Boilerplate for WNYSTEM resources in grant proposals.

WNYSTEM is a NYSTEM-funded facility that provides a variety of services to Stem Cell Researchers. The services provided by each of the 4 Core facilities is shown below.

The overall goals of our Center are to promote and facilitate research on Stem Cells with the long term objective of curing human diseases. We do this by providing facilities that will make it faster and more efficient for researchers currently using stem cells to generate, culture, analyze and test these cells both *in vitro* and in therapeutic non-human models. In addition the facilities and training provided promote stem cell usage by new investigators. The Center is composed of 4 interactive and coordinated facilities with the following functions:

1) Stem Cell culture, banking and training facility (SCCF). This facility cultures human and mouse embryonic, induced pluripotent, adult, and cancer stem cells and required feeder cells, performs experiments to differentiate stem cells into defined cell types, maintains frozen stocks of cells, and provides training for personnel who will use or want to use stem cells in their research.

2) Induced Pluripotent Stem Cell Generation Facility (iPSF). This facility generates iPS cells from mouse, human and other sources to create novel models of human disease as requested by users of the facility.

3) Stem Cell Engraftment Analysis Facility (SCEF). This facility uses *in vivo* models of stem cell engraftment to analyze the ability of adult, embryonic, and cancer stem cells to engraft, repopulate and differentiate in animals models. The facility can also analyze behavioral changes in mutant and engrafted mice.

4) Stem Cell Sequencing/Epigenomic Analysis Facility (SCSF). This facility uses next-generation sequencing including ChIP-seq and RNA-seq to analyze gene expression profiles and the global epigenetic state of stem cells and their differentiated progeny.

More Details about the cores if needed:

1) Stem Cell Culture, Banking and Training Facility (SCCF): *Core Goals:* Our goal is to maintain and bank stem cells and to provide training to researchers interested in the using stem cells in their projects.

*Core Functions:* A) Feeder cell isolation and propagation: Mouse and human ESCs are typically maintained on mouse fibroblasts (mEFs). We isolate, expand, irradiate or Mitomycin-treat, and freeze mEFs, which are available for distribution to UB researchers. mEF-conditioned medium (CM) is generated for those wanting to maintain hESCs without feeder cells. Both mEFs and CM are tested prior to their distribution to ensure their suitability for hESC cultivation. Human feeder cells are banked at the facility because some of the available hESC lines require human feeder layers for their growth. Moreover, improved human feeder cell lines may also be derived and tested in this facility in an effort to develop systems for stem cell culture free of xenogeneic agents.

B) Expansion and banking of stem cell lines: Human and mouse embryonic and adult stem cells will be propagated and stored at the facility. We currently maintain H1 and H9 (NIH registry: WA01 and WA09) from the WiCell Research Institute and HUES1, HUES7, HUES9 and HUES16 from the Harvard Stem Cell Institute. The cells will be expanded to sufficiently large quantities to satisfy the needs of researchers interested in subculturing stem cells in their laboratories if authorized. Expanded cells will be tested for contamination (e.g. mycoplasma) genome integrity and normal karyotype in our Affiliated Facilities. Cells from the iPSF will be banked here. The pluripotency of stem cells are determined by: 1) Probing stem cell-specific markers by RT-PCR, high-throughput sequencing, immunocytochemistry and flow cytometry. These procedures are performed within the SCEF and SCSF facilities and Affiliated facilities. 2) Subjecting the cells to *in vitro* differentiation for the generation of multilineage progeny when applicable (e.g. hESCs). mESC and iPSC and adult stem cell engraftment potential will be tested by the SCEF and Affiliated facilities. Cell lines will be stored as frozen stocks and revived according to needs.

**C)** Training: Short courses will be offered to UB and other interested investigators with hands-on training on stem cell culture. Course topics will include: General cell culture techniques (e.g. aseptic handling, cell splitting) in the context of stem cell cultivation, Mouse feeder cell isolation and culture. Mouse EF treatment for growth arrest and assays for quality control will also be covered. Stem cell thawing and freezing, hESC colony picking, hESC splitting (enzymatic or mechanical) and propagation, and General assessment of stem cell quality including detection of stemness markers, in vitro differentiation assays (including embryoid body culture), and karyotyping.

2) Induced Pluripotent Stem Cell Generation Facility (iPSF): *Core Goals:* To develop and use IPS cell lines for basic research and potential therapy and to train investigators to use this technology.

*Core Functions:* A) Generation of iPS Cells. We will survey Center users to generate a list of commonly needed iPS cells. After satisfying this priority, we will serve individual user’s needs on a first-come first-serve basis. Users are responsible to identify or provide the source cells for the facility to reprogram, using the best method available. Fees during the initial operating period supported by NYSTEM will be subsidized to increase user base and reduce per use charge. When a large user group is established, fees will be structured on chargebacks and institutional support.

B) Testing of iPS Cells. Generated iPS cells will go through basic quality control tests to ensure pluripotency in the SCCF and SCSF. These include staining with embryonic stem cell specific marker such as alkaline phosphatase and various species-specific surface antigens. RT-PCR will be performed to ensure the activation of endogenous ES cell genes such as Sox2, Oct4 and Nanog, as well as the silencing of exogenous viral genes. We will also perform *in vitro* differentiation using the embryoid body method to test whether cells of all three germ layers can be differentiated from iPS cells. CGH and karyotyping will be performed by our Affiliated Facilities to assess genome stability. At the user’s request, *in vivo* differentiation potential of iPS cells can be evaluated by the SCEF. After iPS cells are generated and tested, they will be expanded and banked for distribution by the SCCF*.*

C) Technology Development. Reprogramming somatic cells to iPS cells is a rapidly evolving field. Currently, we are using lentivirus to introduce human Sox2, Oct4, klf4 and c-myc to human primary skin fibroblast cells to generate iPS cells. The diverse needs of facility users makes it necessary to develop more efficient method to reprogram somatic cells from human, mouse, and other mammalian species (such as pig and rat). We will test other factors in addition to the original four factors to improve reprogramming efficiency, especially for adult somatic cells, which are known to be more difficult to reprogram. These factors include SV40 Large T antigen, catalytic subunit of telomerase (TERT), and C/EBPα. We will test various chemicals to enhance reprogramming efficiency, including ROCK inhibitor, DNA methylase inhibitor. We will also test various sources of cells, e.g. skin fibroblast, EBV-immortalized B-lymphoblasts, to develop optimized methods for different source cell types. We will also explore use of different feeders including (MEF cell lines such as SNLH9. As better methods become available, either through publication or collaboration, we will test these methods for adoption by the facility.

3) Stem Cell Engraftment and *in vivo* Analysis Facility (SCEF): *Core Goals:*  i) Stereotaxic and other surgical engrafting of ESC, iPSC, ASC and cancer stem cells into mice and other model systems. ii) *In vivo* gene transfer into endogenous stem cells in brain, heart and other organs. iii) Histochemical qualitative and quantitative stereological evaluation of stem cell survival, differentiation (morphological and biochemical) *in vivo* and *in vivo* integration of grafted or endogenous stem cells. iv) Clinical behavioral, evaluation of stem cells therapy in animal models – translational research.

*Core Functions:* A) Stereotaxic brain engraftment and gene transfer (brain ventricles and specific brain regions) cell grafting, gene transfers, brain lesions in mouse, rat and other rodents. Stem cell engraftment and gene transfer for other disease models: genetic/surgical – Cancer, diabetes, heart disease, skin diseases, retinopathies and tumors. Teratoma formation to assess hESC potential.

B) Quantitative histology and stereology Analyses of endogenous and grafted stem cells in the brain and other organs as required by users. In vivo cell labeling with BrdU, immunocytochemical analyses of stem cells development using double and triple immunostaining and confocal microscopy coordinated with the In vitro Analysis Facility and Affiliated Histology Core Facility. Quantitative stereological analyses (cell numbers, size, morphology, protein expression, etc.) of endogenous and grafted stem cells in the brain, heart, skin, pancreas, retina and other organs.

C) Neurochemical and behavioral evaluation of neural stem cells therapy in mice. The Wringer (Dept. Psychology) and Stachowiak labs developed brain microdialysis technology for in vivo measurement of neurotransmitter release and a common behavioral lab to analyze animal disease models (stroke, Parkinson's, schizophrenia, autism) and the results of stem cell therapies. These laboratories can analyze dopamine release in mouse Parkinson’s model and behaviors: sensory-motor processing, motor functions, social behavior, learning and memory.

4) Stem Cell Sequencing/Epigenomics Analysis Facility (SCSF): *Core Goals:* To characterize and identify epigenetic and transcription signatures of stem cells and their derived progeny. This facility will have two major goals. i) to characterize the transcriptome and epigenome of all stem cell types and their derived lineages. ii) to facilitate investigator initiated research on stem cells by providing experimental and computational support for genomic expression or epigenetic experiments.

*Core Functions:* The facility will function as the centralized resource for all epigenomic analysis on stem cells and function interactively with the other stem cell facilities. This center will have the capabilities to determine gene expression profiles, microRNA expression, genomic DNA methylation, histone modifications, nucleosome positioning, and genome wide protein-DNA localization though high-throughput next generation sequencing technologies. Expression profiles will be determined using RNA-seq. DNA methylation pattern will be determined genome-wide by methylated DNA immunoprecipitation and sequencing. Genome-wide histone modifications and protein-DNA localization we be determined using ChIP-seq. Nucleosome positioning will be determine using gel-excised mono-nucleosomes and high throughput DNA sequencing.

A) Epigenetic characterization of stem cells. Dr. Buck has extensive experience mapping epigenetic signatures in cells and the SCSF will use this expertise in multiple methods including, chromatin immunoprecipitation (ChIP) using antibodies specific for histone modifications, nucleosome mapping using micrococcal nuclease isolated mono-nucleosomes, and identification of nucleosome depleted regions using Formaldehyde Assisted Isolation of Regulatory Elements (FAIRE). The DNA from these experiments will be quantified by next-generation sequencing using an Illumina Genome Analyzer IIx.

B) Transcription signatures of stem cells. Both mRNA and microRNA expression profiles will be determined as desired by researchers by next-generation sequencing. Dr. Gill has significant experience with transcriptional profiling of eukaryotic and prokaryotic cells, using both microarray and next generation sequencing based technologies. The SCSF will isolate the RNA from cells, create the sequencing libraries, and sequence the samples on the GAIIx.

C) Genome wide localization of transcription factors of interest in stem cells. Dr. Buck has extensive experience with ChIP experiments and the SCSF will analyze ChIP-seq experiments. The SCSF will validate common antibodies for stem cell specific transcription factors as ChIP grade, advise in the performance of and analyze the DNA of ChIP-seq datasets. Mapping of transcription factor binding at the genome level, when combined with expression profiling, gives the most precise characterization of cell lineage location and commitment in stem cells and their differentiated progeny.