Authentication of Key Biological and/or Chemical Resources

In this proposal, we will be evaluating expression and functional role of an AAA ATPase enzyme TRIP13 in breast cancer. There are multiple reagents that we will be using and generating during the course of the proposal. We will authenticate all the reagents we generate. Below, we describe the biological and chemical resources and authentication.

Biological Resources:

A) Antibodies. We will be using multiple antibodies in the project including TRIP13, EGFR, phospho-EGFR among others. TRIP13 is not a well worked out protein and hence we evaluated all the available antibodies and tested them by using cell lysate form TRIP13 specific RNA interference using both siRNA and shRNA. Additionally, we overexpressed TRIP13 and immunoblotted with TRIP13 antibodies and identified 2 specific rabbit polyclonal antibodies targeting TRIP13. We will use both the antibodies in multiple experiments. IN addition, as mouse or rabbit monoclonal antibodies that can specifically recognize TRIP13 become available, we will confirm critical data using those antibodies. Similar strategy will be applied for any other antibodies that are not well characterized previously. CIP4 antibody has been well cited and we will perform additional checks by siRNA knockdown. The EGFR, phosphor-EGFR, ERK, phosphor-ERK, b-actin, antibodies are well characterized, cited and commercially available antibodies from cell signaling technology.

B) DNA constructs. We have already constructed TRIP13 in adeno and lenti-viral vectors and sequence confirmed them as wild type. We have also generated mutant constructs for overexpression and sequence verified the mutations. We have used commercially available siRNA for TRIP13 knockdown and tested their effectiveness by immunoblots. We used these siRNA sequence and constructed shRNA through system biosciences and again sequence confirmed the constructs. CIP4 construct has been sequence confirmed. We will make all these constructs available along with the sequences for researchers as per resource sharing plans.

C) Cell lines. All the cell lines being used are obtained from American Type Culture Collection **(**ATCC) or Lonza. Furthermore, we will genotype each of the cell lines we use in the proposal to authenticate these cell lines at the UAB and Indiana University genomic core.

Chemical Resources:

A) Erlotinib, Rapamycin, PD0325901 (MEK inhibitor), JH295 (NEK2 Inhibitor), OTSSP167 (MELK inhibitor) and ZM 447439 (Aurora Kinase B inhibitor), CCT137690 (Aurora Kinase inhibitor) and EZH2 inhibitor GSK126 will be used in the study. Multiple commercial vendors sell high purity inhibitors for *in vitro* and animal experimentation (We will obtain these compounds from Cayman Chemicals (Ann Arbor, MI), BioVision (Milpitas, CA) and EMD Millipore (Billerica, MA).