YPTS  Yale Pathology Tissue Services

STSL – Specialized Translational Services Laboratory

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SERVICES OFFERED:

ANTIBODY VALIDATION:
Antibody validation is a critical important step in all aspects of scientific discovery, but it is often overlooked. The validation required is a function of the ultimate application. So, for example optimal validation for Western Blot is different than[1-3] validation for IHC. Below is a list of validation options:

- **Level BRONZE:**
  Western Blot with 12 cell lines will be performed.
  Specific staining of the antibody will be evaluated during titration (see below).

- **Level SILVER:**
  Western Blot with 12 cell lines will be performed. A TMA containing the same cell lines and 40 surgical specimens will be stained, protein expression levels as assessed by quantitative immunofluorescence will be compared to the bands detected by Western Blotting.

- **Level GOLD:**
  Western Blot with 12 cell lines will be performed. A TMA containing the same cell lines and 40 surgical specimens will be stained, protein expression levels as assessed by quantitative immunofluorescence will be compared to the bands detected by Western Blotting.
  shRNA knockdown of the suggested protein will be performed and quantified with IF.
**ANTIBODY TITRATION:**

Antibody titration is a necessary and critical step to evaluate specificity and sensitivity of an antibody. It can be performed using conventional DAB based IHC methods or quantitative immunofluorescence (QIF). QIF based antibody titration allows us to generate an expression range graph facilitating objective evaluation of the best signal to noise ratio and optimal dynamic range at different antibody concentrations.

Once optimal staining conditions are established specificity and sensitivity of the antibody can be determined as well as assay reproducibility [3].

- **Level BRONZE:**
  - Confirm client titer on a test TMA (40 spots).

- **Level SILVER:**
  - The suggested antibody will be tested with 5 different titers on test TMAs (40 spots each).

- **Level GOLD:**
  - The suggested antibody will be tested with 8 different titers on test TMAs (40 spots each).
  - Reproducibility will be assessed for the 2 most promising titers.

**QUALITATIVE ASSESSMENT OF PROTEIN AND m-RNA EXPRESSION LEVELS IN TISSUE:**

- **DAB based IHC staining performed on whole tissue sections (WTS):** Expression of the suggested protein(s) is assessed by a pathologist according to standardized recommendations for the protein(s) of interest.

**QUANTIFICATION OF PROTEIN EXPRESSION LEVELS IN TISSUE:**

- **Quantitative Immunofluorescence Analysis:** Expression of the suggested protein(s) is measured on TMAs or whole tissue sections (WTS) using quantitative immunofluorescence (quantified by AQUA or the Vectra System) [4, 5]. Assay reproducibility is assessed with test TMAs (40 spots each), positive and negative controls are included.

- **Digital Image Analysis and Quantification of DAB based IHC:** Expression of the suggested protein(s) is measured on TMAs or whole tissue sections (WTS) using semi-automated quantification of DAB based IHC (quantified by the Aperio Technology System or the Vectra system). Assay reproducibility is assessed with test TMAs (40 spots each), positive and negative controls are included.

**IN SITU QUANTIFICATION OF m-RNA LEVELS:**

- **Quantitative Immunofluorescence Analysis:** Using the RNA scope assay modified for IF and quantified by AQUA or the Vectra System, mRNA levels are measured on TMAs or WTS [6]. Ubiquitin is assessed on each spot/WTS to confirm sufficient RNA quality of the tissue. DapB
staining (a bacterial vector) is used to define threshold for positivity. Assay reproducibility is assessed with test TMAs (40 spots each).

- **DAB based assessment of mRNA:** Using the RNA scope assay for DAB staining, digital image analysis of mRNA on TMAs or WTS will be performed using the Aperio Technology System or the Vectra System. Ubiquitin is assessed on each spot/WTS to confirm sufficient RNA quality of the tissue. DAPB staining (a bacterial vector) is used to define threshold for positivity. Assay reproducibility is assessed with test TMAs (40 spots each).

**INDEX-ARRAY DEVELOPMENT:**

- A 40 to 60 spot TMA will be constructed consisting of patient tissue and cell lines with different expression levels of a protein in question. Western Blot of the same cell lines with known protein concentration will be performed and allows absolute protein quantification of patient samples and cell lines represented on the TMA.

**ASSAY DEVELOPMENT:**

- Preliminary data can be submitted in order to develop assays for use in research/clinical settings. These assays will be performed using automated, standardized and tightly controlled procedures.

**CUSTOM SERVICES**

- Custom services are available upon inquiry.
- These services include cohort collection upon request for analysis of WTSs (to assess heterogeneity of a given marker and expression levels at the invasive margins of the tumor).
- Evaluation of immune checkpoint markers using a multiplexed IF based staining approach which facilitates evaluation of different immune checkpoint panels [7, 8].
REFERENCES: