After recovery from cryopreservation, newly established MSC lines are subjected to mycoplasma screening followed by karyotypic analysis at cell culture passage 4. Customers requesting MSC receive a flask of near confluent cells at passage 4 or 5. UCB-MSC typically can be cultured for more than 10 passages.

Over the past year, basic and clinical research involving MSC has increased dramatically, and includes potential applications for treatment of graft-versus-host disease (LeBlanc et al., 2004; Ringden et al., 2006), Crohn's disease (Garcia-Olmo et al., 2005), wound repair (Falanga et al., 2007) and repair of joint (Wilke et al., 2007), bone (Ohgushi et al., 2005, and myocardial (Katritsis, et al., 2007) injuries. In addition, there is recent evidence that MSC may be beneficial in repair of neurodegenerative diseases (Trzaska and Ramseshwar, 2007) and spinal cord injuries (Moviglia et al., 2006). The growing evidence that UCB-derived stem cell products have multiple potential clinical applications will further increase the usefulness of NJSCR, not only statewide, but nationally as well.

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New Jersey Stem Cell Resource

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We hope to extend the resources available through NJSCR to include placental mesenchymal stromal cells (P-MSC). The NJSCR staff will establish protocols to process placental specimens in order to culture P-MSC. These cells will be expanded, cryopreserved and made available to researchers as part of the NJSCR inventory. In addition, Dr. Keller plans to collaborate with Dr. Biagio Saitta (Coriell) to explore the distinctions between P-MSC and UCB-MSC. Specifically, they are interested in comparing their differentiation and proliferation capabilities as well as their cell surface marker and mRNA expression patterns. Such information will aid researchers in choosing which type of cells to utilize to answer particular scientific questions (Keller etal., 2006).

Scientists can learn more about biomaterials offered by NJSCR by visiting the Coriell website (www.coriell.org) and clicking on the link to "Stem Cell Resource". The site contains a description of available products, downloadable forms and contact information. Unlike other Coriell repositories, the items in the NJSCR inventory currently are not listed in the CCR catalog. Interested researchers can send an email message to Dr. Keller (mkeller@coriell.org) or fax a completed Registration form to (856) 757-9737.

Highlight on Dr. Margaret Keller

Dr. Keller was recruited to direct the resource in the fall of 2006. Dr. Keller is a molecular biologist whose recent research has focused on lineage commitment of hematopoietic stem cells. Dr. Keller came to Coriell from Thomas Jefferson University (TJU), where she was a Research Assistant Professor in the Cardeza Foundation for Hematologic Research in the Division of Hematology in the Department of Medicine. Her research at TJU involved isolating, culturing and analyzing transcriptional and epigenetic regulation of stem cells from peripheral and umbilical cord blood (UCB) with the goal of deciphering the transcriptional regulatory networks involved in commitment of the hematopoietic progenitor cell (HPC) to the erythroid lineage. (Keller et al., 2006) In collaboration with TJU scientists, she is extending this work to examine the gene regulatory networks involved in the choice of erythroid versus megakaryocytic lineage commitment of UCB-HPC. In collaboration with Coriell and UMDNJ-SOM faculty, Dr. Keller is interested in the dynamics of epigenetic regulation during lineage commitment of UCB-mesenchymal stromal cells (MSC). Dr. Keller has extensive administrative experience, having led both research core facilities and clinical molecular diagnostics laboratories at TJU and at A. I. DuPont Hospital for Children.

In her role as Director of NJSCR, Dr. Keller has given several oral presentations highlighting the biomaterials available from the NJSCR. These included presentations to Stem Cell Institute researchers at the Keck Center at Rutgers University in Piscataway, New Jersey in December 2006 and at a workshop sponsored by Coriell and the New Jersey Commission on Spinal Cord Injury Research in March 2007.

In addition to her responsibilities as Director of NJSCR, Dr. Keller is Associate Director of the Cell Culture Laboratories at Coriell and is co-principal investigator of the National Institutes of Neurologic Disease and Stroke (NINDS) Human Genetics Resource Center DNA and Cell Line Repository.

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Stem Cell Biomaterials Available by Custom Order

- Unprocessed cord blood
- Cord blood mononuclear cells
- CD34-enriched cells from cord blood
- CD34-depleted cells from cord blood
- Cord blood mesenchymal cells

For more information on placing a custom order, contact Dr. Keller at mkeller@coriell.org or visit the stem cell web page at www.coriell.org/

The NINDS Repository: Cracking Complex Neurological Disease

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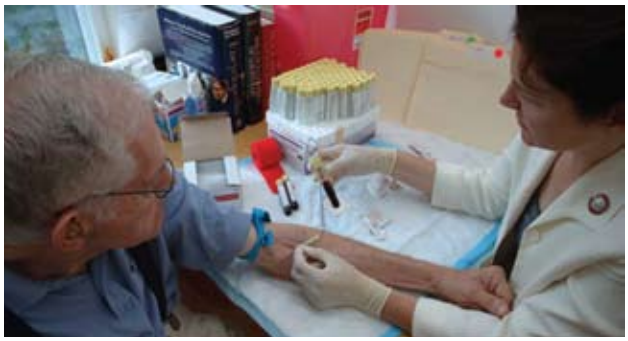
Neurodegenerative disorders affect more than one in three people at some point during life. Most forms of neurodegenerative diseases display a significant degree of heritability, and the majority of heritable human disease is genetically complex. That is, rather than being caused by mutations in a single gene, most heritable disease involves more than one gene. In some cases multiple genes with many different variants or mutations can act alone or together in a variety of combinations to cause clinical symptoms that are virtually indistinguishable. Add to this multiplicity the interaction between the inheritance of these gene variants and environmental factors, for example diet or airborne exposure to toxins, and the path to neurodegenerative disease is even more complex. This complexity makes it challenging to discover genetic susceptibility, and thus slows the search for successful therapeutic intervention. At present the best strategy available to solve genetically complex disease involves high throughput genetic studies of large numbers of subjects.

The goal of the NINDS Human Genetics Resource Center DNA and Cell Line Repository is to offer a centralized source of samples from thousands of patients and control individuals that can be used for high throughput discovery of genetically complex causes of neurodegenerative disease. Coriell's history of high-quality biomaterials, efficiency, and world-wide distribution place it in an ideal position to support NINDS in this endeavor. Enthusiasm is high among clinician scientists and healthcare providers who are a part of this worldwide collaborative effort to enroll tens of thousands of individuals. At present the major disorders represented in the NINDS Repository are Amyotrophic Lateral Sclerosis (ALS, or Lou Gehrig's Disease), epilepsy, Parkinson disease, cerebrovascular disease (stroke), and Tourette Syndrome. Since its inception in the fall of 2002, the NINDS Repository has banked samples from more than 20,000 subjects, making it the fastest growing repository in the history of Coriell. Moreover, sales of NINDS Repository DNA and cell lines have more than doubled in the past year, and in less than five years more than 10,000 DNA samples,



400 lymphoblastoid cell lines, and 100 DNA panels have been distributed.

In addition to this groundbreaking effort in high throughput repository science, the NINDS Repository at Coriell is among the first publicly available sources of biomaterials associated with both phenotypic and genotypic data. Each sample is linked to clinical data including, for example, gender, race, age, diagnosis, and medical history for hypertension, heart disease, cancer, and neurological disorders. In addition, a series of samples are available that have been characterized for mutations known to be associated with Parkinson disease (e.g. α-synuclein triplication) and ALS (e.g. superoxide dismutase), and genome-wide single nucleotide polymorphism (SNP) data are available for many samples. To increase the rate of discovery, all of these data are freely and publicly available via the NINDS Repository catalog (http://ccr.coriell.org/Sections/Collections/NINDS/Motor.aspx?PgId=192&coll=ND).

Samples and data from the NINDS Repository have already been used for more than a dozen studies published in peer-reviewed scientific journals including Science and The Lancet Neurology. The pace of discovery is rapid, with reported results ranging from the discovery of disease-associated mutations (e.g. Simon-Sanchez et al., 2007) to genomics tool development (e.g. Purcell, Sham et al., 2007). This state-of-the-art combination of biology and bioinformatics sets the stage for breakthroughs that can translate the public investment made by NINDS into improved diagnostic and prognostic tools, new targeted therapeutics and, ultimately, an improved clinical outlook for millions that suffer the consequences of neurodegenerative disease.

Selected publications that use NINDS Repository resources:

Purcell, S., Benjamin, N., Todd-Brown, K., Thomas, L., Ferreira M.A.R., Bender, D., Maller, J., de Bakker, P.I.W., Daly, M.J., and Sham, P.C. (2007) PLINK: a toolset for whole genome association and population-based linkage analyses. American Journal of Human Genetics.

Simon-Sanchez, J., Scholz, S., del Mar Martarin, M., Fung, H.-C., Herandez, D., Gibbs, J.R., Britton, A., Hardy, J., and Singleton, A. (2007) Genomewide SNP assay reveals mutations underlying Parkinson disease. Human Mutation.

For a full listing of publications that use NINDS Repository samples and data please visit:

http://ccr.coriell.org/Sections/Collections/NINDS/Publns.aspx?PgId=490&coll=ND

COHORT Collaborative For Discovery of Huntington Disease Markers: Call for Participants

Cooperative Huntington's Observational Research Trial (COHORT) is a large worldwide collaborative research effort by Huntington Study Group (HSG) research centers. The goal of this study is to discover phenotypic and biological markers of Huntington disease (HD) that can be used to improve the treatment and prognosis of HD.

Advances in understanding the pathogenesis of HD have largely been limited by the lack of availability of suitable phenotypic data that are linked to biological markers for disease progression in individual patients. COHORT addresses this need by collecting biological samples and phenotypic data from consenting individuals who are affected by HD and who are part of an HD family. The large descriptive COHORT phenotypic database is used to study the natural history and progression of HD, and biological specimens are used to investigate the relationships among HD genotypes and phenotypes. Biological specimens are also provided to scientists to identify useful biomarkers for HD.

Renewable biological resources for HD in the COHORT Repository, together with corresponding clinical data, are a rich resource designed to stimulate worldwide efforts for biomarker discovery in HD.

Who is eligible to participate in COHORT?

For those 18 years of age and older the following individuals may participate:

- Individuals who have HD or tested positive for an HD gene expansion.
- Parents, children, and siblings of individuals who have HD or tested positive for an HD gene expansion. Grandparents and Grandchildren of those individuals participating in COHORT who have HD or tested positive for an HD gene expansion.
- Spouses of those individuals participating in COHORT who have HD or tested positive for an HD gene expansion.
- HD family members who have tested negative for an HD gene expansion. These individuals must have a family member who has HD or tested positive for an HD gene expansion participating in COHORT to be included.

For those under the age of 18, only individuals who have HD are eligible to participate.

How to register to participate and to provide a sample for inclusion in COHORT

The Huntington Study Group (HSG) is an international association of more than 200 clinical investigators, coordinators, scientists and staff from 55 participating hospitals and universities in North America, Europe and Australia. Formed in 1993, the HSG strives to advance knowledge about the cause, process and clinical impact of HD in order to develop and test promising therapeutic interventions.

If you are interested in learning more about this study, contact the Huntington Study Group at 800-487-7671 or www.Huntington-Study-Group.org or visit the Huntington Project web site at www.huntingtonproject.org. The HSG and the Huntington Project are supported by the Huntington's Disease Society of America, the Hereditary Disease Foundation, the Huntington Society of Canada, and the High Q Foundation.

Highlight on Roderick A. Corriveau

Dr. Roderick A. Corriveau received a PhD in neurosciences from the University of California, San Diego in 1994. Rod was recruited to Coriell in the fall of 2006 as a Scientific Program Manager and Principal Investigator for the NINDS Human Genetics Resource Center DNA and Cell Line Repository, as well as for the COHORT Repository.

As a student and a young investigator Rod focused on how the electrical activity that is present in emerging neural circuits regulates molecular mechanisms of brain development. Rod's laboratory demonstrated that endogenous electrical activity plays a critical role in regulating naturally occurring cell death in the developing brain. His earlier postdoctoral work with Dr. Carla Shatz resulted in the seminal discovery that class I major histocompatibility complex molecules are expressed in developing neurons, where these classic immune molecules play an unexpected role in synaptic development and plasticity. Rod has ongoing collaborations with Dr. Andrei Belousov (University of Kansas) and Dr. Giles Hardingham (University of Edinburgh) that continue his studies investigating mammalian brain development.

As Principal Investigator of the NINDS Repository, Rod's goal is to work with NINDS, clinicians and clinician scientists, and the Coriell team to develop the tools necessary to discover genetic causes of neurodegenerative disease. The complex genetics of most neurodegenerative disorders, including those banked by the NINDS Repository (ALS, epilepsy, Parkinson's disease, stroke, and Tourette Syndrome), make this both a challenging and rewarding endeavor. At present the best strategy available to solve genetically complex disease is to use the power of analyzing genetic information from thousands of individuals. Therefore, the NINDS Repository banks and distributes biospecimens from large numbers of subjects. Since inception in 2002, the Repository has collected samples from more than 20,000 individuals, and numerous publications demonstrate the positive impact of the Repository and the samples in human genetics of neurodegenerative disease.

IPBIR
Integrated Primate Biomaterials and Information Resource

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In 2001 the Coriell Institute for Medical Research, the Zoological Society of San Diego, the International Species Information System and Princeton University instituted the Integrated Primate Biomaterials and Information Resource (IPBIR) with funding from the National Science Foundation (grants BCS0094928, BCS0094993 and BCS 0629321).

The IPBIR was established to provide a large accessible repository of non-human primate DNA with associated information that will advance our understanding of human origins, the biological basis of cognitive processes, evolutionary history and relationships, social structure, and provide critical scientific information needed to facilitate conservation of biological diversity. Population samples are available from a few species. Tissues collected from captive and wild animals are used to establish cell lines.

Since its inception, IPBIR has provided high quality DNA from over 40 species of nonhuman primate to researchers studying everything from molecular phylogenetics to investigating the presence or absence of genes across species. Well-characterized DNA samples from these cell lines, along with associated data, are available to the broad scientific community who agree to restrict use to non-commercial purposes.

New additions to the Integrated Primate Biomaterials and Information Resource

The Integrated Primate Biomaterials and Information Resource is expanding its collection of DNA available to researchers to include several new species:

Strepsirrhines (Prosimians)

Eulemur coronatus
Propithecus verreauxi
Propithecus tattersalli
Propithecus diadema
Nycticebus pygmaeus

Platyrrhines (New World Monkeys)

Alouatta palliata
Alouatta sara
Aotus nancymaae
Aotus azarai (multiple individuals)
Callicebus cupreus
Callithrix jacchus

Catarrhines (Old World Monkeys, Apes and Humans)

Cercobecus torquatus
Cercopithecus hamlyni
Cercopithecus mitis
Cercopithecus neglectus

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IPBIR

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Biomaterials currently available in the Integrated Primate Biomaterials and Information Resource

1) DNA panels. The IPBIR presently offers three DNA panels:
PRP00004 Great Ape Panel consisting of 5 µg of DNA from each of the following:

Bornean orangutan (Pongo pygmaeus pygmaeus)
Sumatran orangutan (Pongo pygmaeus abelii)
Common chimpanzee (Pan troglodytes)
Pygmy chimpanzee (bonobo; Pan paniscus)
Lowland gorilla (Gorilla gorilla)
And as an outgroup an olive baboon (Papio anubis)

PRP00005 Phylogenetic Footprinting/Phylogenetic Shadowing Panel consisting of 5 µg of DNA from each of the following:

Lowland gorilla (Gorilla gorilla)
Mustached guenon (Cercopithecus cephus)
White-faced saki (Pithecia pithecia)
Small-eared galago (Otolemur garnettii)
Brown lemur (Eulemur fulvus)

PRP00006 Chimpanzee Panel consisting of 5 µg of DNA from 17 chimpanzees (Pan troglodytes; 8 male, 9 female)

2) DNA from Wild Populations. The IPBIR currently offers DNA obtained from primates living in wild populations:

10 yellow baboons (Papio cynocephalus) from Amboseli National Park, Kenya
55 yellow baboons (Papio cynocephalus) from Mikumi National Park, Tanzania
10 olive baboons (Papio anubis) from Masai Mara National Reserve, Kenya
12 vervets (Chlorcebus aethiops) from Kenya

3) Whole Genome Amplified DNA from a Philippine Tarsier (Tarsius syrichta)

4) Individual DNA samples. The IPBIR currently offers DNA obtained from 117 individuals representing 65 nonhuman primate species including:

9 species of hominoids (greater and lesser apes)
28 species of old world monkeys
11 species of new world monkeys
17 species of prosimians

To place an order, please visit the IPBIR catalog at www.ipbir.org. In addition to submitting the order form, we request that customers fill out an assurance form and statement of research intent form, all of which are available on our website.

In some cases cell lines can be made available on request. Please contact us (ipbir@coriell.org) if you are interested.

Funding for the IPBIR is provided by the National Science Foundation through the Division of Behavioral and Cognitive Sciences (BCS) within the Directorate for Social, Behavioral and Economic Sciences (SBE) through grants BCS0094928, BCS0094993 and BCS0629321.

Notes from the field

The contributors to IPBIR are doing a great service by making available precious resources for the research community. On occasion we will highlight the research of various contributors in order to educate the community on the origin of IPBIR samples and the research in which they play important roles. In this issue we hear from Dr. Trudy Turner, Professor of Anthropology at the University of Wisconsin-Milwaukee.

Geographically wide spread species of primates can afford biologists insights into evolutionary processes such as gene flow, speciation and genetic drift. Wide spread species may show considerable phenotypic variability and adaptation to local environments. Movement of animals between local groups have kept the populations from speciating. It is with these wide spread species that we can begin to measure the forces of evolution in natural populations. My colleague, Dr. Paul Grobler of the University of the Free State in Bloemfontein, South Africa and I in conjunction with Dr. Joe Lorenz at Coriell, have, for the past several years, been engaged in sampling one of these wide spread species. Vervet monkeys are widely distributed across sub-Saharan Africa. They are extremely adaptable, living in environments that range from semi-arid to temperate conditions. While they exhibit considerable phenotypic variation, animals living thousands of miles apart seem to be fairly similar genetically. There has, however, been considerable debate about their taxonomic status at the generic, species and subspecies level.

Since vervets are so adaptable, they routinely come into conflict with humans. In South Africa, they have been treated as pest species and in the past, they were routinely killed. Sanctuaries exist where orphaned animals are housed. These sanctuaries have been very successful at forming social groups and the owners hope to release animals back into the wild. However, this has led to conflict with local farmers. In addition, care is needed to ensure that animals are not released into areas where other animals, that may not be genetically similar, already live. In the past, vervets in South Africa were placed into several different subspecies. Our work on vervet population genetics and taxonomy has been used to address the practical problem of what to do with animals in sanctuaries. We have been able to compare vervets in Kenya and South Africa to see how closely they are related. We have also been able to compare vervets living in multiple locations in South Africa to each other and determine the degree of genetic distance between local populations. Our work has been used by nature conservation authorities as they establish regulations for the release of animals.

Contract Services from the Coriell Institute

The Coriell Institute for Medical Research offers a menu of services employing protocols that are continually refined and validated by the experience of the Coriell Cell Repositories.

Biomaterials Banking

- Banking, Cryopreservation and Fail-Safe Storage of cell lines, DNA and other biomaterials

Cell Culture

Establishment of Cell Cultures

- Primary cultures:
  endothelial
  epithelial
  fibroblast
  smooth muscle
  EBV transformed lymphoblast cultures

Maintenance of seed and distribution stocks

- Growth of cultures to expand cell stock, for molecular and cytogenetic analyses

Immunofluorescence characterization

Detection of mycoplasma:

- PCR or microbiological assays
- Identification of mycoplasma species

Genotyping/Microarray

Affymetrix GeneChip™ Systems for Genotyping
Gene Expression Analysis
Exon-level Gene Expression Analysis
CustomSeq™ Resequencing Arrays
Whole Genome Tiling Arrays

Cytogenetics

Karyotype analysis

- Human
- Mouse (especially mouse embryonic stem cell lines)
- Rat
- wide range of mammalian species
- wide range of cell types

Fluorescent In Situ Hybridization (FISH)

- Transgene insertion site mapping

Spectral Karyotype Analysis (SKY)

- Human
- Mouse
- Rat

Molecular Biology/ Nucleic Acids

Isolation of high molecular weight DNA

Isolation of polyadenylated (mRNA) and total RNA

Whole Genome Amplification (WGA)
DNA Sequencing

Stem Cell Biomaterials Available by Custom Order

- Unprocessed cord blood
- Cord blood mononuclear cells
- CD34-enriched cells from cord blood
- CD34-depleted cells from cord blood
- Cord blood mesenchymal cells

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