STANDARD OPERATING PROCEDURE

Title: Validation samples preparation (Assessment of repeatability)

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Purpose

The purpose of this document is to describe the characterization of a set of assays according to its repeatability of measurement over 5 days. This is to estimate the performance of the assay measured in a complex sample over multiple days.

Scope

This procedure covers overall preparation and running of samples for generating the validation samples with regards to CPTAC Assay Characterization Guidance experiment #2.

Responsibilities

It is the responsibility of person(s) performing this procedure to be familiar with laboratory safety procedures. The interpretation of results must be done by a person trained in the procedure and familiar with such interpretation.

Materials

- HPLC water: Fisher Cat# W6-4
- Formic Acid: EDM Cat# 11670-1
- Acetonitrile: Fisher Cat# A955-4

Reagents

Standards:

- Heavy stable isotope-labeled standards (SIS) were synthesized with a fully atom labeled 13C and 15N isotope incorporated at the C-terminal lysine (K) or arginine (R) position of each (tryptic) peptide, resulting in a mass shift of +8 or +10 Da, respectively. Pure standards were purified to >95% purity by HPLC and quantified

by amino acid analysis. Aliquots were stored in 3% acetonitrile/0.1% formic acid at -80°C until use.

Matrix:

- An equal mix (by total protein mass) of lymphoblast cell lines (LCL, Coriell, GM07057, GM07026) treated and untreated by 10Gy ionizing radiation, was used as a background matrix. Lysates were prepared as described in SOP P-02 (Cell line lysis). The pooled lysate was aliquotted and independent digestions were performed in an automated fashion as described in SOP D-05 (Trypsin digestion of cell lysate) for each replicate of concentration points to make complete process replicates for each response curve. Following digestion, heavy synthetic peptide was added to the samples at one of eight concentrations, the samples were desalted and the phosphopeptides enriched by IMAC as described in SOP P-04 (Peptide immunoaffinity enrichment). Measurements of heavy:light (endogenous) ratio were used to characterize the assays at the concentrations described below. Digests for different days are performed >= 24 hours apart.

Procedure

Heavy SIS spike levels and preparation of samples

1. Heavy peptides were spiked into 500µg of the digested lysate at three concentrations relative to the SIS mastermix: low, medium and high. Light peptides are added at 150 fmol.
    a. High – 10x
    b. Med – 1x
    c. Low – 0.1x
2. Each sample is independently digested (see SOP D-05 for digestion protocol) to create three replicates of low, med, and high concentrations of validation samples for each day. Each aliquot of background matrix contains 500ug of total protein.
3. The samples are processed as described in accompanying SOPs.
4. Samples were run in increasing order of concentration, with wash and blank runs between each set of replicates. 'Day' replicates were run on separate days.

Referenced Documents

SOP D-05 Trypsin digestion of cell lysate.pdf
SOP P-02 Cell line lysis.pdf
SOP P-04 Peptide immunoaffinity enrichment.pdf