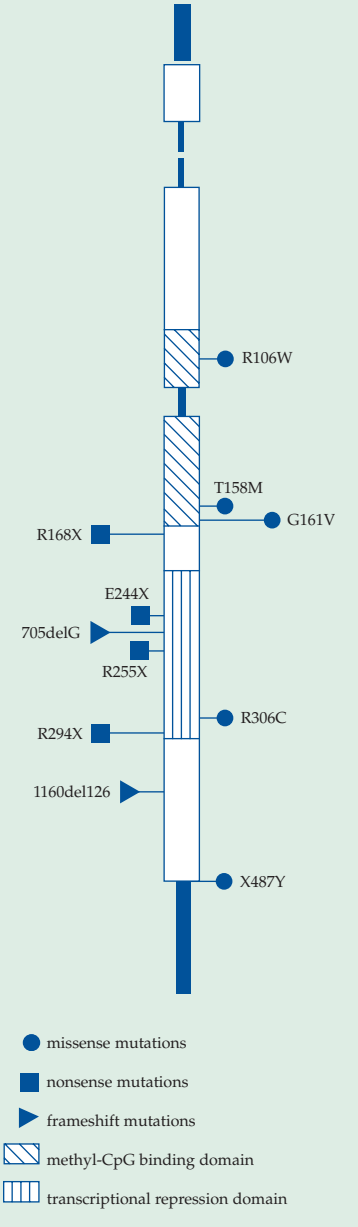
CORIELL INSTITUTE for Medical Research

Cell Collections

A publication of the Coriell Cell Repositories 2003/2004

What's New at Coriell?

Distributions of MeCP2 Mutations in Samples in the NIGMS Human Genetic Cell Repository



Human Variation Panels

In addition to its panels of 50 and 100 African Americans and Caucasians, the NIGMS Repository has added two additional panels of 100 individuals: the Mexican-American Community of Los Angeles Panel of 100 (HD100MEX) and the Han People of Los Angeles Panel of 100 (HD100CHI). The Mexican-American Community of Los Angeles Panel of 100 is a selection of 50 male and 50 female samples. Each sample is from an individual unrelated to all others in the Panel and has either three or four grandparents born in Mexico. The Han People of Los Angeles Panel of 100 is a selection of 50 male and 50 female samples. Each sample is from an individual of Han ethnicity unrelated to all others in the Panel and has all four grandparents born in Taiwan, China, or Hong Kong.

Expansion of the Caucasian Panel [Caucasian Panel of 200 (HD200CAU)]: An additional 100 individuals were added to the Caucasian panel of 100 to create the Caucasian Panel of 200. This panel consists of 200 self-declared Caucasians who are unrelated and are apparently healthy. There are 99 females and 101 males in this panel. The individual cell cultures of this panel are labeled GM17201-17300 and GM18001-18100; the corresponding DNA samples are labeled NA17201-17300 and NA1801-18100. Samples labeled GM17201-17300 represent those from the Caucasian Panel of 100; samples labeled GM18001-18100 represent an additional 100 individuals (an expansion of the Caucasian Panel of 100) and can be ordered separately from the original Caucasian Panel of 100.

In This Issue:

Longevity Collection

Studies on the genetics of aging depend on key resources, including cells and DNA from the rare premature aging syndromes which are already available from the NIA Aging Cell Repository. For studies on successful aging, new resources of cells and DNA from men and women who have reached great age and whose current health is good, though not necessarily perfect, are required. These are being assembled and are available from the Aging Cell Repository. Samples that are currently available are from octogenarians, nonagenarians and centenarians of Caucasian background (collected largely from North America and Italy) and a few

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What's Coming?

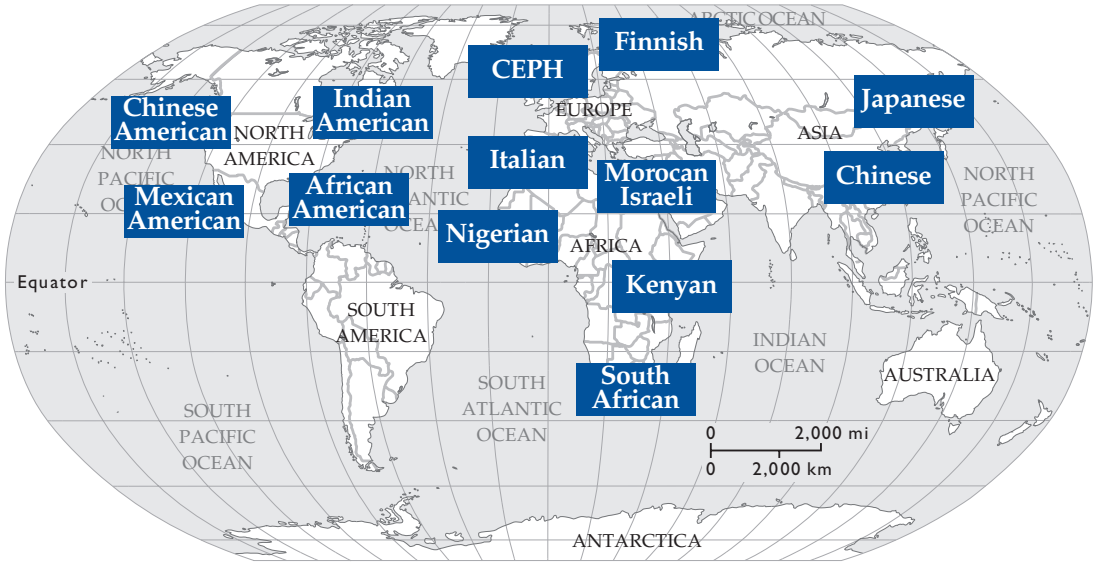
Distribution from the Integrated Primate Biomaterials and Information Resource (IPBIR)

Over the past two years, the Coriell Institute for Medical Research has been assembling and characterizing a collection of samples from non-human primates in collaboration with its partners: the Center for Research in Endangered Species (CRES) at the Zoological Society of San Diego, Princeton University, the International Species Information System (ISIS), and the San Diego Supercomputer Center, with funding from the National Science Foundation, primarily through the Division of Behavioral and Cognitive Sciences (BCS) within the Directorate for Social, Behavioral and Economic Sciences (SBE). IPBIR has received and processed more than 570 samples from 390 unique individuals representing 34 genera and 61 species of primates. These include 52 apes, 131 Old World monkeys, 37 New World monkeys, and 170 prosimians. DNA will be prepared from these samples as each cell line completes the quality control process and this DNA will be openly available to the broad scientific community for non-commercial use. In addition to providing individual DNA samples, IPBIR will assemble samples into panels, e.g., a great ape panel which will include a male from each of the great apes, with a baboon as the outgroup (see article page 20).

The Haplotype Mapping Project

An international research consortium has launched a public-private effort to create the next generation map of the human genome. Called the International HapMap Project, this new venture will provide information to understand predispositions to common illnesses such as asthma, cancer, diabetes, and heart disease. Samples will be collected from many different populations, including the Yorubas in Nigeria, Japanese, Chinese, and U.S. residents with ancestry from northern and western Europe (CEPH), China, India, and Mexico. Once lymphoblastoid cell lines have been established and DNA has been isolated from them, these samples will be stored and distributed by Coriell to researchers who will generate the HapMap.

HapMap Populations



2003: A Powerful Year of Confluence

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In 2002, Coriell celebrated the 30th Anniversary of the NIGMS Repository

- The NIGMS Repository has been continuously funded since it was established in 1972.
- As a founding scientific advisor, Dr. Kurt Hirschhorn still actively guides the Repository.
- Dr. William Mellman was the Repository's first submitter.
- The 1972 catalog had 21 pages and listed 110 cell lines; the current web catalog has 11,000 pages and lists 8,000 cell lines.
- 144 samples were distributed in 1972; over 60,000 samples were distributed last year.
- The Repository has pioneered cell culture media and techniques; protocols for cell culturing, including B-lymphocyte transformation; strategies for cryopreservation, including freezing and safe-storage; and quality control standards. Most of these have become standard practice in the field.

In 2003 the scientific community, and possibly the world beyond, will celebrate a number of powerful milestones. In April, we will celebrate completion of the finished sequence of the human genome and the 50th anniversary of the elucidation of DNA structure by Watson and Crick. In April, we will also celebrate the 31st anniversary of the establishment of the Human Genetic Cell Repository by the National Institute of General Medical Sciences (NIGMS), and in November, we will celebrate the 50th anniversary of the founding of the Coriell Institute for Medical Research.

The reader can decide for himself whether there might be any linkage between these events, but the similarity in the dates certainly suggests more than coincidence. At the same time that Drs. Watson and Crick were trying to associate structure with function in the genetic material, many scientists were also pondering the structure/function relationships in cells, and how to grow cells became a critical question. The early work of the Institute's founder, Lewis L. Coriell, M.D., Ph.D., provided the basic cell culture technology that enabled Drs. Salk and Sabin to develop the polio vaccine. Dr. Coriell and his team, working at the Institute he founded in 1953, made other critical contributions to cell biology in the areas of biological containment systems, cell culture strategies, cryopreservation of cells, and cell characterization. Use of these tools is standard practice in cell biology labs all over the world today.

The confluence of cell biology and molecular genetics made it possible to establish cell cultures from patients with genetic diseases and search for the genetic causative defects. In the early 1980s, genes for Huntington Disease and cystic fibrosis, among many others, were teased out, and the enormous collection of cells at the Coriell Institute from patients and their families representing thousands of genetic defects became the essential raw materials for the race to find and understand human genes. On April 1, 1972, the identification and isolation of hundreds of genes depended on the cell collection established by the NIGMS. Today, as we prepare for the roll out of the completed human genome sequence, that collection continues to be a central resource in human genetics, providing investigators all around the world access to many specialty subcollections, including the Polymorphism Discovery Resource, the Centre d'Etude du Polymorphisme Humain (CEPH) collection, and most recently, the Haplotype Mapping (HapMap) Resource.

The Institute established by Dr. Coriell will celebrate its 50th anniversary on November 17, 2003. During that half century, the Institute conducted research on the causes of cancer, the detection of genetic defects in cells, the association of genetic defects and environmental carcinogens, and the causes of human genetic disease. The Institute developed one of the earliest breast cancer research programs.

Today, the Coriell Institute's core expertise is still the culturing of human cells, and research programs are focused in two areas: stem cell biology and genomic diversity. Stem cell programs are aimed at progenitor cells from neuronal, hematopoietic, adipose, pancreatic, and muscle tissues. Institute labs are also studying the genes in special populations from around the world, from non-human primates, and in ancient human remains.

The confluence of these dates may have been chronologically predictable, but the import they would have for science was

not. The Human Genetic Cell Repository will continue to evolve and serve the broader scientific community, and the Institute's cell collections and research programs will focus on the increasingly complex diseases and scientific problems. The National Institute on Aging Cell Repository, which will celebrate its 30th anniversary in 2004, provides essential support for research programs on aging, surely one of the most complex issues facing us. Newer collections are also focused on complex diseases; for example the National Institute of Neurological Disorders and Stroke established in 2002 a repository of cells from patients with Parkinson's disease, epilepsy, and stroke. On the research side, understanding the complex developmental program of stem cells and how that program might be managed for therapeutic uses may well occupy scientists at Coriell and elsewhere for many years to come.

Inherited Diseases New to the National Institute of General Medical Sciences (NIGMS) Collection

- Apert Syndrome (101200)
- Cerebellar Hypoplasia (213000)
- Cerebrooculofacioskeletal Syndrome (214150)
- Cleft Hand and Absent Tibia (119100)
- Cleidocranial Dysplasia; CCD (119600)
- Emery-Dreifuss Muscular Dystrophy (300384)
- Hyaluronidase Deficiency (601492)
- LIG4 Syndrome (606593)

What's New at Coriell

(continued from page 1)

from individuals of Oriental and African American ethnicity. Each subject is accompanied by a brief statement of major health problems over the lifetime. Wherever possible these medical histories have been provided by a physician, but many are self-reported, as are instances of longevity in close relatives. It is intended that these longevity samples will be useful in the discovery of single nucleotide and other polymorphisms in a wide range of genes that influence the aging process.

18q- Syndrome

The 18q deletion (18q-) syndrome (OMIM #601808), caused by deletion of the distal long arm of chromosome 18, is a terminal deficiency or macrodeletion syndrome characterized by mental retardation and congenital malformations. It can be interpreted as having its basis in haploinsufficiency of multiple genes. The phenotype is highly variable, but is characterized by mental retardation, short stature, hypotonia, hearing impairment, and foot deformities. The syndrome is often accompanied by selective IgA deficiency and associated autoimmune disease. The contribution of individual genes within the region to specific phenotypic features can be modeled, because the occurrence or severity of these symptoms correlate with the extent of the deletion. The power of such studies depends on the size, diversity, and level of characterization of the study population. For some time the NIGMS Repository has held and distributed a limited number of 18q- lymphoblastoid cell lines (LCLs) and human-rodent somatic cell hybrids (SCHs) which include the 18q deletion chromosome. Now with the acquisition of a unique collection of seventy-seven (77) 18q (LCLs) and twenty-seven (27) SCHs from Dr. Joan Overhauser this particular NIGMS resource is greatly enhanced.

The value of the newly acquired materials lies in the extensive characterization of their deletion breakpoints by molecular cytogenetics and molecular genetics methods, together with the detailed clinical documentation of the donor phenotypes that is available. Given the great diversity of phenotypes encompassed in this condition, this collection is the definitive resource for genotype-phenotype studies of the 18q deletion syndrome and also for gene discovery.

A Brief History of IMR-90

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In 1975, the Coriell Institute for Medical Research, under contract to the National Institute on Aging (NIA) to establish and maintain an Aging Cell Repository, was directed to establish and bank for future research a new human diploid cell line as a replacement for the cell line known as WI-38. WI-38 had been established and characterized in the early 1960's as a standard (reference cell line) in the burgeoning field of cell biology; but in the early 70's, the NIH stock of low passage WI-38 cells had become seriously depleted. The cell line developed at Coriell, identified as IMR-90 (1), was the first of several lines planned in support of NIA research programs and general cell biology research. IMR-90 was developed and characterized in such a way as to parallel WI-38 as closely as possible to minimize the variables in replacing WI-38 within ongoing laboratory programs. Extensive frozen stocks of IMR-90 at various passages were laid down to ensure future resources for cell biology research.

The IMR-90 cell line, like WI-38, was derived from lung tissue of a human female embryo following therapeutic abortion. The fibroblast culture was established using standard protocols (Maitland method) in McCoy's 5A medium with 20% FBS and penicillin and streptomycin. Forty-five T25 flasks were set-up initially. After five days, the cells were sub-cultured to T75 flasks and frozen five days later. The pooled cell count was established to be 42.8 x 10^7 cells, with a viability of 93-96% by trypan blue exclusion. The pooled cells were divided into two lots for freezing: Freeze A with 357 ampules at 728,000 cells per ampule and Freeze B with 305 ampules with 506,000 cells per ampule. As it is not possible to determine population doubling levels from the time of establishing the explants until the first passage, the two doublings that occurred between the passage into the T75 flasks and the harvest for the freeze was used as the reference PDL for this cell line. Thus, the first freeze of IMR-90 was designated Passage 1, PDL2. Coriell has kept track of the cumulative PDL for all expansions since that time.

These two original freezes were intended to be the Master Cell Bank for IMR-90 to be recovered and expanded only for quality control testing and for replenishment of the distribution stock. Of the original 662 ampules of IMR-90 frozen at passage 1, seven were used for characterization of the cell line and eight for additional expansions for distribution cell stocks. The remaining cell stock has been divided among a number of fail-safe storage locations to provide maximum security for this valuable cell line.

The characterization and quality control of this cell line has been extensive. Much of the testing is routine Coriell quality control for all cell lines established at the Institute and distributed to the research community. The karyotype completed in the Coriell Cytogenetics Laboratory using the trysin-Giemsa method revealed a normal human female karyotype. The cells were assayed for bacterial, mold, yeast, and mycoplasma contamination by growth on agar plates and in broth tubes at various temperatures. All tests made at the time of the original freeze and at each subsequent expansion have been negative. The cell line was also examined by isoenzyme electrophoresis as a way of verifying species of origin and to verify cell identity during culture for expansion. The results for IMR-90 showed normal human isozyme patterns for lactate dehydrogenase, malate dehydrogenase, 6-phosphogluconate dehydrogenase, and glucose-6-phosphate dehydrogenase at the 10th and 30th population doubling levels.

REFERENCES

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The goal of creating a very well-characterized diploid cell line for use in vaccine production has meant that IMR-90 has undergone a number of tests in addition to the routine quality control of CCR cell lines. Electron microscopic examinations and reverse transcriptase assays to detect virus infection were also negative. Sister chromatid exchanges of both early and late passage IMR-90 cells were found to be within the normal limits of 3 to 12. Several additional assays were performed at collaborating institutions, including HLA typing, lifespan determinations, and virus susceptibility. HLA type, which can be useful in distinguishing among cell lines with the same karyotype, was shown to be A2, A9, BW40, B5 for IMR-90. The growth characteristics of IMR-90 were compared in several different laboratories using different media and culture practices as might be encountered throughout the research community. This cell line proved to be a robust and consistent performer with a lifespan of between 58 and 73 population doublings through various medium, seeding density, and subculture conditions.

Since the goal of establishing this cell line was as a replacement for WI-38 in vaccine production, virus yields (plaque-forming units) were compared for IMR-90, WI-38 and MRC-5 for a number of different viruses including varicella zoster, herpes simplex, vesicular stomatitis virus and cytomegalovirus. In all cases, yields from IMR-90 were comparable to those from the other cell lines, confirming its utility in this role (2).

A rigorous distribution policy was developed by NIA and Coriell to preserve the original cell stock for as long as possible while ensuring ready availability of the cell line to the research community. The original low passage "freezes" were restricted for expansion of cell stock only. Distribution freezes of PDL varying from 11 to 39 are made available through the NIA Web Catalog and replenished as necessary. Requests for other low passage cells (approximate PDL 2 to 5) are reviewed by NIA on the basis of scientific merit.

Since 1978, there have been 2,402 individual shipments of IMR-90, 130 of these to 18 foreign countries. As a result of these shipments, 299 publications which cite the use of IMR-90 have been added to the database. In addition to its use for vaccine production and as a reference cell line for functional studies, it has been used in a variety of other studies, including senescence, cellular transport, and DNA repair. Furthermore, insertion of the catalytic subunit of telomerase, hTERT, has resulted in the production of a cell line with an extended life span (3).

The cell line IMR-90 has clearly fulfilled the goal of the original proposal: a highly characterized, rigorously growing primary human fibroblast cell line that has become a cell culture standard suitable for a wide variety of cell biological research applications.

Secrets for the Successful Transformation of B-Lymphocytes

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As the custodian of several national repositories, the Coriell Cell Repositories process thousands of blood samples each year to establish lymphoblastoid cell lines. Coriell uses standard Epstein-Barr virus transformation protocols which include cell separation by gradient centrifugation and lymphocyte growth enhancement by the mitogen phytohemagglutinin (PHA). In addition, all cell cultures are grown in the absence of antibiotics to reveal any occult microbiological contamination. In reviewing the data generated during the transformation process, we have identified several parameters which appear to influence successful transformation of B-lymphocytes. These include the anticoagulant, volume of blood, the time between collection and processing, ethnicity, age, and disease status of the donor subject at the time of sampling. We will review here our experience with transformation success for each of these variables. Of note, for a transformation to be successful, the cells must be viable, i.e., recover after cryopreservation, and free from contamination with bacteria, fungi, mycoplasma, and other cell lines.

In a study using residual blood from clinical laboratories to establish stable EBV transformed cell lines for performance evaluation and quality assurance for diagnostic molecular genetic testing, we have observed that sample age, i.e., the time between collection and processing, and anticoagulant (ACD or EDTA) were statistically significant predictors of transformation success. Successful transformation was achieved in samples up to 14 days old with as little as one milliliter of blood. However, we observed that the success rate for transformation was significantly less with blood collected in EDTA tubes (29%) compared to ACD (59%) up to 21 days. To quantify the parameters for this difference, we undertook a systematic study to control variables such as volume of blood and time between collection and processing. To this end, parallel blood samples were collected in both ACD and EDTA tubes from a number of healthy volunteers between the ages of 25 and 55. To examine the effect of cell number on transformation success, transformation was initiated 24 hours after collection, with the total number of white blood cells (WBC) ranging from 2.5 to 40 million (approximately 0.5 – 8 ml of blood). All samples transformed regardless of the number of cells used and the anticoagulant. To assess the effect of aging the blood, blood collected in ACD and EDTA tubes was held at room temperature for one day, four days, and seven days before initiating transformation. After a one-day incubation, the total number of viable cells was similar in both anticoagulants [Mean + SEM (ACD: 10.36 + 1.56 x 10^6) and (EDTA: 9.63 + 1.05)]. After aging for four days, the number of viable WBC in the ACD tubes was 51% of that determined after one day of incubation, while the number of WBC in the EDTA was 25% of that observed after one day in the anticoagulant. The number of viable cells in the ACD was significantly greater than those in the EDTA tubes after a four-day incubation [5.26 + 0.96 x 10^6 (ACD) and 2.42 + 0.36 (EDTA); $p<0.01$]. Transformation success in ACD tubes is independent of the time between collection and processing up to seven days; however, with EDTA, success rates diminish over time. These data suggest that the anticoagulant to which the cells are exposed prior to the initiation of transformation may influence transformation success by affecting white blood cell viability.

In another study, cell lines were established from freshly isolated lymphocytes from more than 8,000 bloods collected from affected and unaffected individuals of families with type 2 diabetes using standard Epstein-Barr virus transformation protocols. Among Asians, African Americans, Caucasians, and Hispanics, we found that ethnicity was an important predictor of transformation success, with Hispanics having the highest success rate (95.9%) and Asians the lowest success rate [90.4% (Pearson Chi Square Analysis p=0.000], while gender and disease status did not appear to affect outcome. The time to achieve a successful transformation was dependent on ethnicity, age, and disease status of the donor subject, but was independent of gender. Time to transformation was 36.4 + 0.2 days (Mean + SEM) for individuals affected with type 2 diabetes and 35.4 + 0.3 days for unaffected individuals ($p<0.036$). Younger donor subjects (18-39 years of age) required less time in culture (34.1 + 0.7 days) compared to older donor subjects (80-98 years of age; 37.3 + 1.8 days). Asian donor subjects required an average of 38.5 + 1.8 days in culture, while African Americans required 35.4 + 0.4 days. Caucasians and Hispanics had similar times to transformation [34.0 + 0.2 and 34.0 + 0.3, respectively].

In addition to ethnicity, age of the donor subject is also an important predictor of transformation success. Comparing data for 144 apparently healthy children between the ages of 6-14 years of age with those for 232 older donor subjects (80-101 years of age), the success rate for transformation for each tube of blood was 94.4% for the younger donors compared to 80.6% with the older individuals. In addition, the time to achieve a successful transformation was significantly increased in the older groups [32.3 + 0.3 (younger donors; Mean + SEM) versus 51.2 + 1.5 (older donors)].

We have also observed that transformation success varies with the disease of the donor subject, with schizophrenia and Alzheimer disease (single culture transformation success rate of 69.2% and 78.2%, respectively) having the lower success rates and anorexia and Parkinson's disease exhibiting the higher success rates (95.6 and 96.2% respectively).

The data presented suggest that when designing protocols for studies in which lymphoblastoid cell lines will be established, it is important to understand the composition of the study population. Based on our experience, when collecting blood, if the goal is the establishment of lymphoblastoid cell lines, we would recommend the following procedures:

- collect blood in ACD (Acid Citrate Dextrose) tubes
- collect a minimum volume of 4 ml (the preferred amount is greater than 5 ml)
- store at ambient temperature until processed
- isolate the white cells within seven days
- if the donor subject cannot be re-contacted, collect several tubes of blood to ensure successful transformation

Coriell Launches Neurological Disorders Collection

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Background

During the last decade, many Mendelian causes of neurological illnesses have been identified. For most cases of Parkinson's disease, epilepsy, and stroke, as well as for other neurological disorders, however, complex genetics plays a role, and discovering the genetic risk factors in these diseases requires a large sample and clinical database. Towards this goal, a contract for the development of a repository of data, cell lines, and DNA samples, for the study of the genetic factors contributing to neurological diseases has been awarded to the Coriell Institute for Medical Research in Camden, New Jersey, by the National Institute of Neurological Disorders and Stroke (NINDS). The mission of this NINDS Repository is to develop a sample resource that can be shared by the research community, while protecting the rights of subjects, in order to expedite discoveries into the genetic causes of neurological diseases. Samples sought will be primarily venous blood although other tissues of particular import to neurological diseases may be included as they are available and as research opportunities demand.

The repository is available for immediate receipt of blood samples collected in the field of Parkinson's disease, epilepsy, and stroke. In the future, other disease categories will also be included. Generally, in order to submit samples, an investigator must have NIH funding (or be specially approved by the Project Officer for submission) and must have IRB approval for banking of samples. Additionally, samples submitted must be accompanied by a minimum of clinical data including age at time of evaluation (sampling), age of onset, disease diagnosis, family history, and minimal but germane clinical data. Samples will be shared anonymously with other submitting researchers after a specified embargo period (defined following discussions with NINDS Program Staff). In return for submission, each submitter will receive a viable immortalized cell line for each sample and a 20µ of DNA, at no cost, and, be eligible to obtain other submitted samples (which have been released from the embargo period) from the repository in their own studies for merely a processing fee.

Parkinson's

James Parkinson (in 1817) noted that 15% of his cases appeared to have a familial susceptibility, and Charcot's student, Leroux (1880) went so far as to write "Une cause vraie de la paralysie agitante et peut être la seule vraie, c'est l'hérédité." However, for the past 40 years, on the basis of twin and epidemiological studies, a genetic component had been thought improbable. These studies revealed that Caucasian ancestry, herbicide/pesticide exposure, rural residency and age are associated with a higher incidence of disease. In the past two years, this view was re-evaluated, and multiple loci (including mutations in five genes, alpha-synuclein, parkin, ubiquitin-C-terminal hydrolase, DJ-1 and tau) have been implicated in familial parkinsonism. There is evidence to suggest many more susceptibility loci are to be discovered. As a result, there has been a revolution in the study of Parkinson's disease and in the ways we think about environment, susceptibility and inheritance.

Stroke

Stroke is the third leading cause of death and a leading cause of serious, long-term disability in the United States. It is clear that family history plays a role in the risk for stroke, suggesting the influence of

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The BRCA Fanconi Connection

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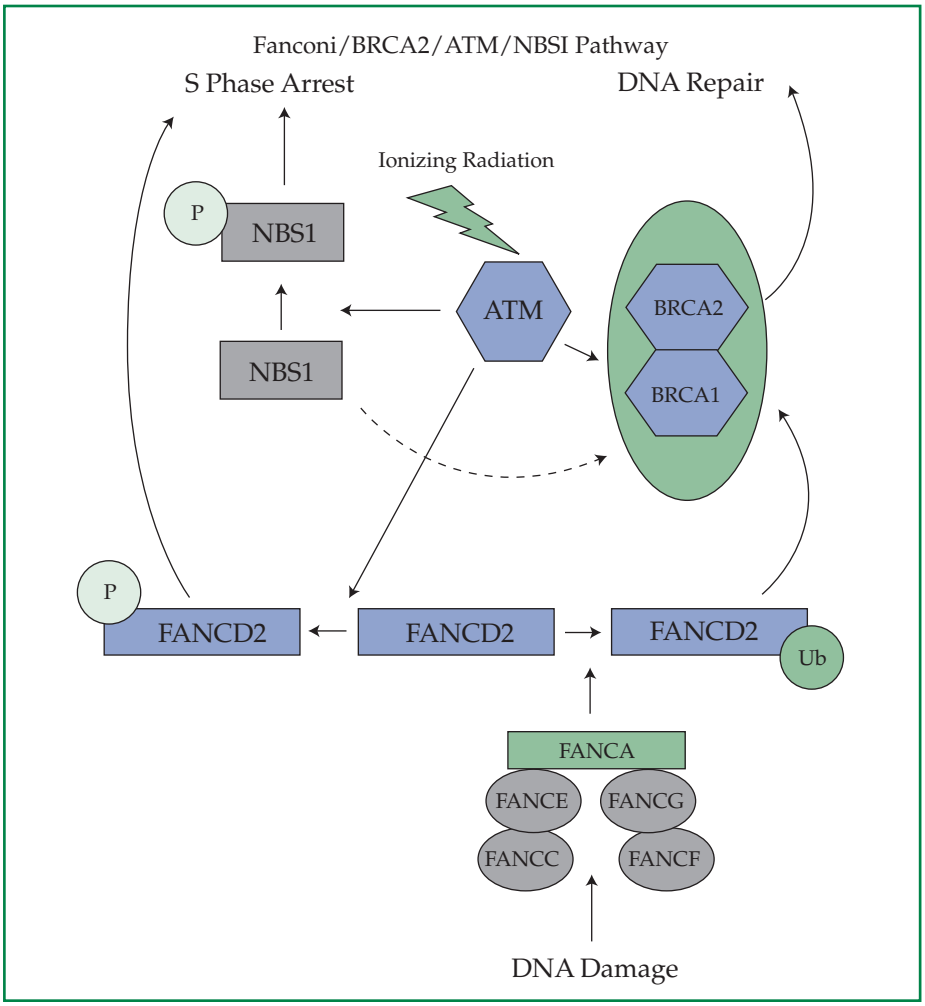
Connections Between Fanconi Anemia, Ataxia-Telangiectasia, Breast Cancer, and Nijmegen Breakage Syndrome

In the last few years there has been major progress in cloning and characterizing the genes responsible for the predisposition leading to these five disorders. The study of cell lines derived from patients with these disorders has demonstrated they are all involved in common pathways regulating DNA repair and S phase arrest. A defect at any step of these pathways leads to a failure to repair DNA damage efficiently and thus to a predisposition to a variety of cancers and other disorders. Some of the most important interactions in this pathway are shown in Figure 1. Recent progress has been reviewed (1,2,5).

Fanconi anemia has 8 identified complementation groups (A, B, C, D1, D2, E, F, and G) and genes for at least 7 of these groups have been identified. Most excitingly in 2002, two of the Fanconi anemia genes, *FancB* and *FancD1,* have been identified as the Early Onset Breast Cancer gene *BRCA2* (3). Through studies of protein-protein binding, made possible by the cloning of the genes it has been shown that five of the Fanconi anemia genes *(FancA, FancC, FancE, FancF, and FancG)* form a complex which interacts with DNA and leads to the mono-ubiquitination of the *FancD2* protein. Through an association with *BRCA1* and *BRCA2* in nuclear loci (represented by the light blue area in Figure 1) this leads to activation of the processes that lead to

Figure 1



Gene	OMIM Phenotype	OMIM #
ATM	Ataxia-Telangiectasia	208900
BRCA1	Breast Cancer, Type 1	113705
BRCA2	Breast Cancer, Type 2, early onset	600185
FANCA	Fanconi Anemia, complementation group A	607139
FANCB	Fanconi Anemia, complementation group B, BRCA2	227660
FANCC	Fanconi Anemia, complementation group C	227645
FANCD1	Fanconi Anemia, complementation group D1, BRCA2	605742
FANCD2	Fanconi Anemia, complementation group D2	227646
FANCE	Fanconi Anemia, complementation group E	600901
FANCF	Fanconi Anemia, complementation group F	603467
FANCG	Fanconi Anemia, complementation group G	602956
NBS1	Nijmegen Breakage Syndrome	602667

DNA repair. The proteins responsible for FancD2 mono-ubiquitination are as yet unknown, but may include *BRCA1* or other associated proteins. The ubiquitination step is crucial for the activation of DNA repair. The identification of *BRCA2* as *FancD1,* the gene for the D1 complementation group, shows the tight interconnection between the pathways leading to breast and ovarian cancer and those leading to Fanconi anemia.

Interacting with this pathway is serine and threonine protein kinase that is mutated in Ataxia-telangiectasia (ATM). The ATM kinase can be activated by ionizing radiation, which in turn activates many targets. One of these, the *FancD2* protein, is phosphorylated by ATM, which then leads to S phase arrest. Of interest to coffee drinkers may be the fact that the ATM kinase is inhibited by high concentrations of caffeine which accounts for some of caffeine's well known effects on DNA repair (4).

An additional link in this pathway includes the phosphorylation by ATM of the protein encoded by the gene of the Nijmegen Breakage Syndrome *(NBS1)* and *BRCA1.* The *NBS1* protein is part of a complex which in turn also leads to phosphorylation of *FancD2* by ATM. *NBS1* appears to have two independent functions, one in inducing S-phase arrest where *FancD2* is not required and the second in interacting with *FancD2* in promoting DNA repair (see dashed arrow in diagram). Thus *FancD2* is at the cross roads of two pathways — one leading to S phase arrest which functions from ATM through *NBS1* and associated proteins and the other in response to DNA damage acting through the Fanconi complex.

Over the past thirty years the Coriell Cell Repositories have made available, through the NIGMS Genetic Cell Repository and the NIA Aging Cell Repository, cell lines

Mutations associated with BRCA2 [location 13q12.3]

Mutation	Allelic Variant Number	Nucleotide Number	Codon Number	Phenotype [OMIM #600185]
Tyr42Cys		353	42	Breast cancer 2, early-onset
Trp194ter		810	194	Breast cancer 2, early-onset
983del4		983	252	Breast cancer 2, early-onset
5946delCT		5946	1908	Breast cancer 2, early-onset
1-BP Del, 6174T, fS	9	6174	1982	Breast cancer 2, early-onset
6426delTT		6426	2066	Breast cancer 2, early-onset
2-bp del, 6503TT	2	6503	2092	Breast cancer 2, early-onset
Lys3326ter		10204	3326	Breast cancer 2, early-onset

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from most of the syndromes depicted in the figure. Our largest set of cell lines is from patients diagnosed with Ataxia-telangiectasia. Of particular interest will be in understanding how specific mutations lead to different clinical outcomes. Our greatest need is to increase our holdings of cell lines for the specific Fanconi complementation groups. Our collection in Fanconi anemia reflects the clinical abundance of the complementation groups. As additional genes in neighboring pathways are discovered their roles in related syndromes will be revealed through cell lines carrying mutations.

The availability of cell lines derived from patients with these diseases has been instrumental in unraveling these pathways. A large number of mutations have been found for many of these genes, reflecting, in part the size of the genes (up to 350 kD). By investigating a large variety of cell lines with distinct mutations and phenotypes, the importance of different regions will become clear. This, in turn, may permit clinicians to predict the best course of therapy for these patients.

Coriell Launches Neurological Disorders Collection

(continued from page 9)

genetic factors. Gene discovery has already elucidated some causes of stroke and may reveal general biological mechanisms as it has in other neurological illness. Ultimately, the quality of genotype-phenotype characterizations depends on the quality of the phenotypic definitions. The Clinical Data elements to accompany each sample from a stroke subject are being developed now in conjunction with the stroke genetics research community.

Epilepsy

Epilepsy is a diagnosis encompassing more than 40 clinical syndromes consisting of biochemical, anatomic, and physiologic changes that lead to recurrent seizures. Abundant evidence of a genetic contribution to the epilepsies derives from a variety of sources, including familial aggregation studies, twin studies, linkage, association and gene identification studies in human epilepsies, and studies of human Mendelian disorders with seizures as part of the phenotype. As with other complex diseases, finding the genes responsible for epilepsy is a challenging prospect. The basic mechanisms of the epilepsies cover a substantial fraction of the neural processes that have been identified in the normal brain; thus the number of potential genes that could contribute to their expression is vast. Collaborative studies with the Coriell Institute will provide the opportunity to expand available sample size beyond what has been possible in previous studies.

Summary

The Coriell Institute has been awarded a cell line repository contract, focusing initially on Parkinson's, stroke, and epilepsy, but with a goal to expand to include other illnesses. This NINDS repository will allow receipt, storage, maintenance, standardization, quality control, and equitable, ethical distribution of DNA and other resources important to research in neurological diseases. This will allow sharing of resources and thus will encourage work by junior investigators, investigators with novel approaches, and others not included in current collaborations, without excluding those who are established in their fields. It will ensure that research participants will be making a maximal contribution, and decrease duplicative sampling efforts.

Adipose Stromal Cells: A Buried Treasure?

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Fat. Sometimes it almost seems like a four-letter word! Somehow, in spite of our best efforts to the contrary, we manage to accumulate ever more of it. Evolutionarily speaking, this tendency reflects the need to accumulate reserves during times of plenty, in preparation for the times when food is scarce. For most people in the developed world now, scarcity never comes. But at the root of this are cells, thought until the last few years to be merely precursors to fat cells, which appear to have tremendous research and therapeutic potential.

It has been known for some time that there are precursor cells present in human adipose (fat) tissue, and that these small, fibroblast-like cells can be grown and differentiated into mature adipocytes (fat cells) in culture (4,8). Thus, these cells have been generally referred to as "preadipocytes." Although they have not been used as isolated populations in human transplantation, these cells, which may be less susceptible than mature adipocytes to mechanical lysis during re-injection, may contribute significantly to the long-term survival of fresh adipose tissue grafted by injection. In addition, some exciting recent studies have shown "preadipocytes" to be multipotent, capable of differentiation not only into adipocytes, but also into chondrogenic, osteogenic, and myogenic lineages (cartilage, bone, and muscle, respectively; 1,9,3). A very recent publication suggests that it may even be possible to promote neurogenic differentiation of ASC (6). Thus, such cells of the stromal-vascular fraction of adipose tissue are now variously referred to as processed lipoaspirate cells, adipose-tissue-derived stromal cells, adipose-derived adult stem (ADAS) cells, or just adipose stromal cells (ASC). The differentiated cell types obtained from adipose stromal cells to date are a subset of those reported for the more extensively studied mesenchymal stem cells (MSC), suggesting that that ASC and MSC may be quite similar. Thus ASC, readily isolated from "waste" fat obtained from tumescent liposuction procedures, may therefore have the same potential therapeutic applications as MSC, without the ethical complications of embryonic stem cells. Furthermore, most patients in the United States could serve as their own donor, obviating any concerns about graft rejection or disease transmission. Thus, a potentially valuable resource awaits efficient mechanisms for harvesting, culture, differentiation and cryopreservation.

Isolation and Culture of Adipose Stromal Cells

ASC are isolated by a modification of the method of Strutt *et al* (7). The fat is washed with a large volume of buffer, then cleaned of obvious blood vessels or connective tissue, and cut into small pieces with sterile scalpels. The cleaned fat is washed again with saline and the floating, clean fat recovered by centrifugation. The fat is then digested with collagenase to release the ASC from the adipose tissue matrix. The ASC are isolated by centrifugation, after which the mature adipocytes float, whereas the cells of the stromal-vascular fraction, including the ASC, are found in the pellet. The cell pellet is further processed to remove debris, and plated in low serum medium similar to that developed for mesenchymal stem cells (5). Other ASC preparations initiated and subcultured in standard cell culture media containing 10% FBS may have significant fractions of endothelial and smooth muscle cells (9), and may also exhibit inefficient differentiation and a very limited lifespan *in vitro.* The use of low serum or serum-free medium is necessary to retain

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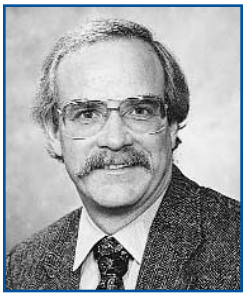
differentiation potential in response to "physiological" inducers, i.e., growth factors, vitamins and other co-factors, without pharmacologic agents (1, 7, 9). The combination of a very low serum concentration and the absence of endothelial cell growth factors allows expansion of ASC but not microvascular endothelial cells that are present in the initial stromal-vascular fraction. The primary cells are expanded and cryopreserved. The cells can then be tested for their capacity to differentiate into adipocytes, chondrocytes, osteoblasts, and muscle *in vitro* using published protocols, or tested for differentiation into other lineages with novel protocols in a manner analogous to the methods used for other stem cells.

The media developed by Lennon *et al* (2) for expansion of rat mesenchymal stem cells and the modification by Reyes *et al* for *in vitro* expansion of human "mesenchymal progenitor cells" do not support vigorous growth of human ASC without the addition of fetal bovine serum. However, the concentrations of fetal bovine serum used in most tissue culture media blocks the efficient differentiation of ASC into adipocytes, and has been reported to change the cell surface phenotype and reduce the lifespan of mesenchymal stem cells relative to that obtained in reduced serum media (5).

The Differentiated Cell Laboratory at the Coriell Institute for Medical Research is working to determine whether additional specific growth factors can substitute for FBS in expansion of ASC. This research involves testing the responses of ASC to various growth factors likely to stimulate growth and maintain stem characteristics, coupled with determination of culture lifespan and analysis of differentiation capacity. Our goal is to develop a completely defined medium to permit expansion of ASC in culture, maintaining their differentiation capacity without phenotypic alterations.

The ability of ASC to grow well *in vitro,* coupled with their multipotential differentiation capabilities, make them a valuable resource for both basic and applied research. The cells could be useful for studies of differentiation into adipogenic, chondrogenic, myogenic, and osteogenic lineages, cellular physiology, and tissue engineering. The ability to switch the cells from growth conditions to conditions favoring differentiation to a specific lineage would enable studies of sequential changes in gene expression at the mRNA or protein level, as well as efforts to improve the differentiation efficiency. The ASC could also be useful for comparative studies with other adult tissue derived stem cells. A number of human adipose cell lines have been established at Coriell from lipoaspirate waste. In addition to our usual quality control assays for microbial and viral contamination, the ASC cell lines are being characterized with respect to culture lifespan and ability to undergo adipogenic differentiation. These cell lines will be available from the National Institute on Aging Repository in the very near future. ASC lines will also be available from non-human primates (baboon and macaque) through the National Institute on Aging Repository and the Integrated Primate Biomaterials and Information Resource (IPBIR). In addition to their utility in the same kinds of investigations as the human ASC, the non-human primate lines would also be useful for genomic and other comparative studies.

Collecting Genetic Materials for Large Epidemiological Studies



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Buccal cell collection using mouthwash lavage is becoming increasingly popular as a source of DNA samples in large epidemiological studies. A properly designed method should be able to be done conveniently in the donor's residence without supervision. This non-invasive method of collection with simple instructions can promote donor compliance and allow the collection of large numbers of samples. The disadvantage of the method, compared to the conventional method of collecting peripheral blood, is the possibility of bacterial and food contamination, limiting amount of DNA, and highly variable yields of DNA (1, 2).

The Coriell Institute collaborates with several consortia in the collection of mouthwash samples to supplement the collection of blood for repository development, and the Institute has designed a kit and a procedure for mouthwash lavage that has been thoroughly tested and validated.

A kit containing a sample size bottle of commercial mouthwash, a receptacle cup, written directions, and shipping materials to return the mouthwash lavage by mail to a processing location is provided to the donor. The ethanol in the commercial mouthwash partially fixes the cells and prevents bacterial growth. Ongoing research efforts at Coriell have been focused on parameters that may affect DNA yield, such as donor age, donor gender, volume of sample collected, time between sample collection and DNA extraction, disease affected versus apparently normal donor status, and supervised versus unsupervised (mailed) sample collection. In addition to measuring total DNA yield from samples, real time-PCR assays have been developed to measure total human DNA and bacterial DNA and to determine whether the variation in yield of total DNA may be attributed to the amount of bacterial DNA.

The protocol for collecting mouthwash was that of Lum and Le Marchand (3),

Table 1

Unsupervised				Supervised			
Sample #	Total Human nDNA, µg	Total Bacteria DNA, µg	% Human DNA	Sample #	Total Human nDNA, µg	Total Bacteria DNA, µg	% Human DNA
1	15.6	0.3	98.3%	1	1.7	3.6	32.1%
2	6.4	3.5	64.6%	2	7.4	7.5	49.7%
3	24.8	3.2	88.6%	3	7.3	1.9	79.3%
4	8.5	1.0	89.5%	4	8.8	1.8	83.0%
5	22.0	3.6	85.9%	5	16.3	10.5	60.8%
6	6.5	1.1	85.5%	6	7.5	3.2	70.1%
7	8.8	3.5	71.5%	7	9.1	1.7	84.3%
8	26.7	4.0	87.0%	8	6.5	0.5	92.9%
9	7.2	23.7	23.3%	9	12.5	0.16	98.7%
10	23.8	2.2	91.5%	10	10.4	2.5	80.6%
Average per sample	**15.03**	**4.61**	**78.6%**		**8.75**	**3.34**	**73.2%**

Results of real time PCR assays measuring human nuclear DNA (nDNA) and *Streptoccus* (bacteria) DNA. Mean yield of human DNA in unsupervised samples was significantly higher at 15.03 µg than the supervised samples at 8.75 µg (p=0.047).

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using Scope for lavage. After straining through cheesecloth, DNA was prepared using Qiagen kits (Qiagen Inc., Valencia, CA) and quantified using SYBR Green (Molecular Probes, Eugene, OR). The measurement of human DNA is based on a real time PCR using a 5′-fluorogenic assay targeting c-myc sequences (4). The measurement of bacteria DNA is similarly a real time PCR using a 5′-fluorogenic assay targeting bacterial 16S ribosomal gene sequences. This assay was developed at Coriell (unpublished) and is designed to detect the ribosomal gene sequences from several *Streptococcus* species *(S. mutans, S. oralis, S. salivarus, and S. pneumoniae).* In our initial study, 389 mouthwash samples were analyzed. The median time between collection and DNA extraction was 3 days (range, 1 to 15) and yields of DNA ranged from 0.01 to 102 micrograms (µg) per subject. The variation in DNA yield does not depend on gender of the donor, volume of sample collected, ethnic origin of donor, and affected versus apparently normal donor status. However, there was a significant difference between samples collected under supervised conditions where a health professional provided directions and was present during sample collection versus unsupervised conditions where participants were provided a kit and performed the mouthwash at their convenience, generally at home, then mailed the sample to a collection point. Median total DNA yields for the samples collected under supervision (n=214) were significantly lower (1.4 µg, range 0.01 to 82.3) than the yield for the samples collected by sending a kit to the participant [n=175 (3.5 µg, range 0.07 to102)]. This result was surprising and raised the possibility that the differences in DNA yield might result from differences in bacteria contamination.

To investigate this possibility, a subset of 20 of the samples with higher DNA yields were further analyzed in the real time PCR methods for quantification of human DNA and bacterial DNA. The results are illustrated in Table 1. The total amount of human DNA and the amount of contaminating bacterial DNA vary greatly and are not proportional. There are samples with large amounts of human DNA and little bacteria DNA and samples with more bacterial DNA than human DNA. However, the difference in yield of total DNA between supervised and unsupervised samples is reflective, on average, of differences in human DNA. The unsupervised samples yield significantly more human DNA than the supervised samples. This result indicates that there may be a behavioral component in the collection of mouthwash that significantly affects the yield of DNA. Possibly, donors rinse less vigorously when supervised as compared to donors in the privacy of their residence. Whatever the reason, the results support the use of mouthwash collection under unsupervised conditions where kits can be mailed to donors, aiding in compliance and reducing expense. Studies are continuing on the behavioral aspects of mouthwash collection under controlled conditions.

Population Genetic Research in the South Pacific

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Funeral dancers in the Lau Lagoon, Malaita, Solomon Islands

Advances in molecular genetics during the past decade have transformed anthropological genetics in two major ways. First, the variety and accessibility of materials has dramatically increased. DNA from a wide variety of sources, including decades old plasmas, hair, urine and fecal materials, as well as blood draws and transformed cell lines, has been used to characterize an ever increasing number of human populations from around the globe. Secondly, long sequencing, especially in the non-recombinant mtDNA and sections of the Y chromosome, has allowed for the detection of genetic lineages via haplotype analysis that was unthinkable even during the pioneering mtDNA work of Alan Wilson and his students at Berkeley. The end result is that we are developing a more comprehensive understanding of normal human genetic diversity than ever before.

Research in this area is focusing on more exhaustive genetic analysis of samples and on more intensive sampling of human populations. Current methodology has moved beyond RFLP analysis of mtDNA to high resolution SNP analysis and DNA sequencing of large DNA segments at multiple loci. An important focus for human sampling is areas where urbanization and modern mobility has not yet obliterated structured patterns of genetic variation across populations (1). Early on, researchers preferred to rely on panels of samples taken from 150-200 individuals from 10 to 20 populations to represent the extent of human genetic diversity, and to characterize variation at a single locus at a time. This approach now is derided as "stamp collecting" within the vast ocean of human variation, tending to exaggerate the discreteness of different populations, and simultaneously to under-estimate the extent of genetic diversity within populations (2).

Our work, in collaboration with investigators at the Coriell Institute, and currently supported by grants from the National Science Foundation, The National Geographic Society, The Wenner-Gren Foundation for Anthropological Research, as well as Temple University, is an example of this more intensive phase of anthropological genetics research (3). We are sampling a highly diverse set of populations living in very close proximity to one another in the Southwest Pacific. We have now amassed a collection of over 3,000 DNA samples from villagers belonging to more than 20 different language groups in Melanesia - specifically in a section of New Guinea, and neighboring islands of New Britain, New Ireland, and Bougainville (see Map 1). This work relies heavily on Coriell's cell line human diversity collection for reference. The people living in Melanesia speak a remarkably diverse set of languages belonging to widely divergent language families, and are culturally and biologically very diverse as well. Human settlement extends back at least 50,000 years, and the diversity has developed as the result of this as well as the influence of subsequent migrations into the region. Along with this biological sample, we have collected information on individual marriage and migration rates among the neighboring areas, so that we can estimate rates of gene flow and the stochastic effects of genetic drift that should predominate in such a situation.

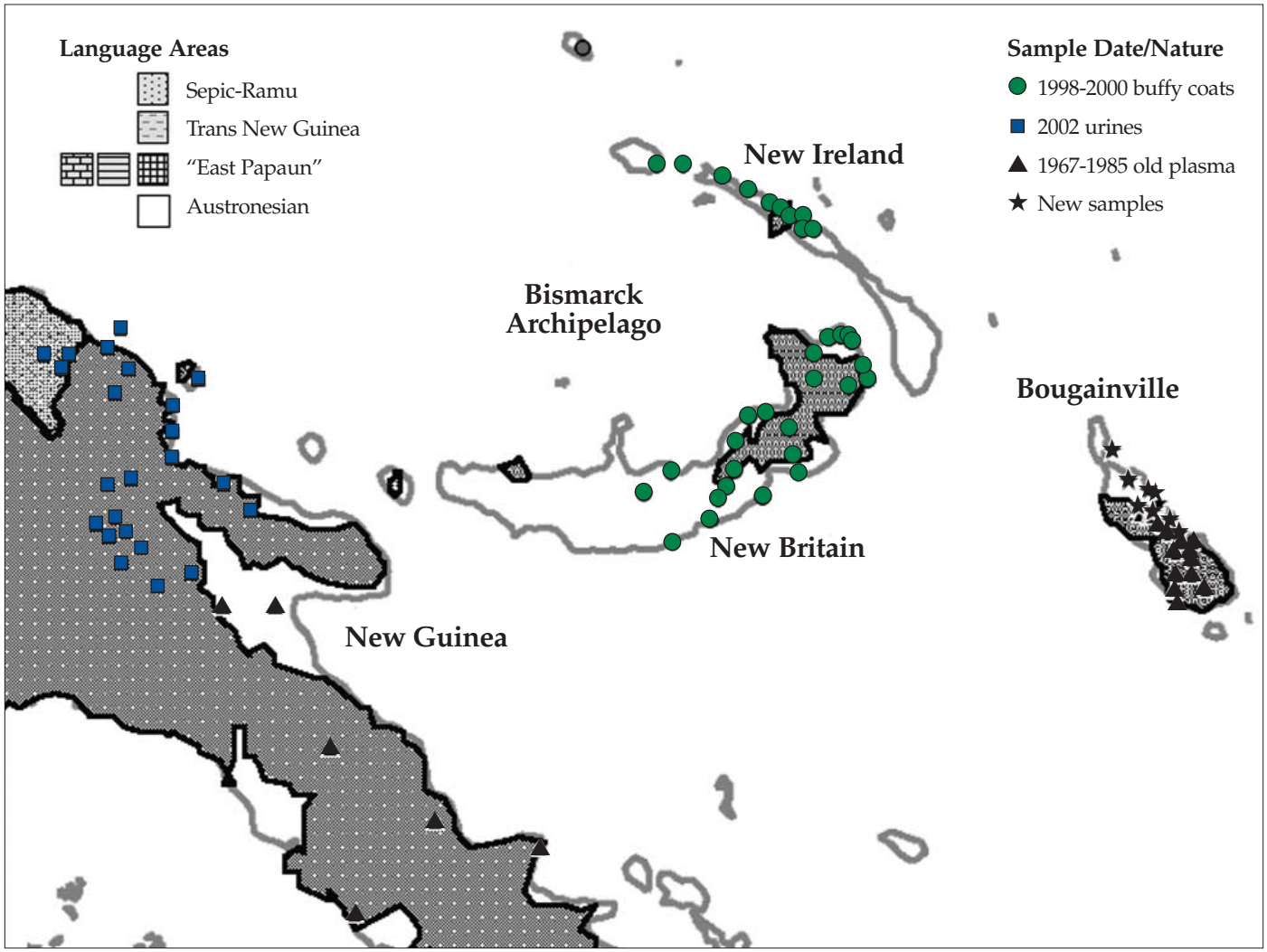
We are well along in the analysis of mitochondrial DNA haplotype diversity. The remarkable diversity is illustrated in Map 2, which shows the frequency of the most common haplotype in Bougainville Island (which belongs to Haplogroup B, or more specifically the "Polynesian motif"). This haplotype was introduced to this

island sometime during the last 3,000 years, and has spread by intermarriage through all but the most isolated mountainous regions of the island during that time. The island is only 125 miles long, but migration rates are exceedingly low and there are 19 different languages spoken only on Bougainville (4). Besides this haplotype, our intensive sampling has uncovered a group of heretofore unknown haplotypes that appear to be specific to this region, and some of which are not related to the ubiquitous Asian haplogroup family, haplogroup M.

This patterned heterogeneity in mitochondrial DNA exists only on Melanesian islands that are as large, or larger, than Bougainville. New Britain, three times the size of Bougainville, is just as diverse, but narrow New Ireland is rather uniform.

At Coriell, Joseph Lorenz and Laura Scheinfeldt (a Temple University Anthropology graduate student) are amassing data on Y chromosome polymorphism variation in the same populations. This will provide complementary information on non-recombining DNA patterns of variation molded by male demographic parameters. A selection of other nuclear variants is being included in the screen, so that an overall picture of genetic relationships will emerge within the near future.

Map 1



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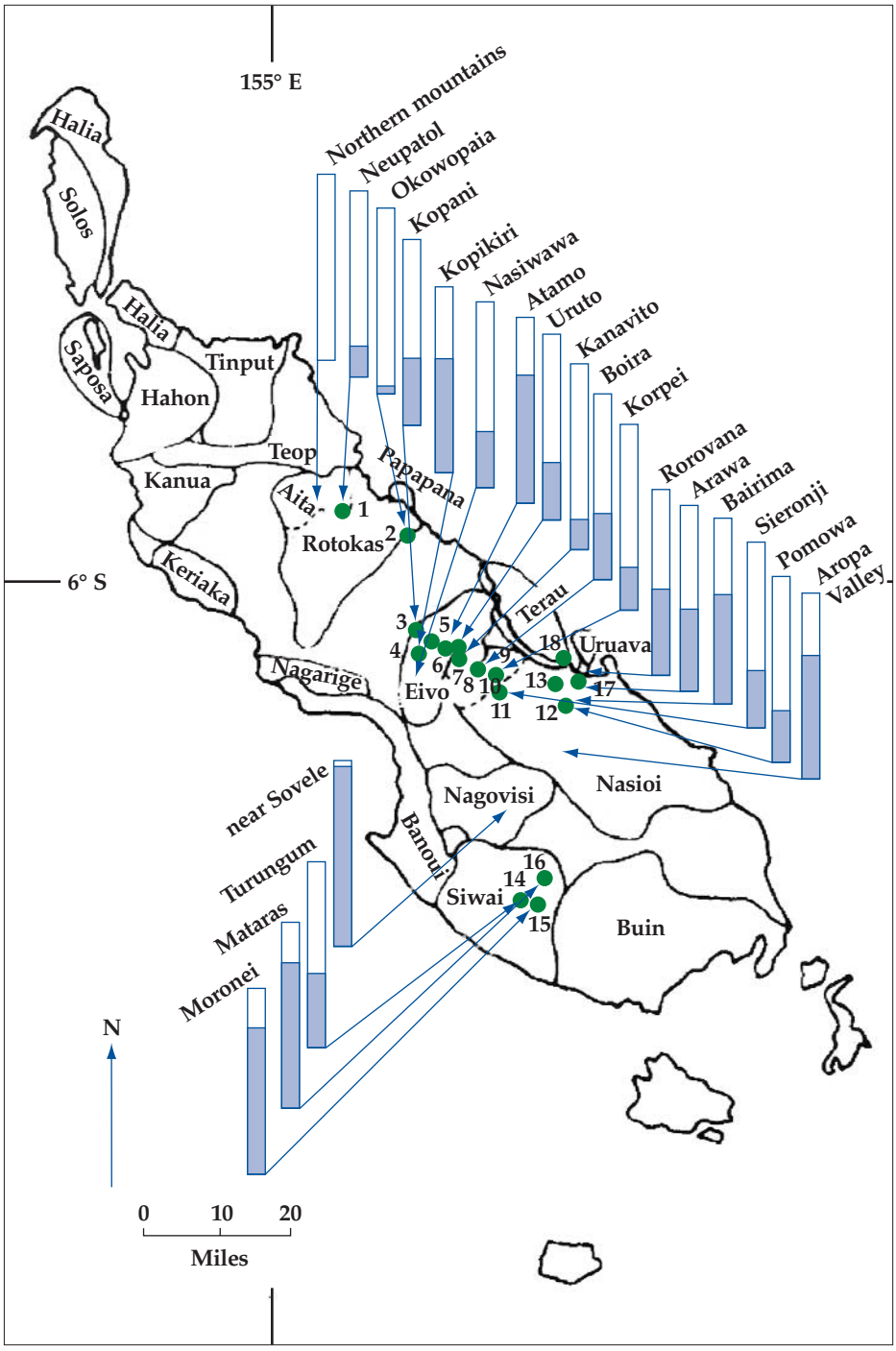
Map 2



Adventures in Kenya

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Five collaborating institutions—the Coriell Institute for Medical Research, the Zoological Society of San Diego, the San Diego Supercomputer Center, the International Species Information System, and Princeton University—have joined together in a National Science Foundation-supported collaboration to establish an Integrated Primate Biomaterials and Information Resource (IPBIR) that will take advantage of the substantial strengths of each organization to meet a pressing national and international need for high quality non-human primate biomaterials.

Many features of the IPBIR make it unlike the other repositories at Coriell. As opposed to collecting just one species (human) or very few (human and rodent), the IPBIR has 61 species to date, and is acquiring more all the time. Familiar protocols have to be modified for each new species, and some new assays have to be developed as the number of species continues to grow.

Another unique feature of the IPBIR is the concept of "capacity building." An important part of the Primate Repository is to give something back to countries from which we collect samples. As no species of non-human primate is indigenous to North America, many of the samples come from captive primates in zoos and research institutions. However, to increase the research opportunities, and therefore the value of the collection, the IPBIR plans to accession samples taken from wild animals living in their natural habitats. Wild non-human primates are often an extremely valuable resource to these range countries, primarily due to tourism. In appreciation and recognition of being able to make use of a country's valuable resources, i.e., skin biopsies or blood from wild animals, we want to reciprocate in some way, primarily through professional collaborations, training in molecular techniques, and providing educational opportunities.

Last summer, Mr. Joseph Mintzer, Chief Operating Officer and Vice President of Coriell and I visited Kenya with the goal of establishing sustainable collaborations with Kenyan institutions and scientists that

"Colobridges" allow Kenyan colobus monkeys to cross the road while remaining high up in the trees, where they prefer to be.

The Integrated Primate Biomaterials and Information Resource (IPBIR) will involve hundreds of samples from zoos, museums, primate centers, and the wild, and is expected to form the basis for comparative genetic studies of human and non-human primates.

would benefit both Kenyan research and the IPBIR in its acquisition of biomaterials. During the two-week visit, we met officially with 27 individuals, all involved with primate research, conservation, and/or the issuing of permits for exporting primate samples, and we visited eight institutions.

The most rewarding aspect of the trip was establishing collaborations with institutions such as the Institute of Primate Research (IPR), the Colobus Trust, the University of Nairobi, and the International Livestock Research Institute (ILRI), and seeing ways in which each of these institutions could collaborate with each other in ways beneficial to each. IPR and the Colobus Trust have become our two main Kenyan collaborators.

IPR is a research institution located on 500 acres in the Oloolua Forest just outside Nairobi that houses 375 non-human primates from seven different species. Most of the animals at IPR are wild-caught as adults, and then assigned to one of the on-going medical research projects. To date, we have acquired blood and skin biopsies from 24 individual primates from IPR. However, of possibly greater importance, we have also established a training collaboration with IPR. A young research assistant at IPR, Mutinda Cleophas Kyama, came to Coriell last year for a three-month internship learning the molecular techniques necessary to extract DNA from various biomaterials such as mouthwash, blood, and feces (from primates). He then returned to IPR and trained other researchers in these






techniques. Since interning at Coriell, Kyama has been accepted into a Master's program at the University Hospital Gasthuisberg in Belgium.

The Colobus Trust provided us with another opportunity to develop mutually beneficial collaborations. The Colobus Trust is a small project focused on the conservation of colobus monkeys inhabiting the forests adjacent to Diani Beach. To support the tourist business of Diani, a major road was built in the middle of the forest that is the home range for many colobus. Whereas the terrestrial baboons in the area adapted to the new traffic in their habitat, the arboreal colobus did not; many were being injured or killed by oncoming cars and trucks. The Colobus Trust, which is manned mainly by volunteers, has built more than 30 "colobridges," rope and plastic bridges that span the road at the tree canopy level. We are in the process of establishing a collaboration with the Colobus Trust in which they will send the Coriell Institute skin biopsies from some of the colobus, baboons, syke monkeys, and vervets collected opportunistically when the animals "pass through" the veterinarians office due to highway injuries or electrocution. In turn, the IPBIR sent a Princeton undergraduate student working with Dr. Jeanne Altmann, one of our IPBIR colleagues, to volunteer at the Colobus Trust last summer. We would like to send more volunteer help in the future. A final example of how the capacity building concept is being developed is the story of how we came to acquire the feces from 50 endangered Tana River red colobus. While we were in Kenya, Mr. Mintzer and I were interviewed for the science section of the *Daily Nation*, East Africa's largest newspaper. A Kenyan graduate student working on his Ph.D. at the University of Miami, Ohio read the article on the internet. He had previously collected fecal samples from these endangered animals in Kenya with the intention of doing molecular and genetic analyses on this population as part of his dissertation. Ultimately, he did not use the samples, but recognized their value; it is impossible to get permits to collect legally any invasive biomaterial from wild, endangered animals. This non-invasive source is the only means of studying the genetics of these wild animals. After reading the article about Coriell and the IPBIR repository, David Mbora contacted us and asked if Coriell would accept these samples into the collection. We did so gladly and are banking them until techniques improve for amplifying the whole genome of extremely limited and valuable DNA sources.

There is still much we are learning, and have yet to learn, about establishing successful and sustainable collaborations with institutions in Kenya, but we hope that in the end, the capacity building we are doing will be of benefit to Coriell, the IPBIR, and to primate research and conservation in Kenya.

Coriell Institute Custom Services

2003/2004

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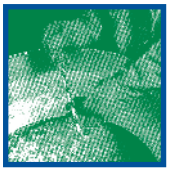
The Coriell Institute has a history of achievement and excellence in cell culture technology spanning nearly fifty years. Not only has Coriell consistently been in the forefront of developing the fundamental technology of cell culture, it has led the design of electronic catalogues for its Repositories and the implementation of relational databases as key parts of its bioinformatics mission. Since its founding in 1953, Coriell Institute scientists have pioneered many solutions to problems in cell culture, cell characterization, and cryopreservation which contribute substantially to the current standard of practice in cellular research.

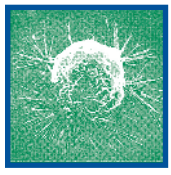
Coriell offers a wide range of services to clients and is committed to a flexible and innovative approach to all of its contract services. This assures that the particular needs of the laboratories requesting services are met.

Coriell Cell Repositories



The Coriell Cell Repositories provide essential research reagents to the scientific community by establishing, maintaining, and distributing cell cultures as well as DNA derived from these cell cultures.

Cell Culture Services



Currently, Coriell stores more than a million vials of cells in liquid nitrogen. Cells that have been stored for nearly 50 years are still viable and available for research purposes today. This is made possible by the techniques that Coriell has developed to meet the requirements of the scientific community. Coriell uses cutting edge strategies to provide the following services:

Establishment of cell lines
- EBV transformed lymphoblast cultures from human blood
- fibroblasts, endothelial, smooth muscle, and epithelial cell cultures from appropriate tissue biopsies

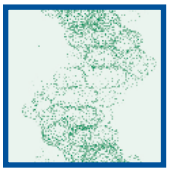
Cell banking of public, private, or specialty collections
- cryopreservation of primary or transformed cell lines
- cryopreservation of peripheral blood mononuclear cells (buffy coats)and selected tissue biopsy material
- maintenance of seed and distribution stocks
- expansion of cell lines for molecular and cytogenetic analysis

Mycoplasma Testing
- detection by PCR or microbiological assays
- identification of mycoplasma species by immunological or PCR methods

Remote Fail-Safe Storage
- cell lines
- DNA
- blood products and other biomaterials



Molecular Biology Services



During the past 10 years, Coriell has distributed nearly 90,000 DNA samples worldwide and currently stores approximately 900,000 samples of DNA (10, 20, 25 or 50 microgram aliquots). Coriell offers many preparative and diagnostic nucleic acid and molecular biology services, all subject to extensive quality controls:

DNA Isolation
- microgram to milligram quantities of high molecular weight DNA isolated from whole blood, cell lines, and tissue biopsies suitable for molecular biology applications

RNA Isolation
- polyadenylated (mRNA) and total RNA from a variety of cell lines and tissues is verified for quality by RT-PCR

Genetic Mapping
- mapping the chromosome location with genetic probes

Whole Genome Amplification (WGA)
- high fidelity amplification of DNA from limited amounts of fresh or frozen genomic materials (e.g. from mouthwash samples)

Gender Analysis
- gender analysis based on the fluorescent detection of sex chromosome specific alleles

Verification of family pedigrees
- genotyping with highly polymorphic microsatellite markers

Cytogenetics

Coriell's cytogenetic research group has three decades of experience with chromosome analysis and, using the most advanced techniques, is fully equipped for bright field and fluorescence microscopy. Digital imaging and analysis are performed using the Applied Imaging Cytovision Ultra and the Vysis Smart Capture Imaging Systems.

Karyotyping (Human)
- standard analysis of metaphase chromosomes using G-banding technique
- high resolution analysis of prometaphase chromosomes using G-banding and other banding procedures as required

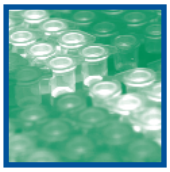
Karyotyping (Mammalian)
- standard analysis of metaphase chromosomes from a wide variety of mammalian species, including but not restricted to mouse, dog, pig, as well as mouse stem cells
- standard metaphase chromosome analysis of various primate species

FISH Analysis
- mapping
- localization of chromosome break points
- estimation of gene copy number in interphase
- determination of interphase zygosity of transgenic mice

Comparative Genomic Hybridization Analysis (CGH)
- assessment of chromosome loss, gain, or amplification in a sample of genomic DNA by competitive hybridization of two genomes against a target cell

Chromosome Aberration Analysis and Micronucleus Assays
- evaluation of genomic damage by a variety of assays to provide genotoxic end points

Research Kits and Reagents



Over the past few years, there has been an increased demand by the research community for specific reagents. To facilitate research, Coriell has developed and now offers to the public the following:

Genotyping Kits
- complete with instructions, all reagents and a control DNA sample of known genotype, this kit will provide confirmation and identity of any cell line using 5 highly polymorphic microsatellite markers

Epstein-Barr Virus
- product: supernatant of cultured B95-8 (transformed marmoset lymphocytes) filtered at 0.22 microns; medium is RPMI 1640 with 10% FBS, 2 mM L-glutamine, 4.5 g/L glucose, 1.5 g/L sodium bicarbonate; no antibiotics.
- quality control: verified to be free of bacterial and fungal contamination; free of mycoplasma by PCR assay; free of contamination with marmoset cells by isoenzyme electrophoresis for glucose-6-phosphate dehydrogenase (G6PDH), lactic dehydrogenase (LDH), and nucleoside phosphorylase (NP).

FISH Probes
- pooled 18S and 28S ribosomal cDNA
- mouse calmodulin gene

C_ot-1 DNA
- human
- mouse
- hamster

CORIELL INSTITUTE *for Medical Research*

For more information on any of these products or services, please call the Business Development Office at (856) 757-9753 or e-mail bmessina@coriell.umdnj.edu

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