Rapid Methods for the Assessment of Tissue Quality

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Biospecimen Services

- ✓ Tissues, blood, and fluids
- ✓ RNA and DNA
- ✓ Protein Extracts
- ✓ Subcellular Fractions
- ✓ Histology
- ✓ Immunohistochemistry
- ✓ In situ hybridization
- ✓ Tumor Cell Lines
- ✓ Primary Tumor Screening
- ✓ Tissue Quality Analysis

Collaborators

Tissue Collections

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Biochemical Assay R&D (ABS**)**

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IHC/ISH

Slidomics, LLC

Reverse Phase Protein Arrays (Center for Applied Proteomics and Molecular Medicine, GMU)

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Computational Analysis

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Jake Chen, PhD (Indiana University)

Presentation Outline

- **What:** Project Goals
- **Why:** Sample Quality Challenges
  - Heterogeneity issues with human biospecimens
    - Population heterogeneity
    - Sample heterogeneity
  - Collection and processing challenges
- **How:** Experimental Approach

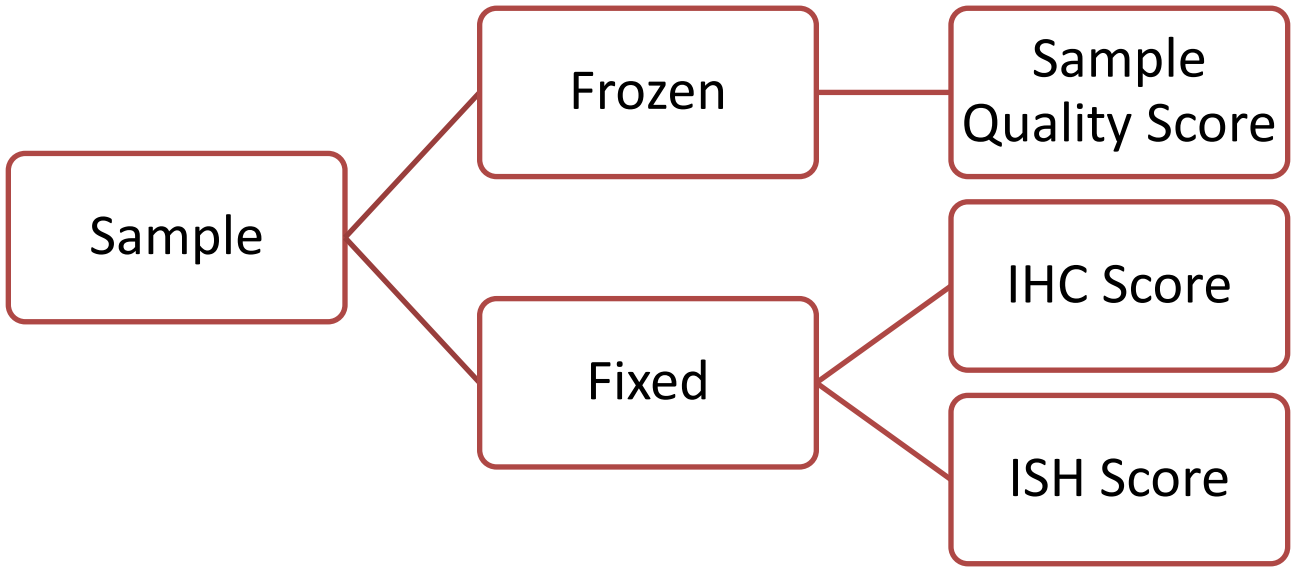
Project Goals

Develop Methods to Quickly Assess the Quality of Frozen and Fixed Tissue Samples

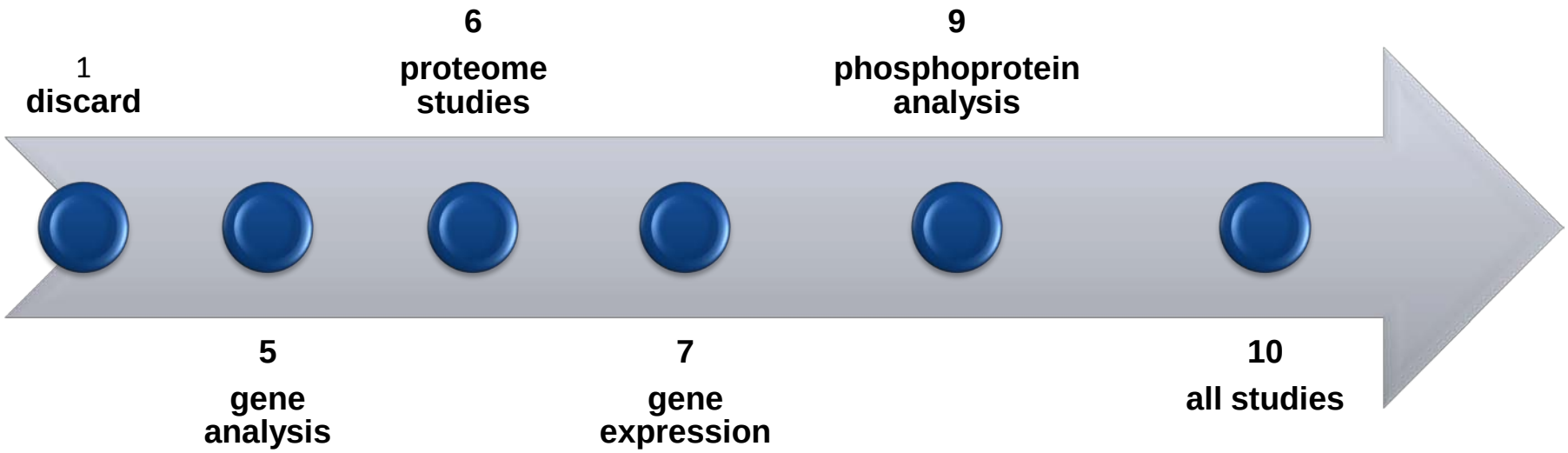
→ **Sample Quality Scores**

- Fast
- Inexpensive
- Use small amounts of tissue
- Easy to Use (mix &read, multiplexed)
- Highly predictive of “Fit for Purpose”

Quality Scores

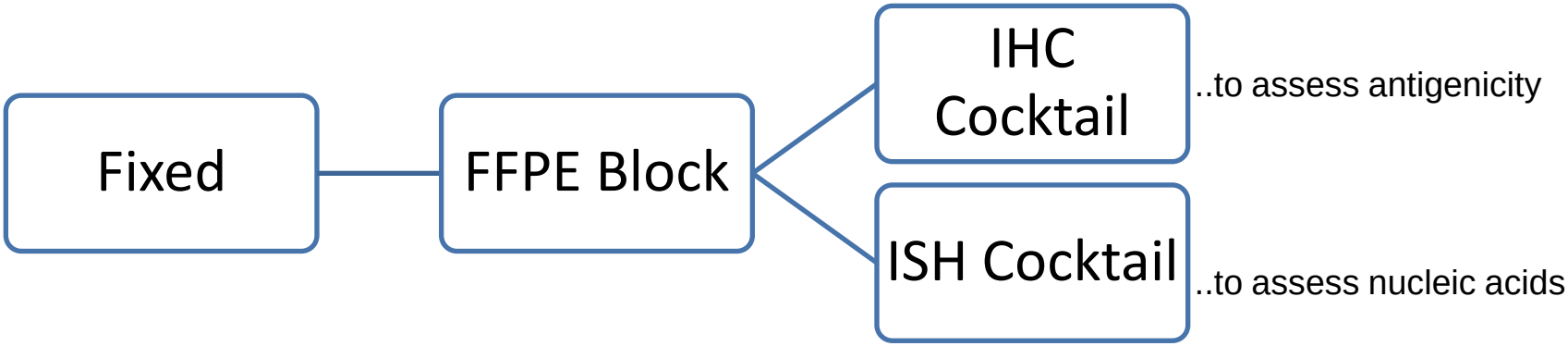


Sample Quality Score (SQS)



hypothetical example

Histological Scoring



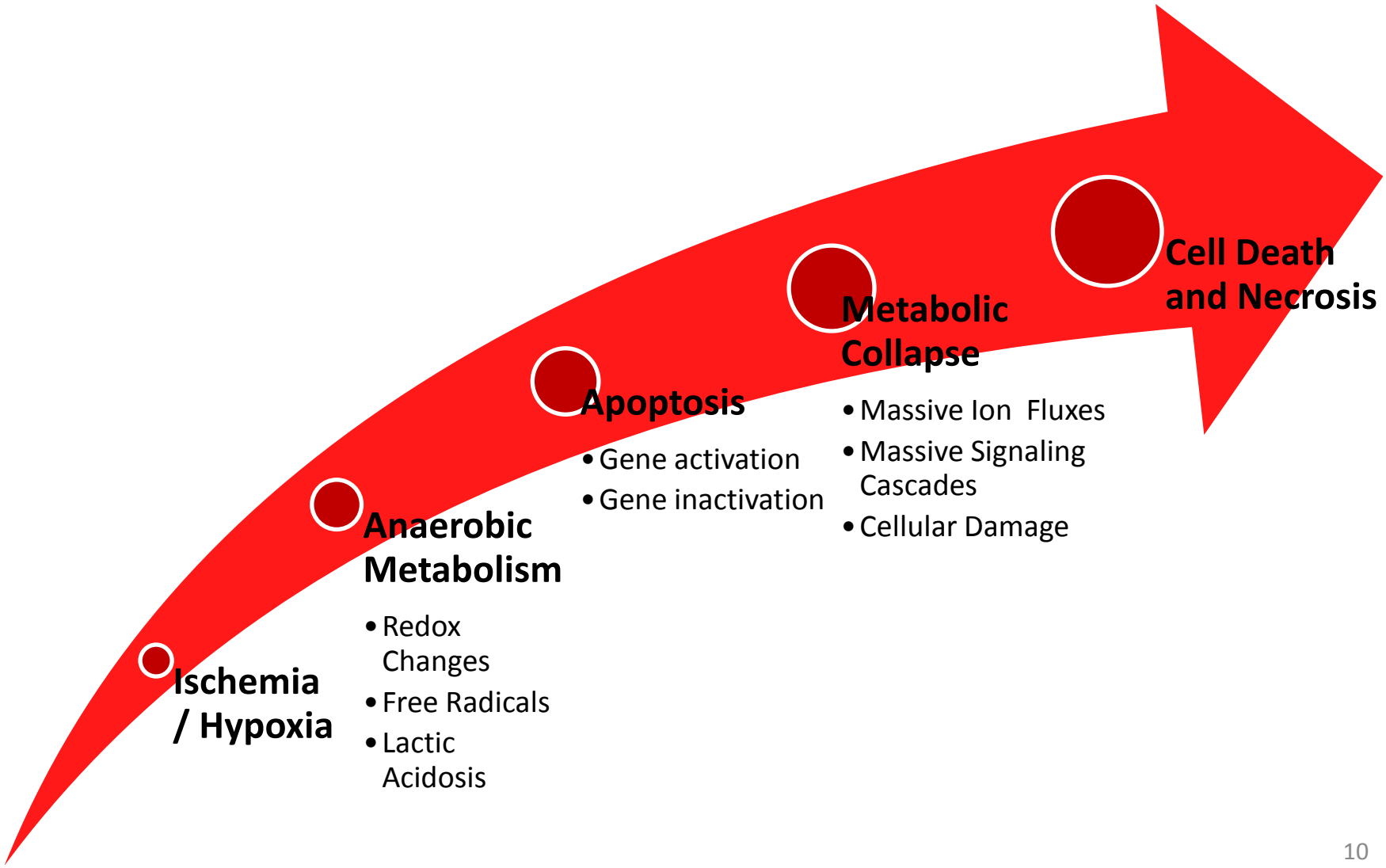
IHC cocktail = mixture of antibodies to label many cell types and proteins (actins, keratins, vimentin, inflammatory cells and proliferating cells)

ISH cocktail = β-actin and GAP-DH, RNA probes

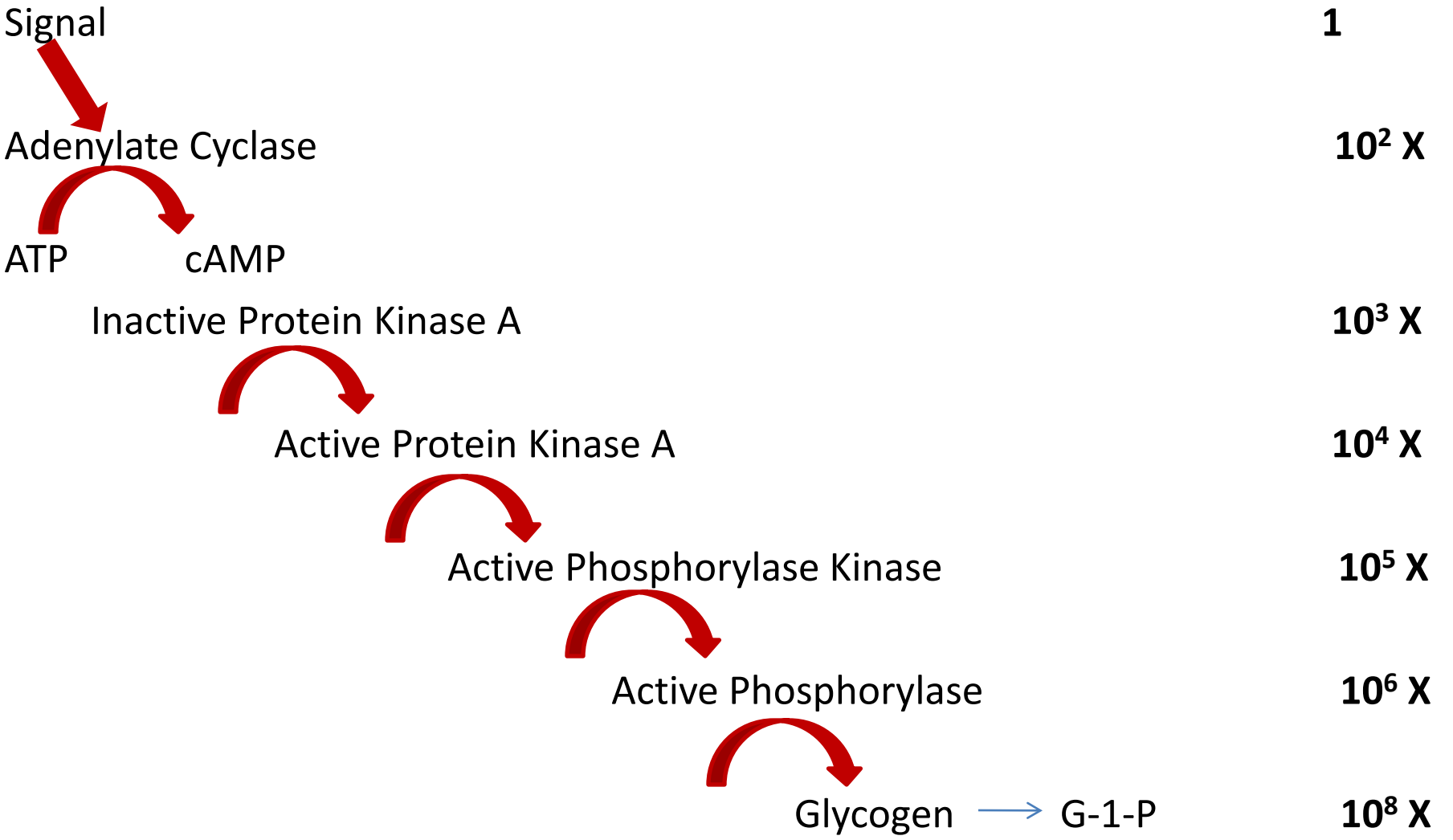
What is Tissue Quality?

- How do you know if a sample is “Good or Bad”**?**
- What does “Fit for Purpose” mean? Can a tissue be OK for one method (IHC) and not for another method (RNA) at the same time?
- How do you know if the signaling pathway (gene, etc.) changes you are measuring are a consequence of the disease or if it is related to hypoxia and/or ischemia, medications, anesthesia, etc.?

Post-Ischemia / Hypoxia Deterioration



Phosphorylation Cascades



Transcription Factors Induced by cAMP or Hypoxia

- Hypoxia Inducible Factor (HIF-1)
- NF-κB
- cAMP Response Binding Proteins (CERB), including Activating Transcription Factor (ATF-1)
- cAMP Response Element Modulator (CREM)
- Steriodogenic Factor (SF-1)

Solutions

- Standardization & Annotation
- Importance of Adjacent Tissues
- Fast, Easy, Reliable Assays
- Multivariate Analysis

Standardization & Annotation

Collection Variables

Clamp time
Time of excision
Time of freezing
Fixation time after excision
Length of fixation period
Approximate sample size
Sample Temperature
Number of sample aliquots

Clinical Variables

Anesthesia
Radiation therapy
Chemotherapy
Pre-surgical medications
Histology
Diagnosis
Tumor Staging
Race, Ethnicity

Importance of Adjacent Tissues

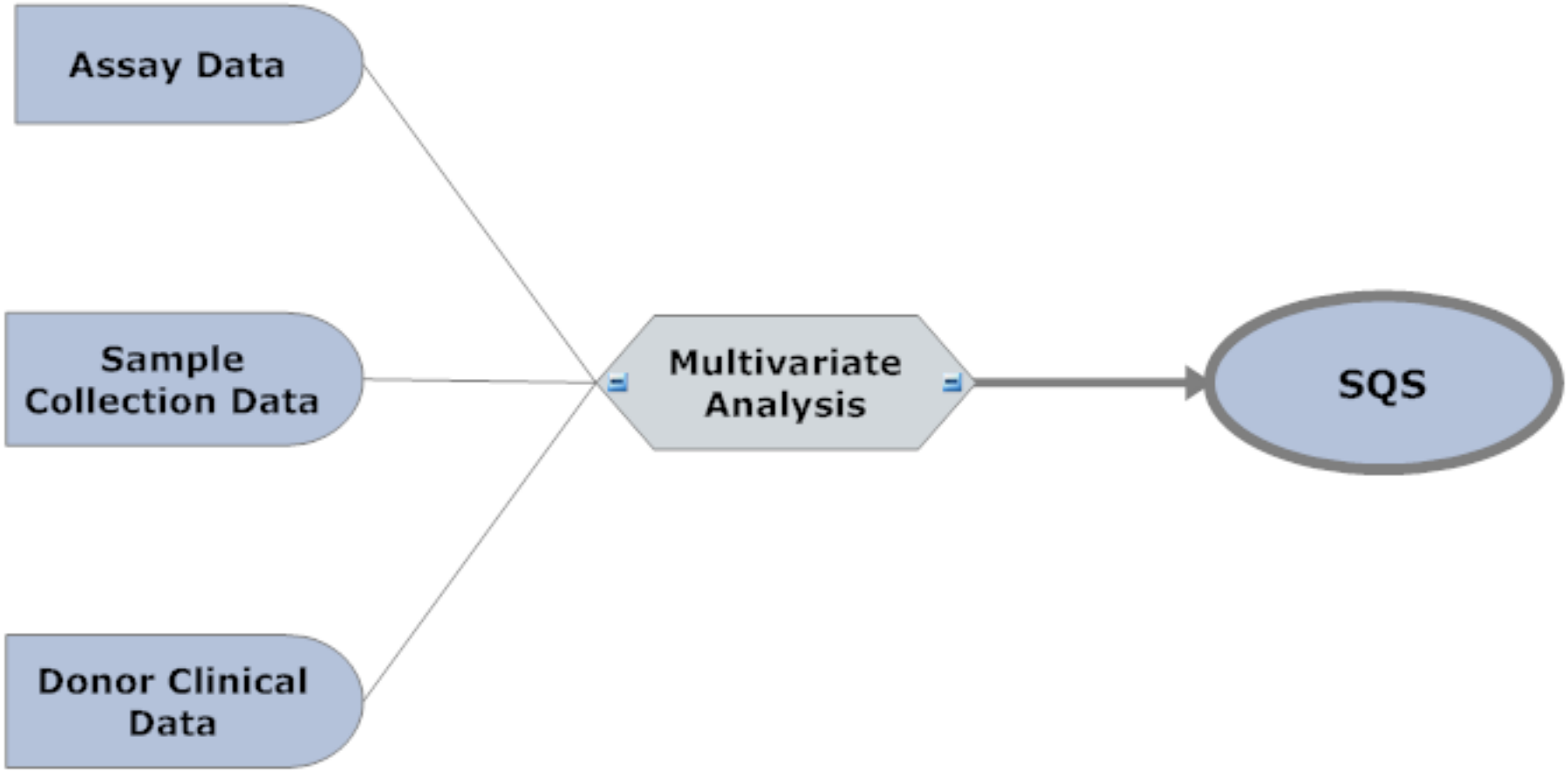
- Not “normal”, but an important internal control
- Exposed to same pre-surgical variables as tumor
- Exposed to same surgical variables (anesthesia, etc.)
- Exposed to similar processing post-excision
- Exposed to same analytical procedures

Sample Criteria

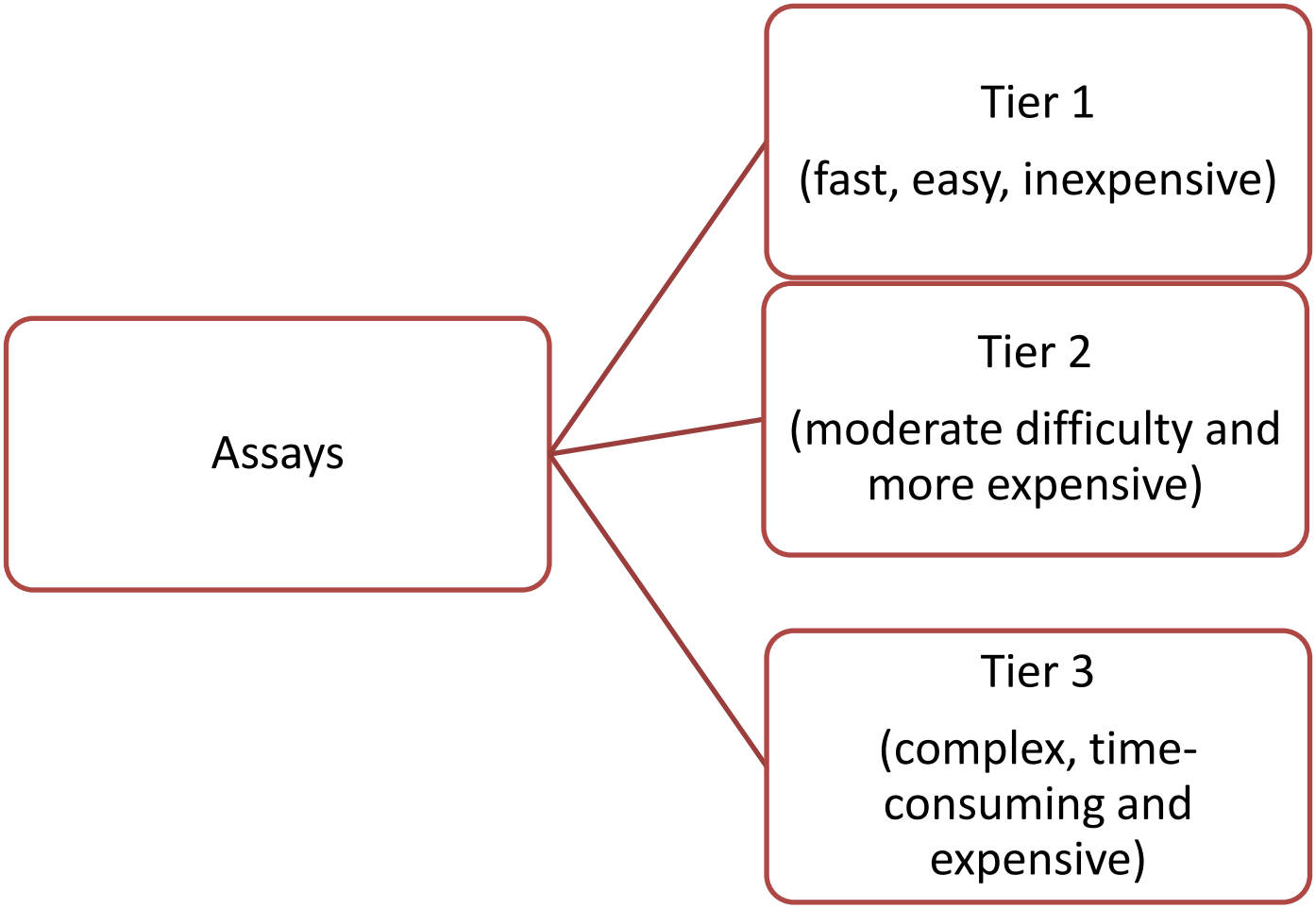
- > 70% viable tumor nuclei
- <20% of the viable cells should be normal stromal, inflammatory or immune cells
- < 20% Necrosis
- < 30 min. Tissue Preservation Interval

  (i.e., warm + cold ischemia time), unless otherwise required for validation studies

SQS Paradigm



Tissue Quality Assays



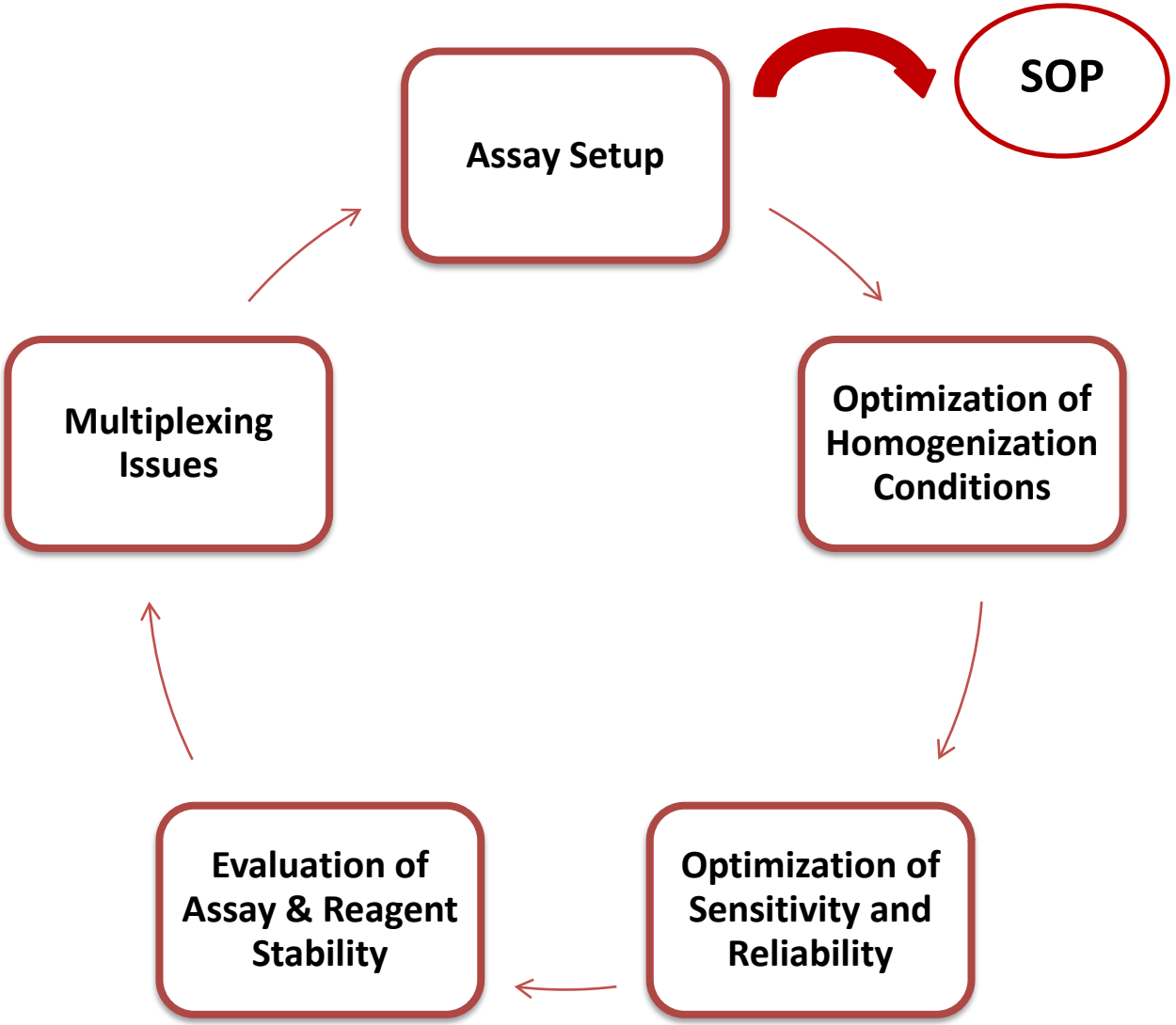
Tier 1 Assay Criteria

- Low Cost
- Mix and Read
- Reagents stable
- No rigorous timing requirements
- Large linear range as function of protein conc.
- Sensitivity (requires < 1 mg tissue, including replicates)
- 384 well compatible
- Multiplexed
- Accurate and Reliable
- **Predictive** (Δ as function of Tissue Preservation Interval)

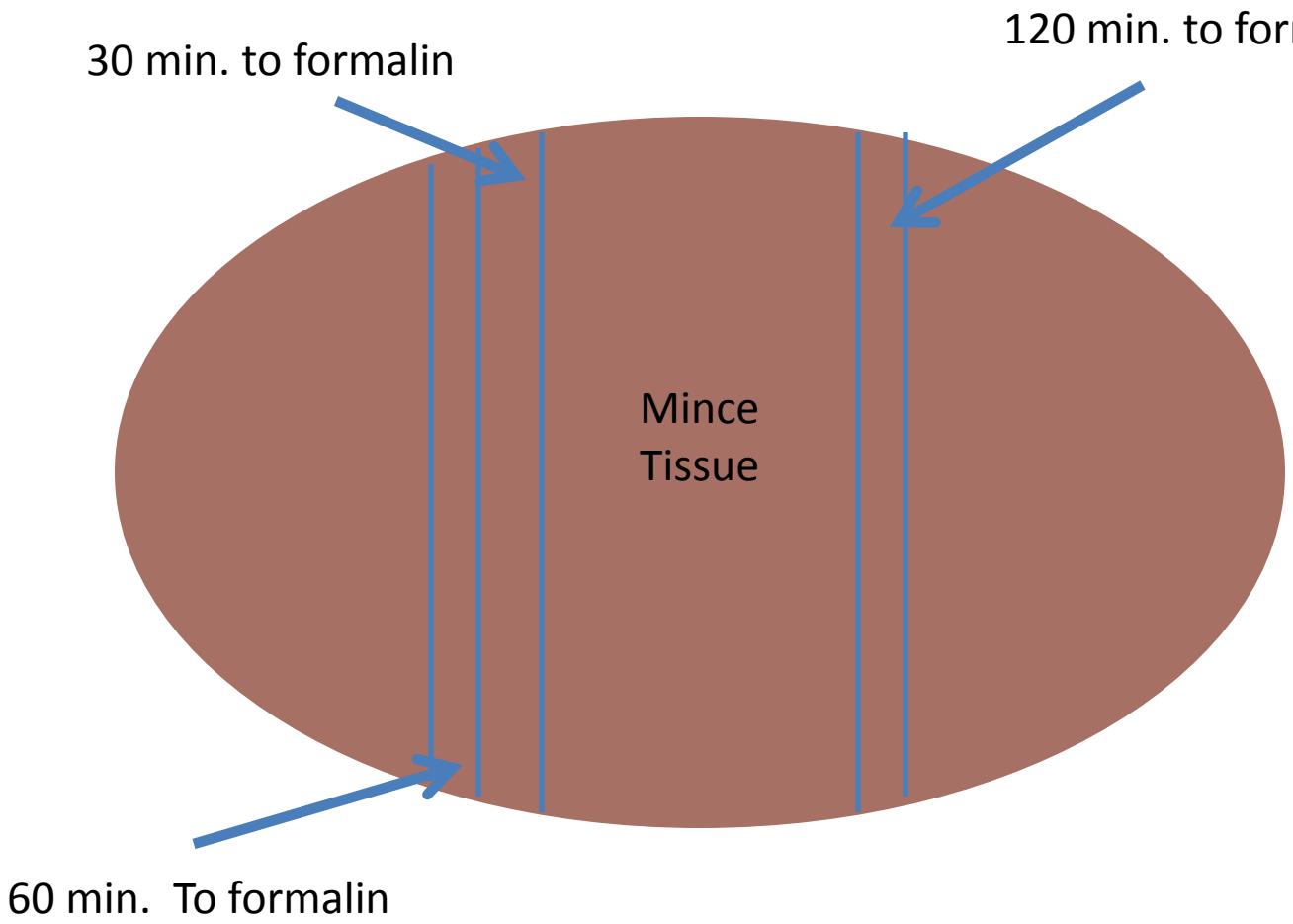
Tier 1 Assays

- pH (microelectrode or fluorophore)
- Lactate
- Oxidation-Reduction state (microelectrode)
- Redox Capacity
- Thiol Status
- Lipid Peroxidation
- Protein Degradation

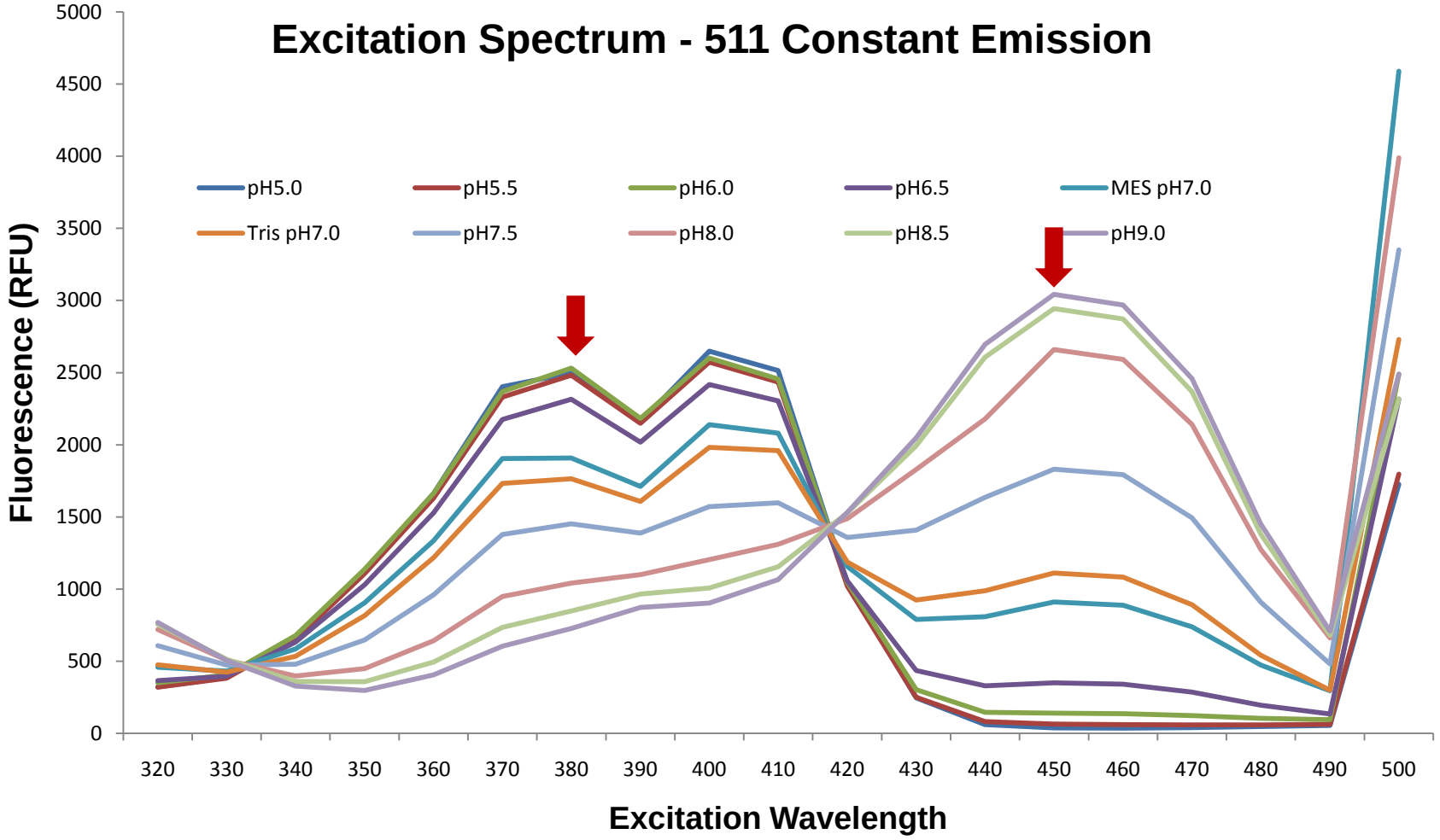
Tier 1 Assay Development Strategy (Iterative Rounds)



TPI Experiments

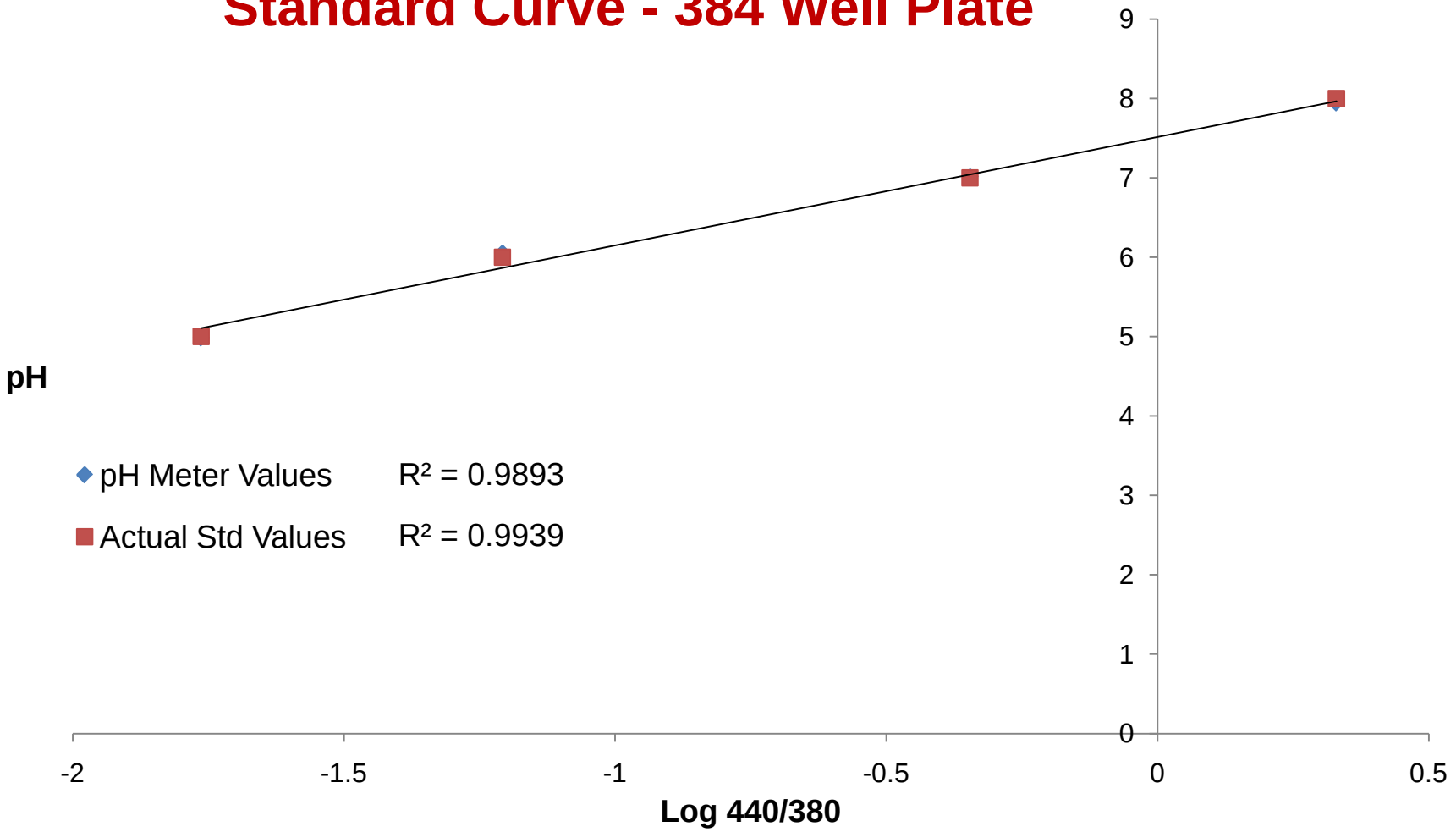


Excitation Spectra of HPTS as a function of pH

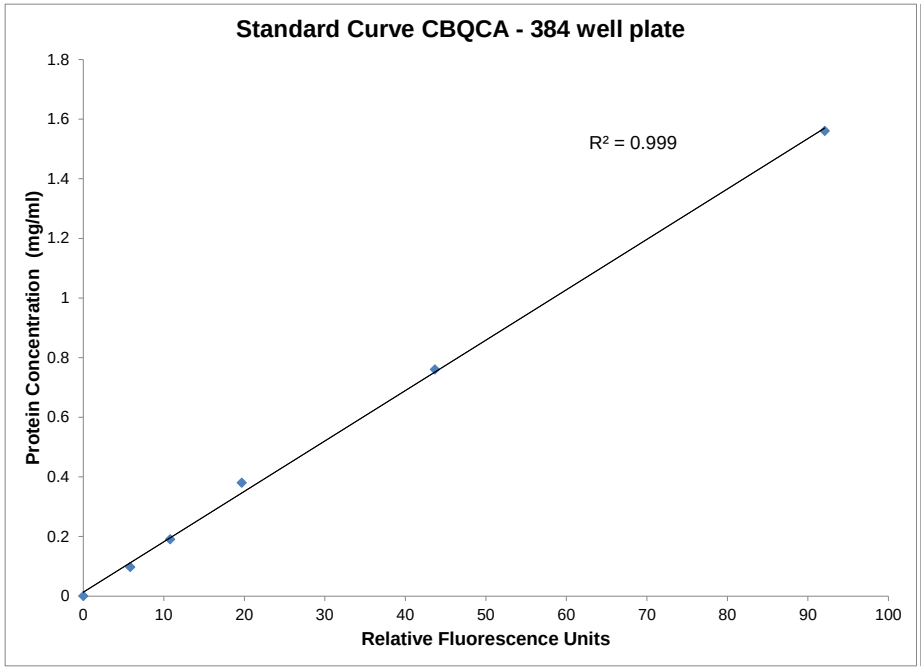


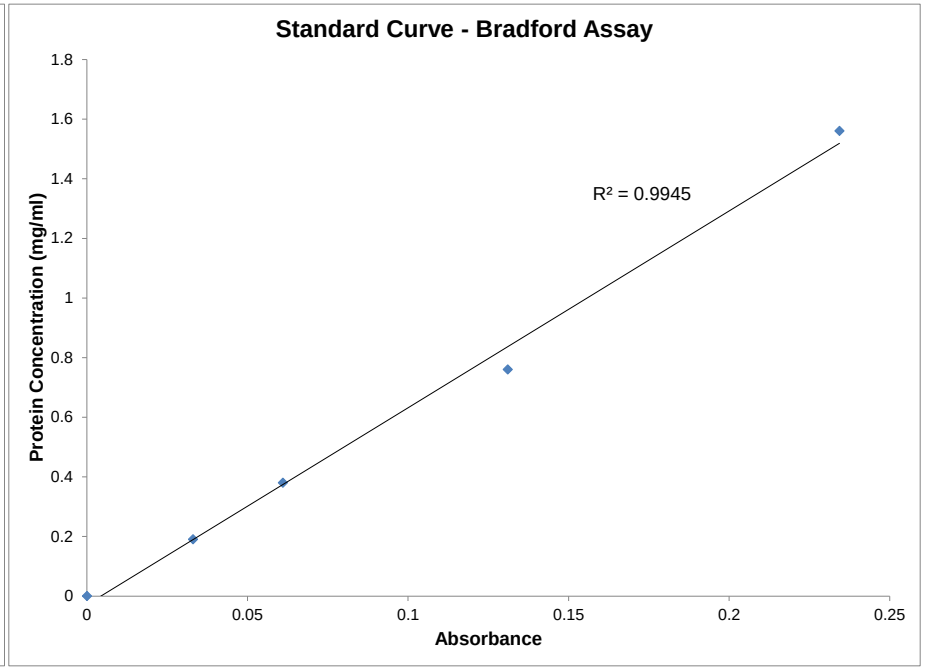
pH with Microelectrode vs. Fluorophore

Standard Curve - 384 Well Plate



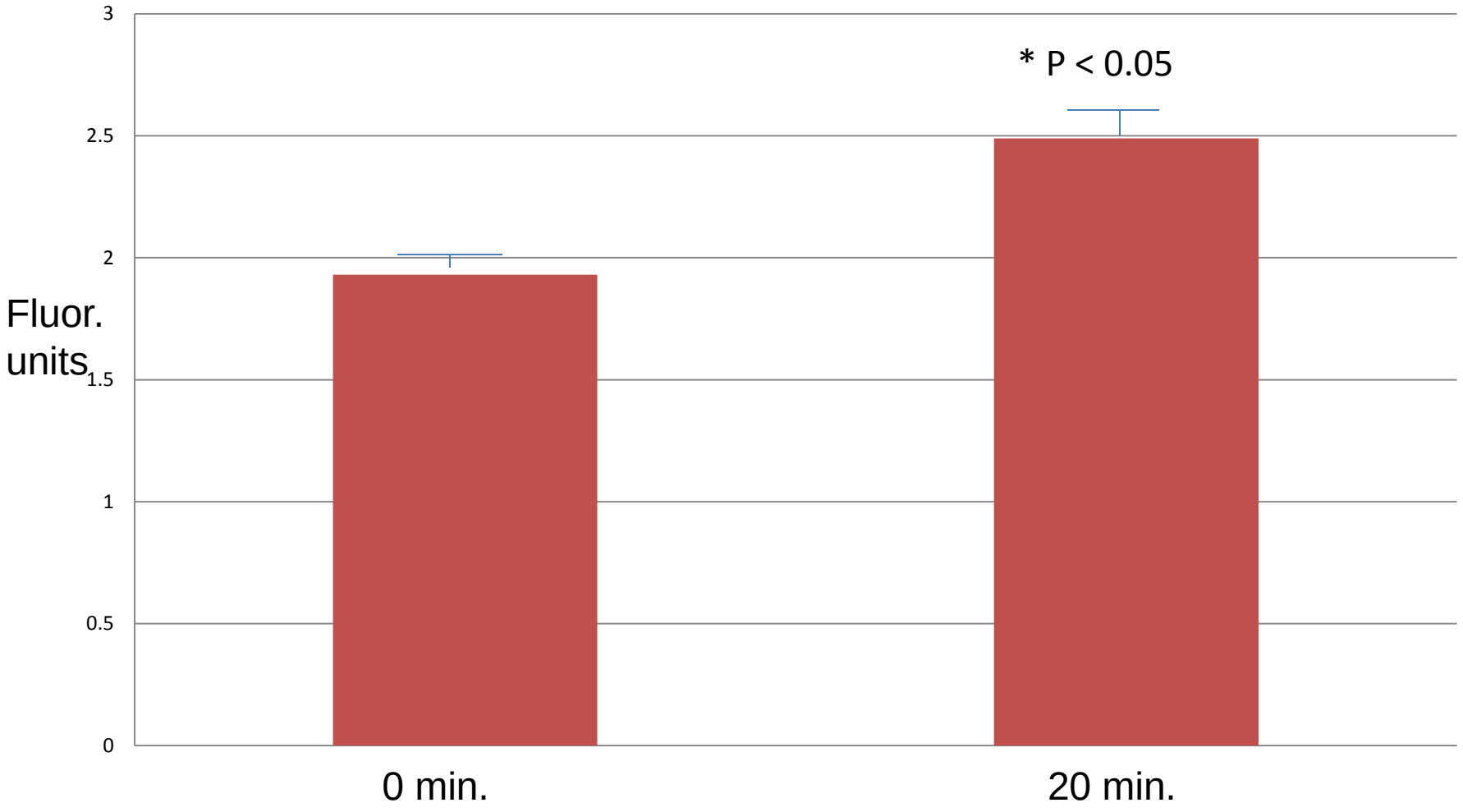
CBQCA vs. Bradford Assays



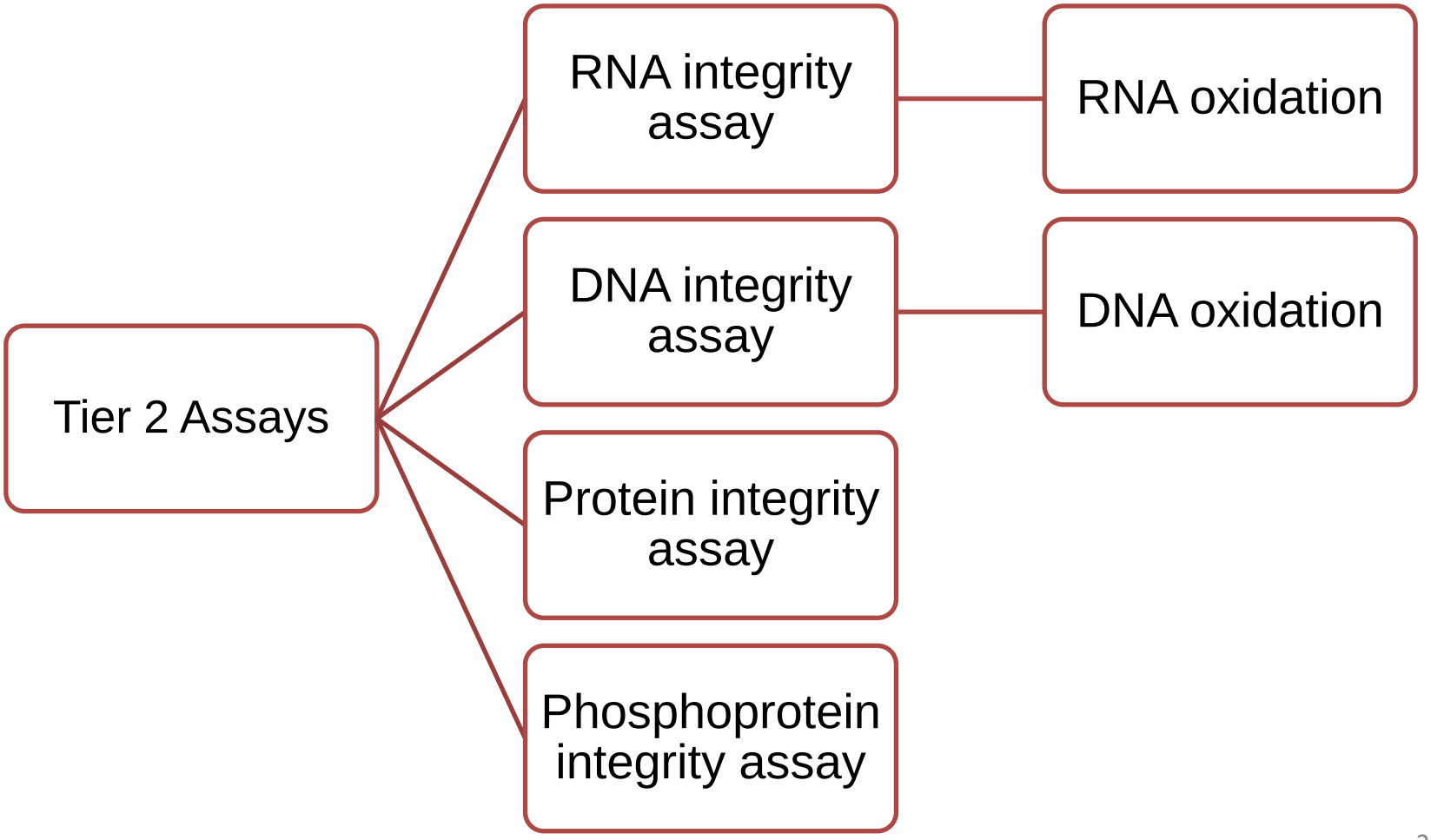


Tissue Type	CBQCA (mg/ml)	Bradford Assay (mg/ml)	CBQCA/ Bradford Assay Ratio
Lung	1.706298	0.999288	1.707514
Pancreas	2.102434	1.734567	1.21208
Colon	0.703509	0.338858	2.076116

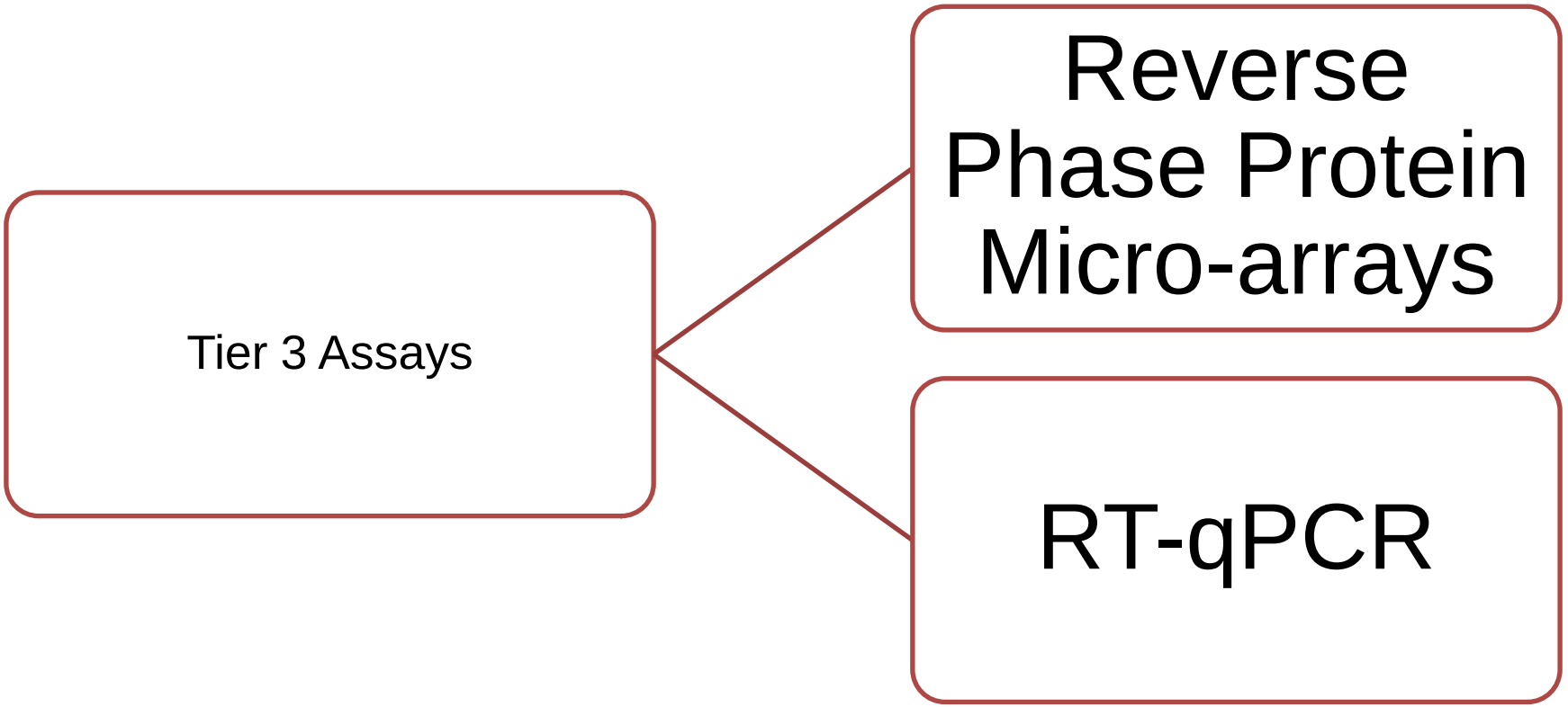
Effect of Incubation on CBQCA Assay



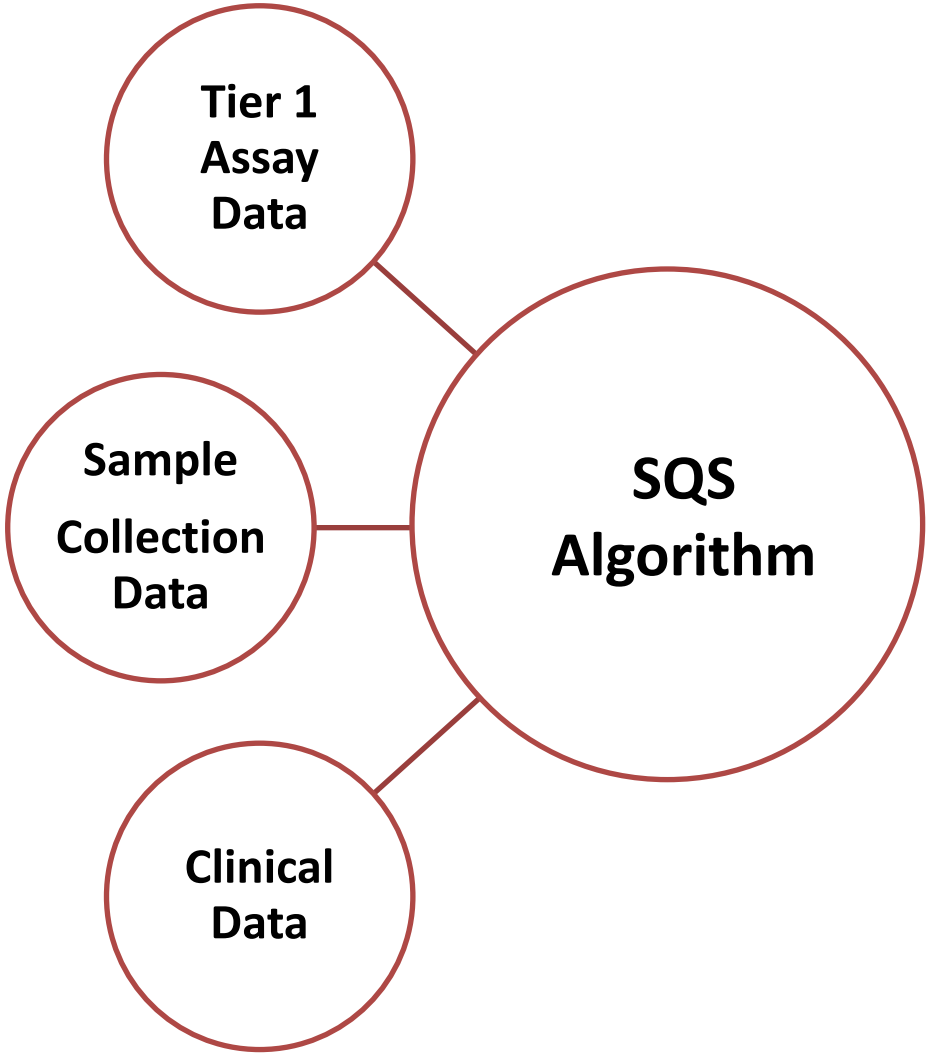
Tier 2 Assays



Tier 3 Assays



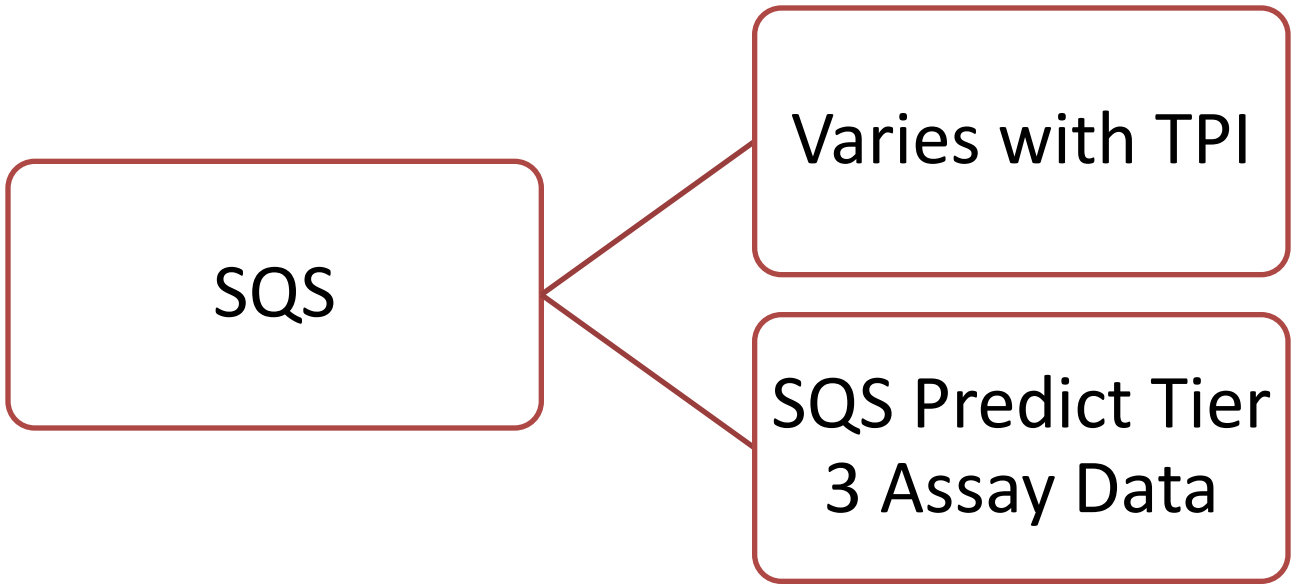
Creation of SQS Algorithm



Data for Multivariate Analysis

Clinical Data	Sample Collection Data	Analytical Data
Specimen Type	Warm Ischemic Time	pH
Preparation (Fixed or Frozen)	Cold Ischemic Time	Lactate
Organ of Origin	Tissue Preservation Interval	Oxidation-Reduction state
Histology Type	Sample Weight	Redox Capacity
Diagnosis	Sample Photography	Thiol Status
Gender	Length Fixation	Lipid Peroxidation
Age at surgery	% Tumor nuclei	Protein Degradation
Race	% Inflammation	
Ethnicity	% Necrosis	RNA integrity
History of Malignancy		DNA integrity
Performance Status		Phosphorylation Status
Tumor Staging (TMN)		
Radiation Therapy		RT-qPCR
Chemotherapy		Protein Microarrays
Anesthesia		
Medications		

SQS Validation



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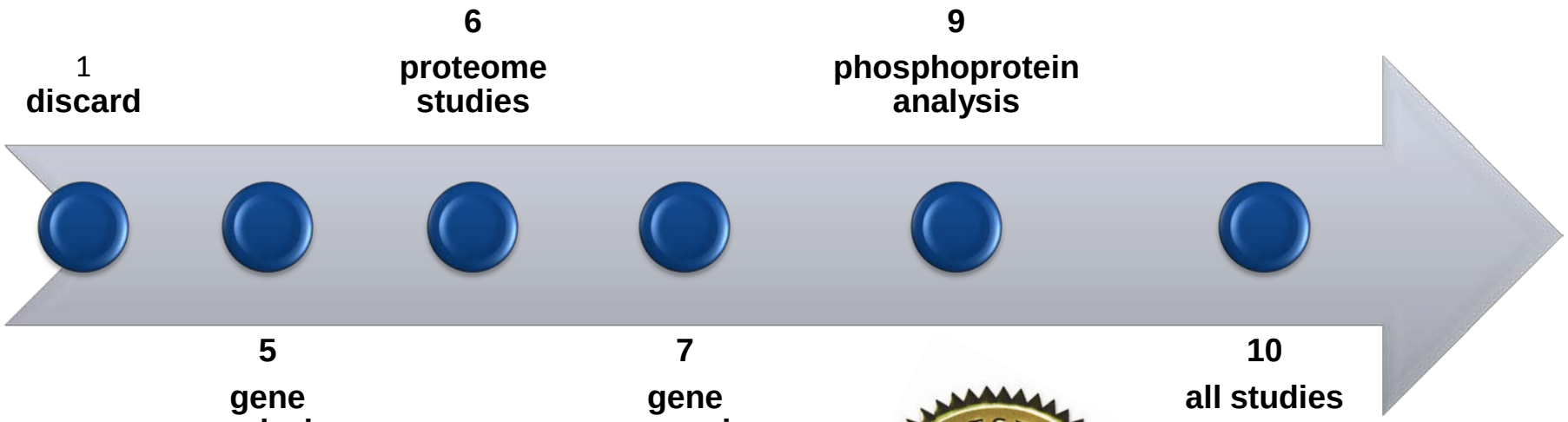

hypothetical example