**Authentication of Key Biological and/or Chemical Resources**

*[Note: This document is not meant to be a “copy-and-paste” resource. Rather, it is meant to be a guideline for the types of biological and chemical resources that might require validation as required by new requirements for NIH grant applications. This document also includes a list of validation tests to consider for each resource. Obviously, it is the responsibility of each Principal Investigator to accurately and honestly describe specific validation plans that will be carried out for each project within the capabilities of the PI’s laboratory. Since each project is different, each authentication plan will be different.]*

**Overview:**

• Briefly summarize the **Key Resources** for the project. Since there is a **one-page limit**, it is important to focus on the resources that matter most for the project.

• Some grantees briefly summarize basic best practices in their laboratory, although this is not requested in PHS 398 guidelines (e.g. data storage, basic experimental design and statistical tests, online educational modules).

1. **Cell Lines**

• name source of cell lines (e.g. ATCC, in-house), and whether vendor sources provide a certificate of analysis

• name method for Authentication.

*[Note: Most common authentication is “Short Tandem Repeat profiling”. Multiple vendors offer this service. There are protocols to perform STR profiling in-house, but see “Notes and Additional resources” for a partial list of vendors that offer this service.]*

• state frequency of testing (e.g. once a year, every X months, every Z passages)

• cell lines with key mutations: describe a test to confirm mutational status of key markers/factors

• some grantees state how many weeks or how many passages will be used before discarding the culture and thawing a new vial (minimize genetic drift).

• Mycoplasma testing: most positively reviewed plans include a statement about mycoplasma. Multiple kits and services are available for testing. State the specific test and the frequency of the test. See *“Notes and Additional resources”*

1. **Antibodies**

* Some grantees provide references of published studies that have validated-vetted specific antibodies.

*[Note: Because this does not necessarily satisfy the issue of lot-to-lot variation from vendors, it is only worthwhile referencing published studies if the lot number is the same between what will be used in the study and the published validation.]*

• list commercial sources (some grantees provide catalog numbers and lot numbers) or state whether in-house antibodies are generated.

• describe a rigorous standard for testing antibody specificity, efficacy

*[Note: The most positively reviewed authentication plans for commercial antibodies did NOT rely solely on vendor certificates of authentication, but instead described one or more in-house validation tests.]*

- immunoblot analysis of cell extracts with target protein overexpressed *(weaker control)* or knocked down *(better control)*. For IHC validation, these cell lines can be formalin-fixed/paraffin-embedded and serve as positive and negative controls.

- immunoblot analysis of epitope-tagged target protein (with known shift in electrophoretic mobility).

- immunoblot analysis using more than one antibody from different sources.

- immunoblot analysis using tissues from Knock-out animals.

1. **Specialty Chemicals**

• if chemical from commercial sources, list vendor and state whether QC authentication is provided

• chemical can be validated by functional tests (describe the test briefly)

• chemical can be validated by mass spectrometry (describe the specific platform)

*[Note: UC Irvine’s mass spec facility in Chemistry has an affordable $5 cost per sample]*

• if synthesizing chemical in-house: state whether synthesis uses commercially available precursors. Intermediates and Final compounds purified by X method (e.g. crystallization? Chromatography?). State test for validation of purity (e.g. LCMS, NMR – describe specific test)

• some grantees state that specialty chemicals will be made available to the research community for additional validation and testing (refer reader to “Resource Sharing Plan” where a similar statement should appear).

1. **Purified Proteins**

• Western blot analysis, Coomassie or silver stain SDS-PAGE analysis

• validation test for enzymatic activity

• expression plasmid sequenced to confirm open reading frame

1. **Vertebrate Animals**

*[Note: unlike antisera, there seems to be greater acceptance of authentication certificates from vendors (at least currently)]*

• state commercial source of animals if purchasing.

• state if/how animals are genotyped and the general method (e.g. genomic DNA from tail tissue).

• for animals with a mixed genetic background, littermates are used to compare genotypes.

* If appropriate, state use of SNP analysis to validate strain background of mice.
* If appropriate, state use of published PCR assay to discriminate whether C57BL/6 mice have *Nnt* mutation from C57BL/6J, which can have significant effect on mutant phenotypes. Also applies to use of hybrid / outbred mice if C57BL/6 strain is in the lineage.

• Xenograft studies: state commercial type and source of immunocompromised mice if purchasing

• PDX studies: SNP analysis can compare PDX tumors to original patient sample if such data is available. State frequency (e.g. X passages).

* If mice are being generated from in-house breeding studies, state methods used to test for pathogens and frequency of testing.

1. **Plasmids**

• state source of plasmid (commercial sources with sequencing validation)

• list vendor used for sequence validation of custom plasmids

1. **Nucleic acids** (e.g. siRNA, shRNA, gRNA, etc.)

• list vendor

• for siRNA/shRNA knockdown, use multiple RNAs for each target gene

• negative controls

• validation tests (e.g. immunoblot, RT-PCR, etc.)

***“Notes and Additional resources”***

1. NIH Information about Authentication on “Extramural Nexus” website: <https://goo.gl/xRK7X4>
2. STR profiling, a partial list:

• University of Arizona (STR profiling): <http://goo.gl/oy2g5B>

• Genetica (STR profiling, AND mycoplasma testing): <http://www.celllineauthentication.com/>

• ATCC (STR profiling, database, FAQs): <http://goo.gl/OcVK48>

*[Note: The Waterman laboratory has used the University of Arizona service to STR profile a library of cell lines. Current cost is ~$40/cell line, data sent within one week, responsive technical service staff]*