



A candidate marker of 5-FU resistance in colorectal cancer

From basic science observation to clinical validation

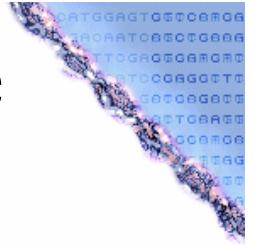
Malachi Griffith

(Dr. Marco Marra lab)

April 4 2009

1

Colorectal cancer and 5-FU resistance

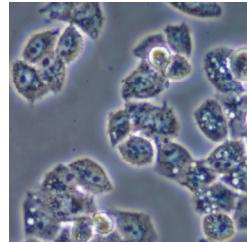


- Fluorouracil (5-FU) is a uracil analog commonly used in treatment of colorectal and other cancers
 - 5-FU & Capecitabine (oral 5-FU)
 - ~1,900 patients last year at BCCA (65% GI)
 - ~1.3 million dollars
- Response rates range from 5% to 50% depending on protocol
 - Intrinsic and acquired resistance...

Genome wide screen for 5-FU resistance genes

Sensitive/resistant cell lines

MIP101



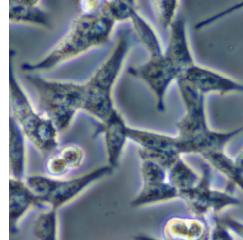
MIP101/5FU



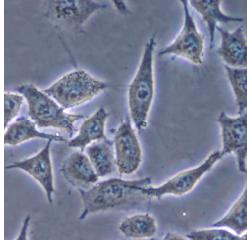
HCT116



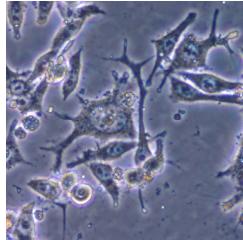
HCT116/5FU



RKO

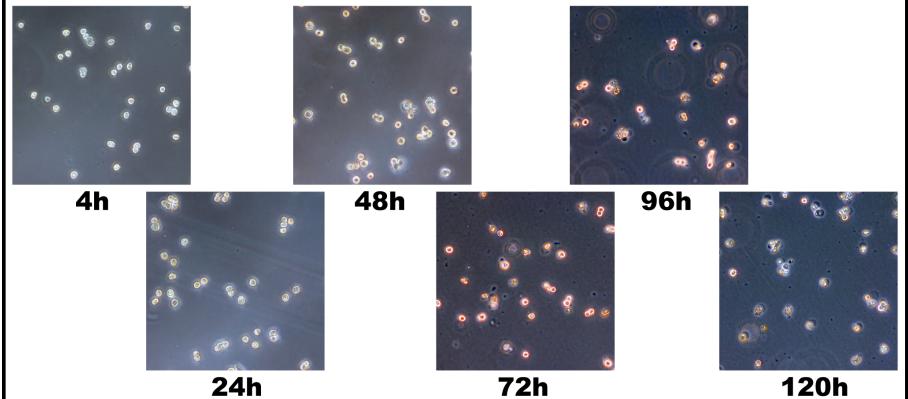


RKO/5FU

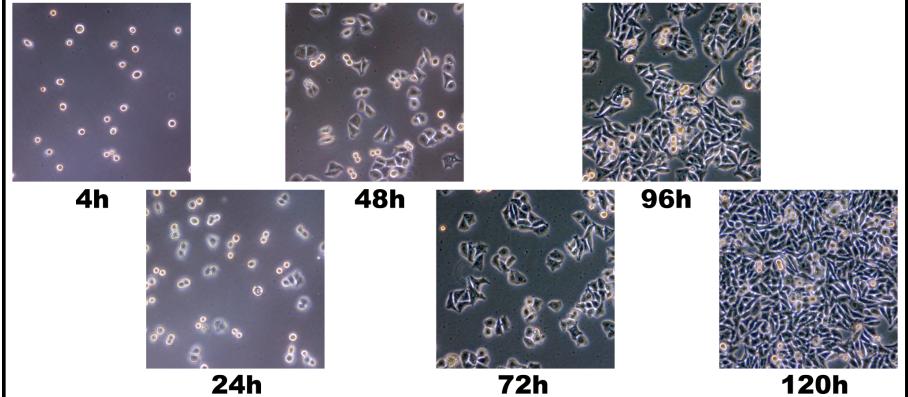


Resistance test

MIP101 (Sensitive) + 5FU Treatment (0.5 mM)

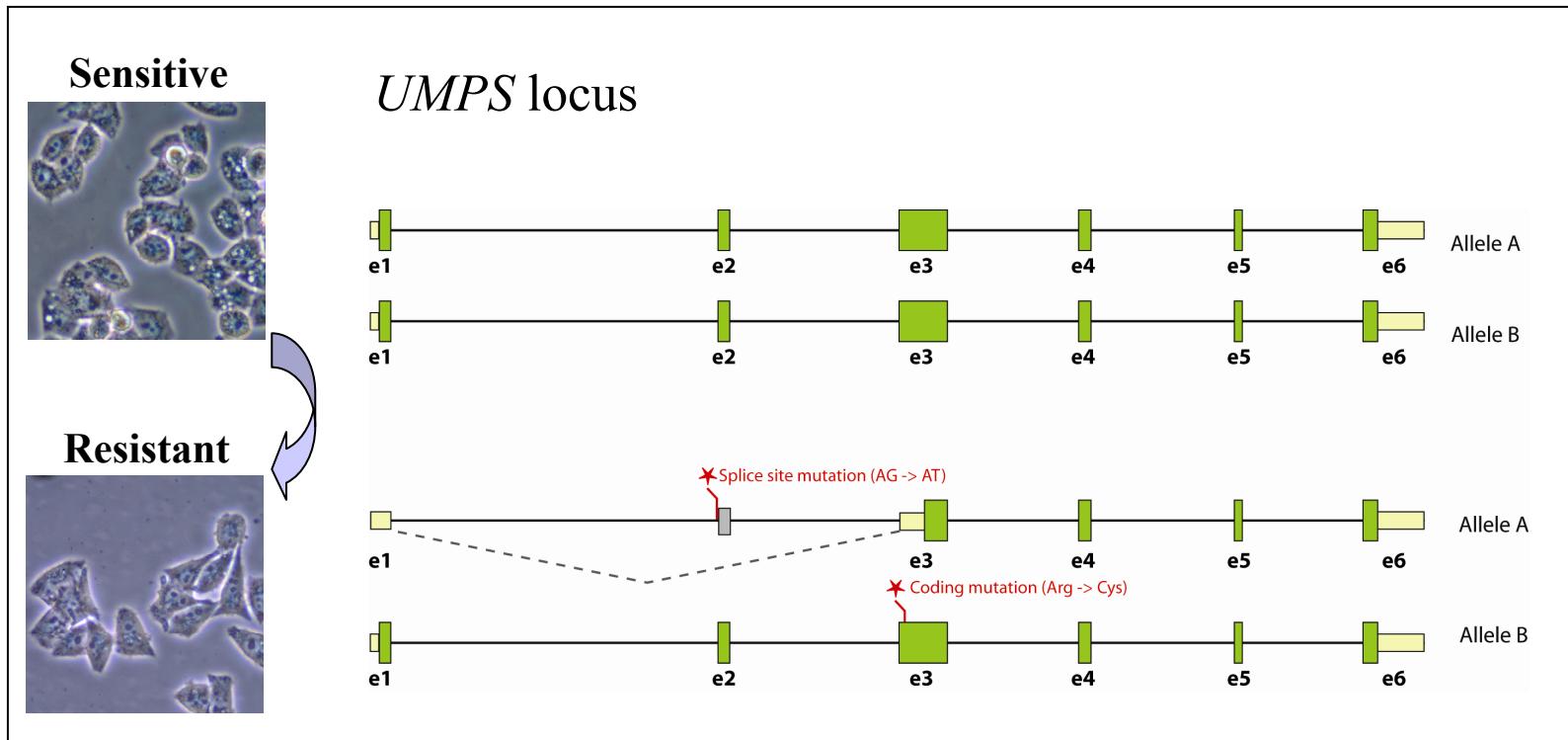
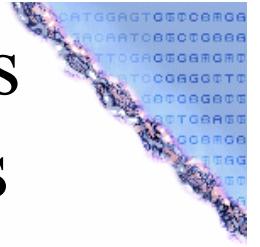


MIP-5FUR (Resistant) + 5FU Treatment (0.5 mM)



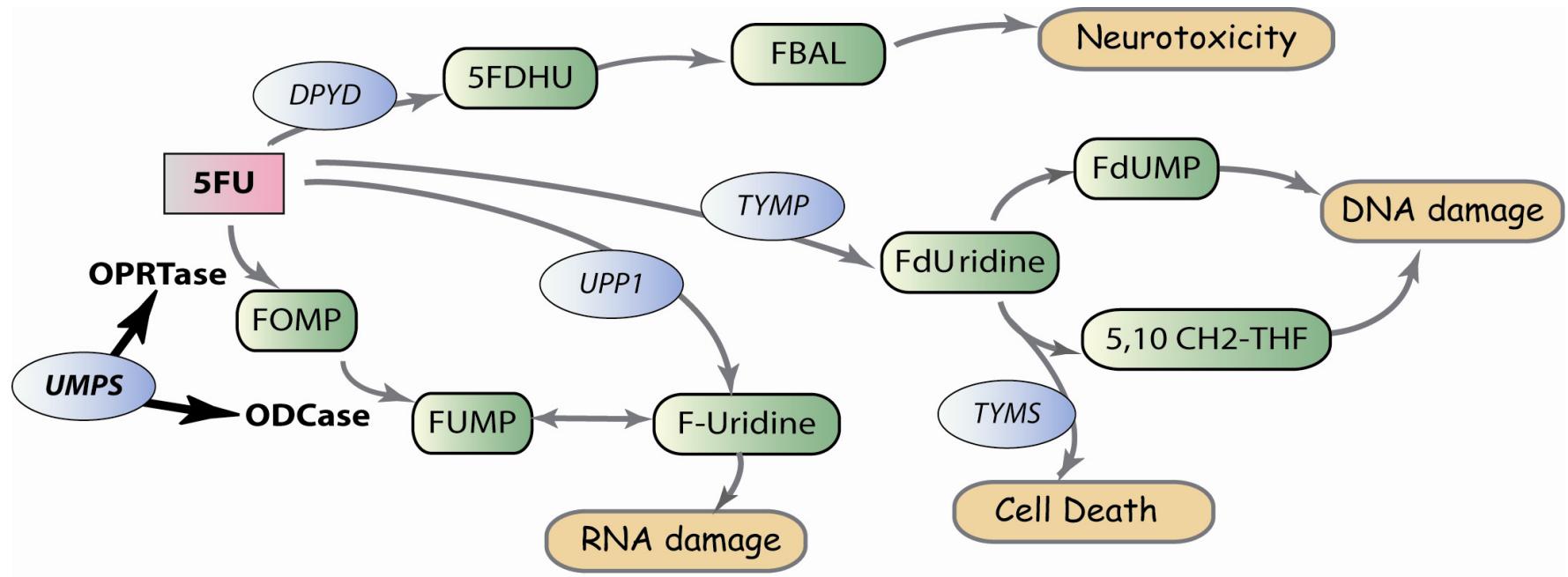
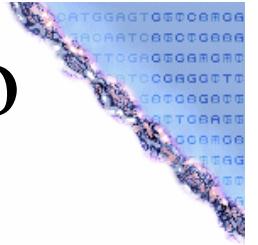
Cell lines developed in Dr. Isabella Tai's lab

Uridine monophosphate synthetase (*UMPS*) was recurrently disrupted in 5-FU resistant cell lines

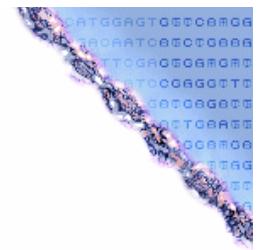


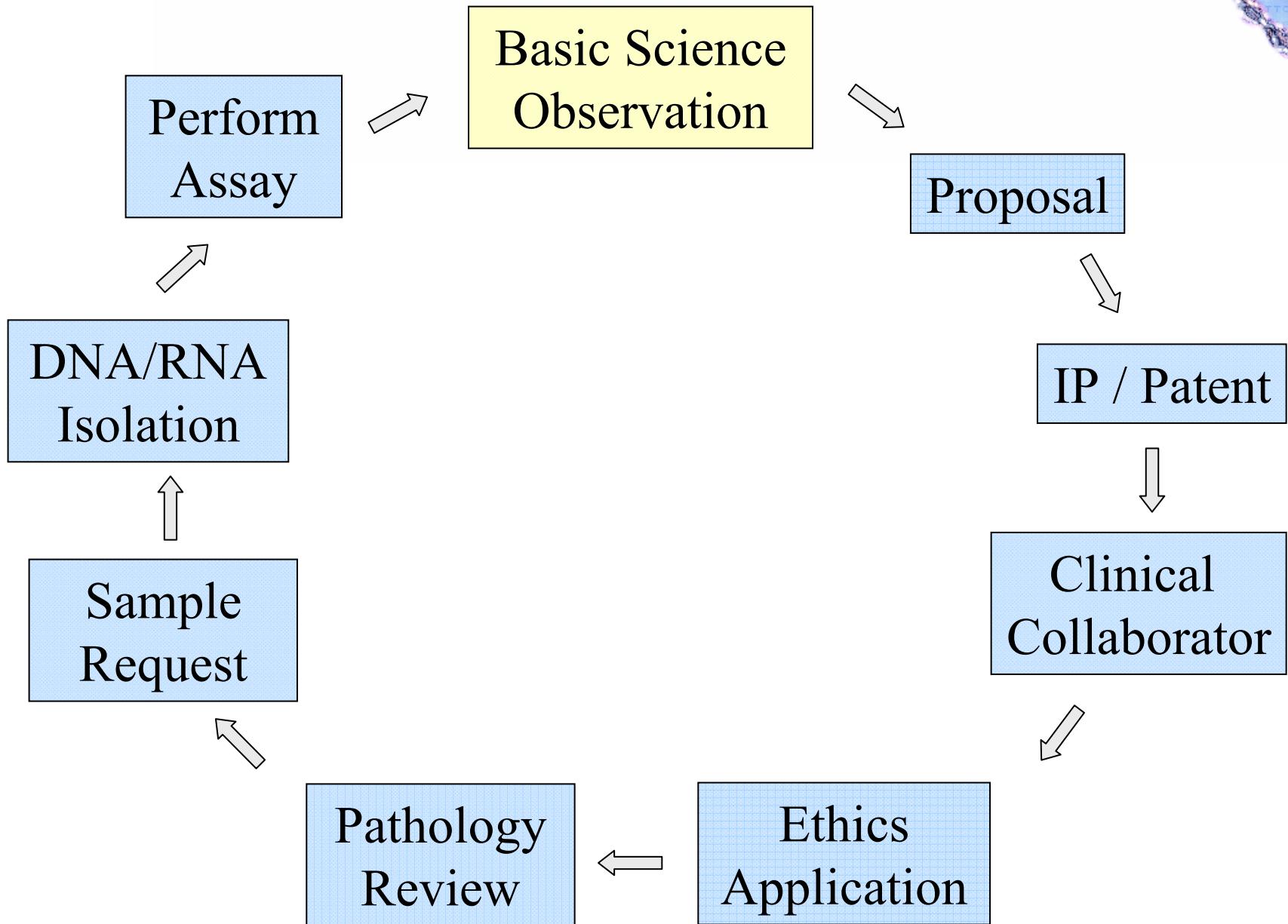
Differential expression, aberrant splicing and mutation of *UMPS* was observed in three 5-FU resistant cell lines

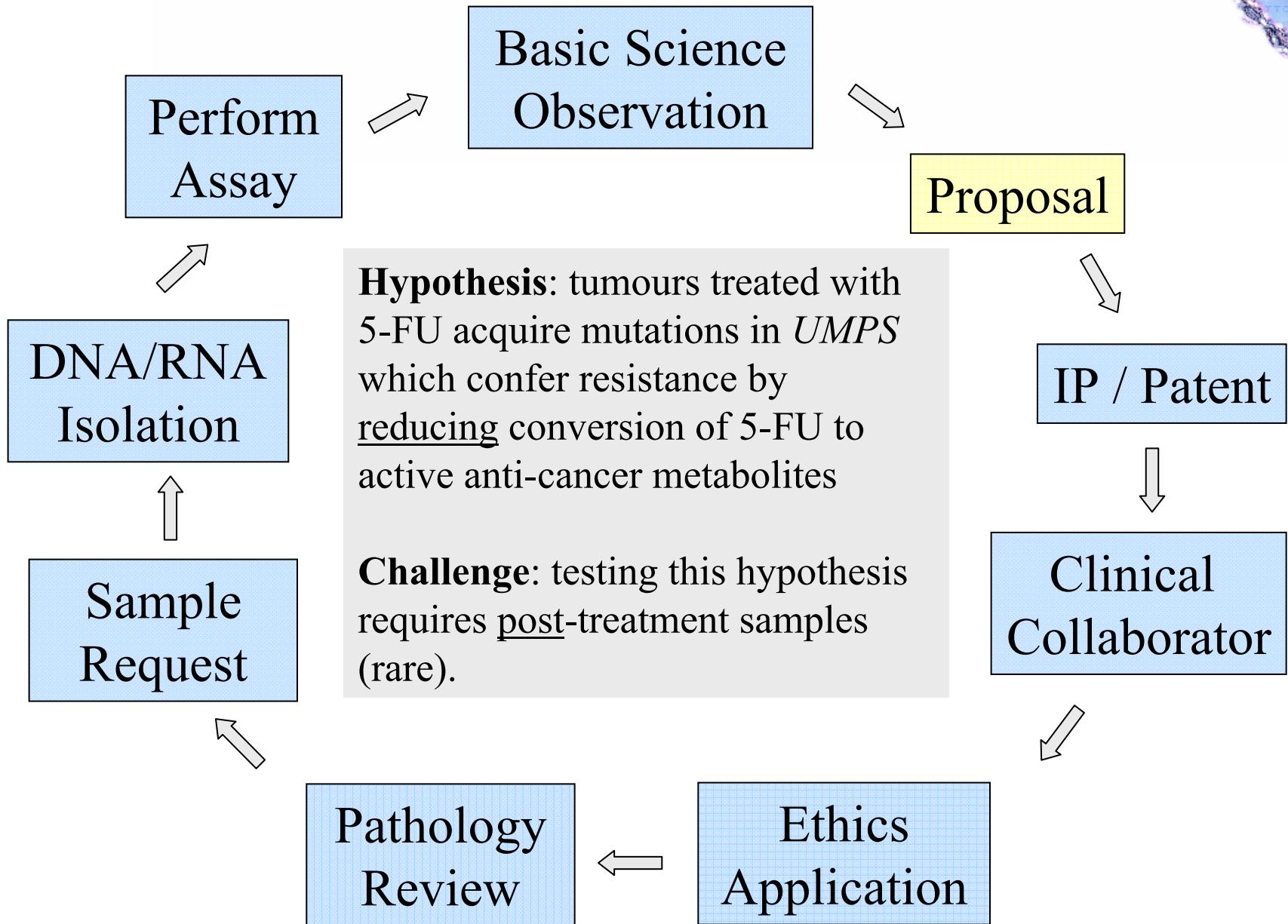
Reduced *UMPS* activity is predicted to result in reduced 5-FU efficacy

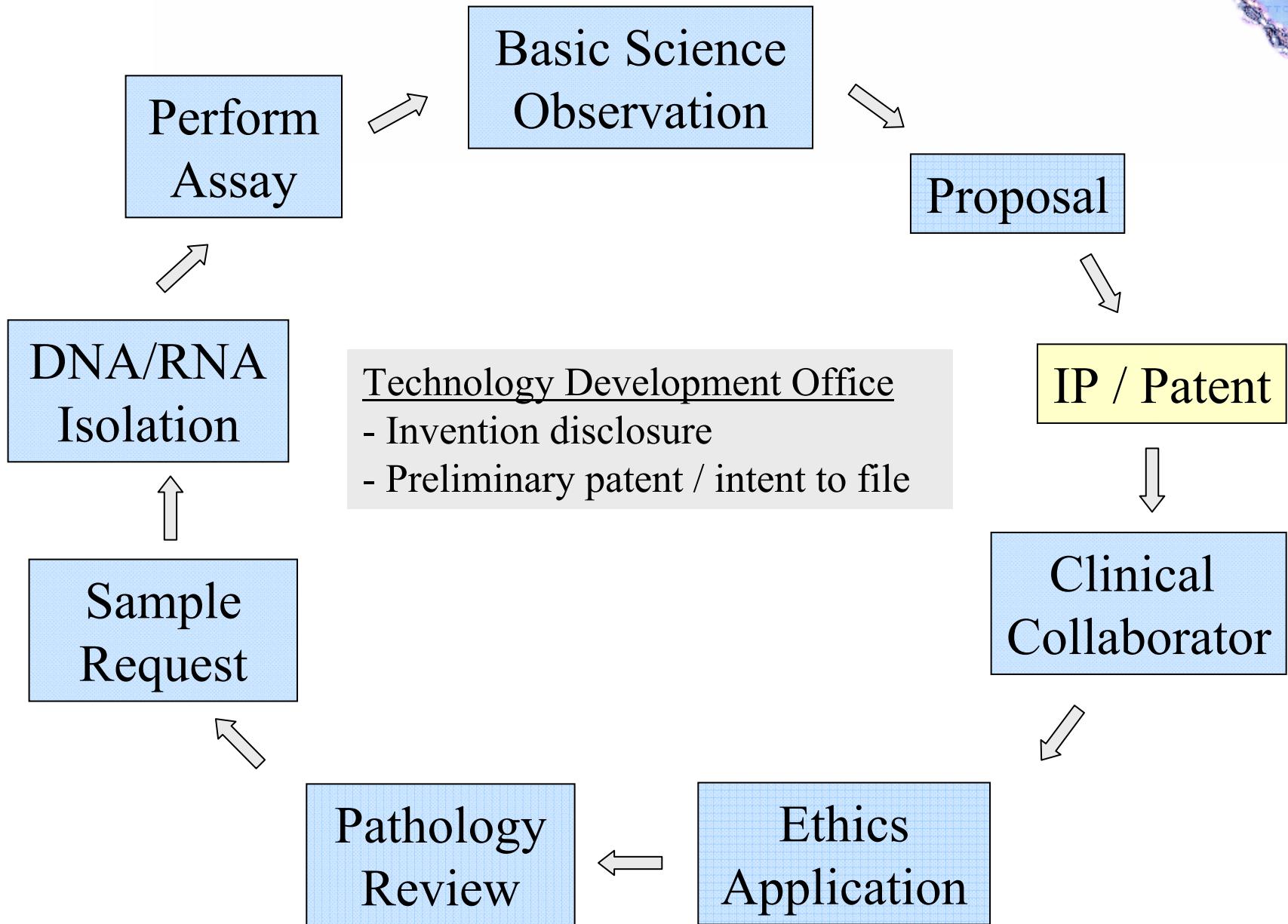


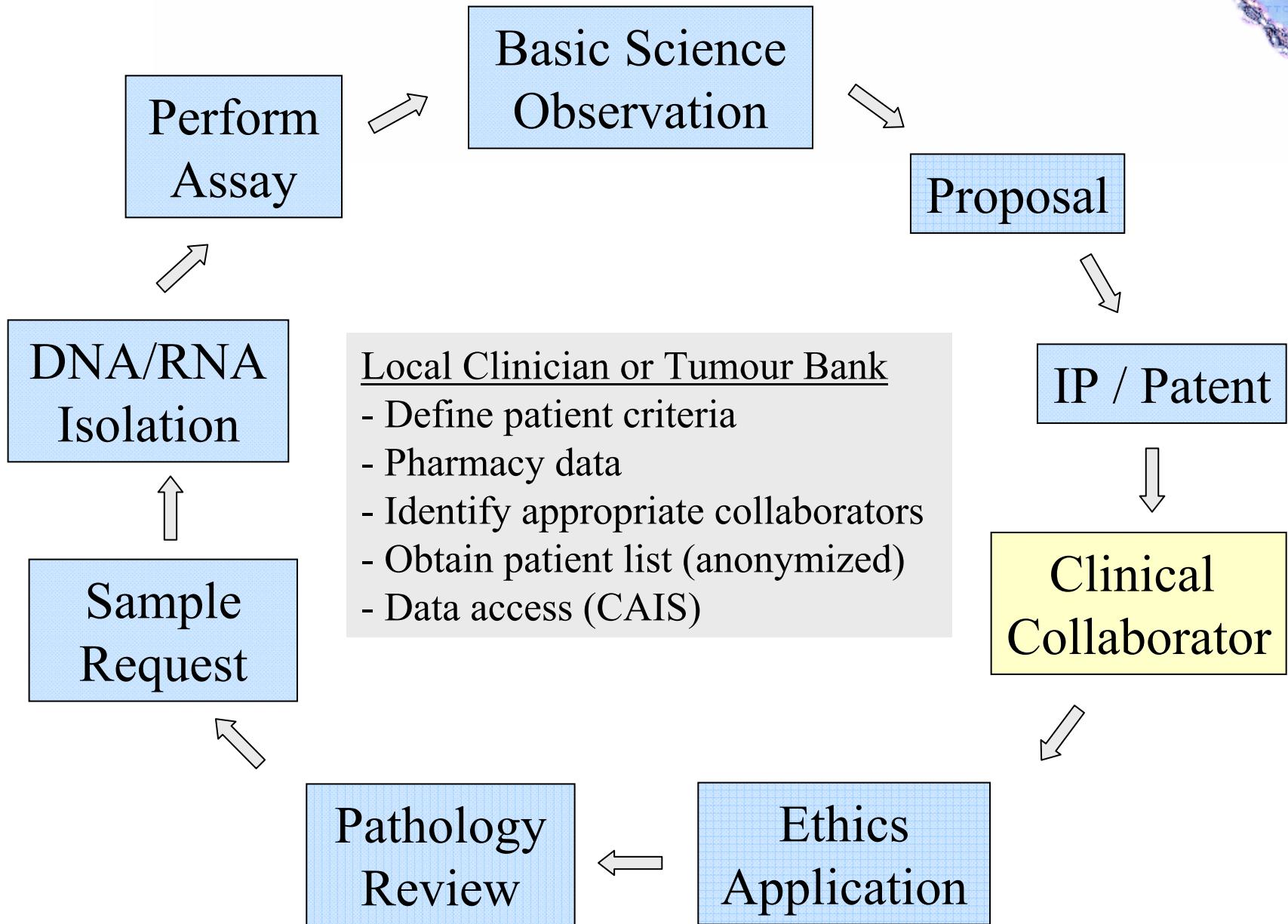
What now?





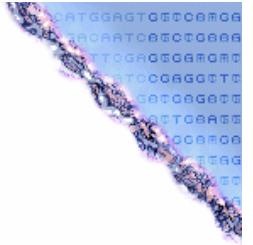


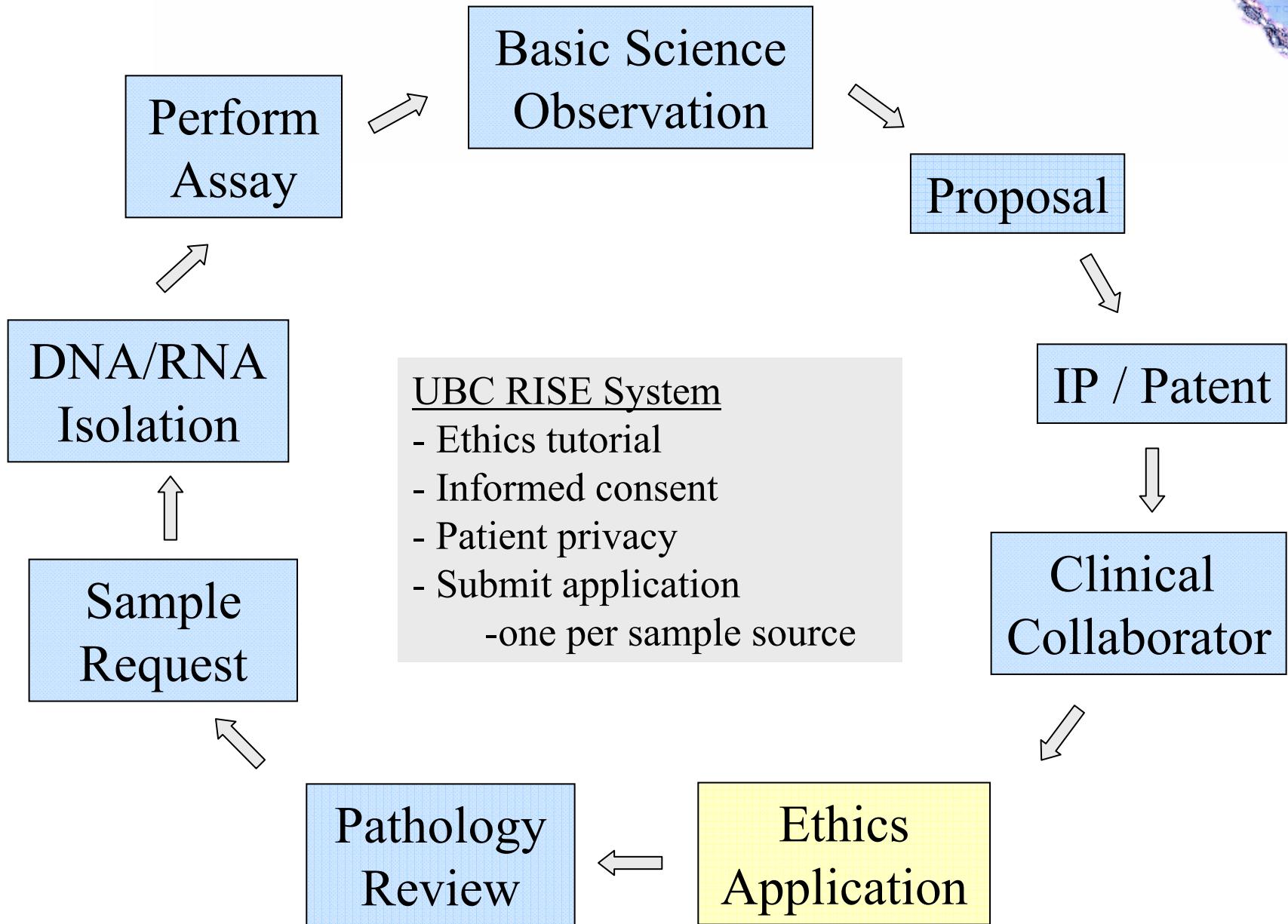


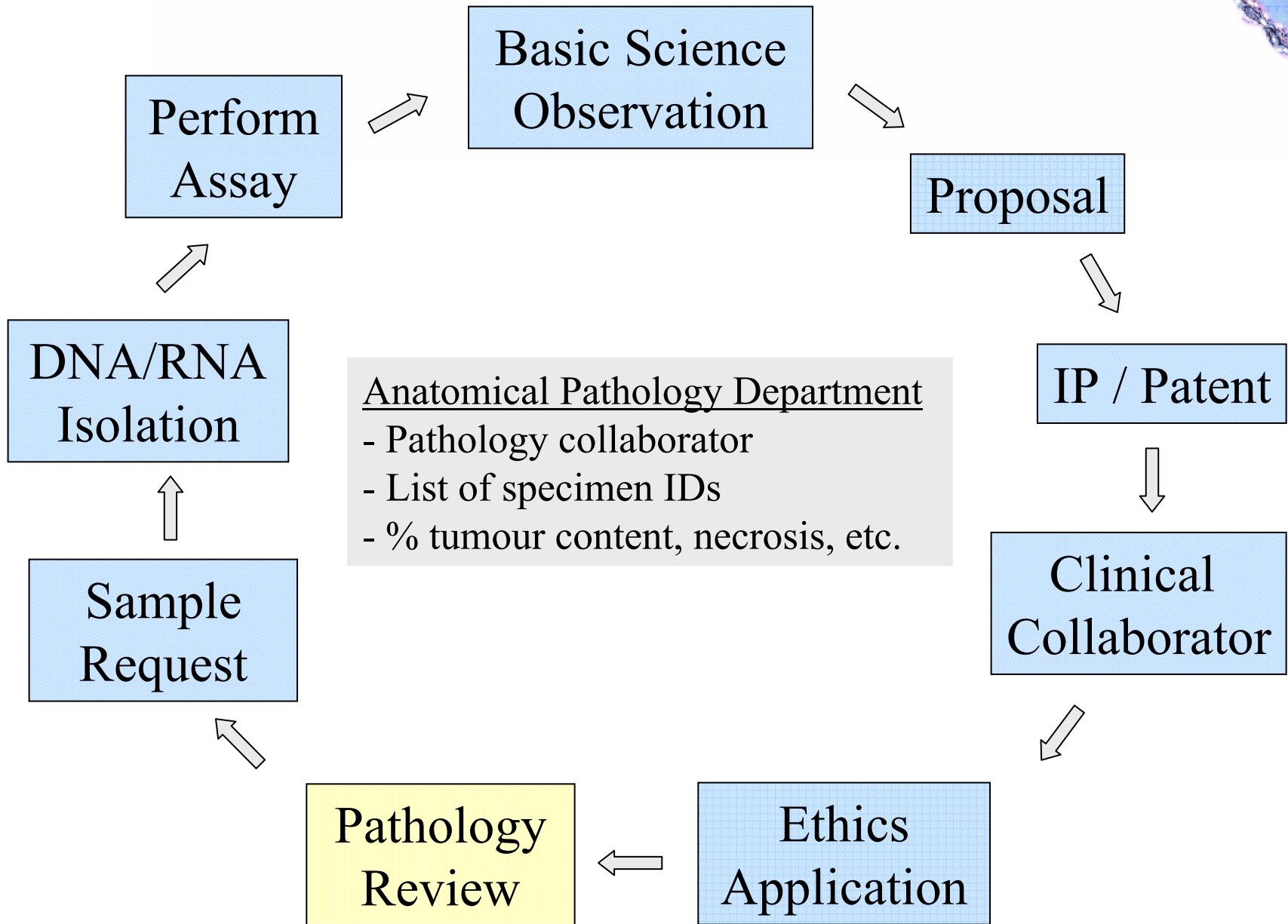


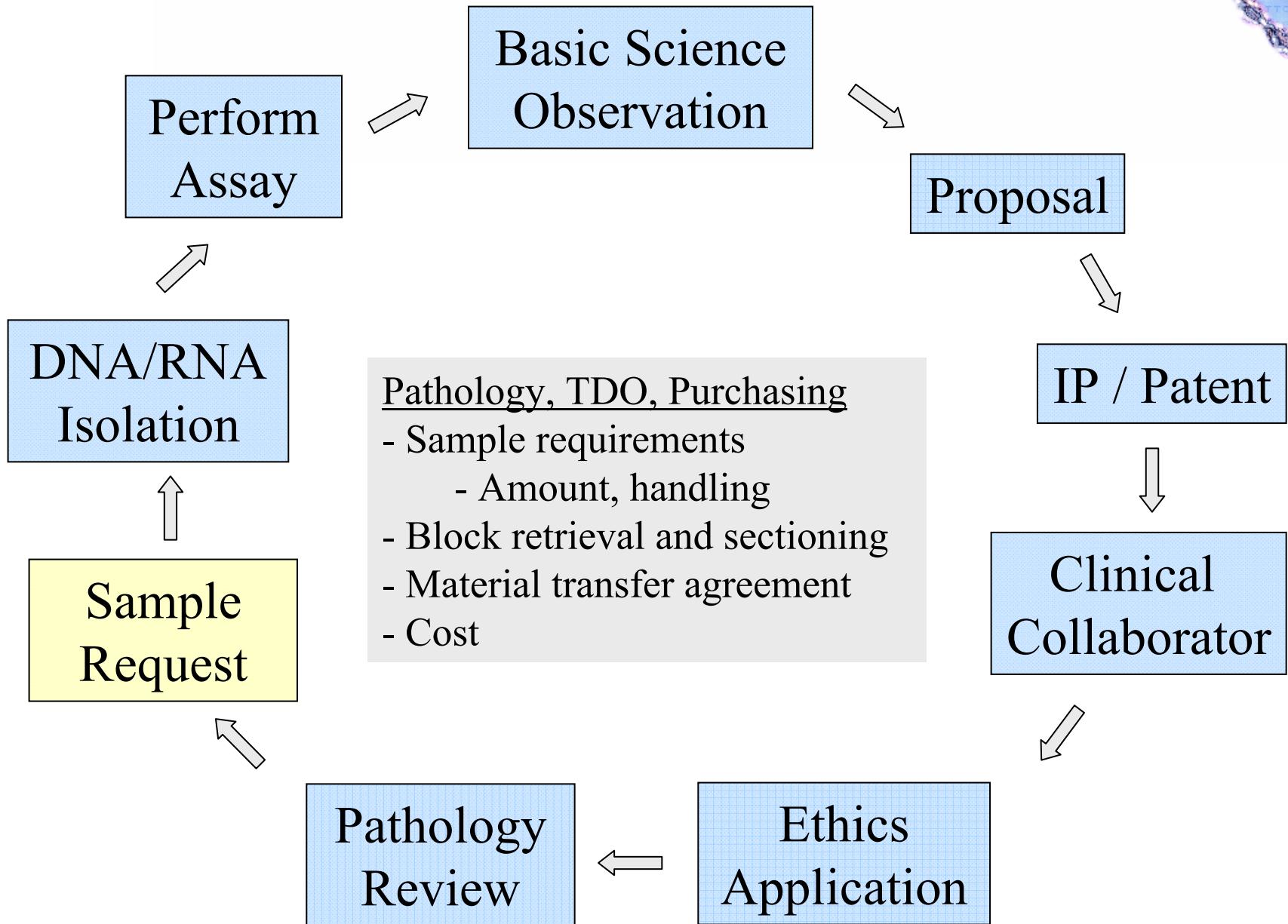
Sample sources

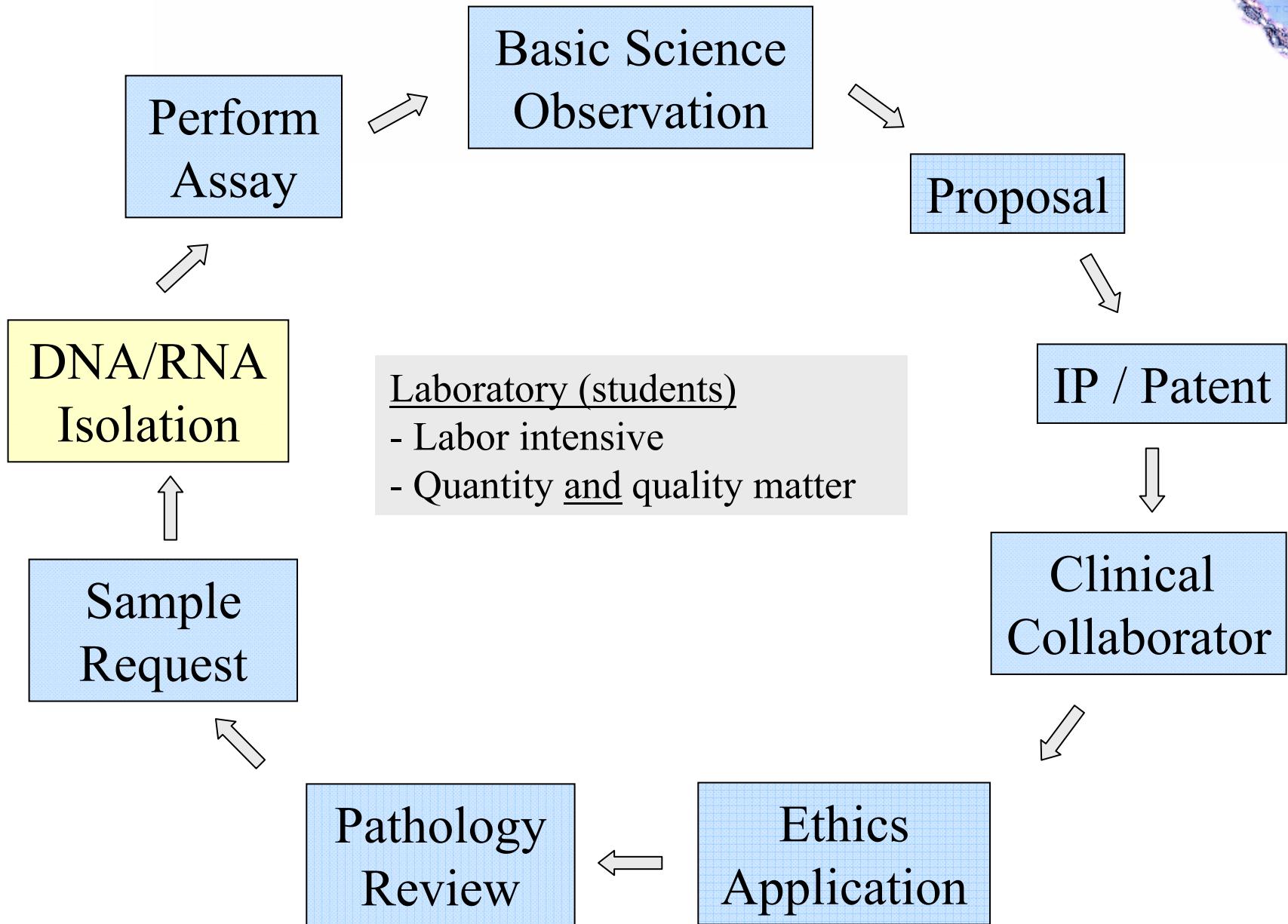
- Ontario Tumour Bank
 - Tumour/normal pairs
 - Mostly treatment naïve
 - Fresh frozen tissue
- Dr. Sharlene Gill (BCCA/VGH)
 - Liver metastases of colorectal cancer
 - Varying degrees of 5-FU exposure
 - FFPE sections
- Dr. Carl Brown (St. Paul's Hospital)
 - Primary tumours of colorectal cancer
 - Neo-adjuvant chemotherapy + radiation
 - FFPE sections







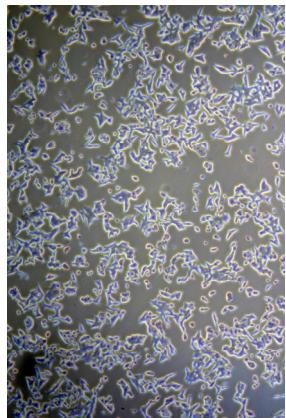




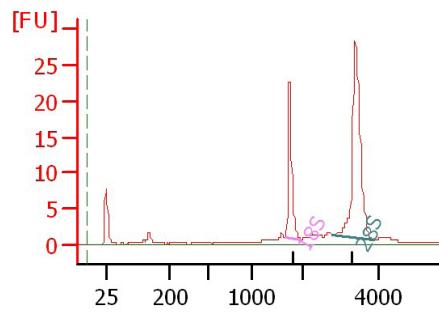
Sample source affects quality



Tissue culture

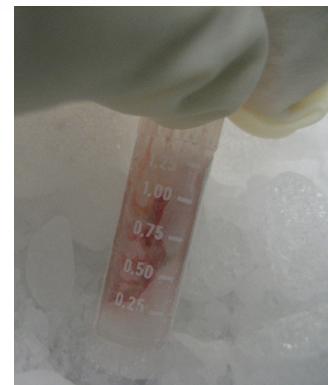


↓
RIN: 10.0

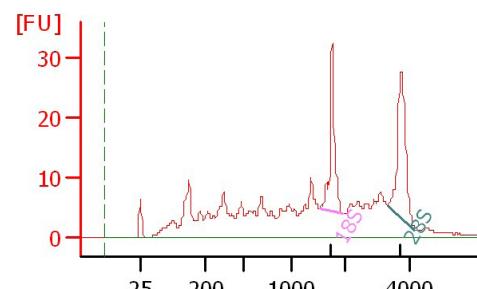


Any assay

Fresh frozen tissue



↓
RIN: 6.5

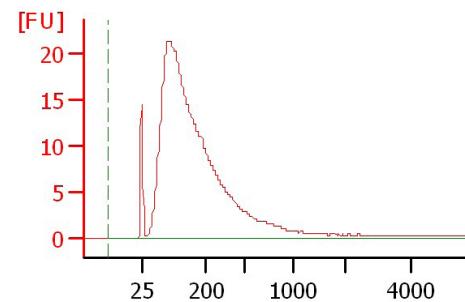


Most assays

Section from block

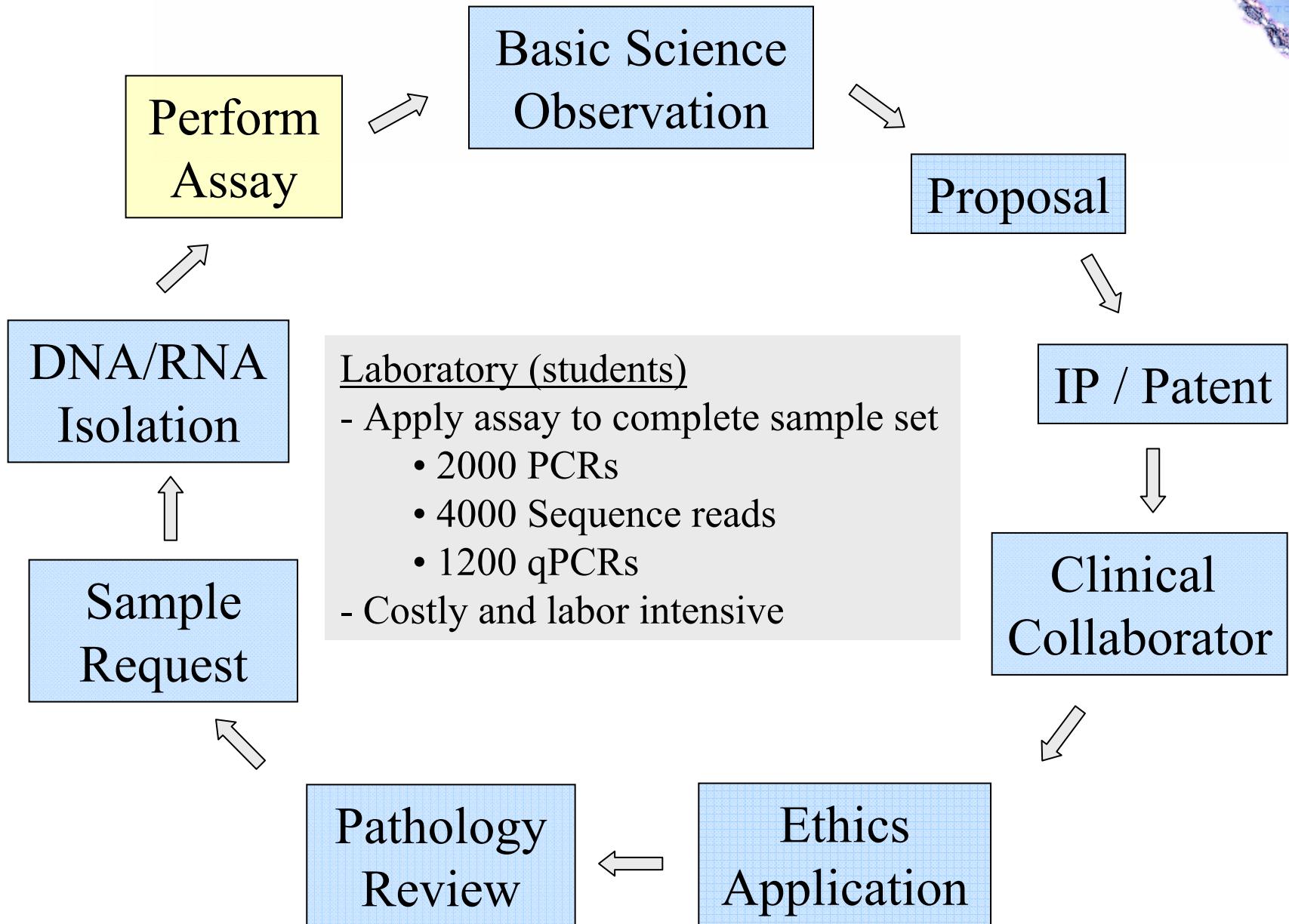


↓
RIN: 2.5

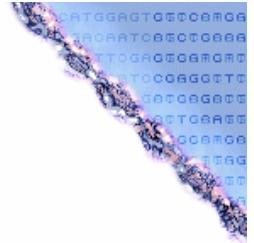


PCR only



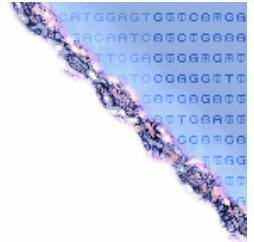


Conclusions



- 100 samples obtained to date
 - Gene expression and sequencing assays underway
- Challenges
 - Complexity
 - Communication
 - Data management
 - Cost
- Future work
 - ‘How-to’ manual

Acknowledgements



Supervisor

Marco Marra

Committee

Joseph Connors
Stephane Flibotte
Steve Jones
Gregg Morin

Bioinformatics

Obi Griffith
Ryan Morin
Allen Delaney

Laboratory

Trevor Pugh
Tesa Severson

GI cancer

Isabella Tai
Michelle Tang

Illumina

Martin Hirst
Thomas Zeng
Yongjun Zhao
Helen McDonald

Affymetrix

Agnes Baross
Susanna Chan
Jennifer Asano
Adrian Ally

Students

Alison Lee
Jessica Paul
Lisa Miao

BCCA

Sharlene Gill
Hagen Kennecke
Susan O'Reilly

VGH Pathology

David Owen
Margaret Luk

St. Paul's Hospital

Carl Brown
Aleksandra Kuzmanovic
Sue Finley
Blair Walker
Douglas Filipenko

BCCA Pharmacy

Riva Pickering

Ontario Tumour Bank

Vanessa Ballin
Jeffrey Yung
Christina Rodriguez
Victoria Shelep

Patent

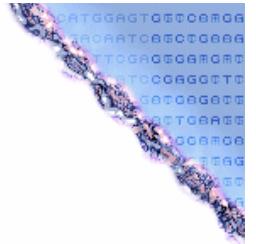
Daniel Polonenko
Erin Pisko

Technology Development

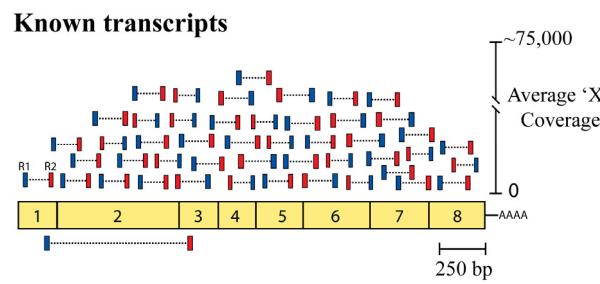
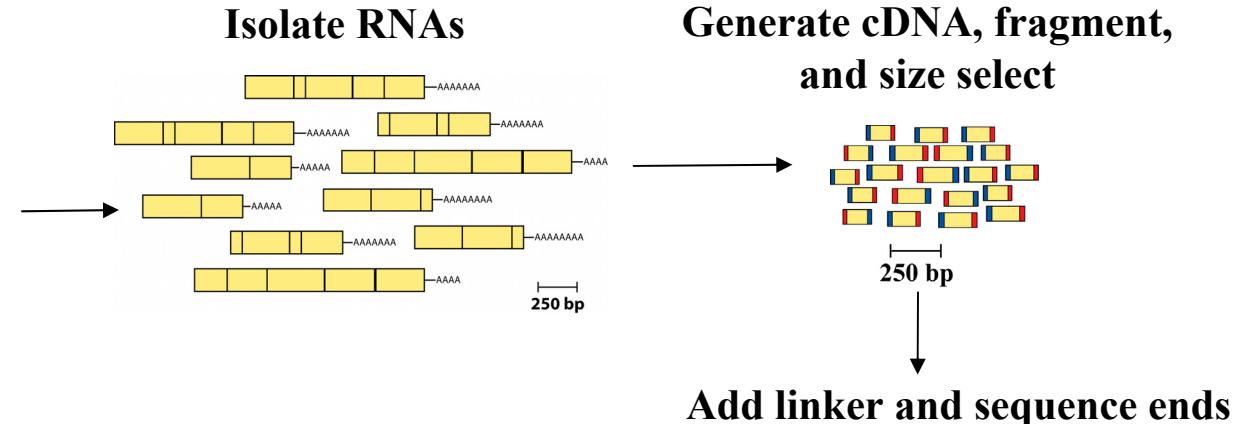
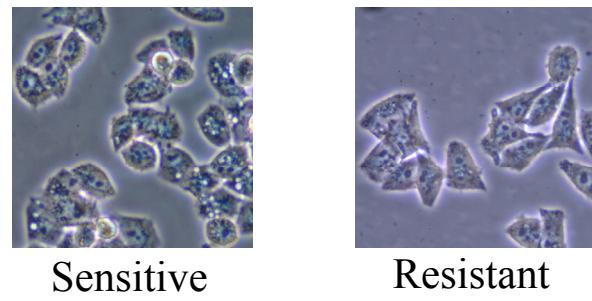
Patrick Rebstein
Kimberly Richmond



5-FU sensitive and resistant cancer cells were profiled by RNA sequencing

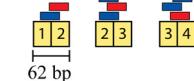


5-FU sensitive/resistant colon cancer cell lines

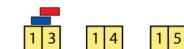


Map to transcripts

Known exon junctions



Predicted exon junctions



263 million paired reads
21 billion bases of sequence

