**AUTHENTICATION OF KEY RESOURCES PLAN**

**Animals:** One of the key biologic resources used in this grant is the unique rat strains that we generate for use in our studies. We are keenly aware of the many factors that can influence the outcome of experiments that utilize these whole animals either for chronic physiological studies, acute physiological studies, or collection of tissues for study.

* Custom prepared diet from the same source, Dyets, Inc. Bethlehem, PA. Is prepared from purified ingredients and shipped quarterly for use in feeding the breeders and weanlings of all the parental SS strain as well as the numerous congenic, transgenic, and knockout strains developed and maintained on the SS background.
* Well-controlled environmental rooms for breeding and colony management. Our Biomedical Resource Center has optimized the conditions under which these colonies live. Highly trained staff and well-regulated temperature, humidity, and lighting settings all contribute to a reproducible environment for the colonies to thrive.
* Avoid bias introduced by batch-to-batch phenotypic variability. We will make every effort to minimize the variability of phenotypes that are inherent to the study of a living organism. We have experienced staff that handle the animals during study and to whom the animals are accustomed. Most importantly, each treatment group will be compared to a control group studied in parallel.
* Validation of strain identity. The genotype of each gene knockout and transgenic rat used in this project will be confirmed by genotyping. Colonies of parental FP and HT rats will be periodically genotyped to confirm their identities using appropriate genomic markers.

**Cell lines:** This project will use endothelial cell lines derived from SS and SS13BN rats for functional assessment of EPC following modulation of Btg2 in Protocol 1.4. These cell lines were produced in our laboratory and have been extensively validated by both genotyping and by expression of CD31 and Dil-ac-LDL uptake. The identity of the cell lines will be authenticated regularly by short tandem repeat (STR) and verification of CD31 expression. STR profiling aids in the detection of misidentified, cross-contaminated, or genetically drifted cells.

**Other biologics:** All oligonucleotides, PCR primers, probes, and transfection constructs will be synthesized based on a set of pre-determined specifications (sequences, types and positions of chemical modifications, level of purity) and purchased at sufficient quantities at one time and properly stored to complete a study. If it becomes necessary to synthesize additional batches, the exact same set of specifications will be adhered to.

We will make every effort to purchase antibodies and other specialty chemicals in sufficient quantities at one time and properly stored to complete a study. Some of these reagents have limited shelf life. If it becomes necessary to purchase additional batches of reagents, we will make every effort to purchase the same product from the same vendor. Again, we will analyze each treatment group in parallel with an appropriate control group to avoid any systematic bias introduced by any batch-to-batch variation in reagents.

**Quality control for RNA samples:** As the experiments in Aim 2 this project are dependent on high quality RNA-seq datasets, we validate the quality of each sample before library preparation. Purity of each sample is assessed by qPCR for both positive and negative control genes. For example, the levels of both NKCC2 (to positively detect mTAL) and Aqp2 (to detect possible collecting duct contamination) are measured by qPCR for each collected sample. Similar positive and negative markers are used for ECs and EPCs in this project. In addition, several quality control steps are included to ensure we have high-quality RNA and DNA samples, libraries, and sequencing data, as described in our publications.

**Analytical and Computational tools:** This project will use several custom and commercial computational tools to analyze data generated. To avoid introduction of variability as versions of programs or databases change we have implemented systems to record the program build number, database version, operating system and input parameters for any analysis. Every effort will be made to be consistent in each of these as we complete analysis at various times throughout the program. Should it become necessary to update the analytical platform, we will first validate previous results on the new platforms before any additional analysis is completed.