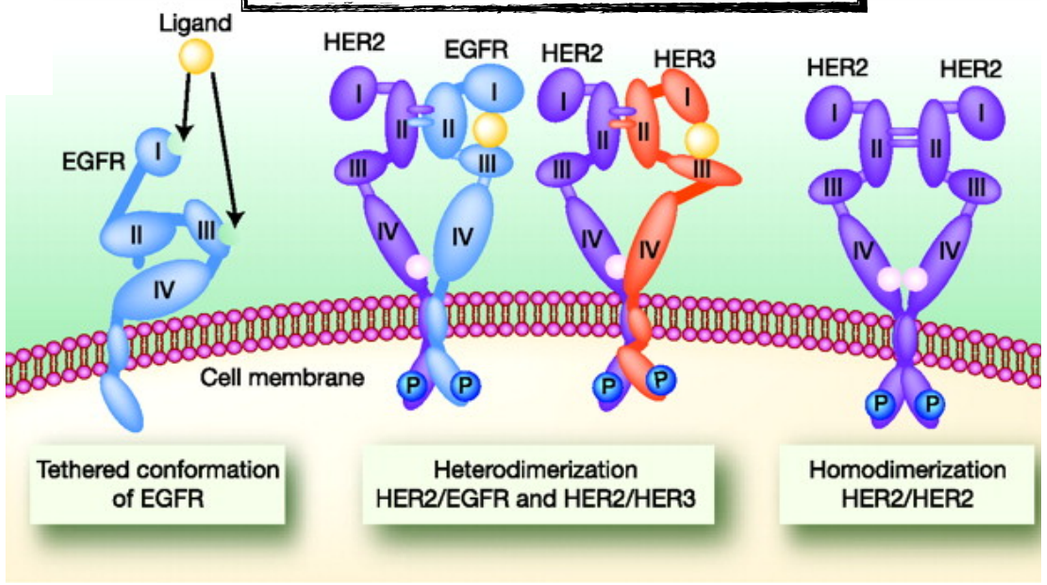
Module 2: Systems Engineering (M2D4)

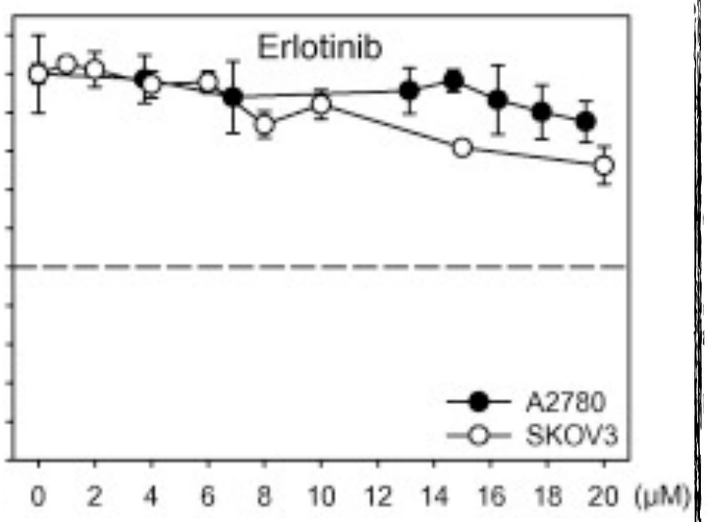
- A few announcements -- if you haven't watched my Oscar-worthy video
- Revisit sequencing analysis
- (Semi)Quantitative methods for measuring pathway activity
- A few hints for lab
- Scale it up!
- Journal club!

Module 2: Systems Engineering

Experimental Context: EGFR System



Design Goal:



Grunt et al. Biochem. Biophys. Res. Commun. 385:454-459(2009)

Overcome resistance to EGFR inhibition in SKOV3 human ovarian cancer cells.

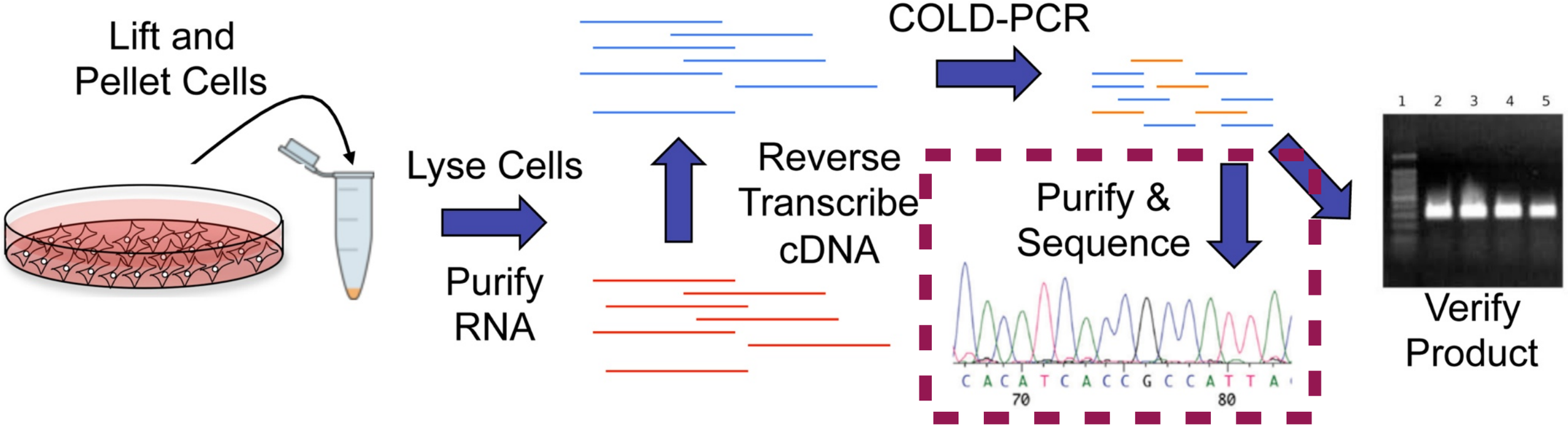
Approach:

Use mathematical models to make predictions and 'high throughput' experiments to test hypothesis.

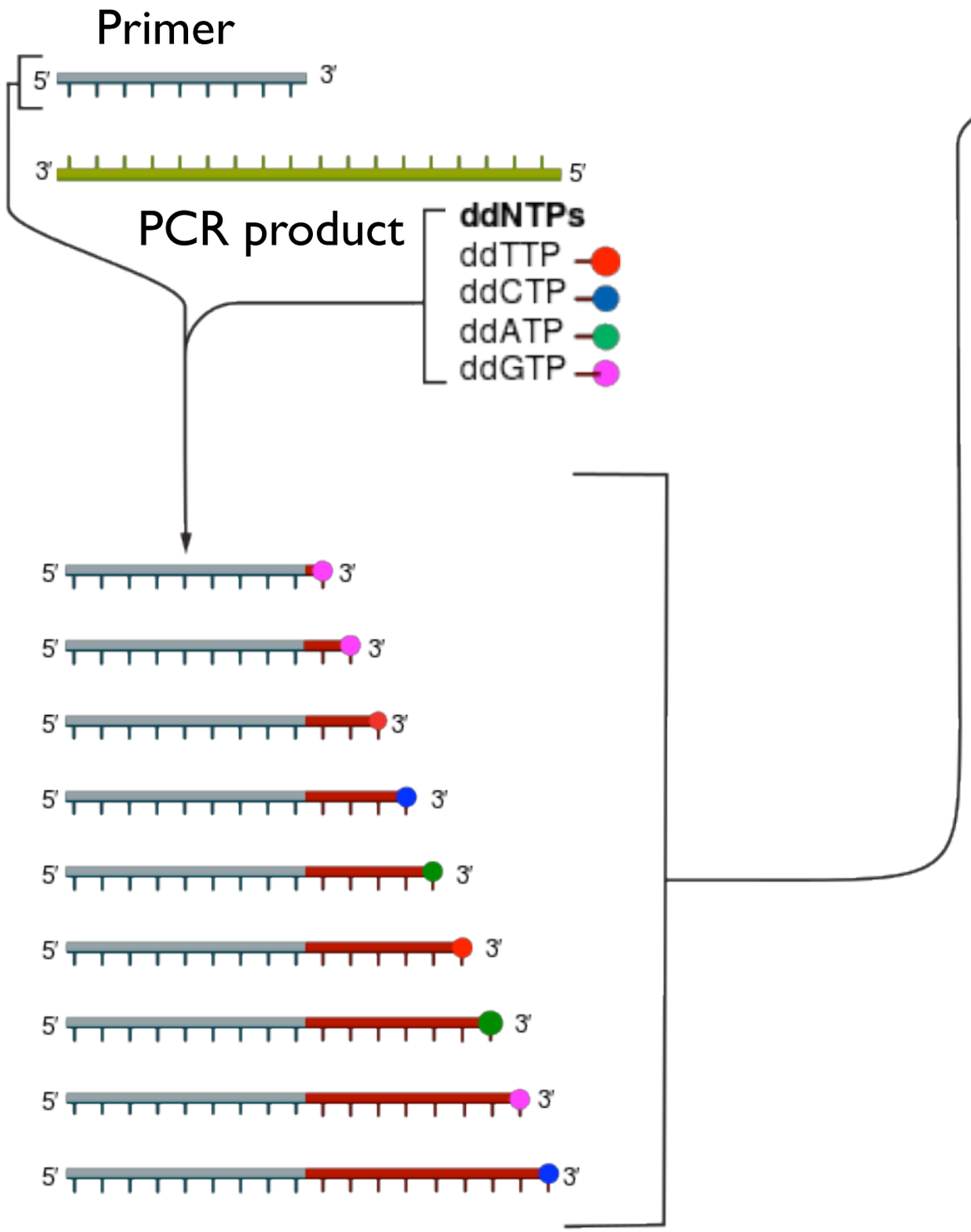
Themes of the module:

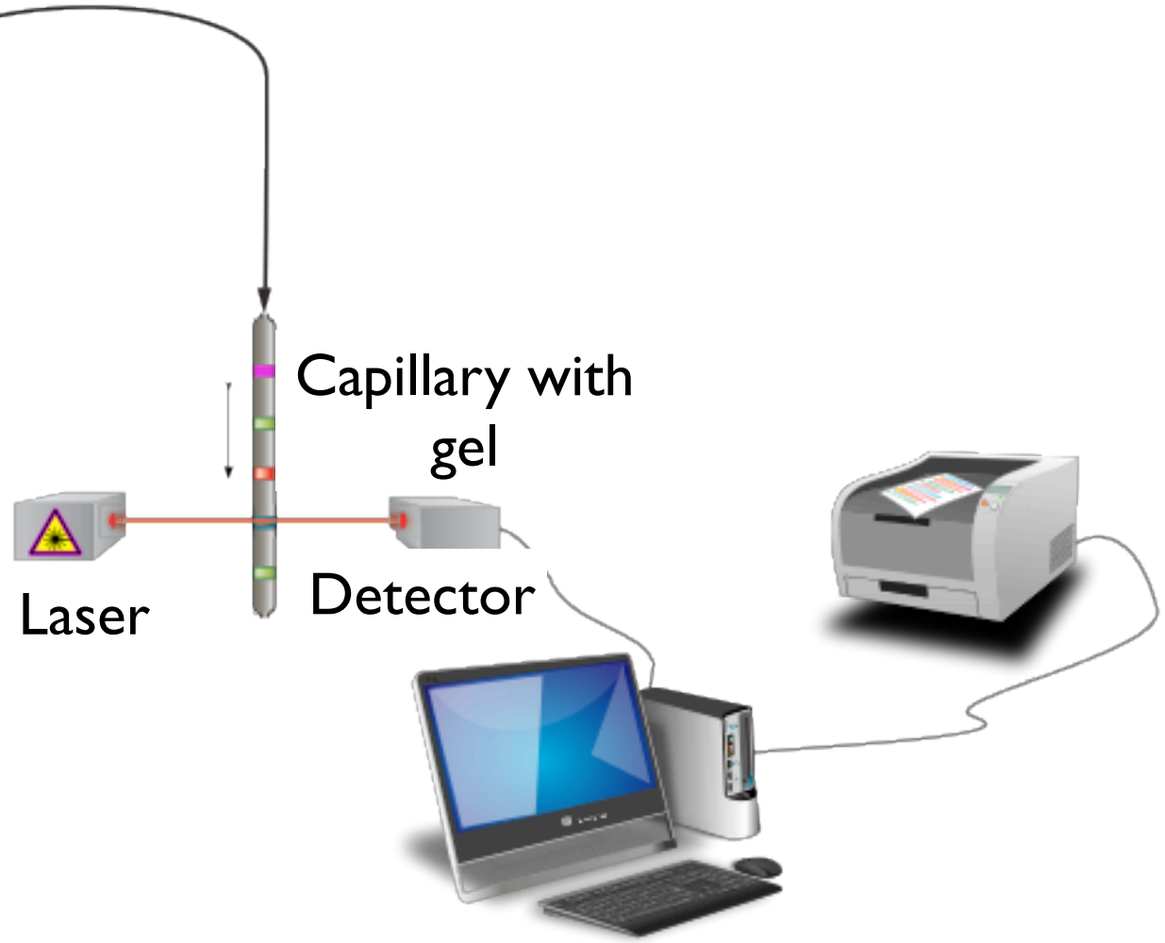
Cancer Systems Biology
High Throughput Screening Technologies

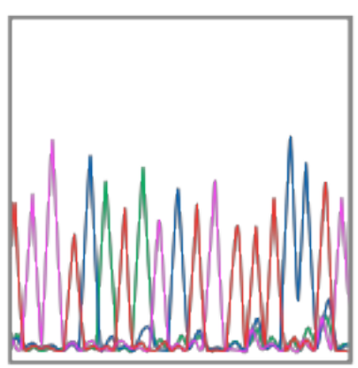
A bit more information about automated Sanger sequencing...



A bit more information about automated Sanger sequencing...





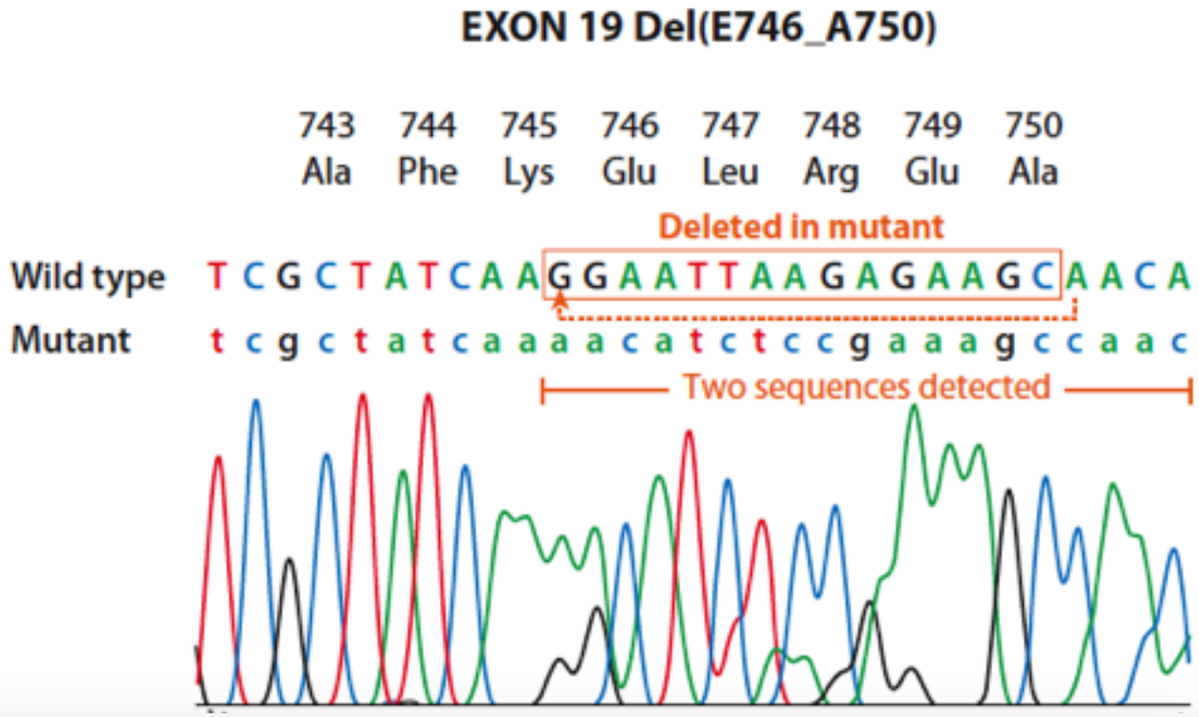


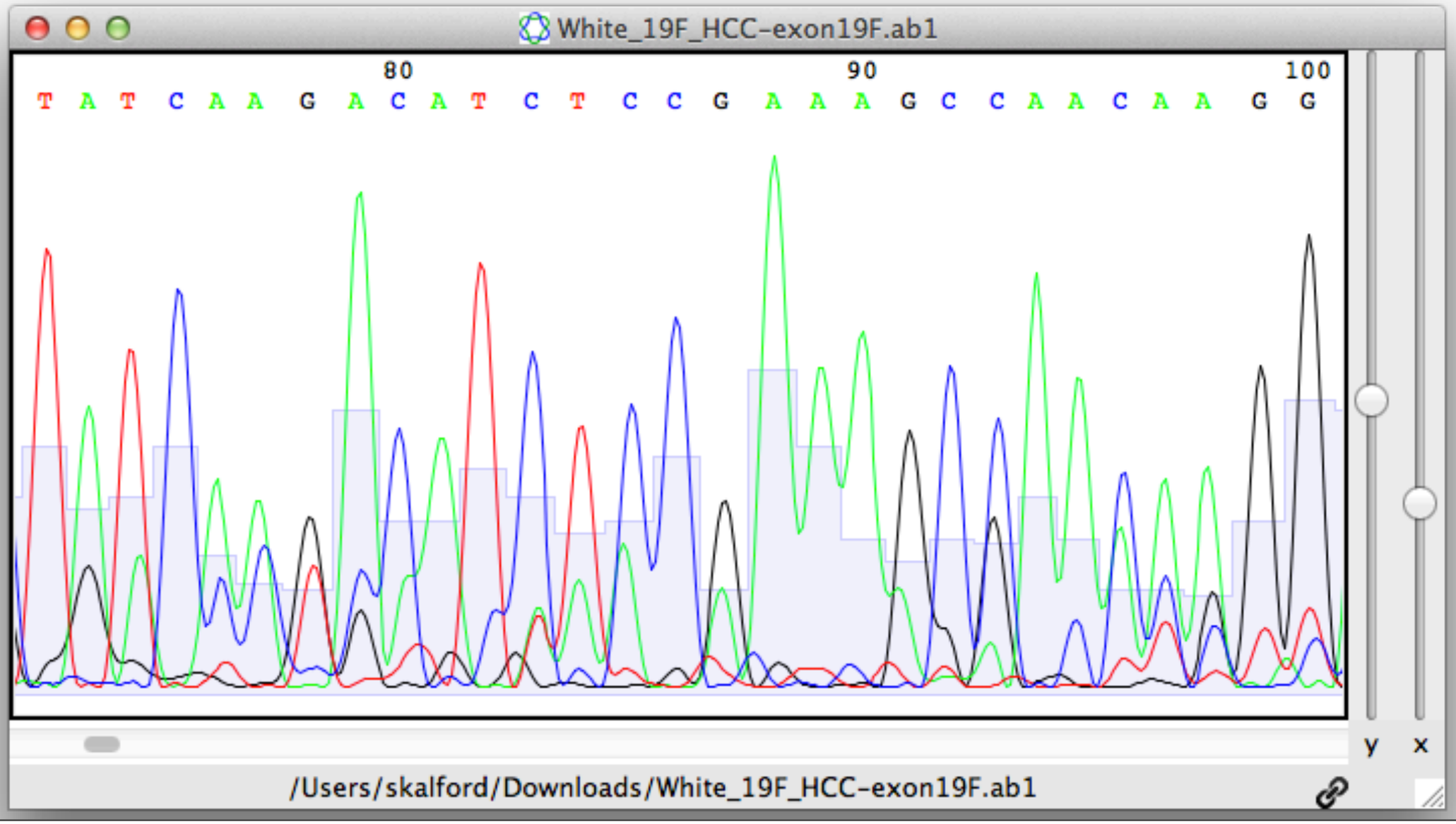
Chromatograph

Modified from Wikipedia Commons

Positive control for exon 19 deletion:

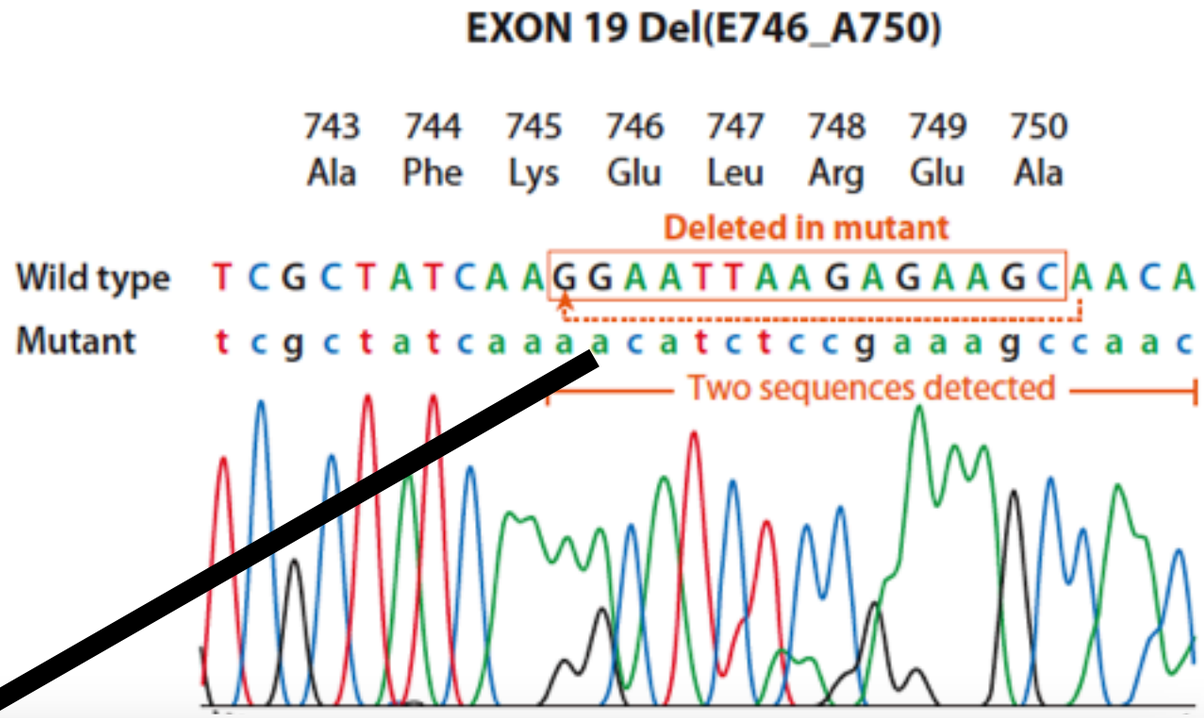
HCC-827

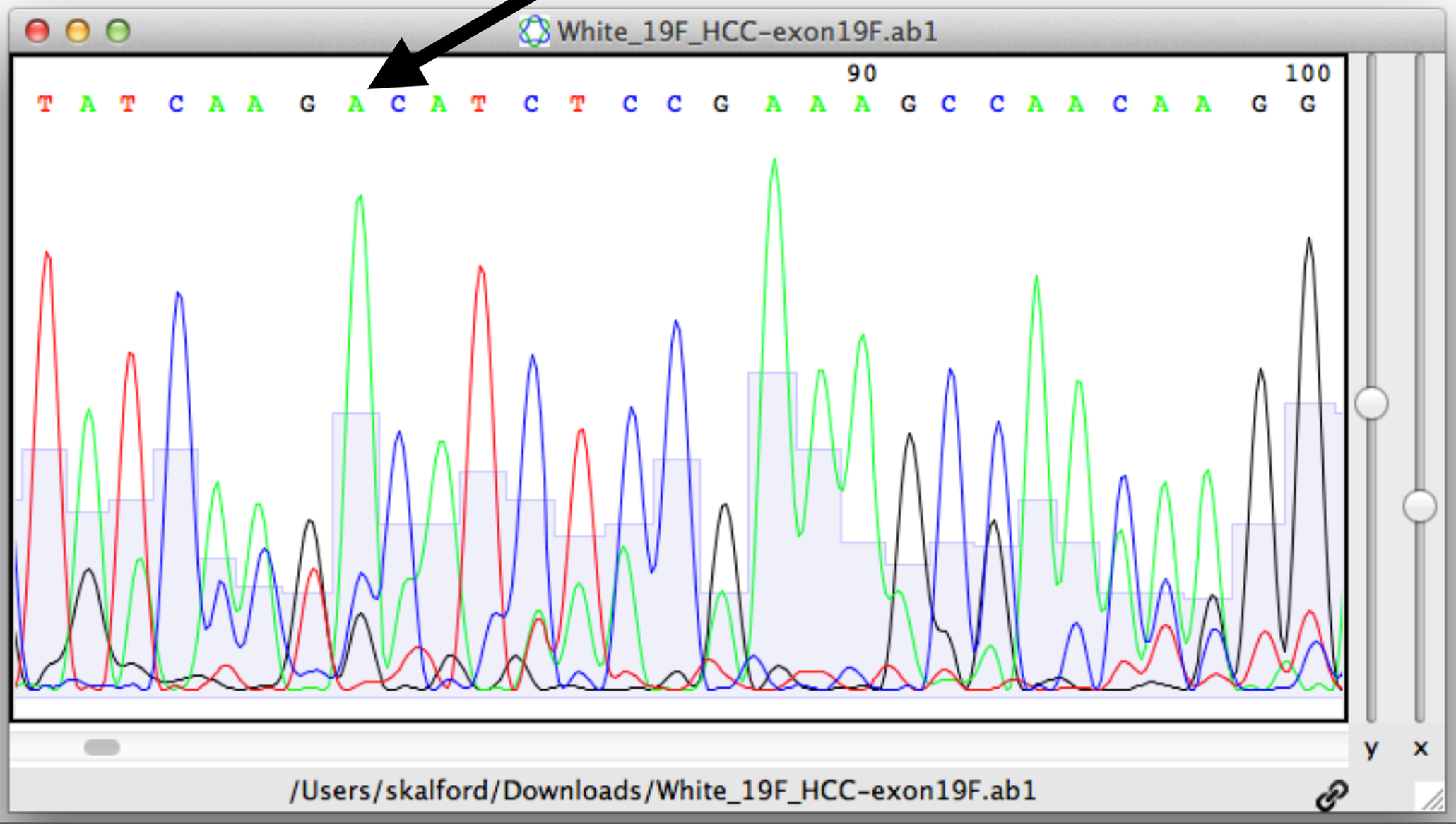




Positive control for exon 19 deletion:

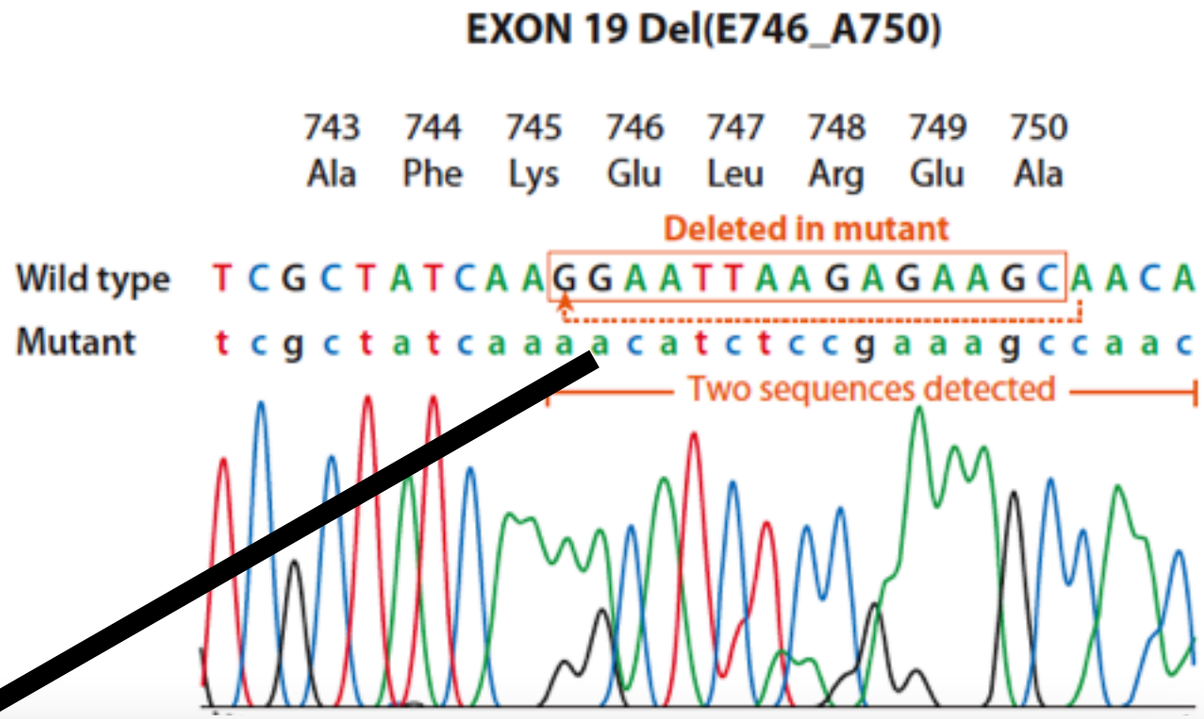
HCC-827

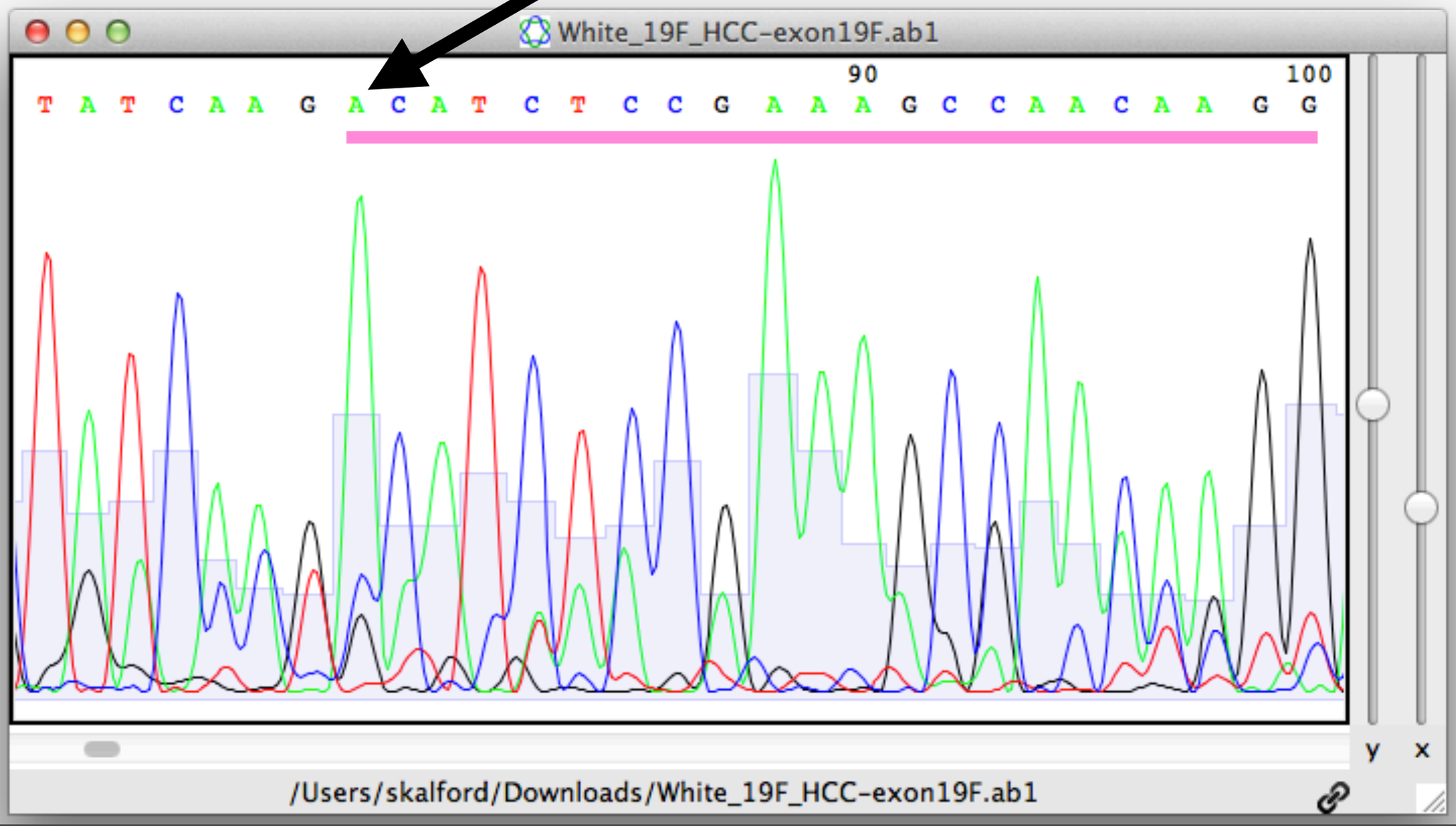




Positive control for exon 19 deletion:

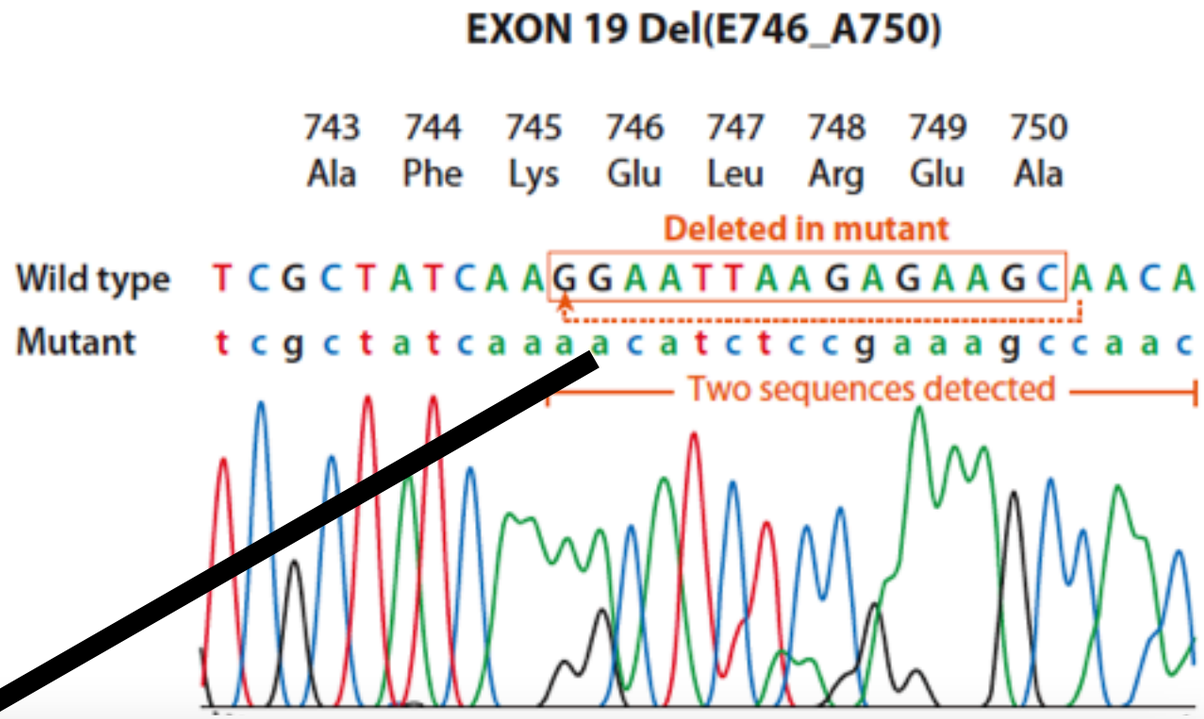
HCC-827

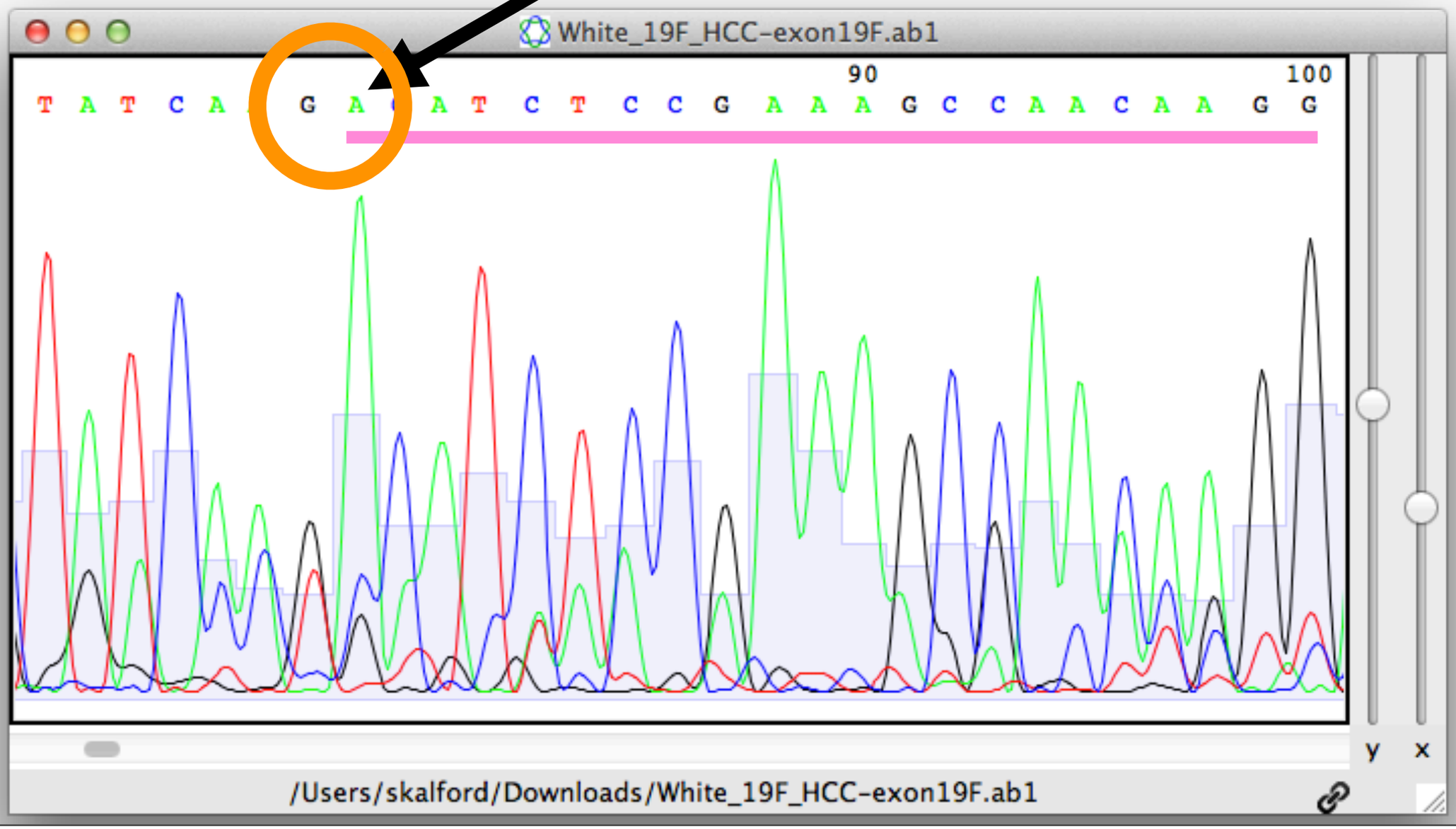




Positive control for exon 19 deletion:

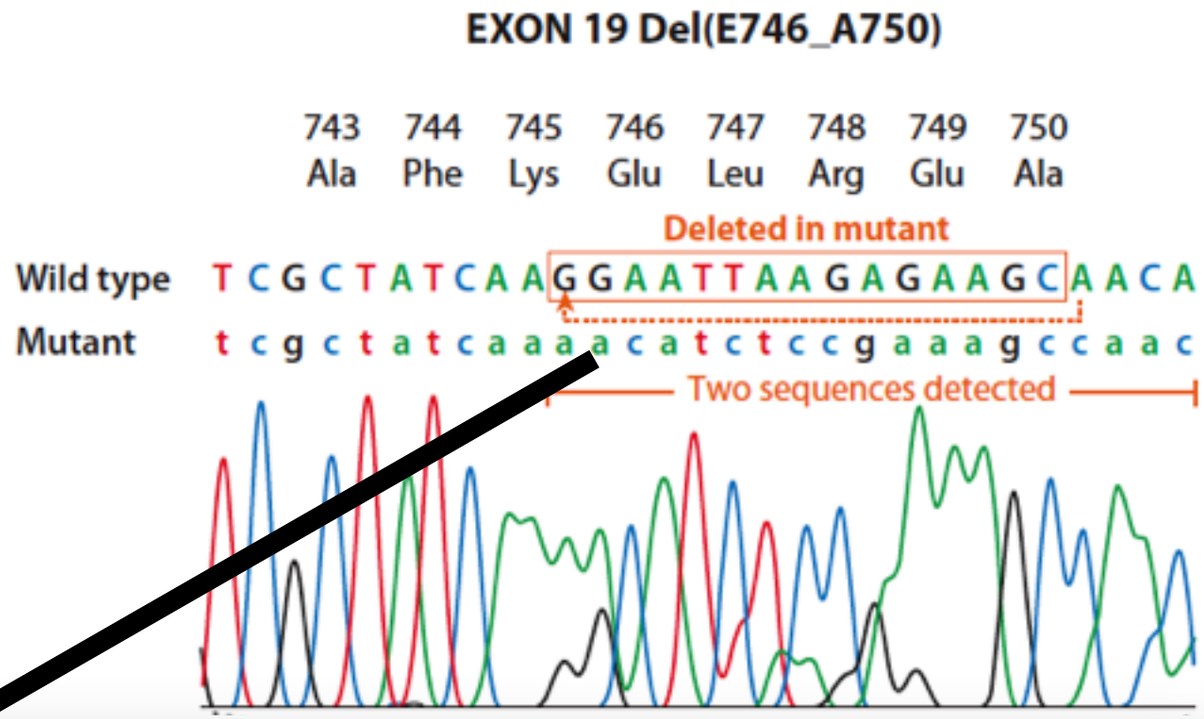
HCC-827

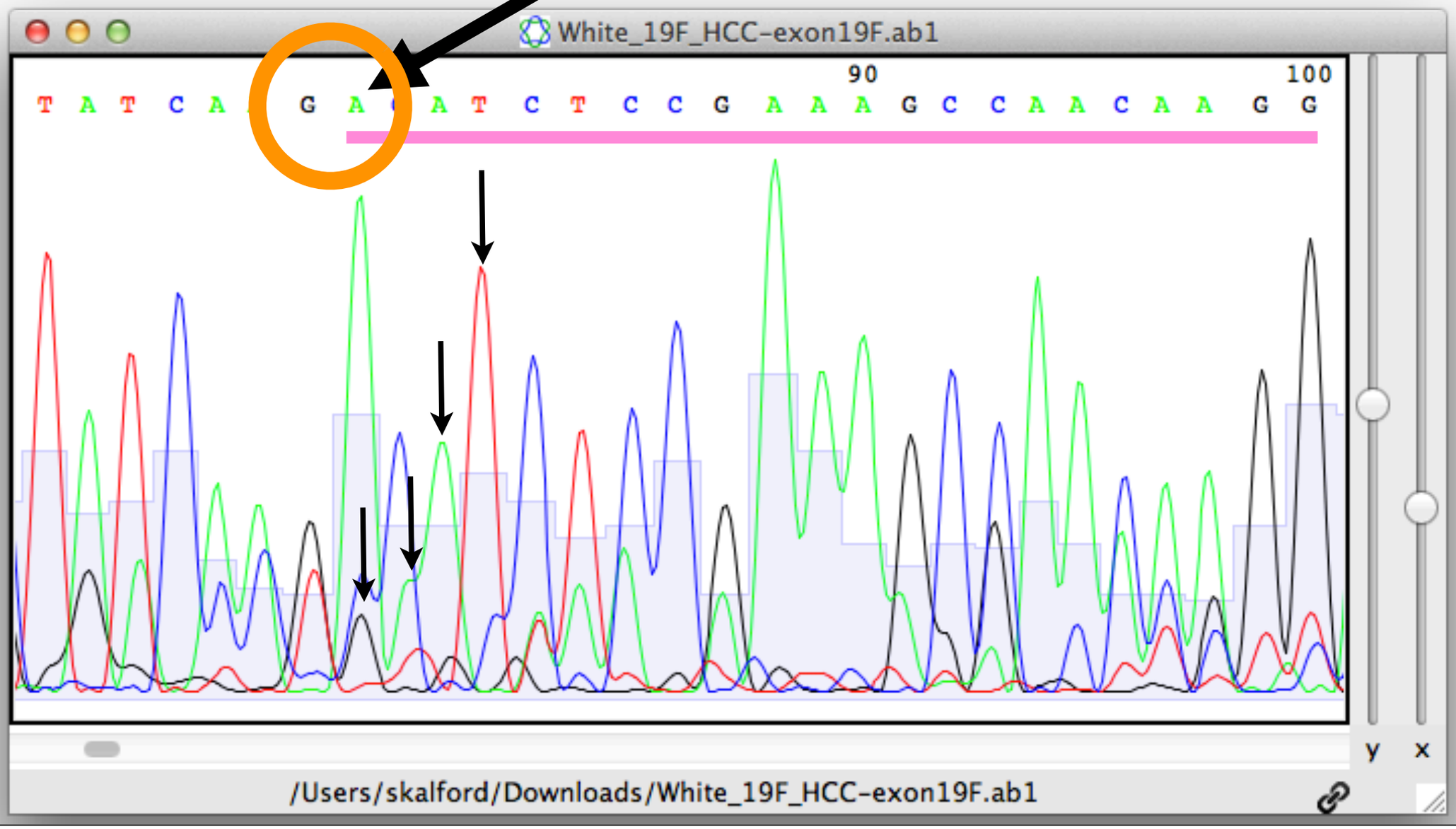




Positive control for exon 19 deletion:

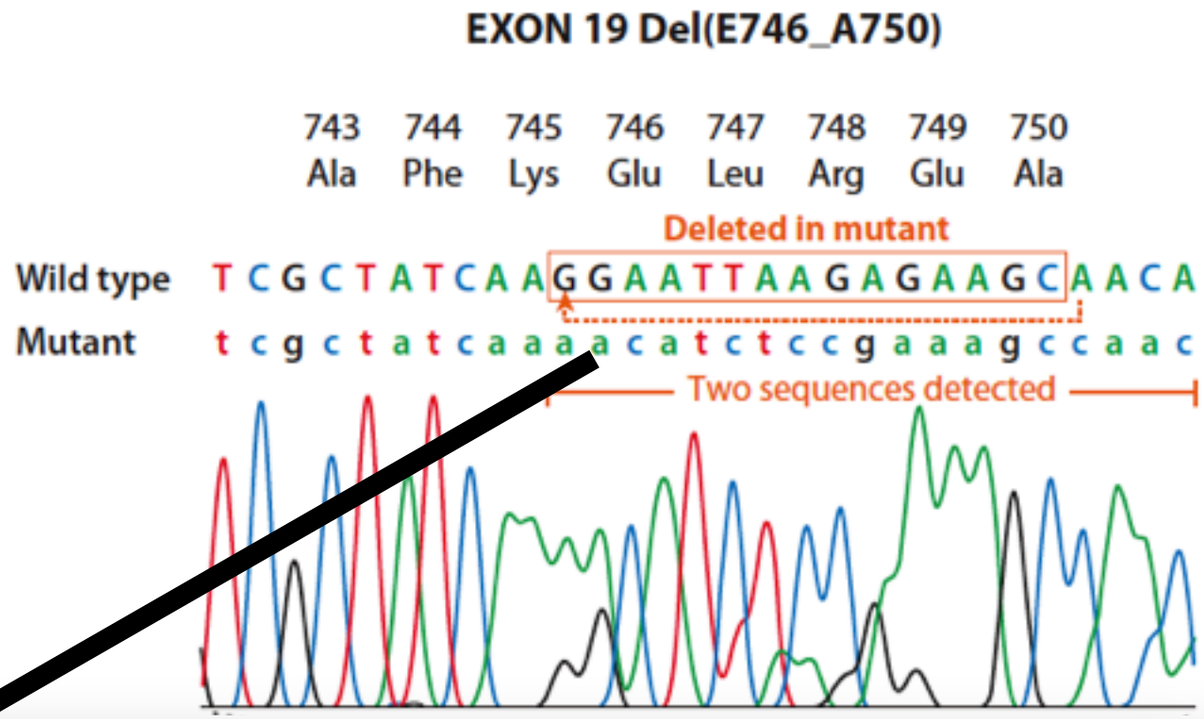
HCC-827

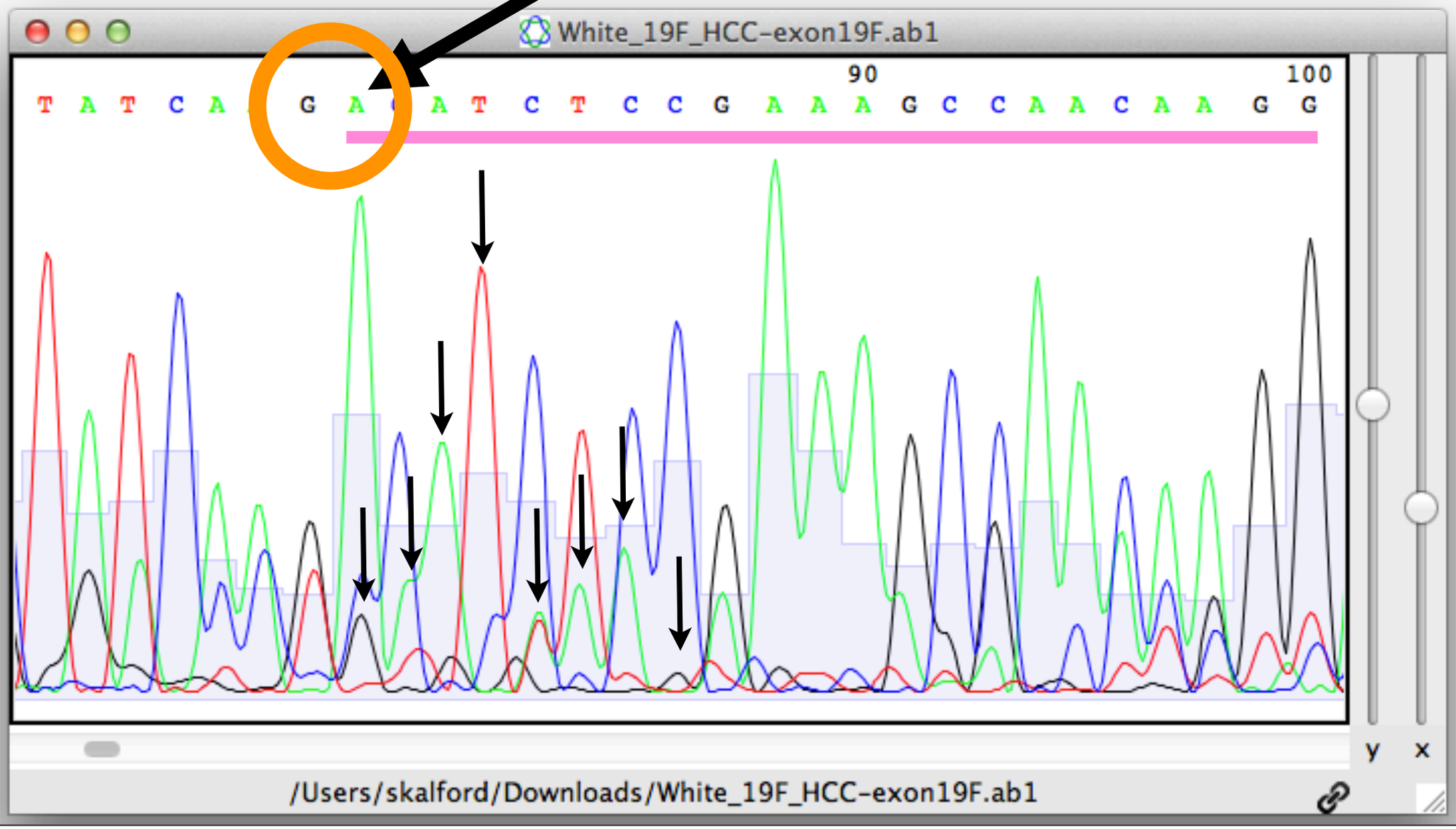




Positive control for exon 19 deletion:

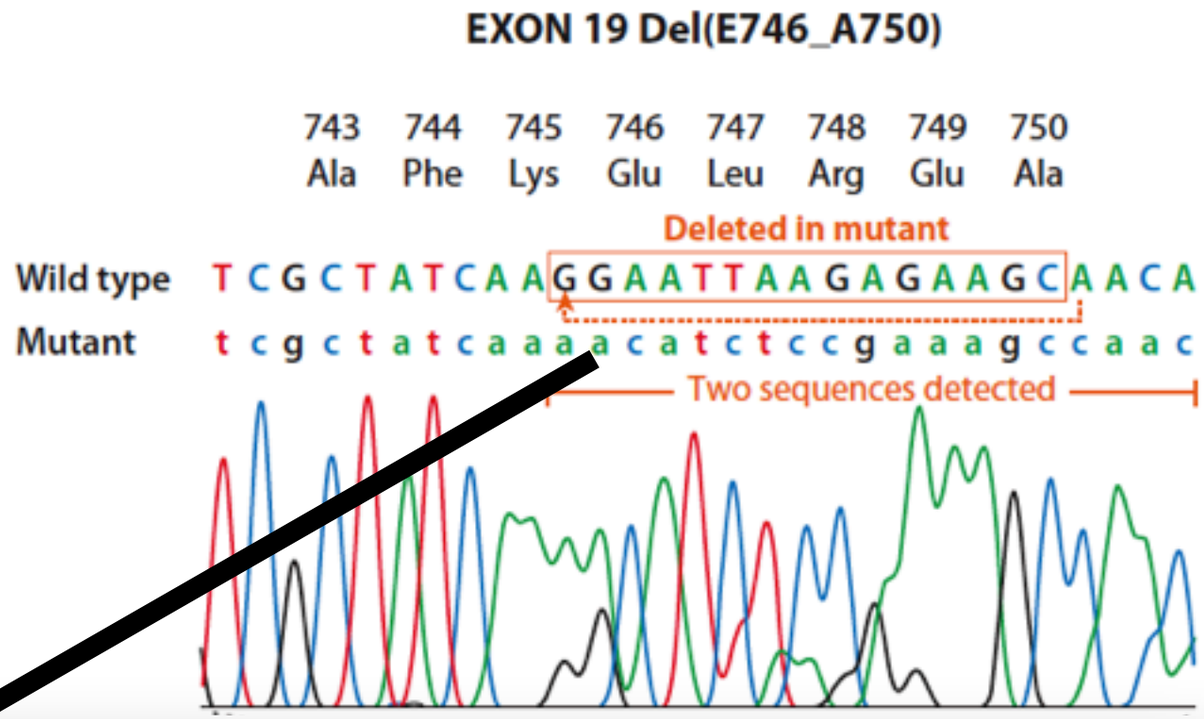
HCC-827

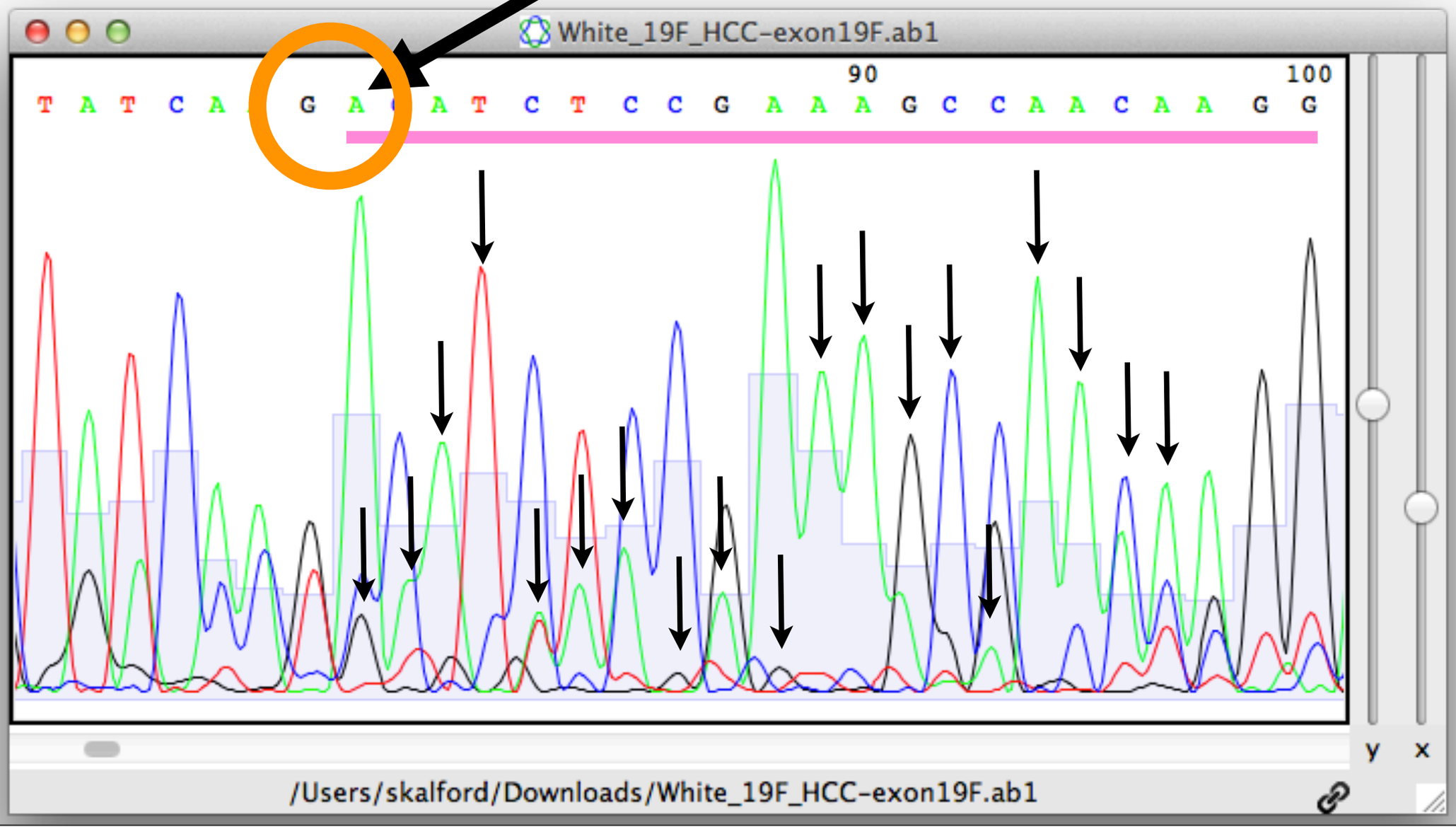




Positive control for exon 19 deletion:

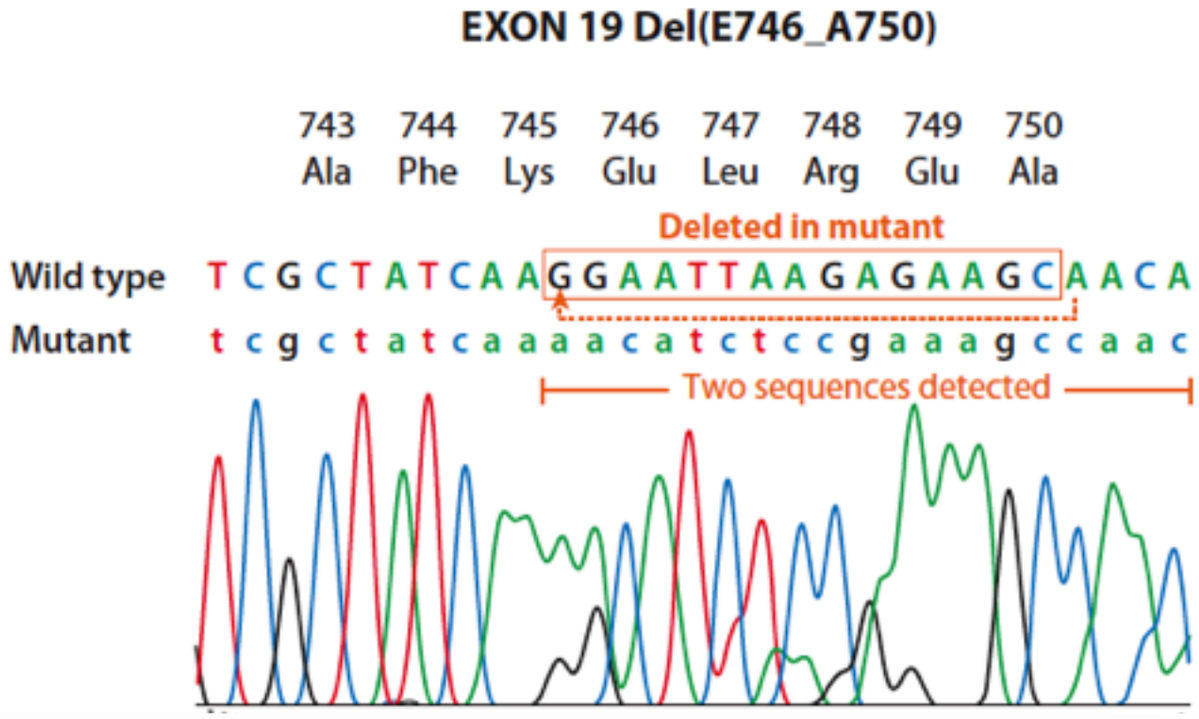
HCC-827

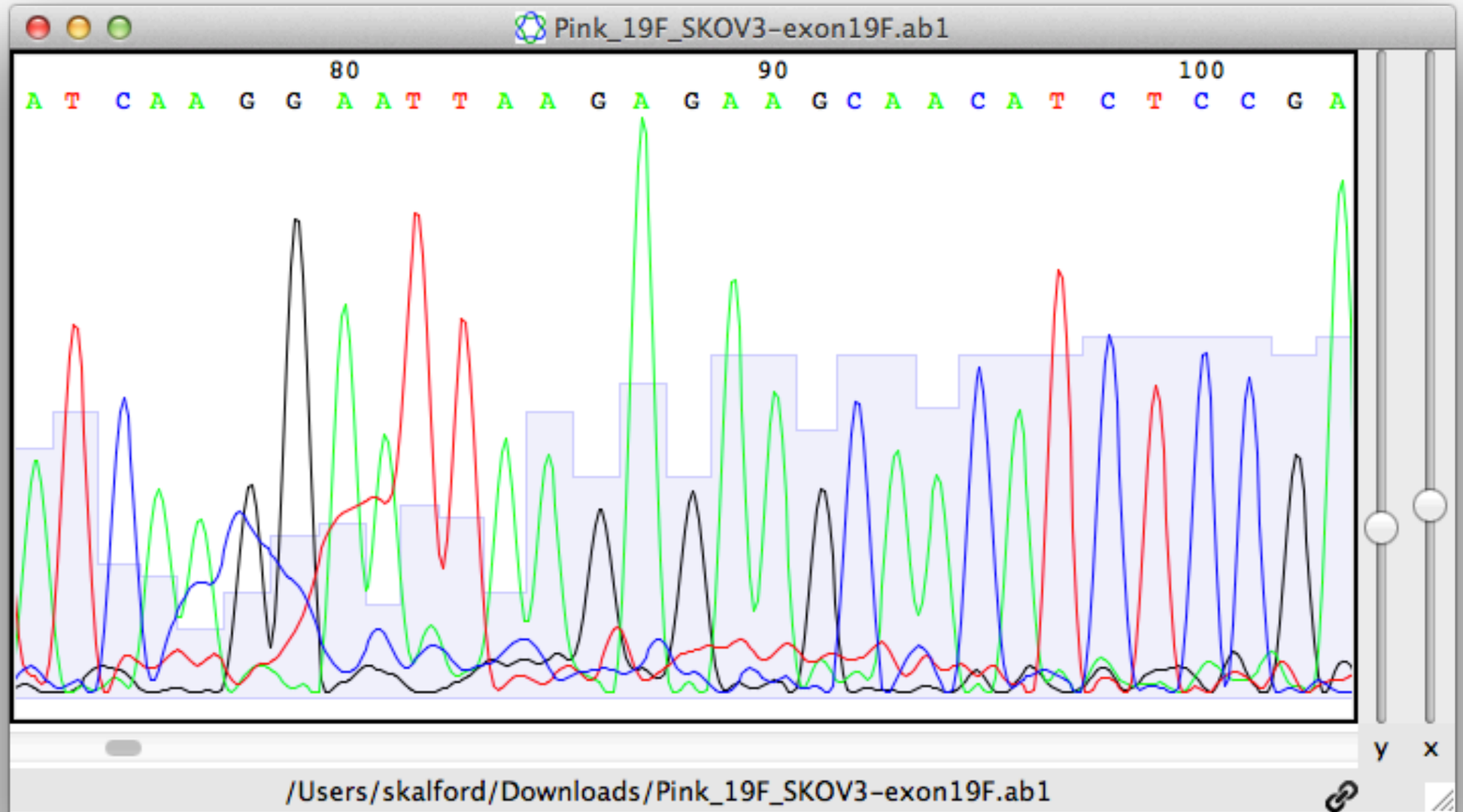




Our cell line of interest:

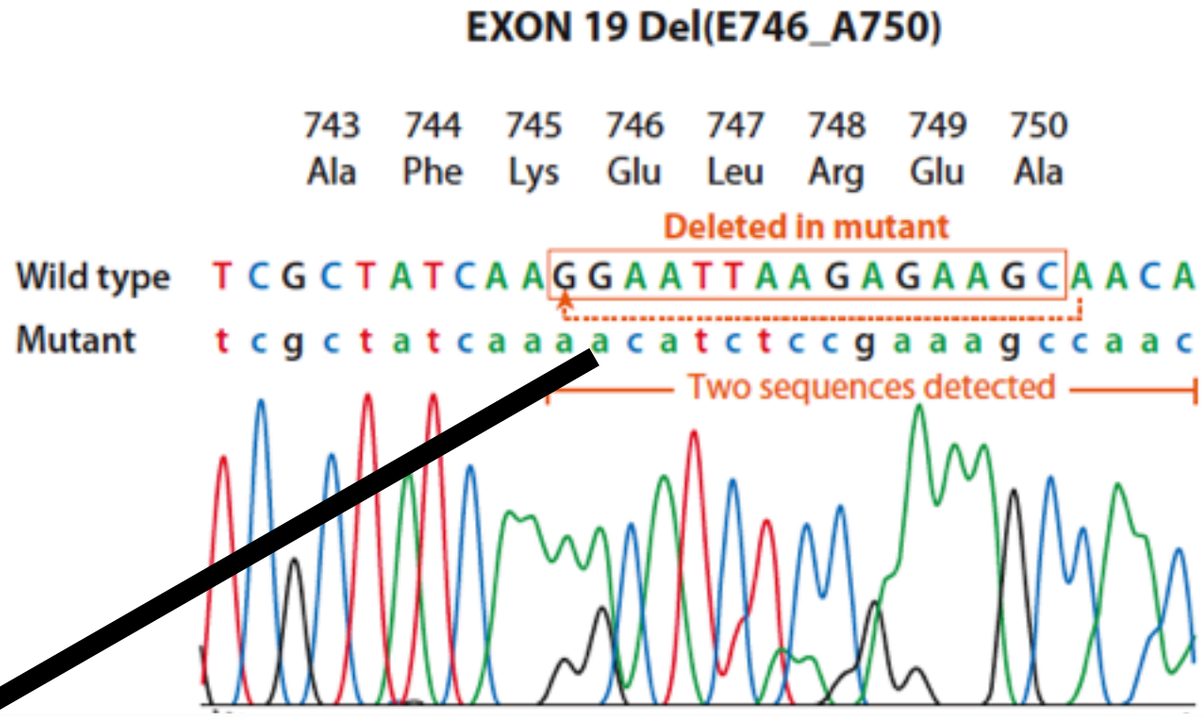
SKOV3

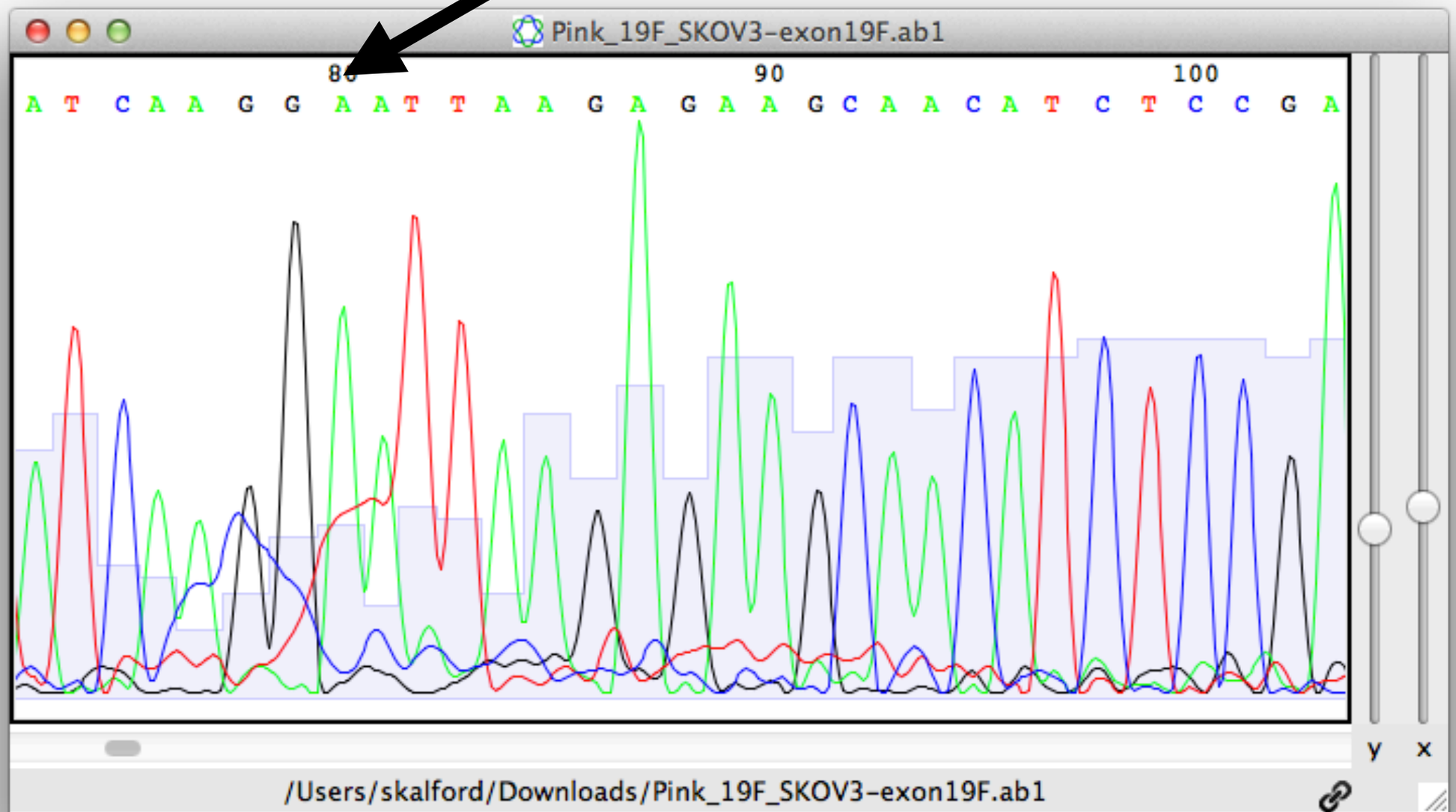




Our cell line of interest:

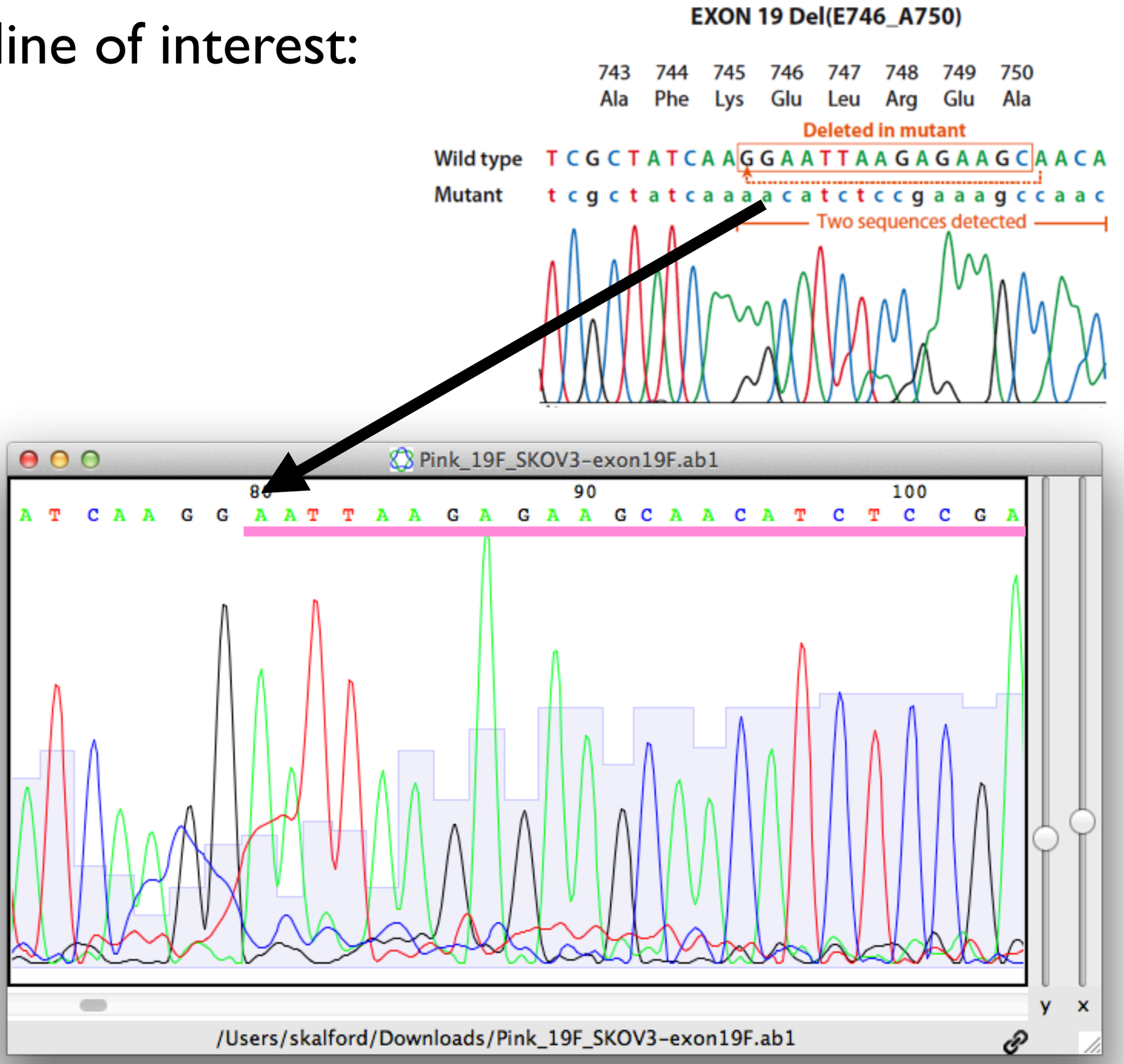
SKOV3



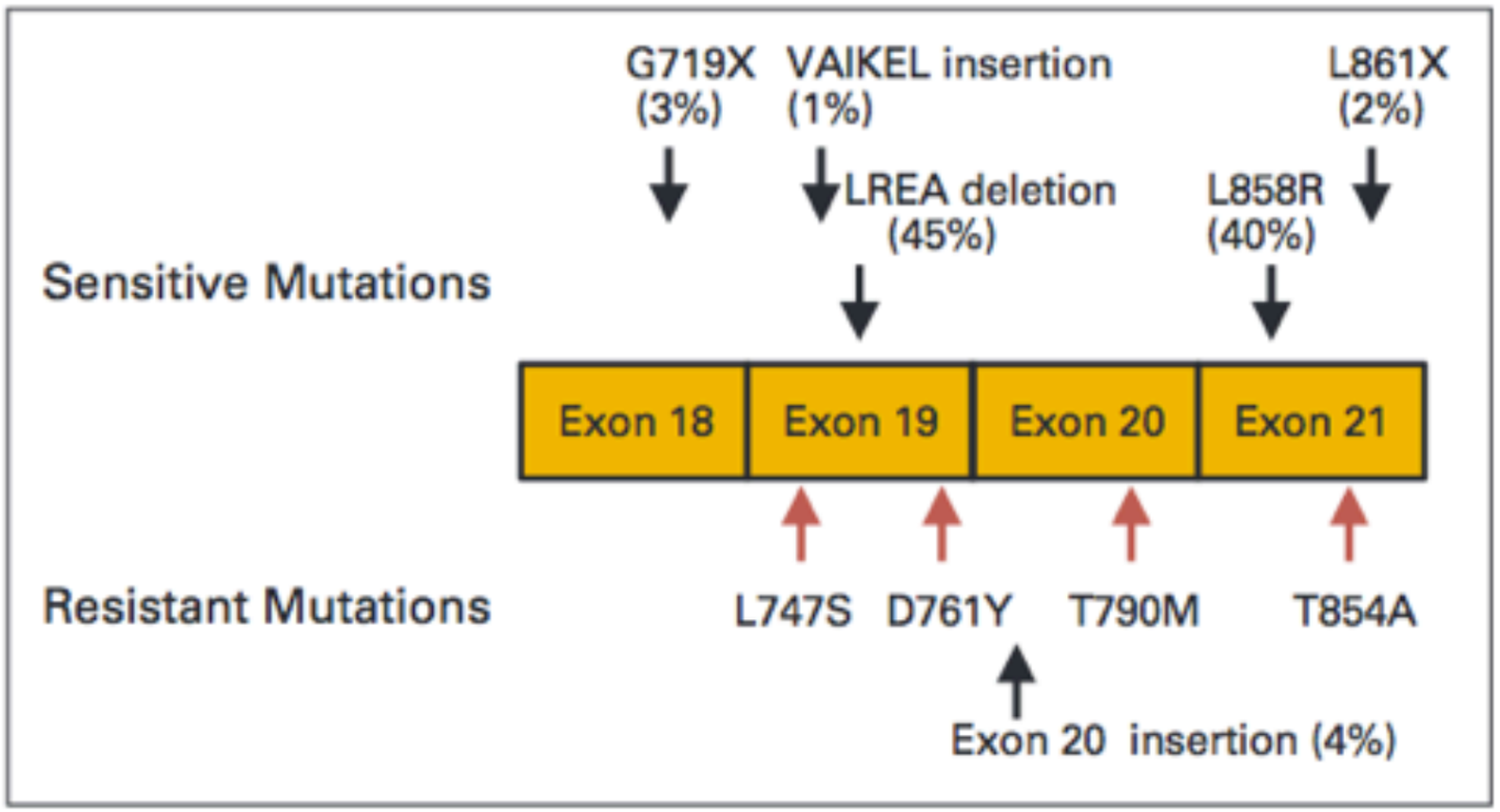


Our cell line of interest:

SKOV3



EGFR mutation predicts drug response.

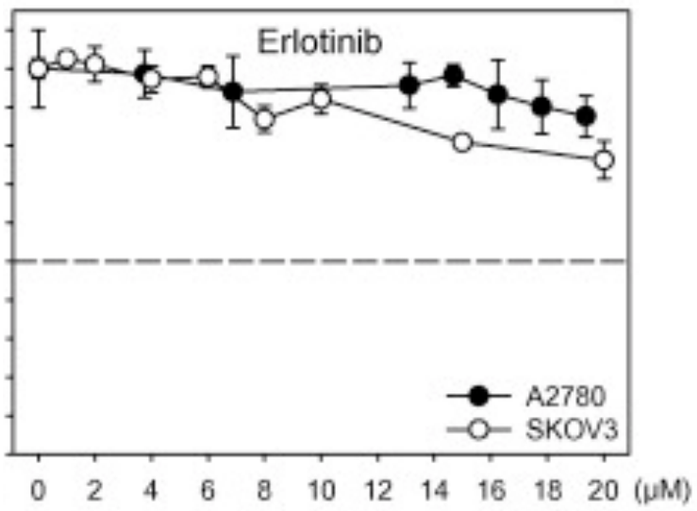


SKOV3?

Epidermal growth factor receptor tyrosine kinase inhibitor-resistant disease. Ohashi K, Maruvka YE, Michor F, Pao W. J Clin Oncol. 2013 Mar 10;31(8):1070-80.

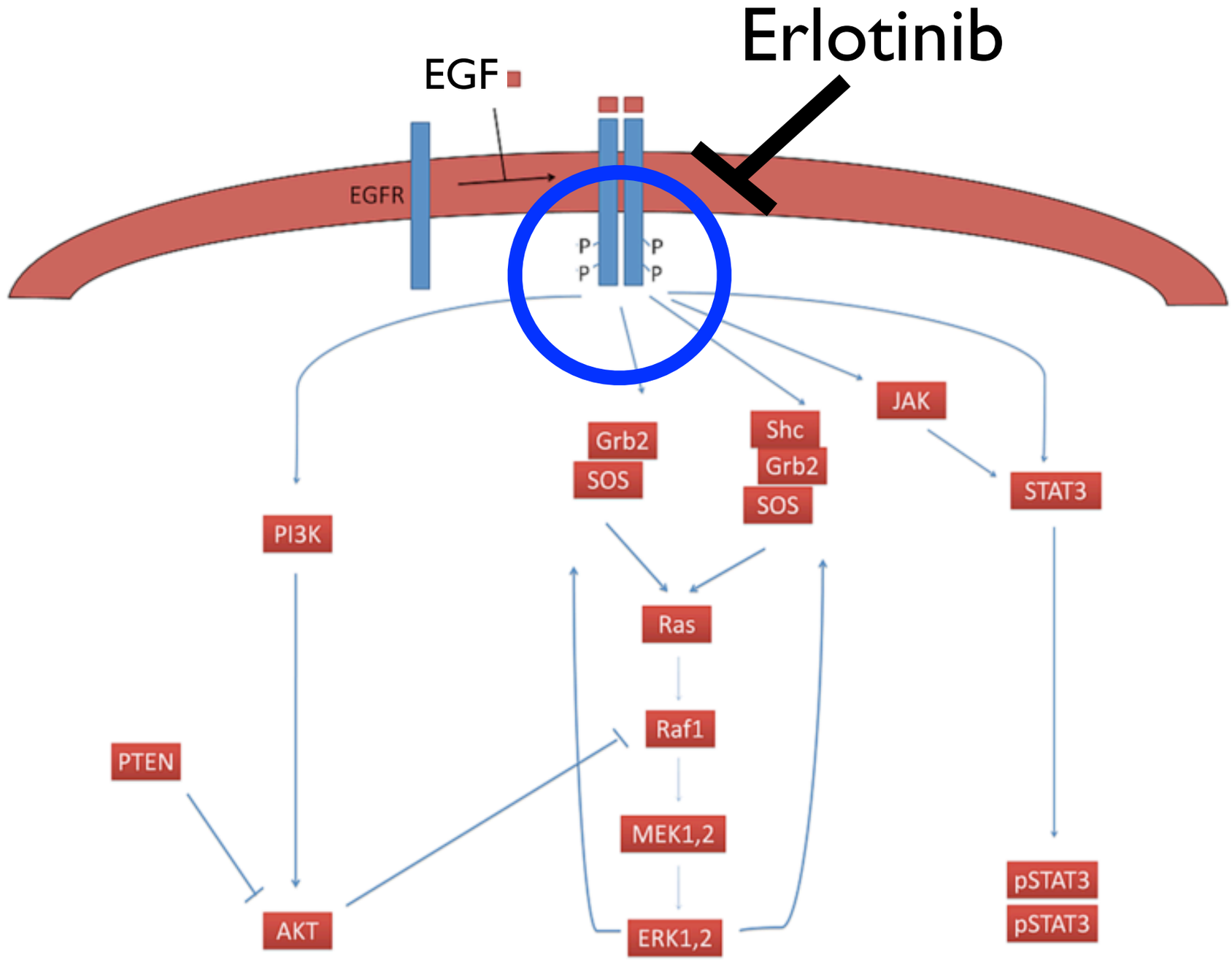
What other information might we need?

Design Goal:



Grunt et al. Biochem. Biophys. Res. Commun. 385:454-459(2009)

Overcome resistance to EGFR inhibition in SKOV3 human ovarian cancer cells.

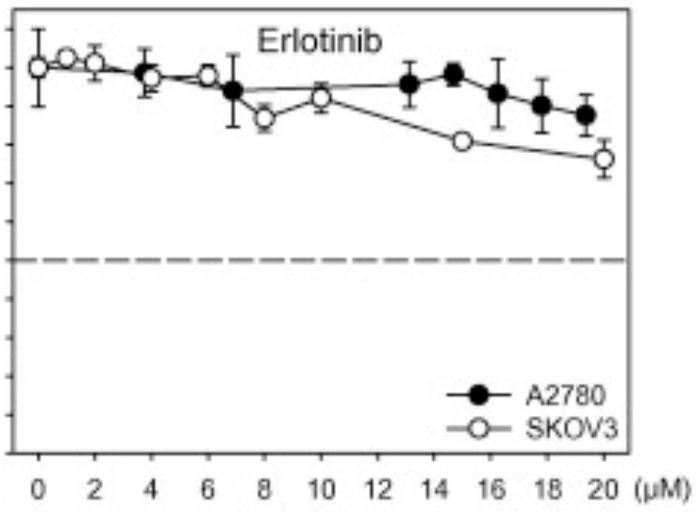


pAkt pErk pSTAT3

Bidkhori G, Moeini A, Masoudi-Nejad A (2012) Modeling of Tumor Progression in NSCLC and Intrinsic Resistance to TKI in Loss of PTEN Expression. PLoS ONE 7(10): e48004

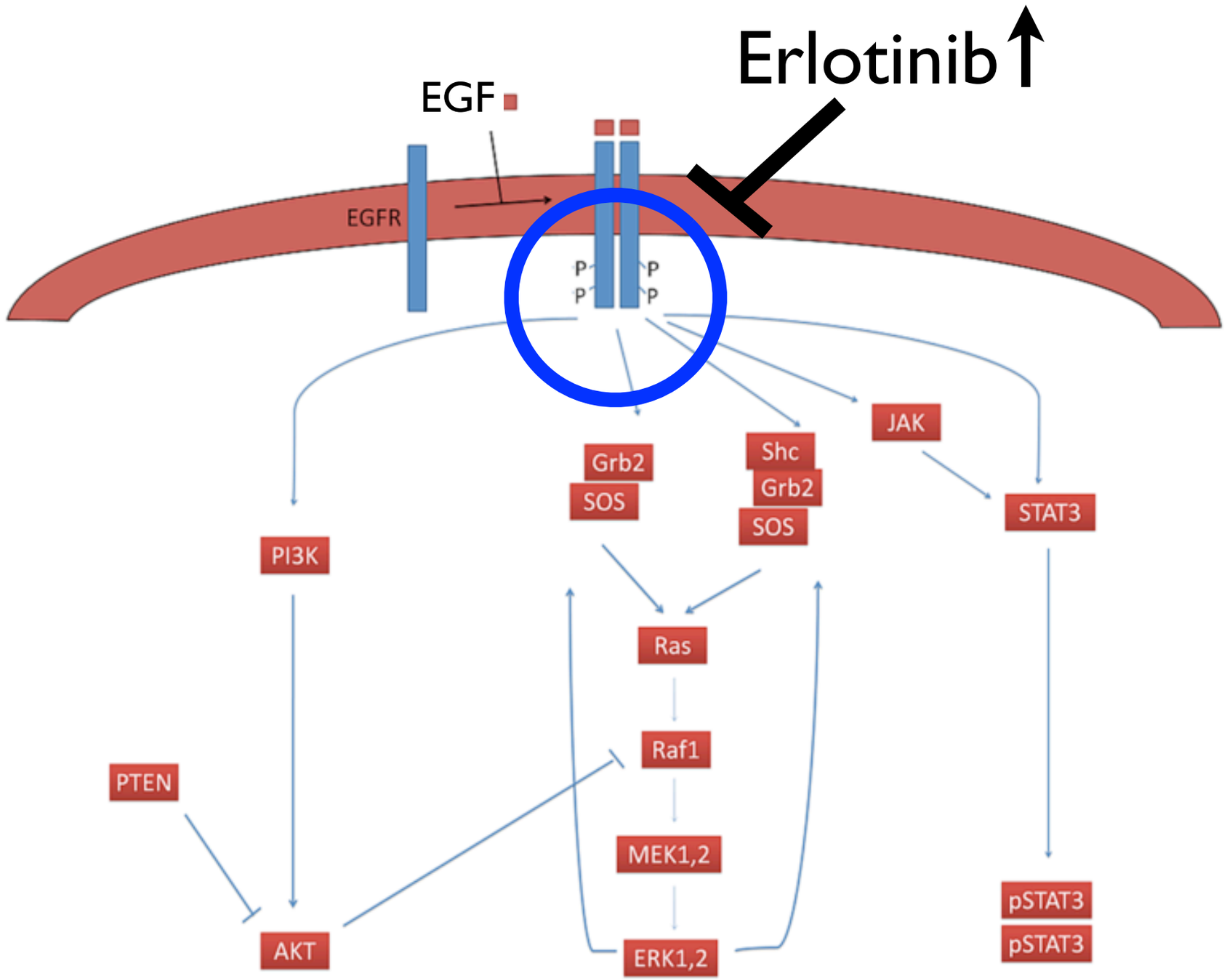
What other information might we need?

Design Goal:



Grunt et al. Biochem. Biophys. Res. Commun. 385:454-459(2009)

Overcome resistance to EGFR inhibition in SKOV3 human ovarian cancer cells.

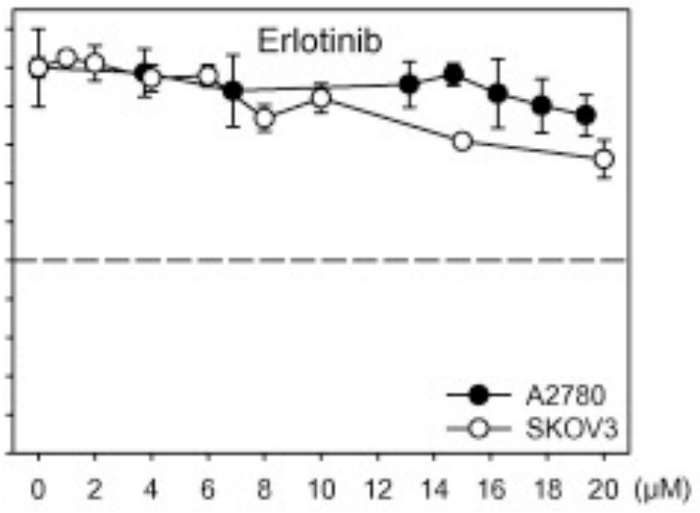


pAkt pErk pSTAT3

Bidkhori G, Moeini A, Masoudi-Nejad A (2012) Modeling of Tumor Progression in NSCLC and Intrinsic Resistance to TKI in Loss of PTEN Expression. PLoS ONE 7(10): e48004

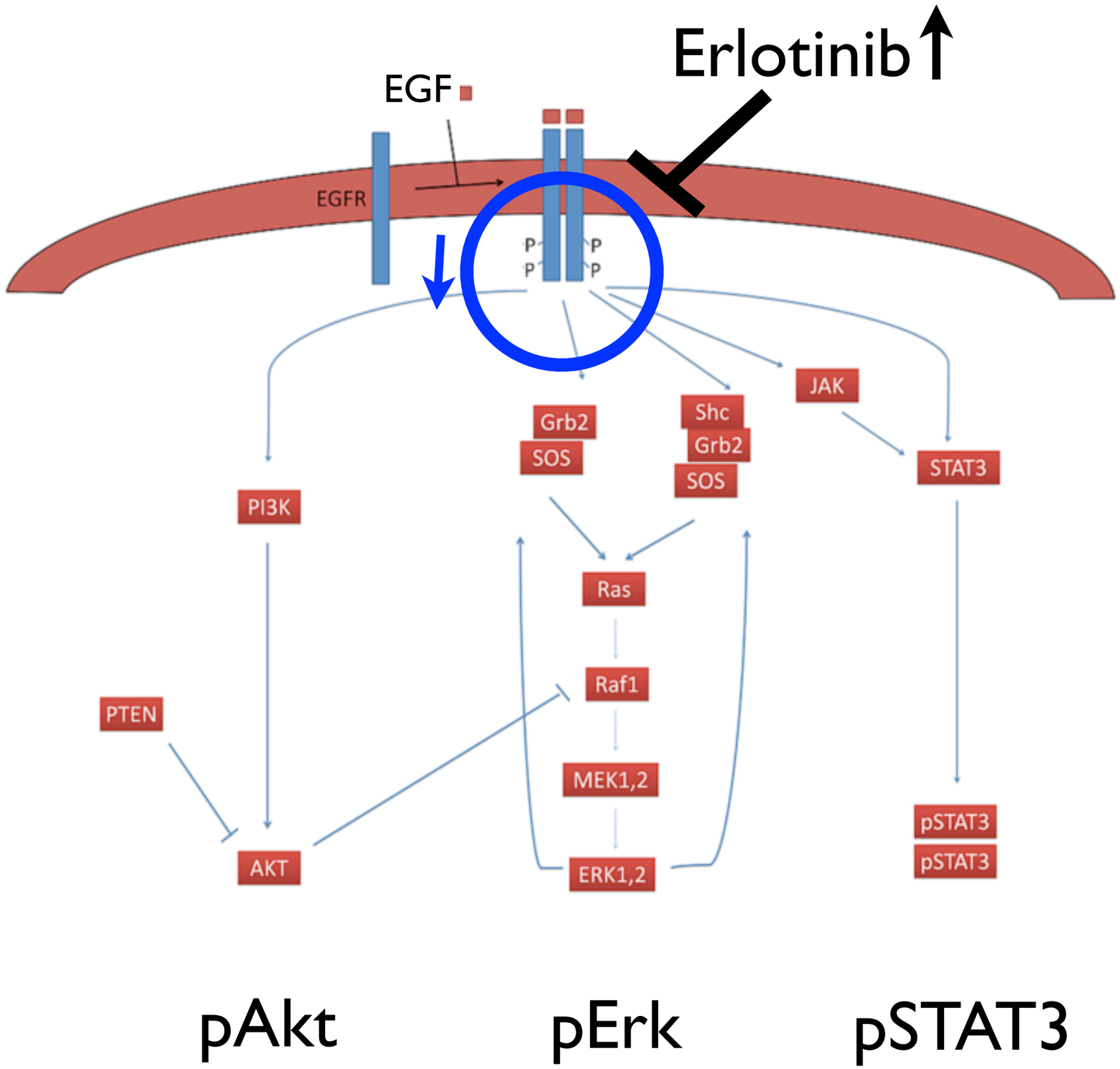
What other information might we need?

Design Goal:



Grunt et al. Biochem. Biophys. Res. Commun. 385:454-459(2009)

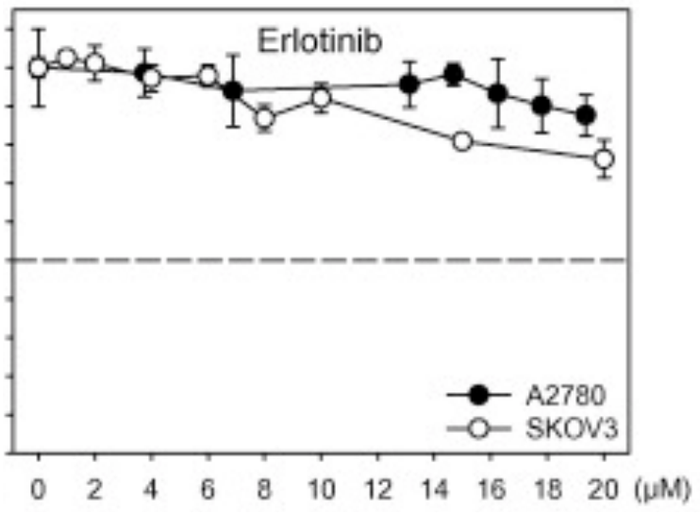
Overcome resistance to EGFR inhibition in SKOV3 human ovarian cancer cells.



Bidkhori G, Moeini A, Masoudi-Nejad A (2012) Modeling of Tumor Progression in NSCLC and Intrinsic Resistance to TKI in Loss of PTEN Expression. PLoS ONE 7(10): e48004

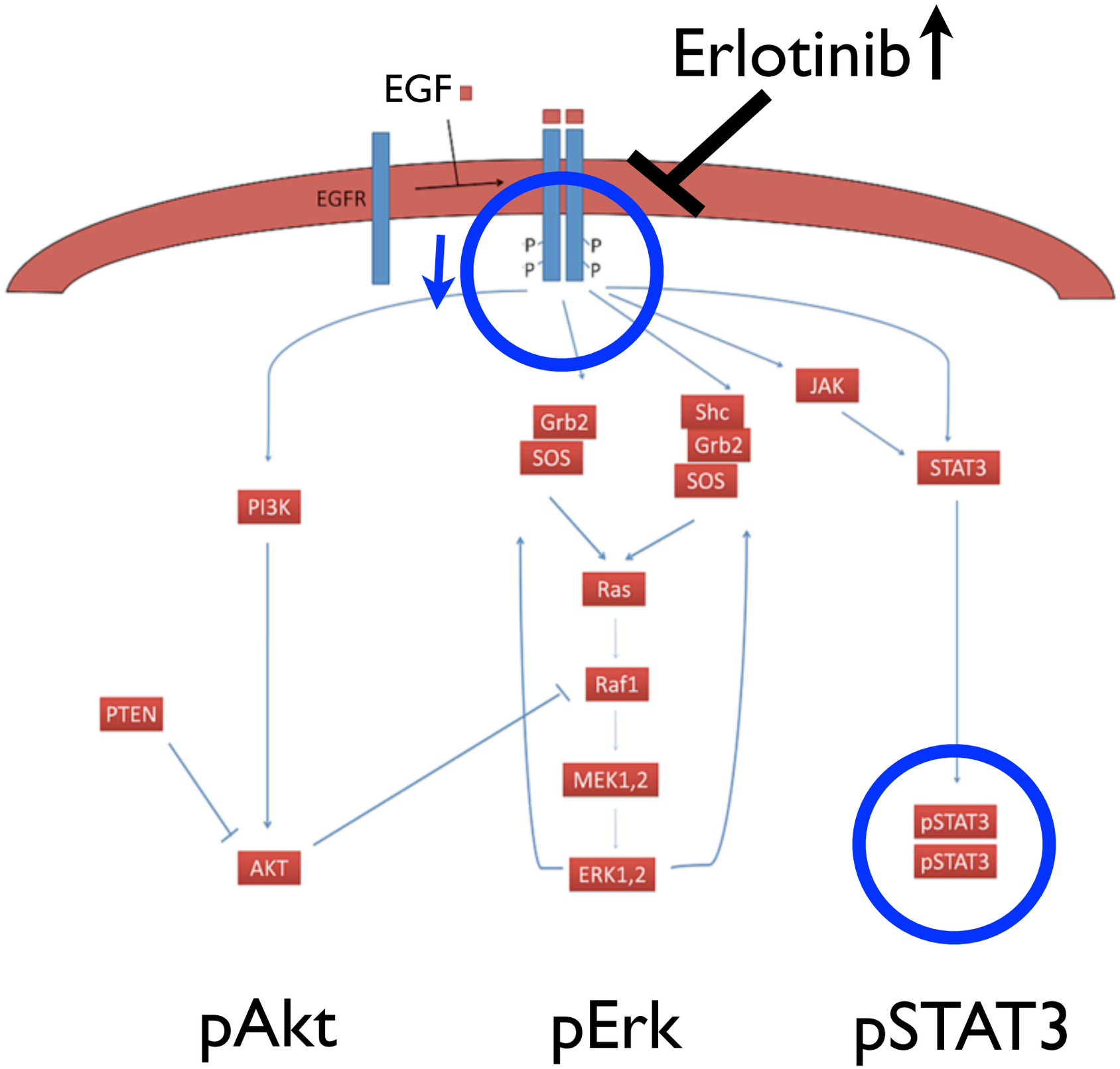
What other information might we need?

Design Goal:



Grunt et al. Biochem. Biophys. Res. Commun. 385:454-459(2009)

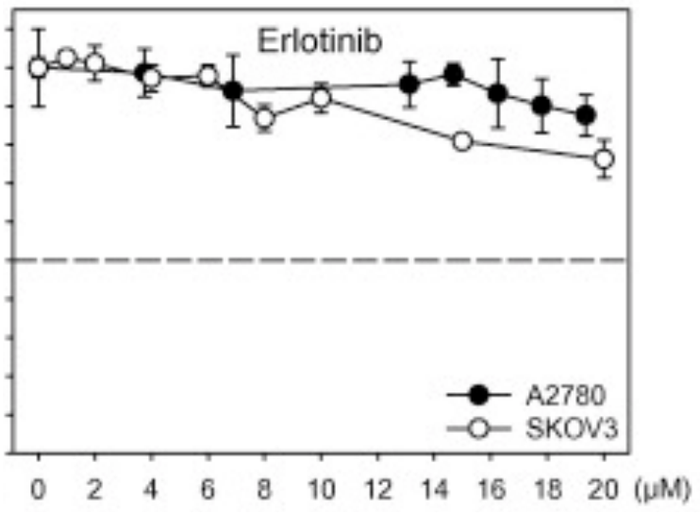
Overcome resistance to EGFR inhibition in SKOV3 human ovarian cancer cells.



Bidkhori G, Moeini A, Masoudi-Nejad A (2012) Modeling of Tumor Progression in NSCLC and Intrinsic Resistance to TKI in Loss of PTEN Expression. PLoS ONE 7(10): e48004

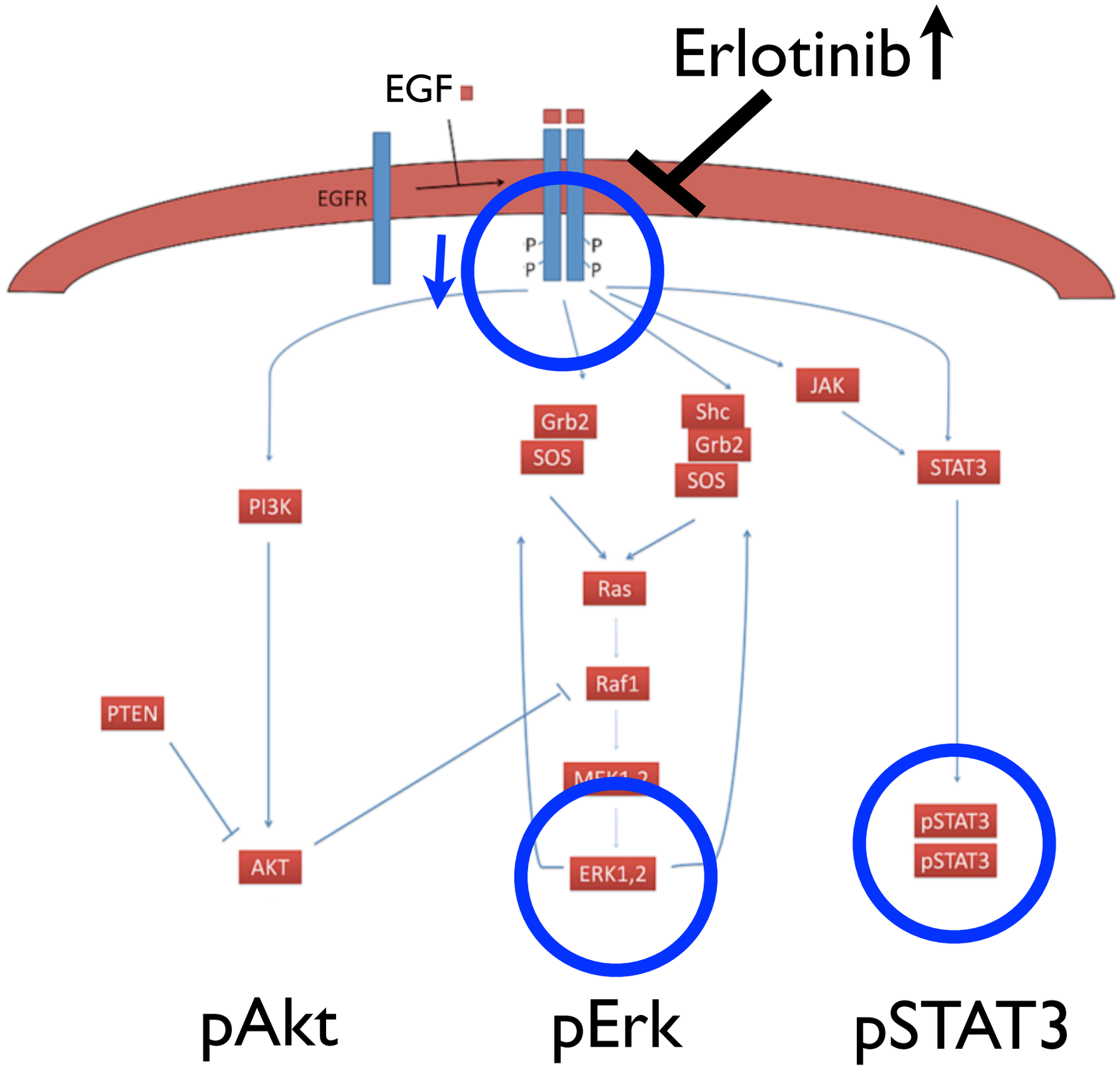
What other information might we need?

Design Goal:



Grunt et al. Biochem. Biophys. Res. Commun. 385:454-459(2009)

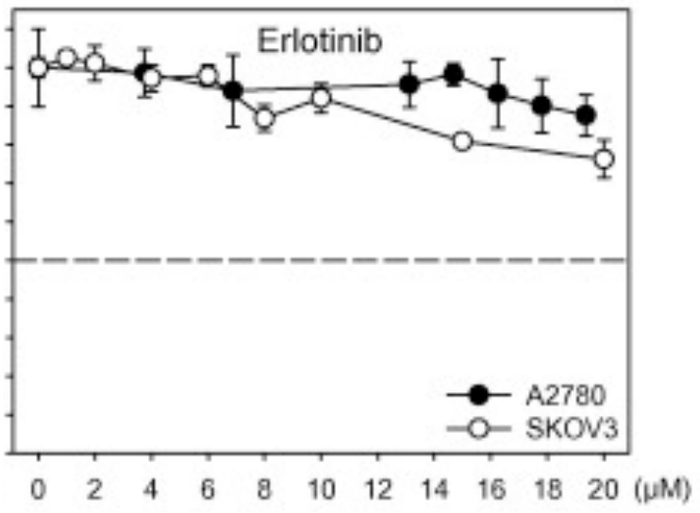
Overcome resistance to EGFR inhibition in SKOV3 human ovarian cancer cells.



Bidkhori G, Moeini A, Masoudi-Nejad A (2012) Modeling of Tumor Progression in NSCLC and Intrinsic Resistance to TKI in Loss of PTEN Expression. PLoS ONE 7(10): e48004

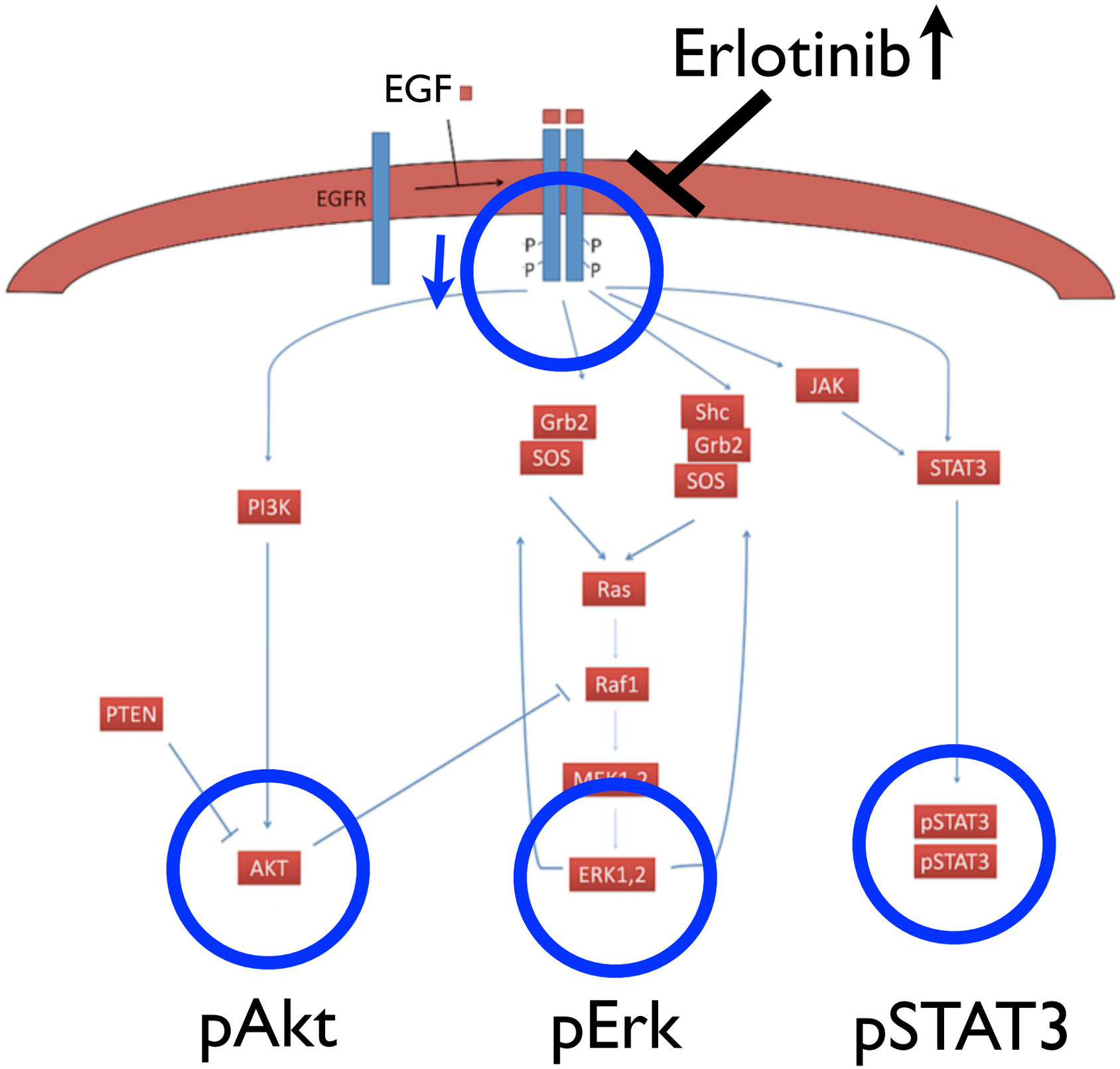
What other information might we need?

Design Goal:



Grunt et al. Biochem. Biophys. Res. Commun. 385:454-459(2009)

Overcome resistance to EGFR inhibition in SKOV3 human ovarian cancer cells.



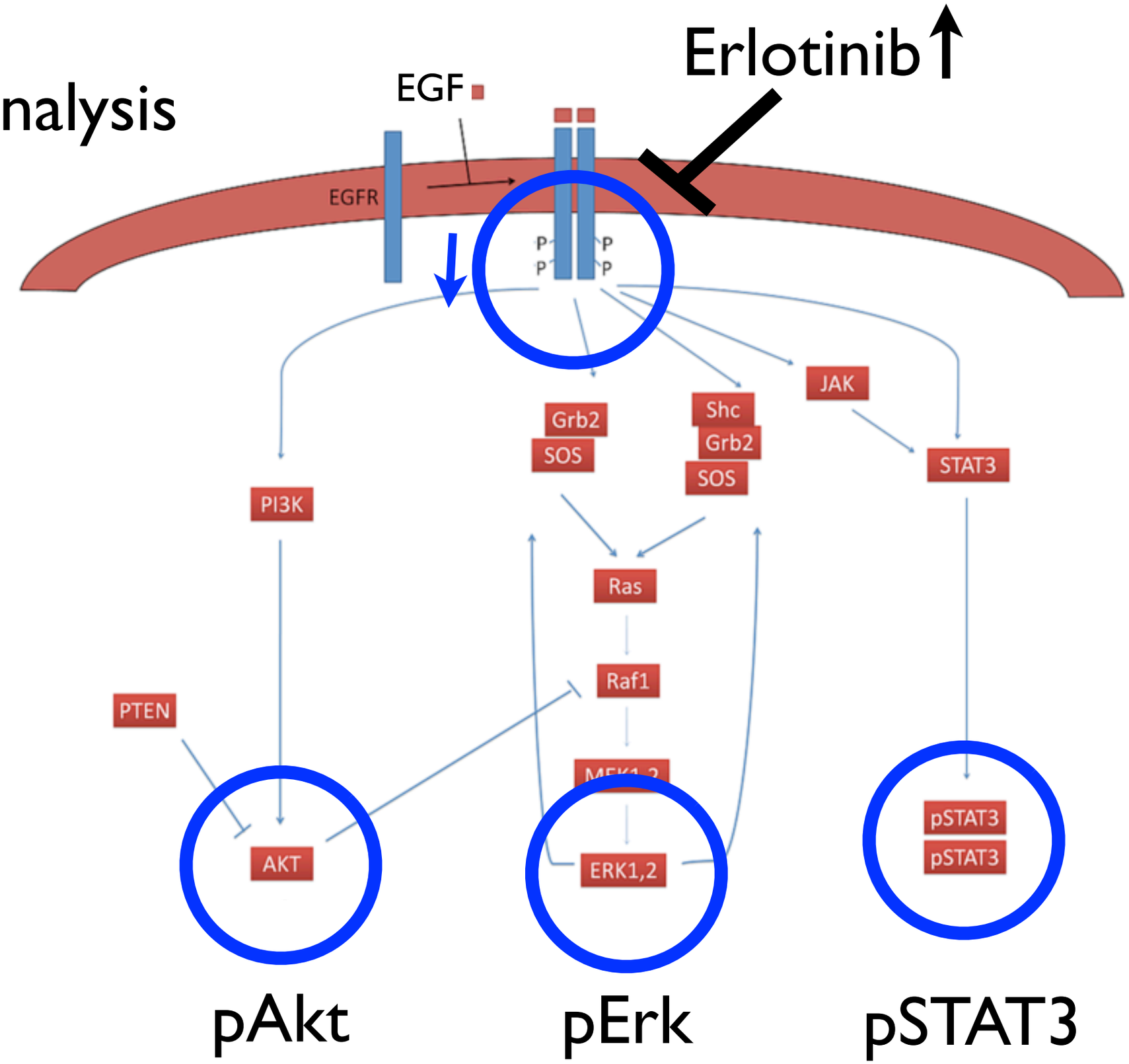
Bidkhori G, Moeini A, Masoudi-Nejad A (2012) Modeling of Tumor Progression in NSCLC and Intrinsic Resistance to TKI in Loss of PTEN Expression. PLoS ONE 7(10): e48004

What other information might we need?

Low throughput analysis

"Easy" with only 1-2 conditions.

How to measure?

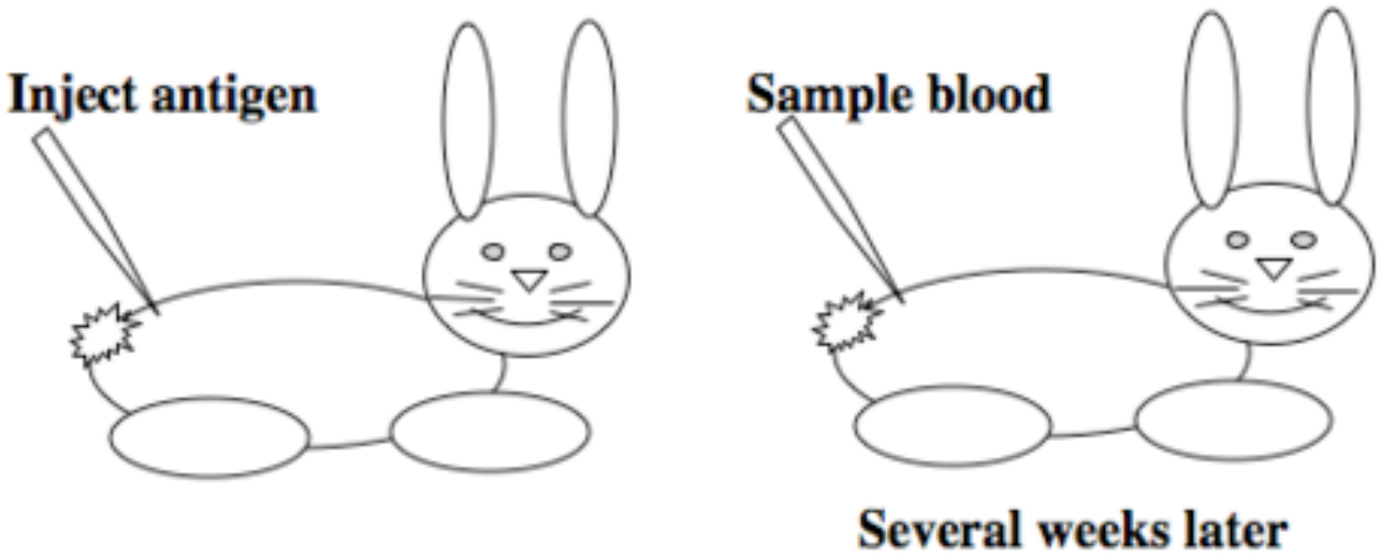


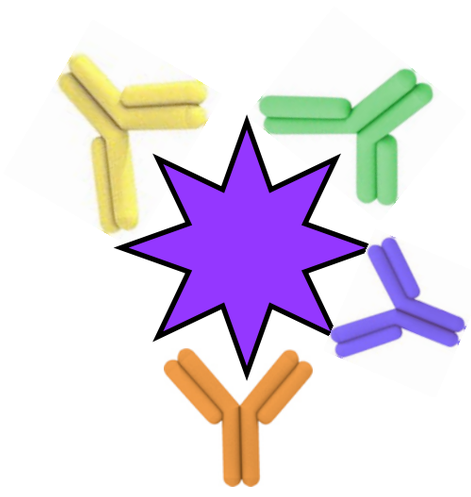
Bidkhori G, Moeini A, Masoudi-Nejad A (2012) Modeling of Tumor Progression in NSCLC and Intrinsic Resistance to TKI in Loss of PTEN Expression. PLoS ONE 7(10): e48004

Antibody-based methods for phosphorylation detection: WB, ELISA, Luminex

How do you make antibodies?

Polyclonal Antibodies



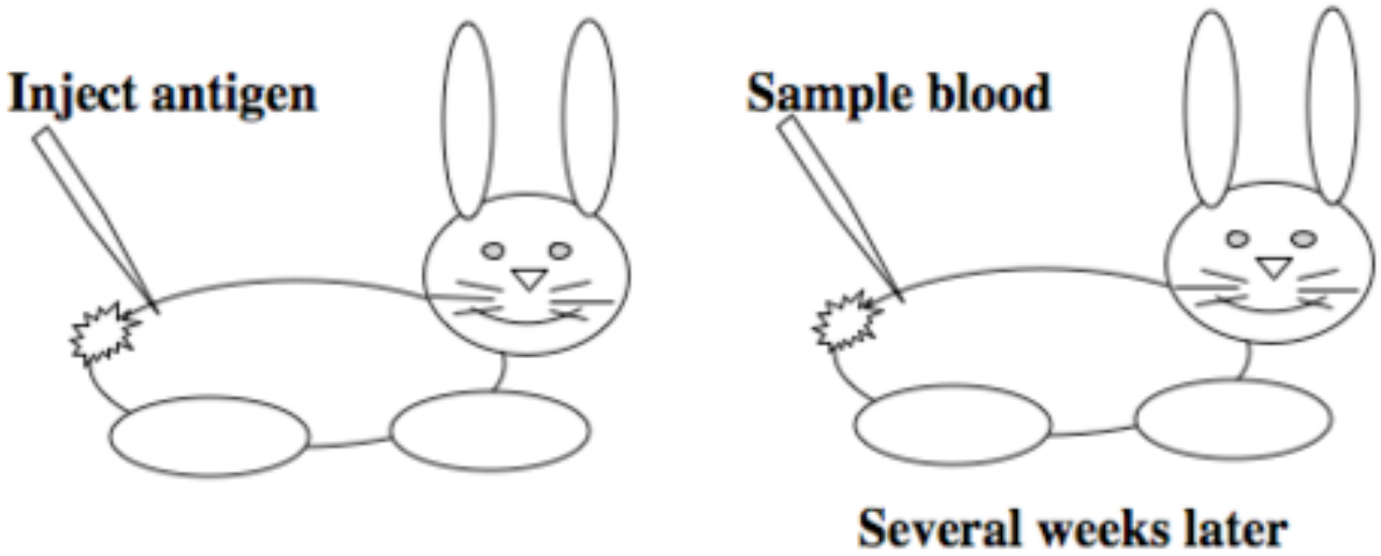


Many antibodies against your target.

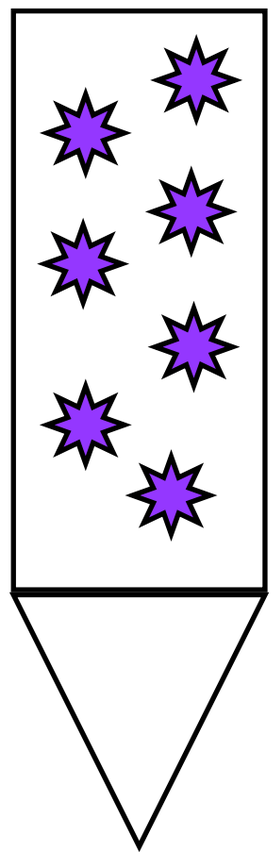
Antibody-based methods for phosphorylation detection: WB, ELISA, Luminex

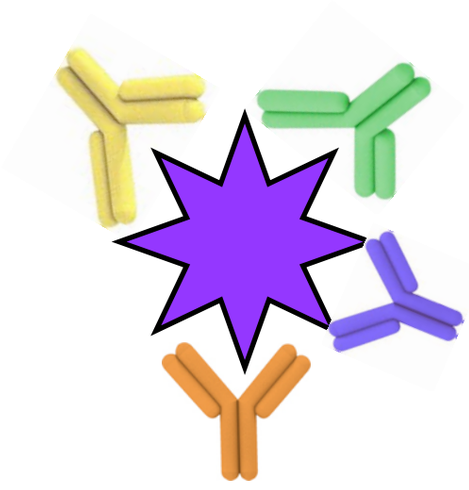
How do you make antibodies?

Polyclonal Antibodies



+ purification



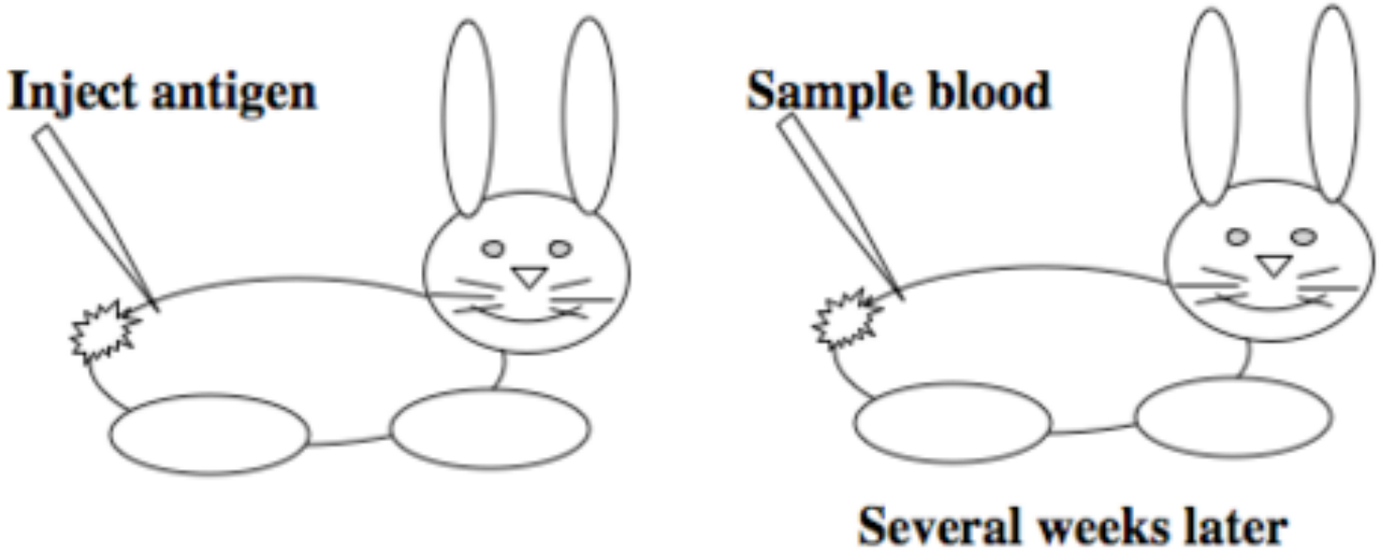


Many antibodies against your target.

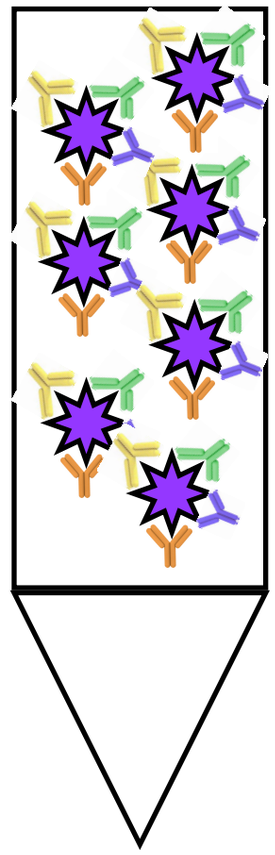
Antibody-based methods for phosphorylation detection: WB, ELISA, Luminex

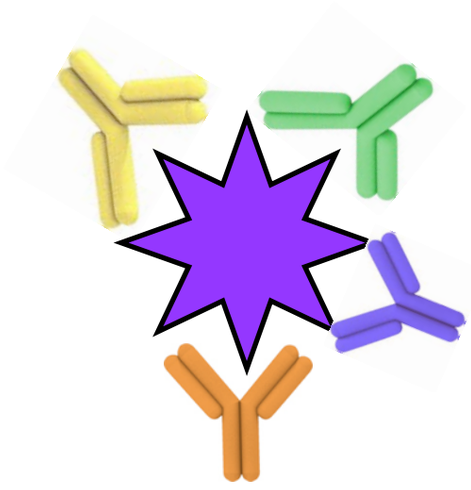
How do you make antibodies?

Polyclonal Antibodies



+ purification



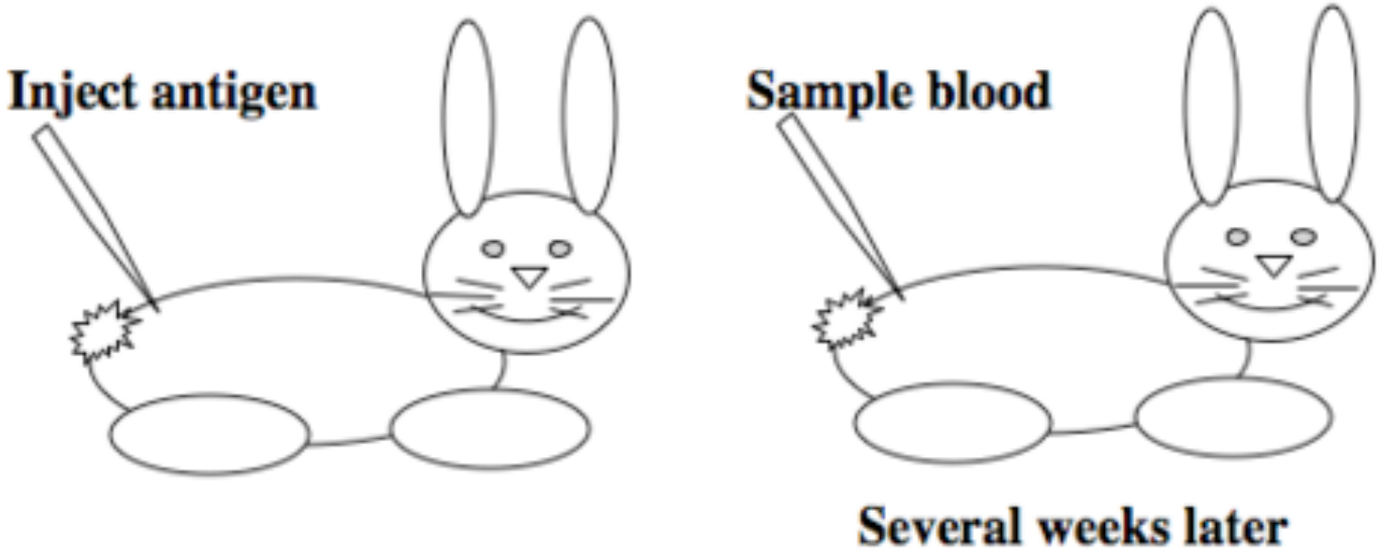


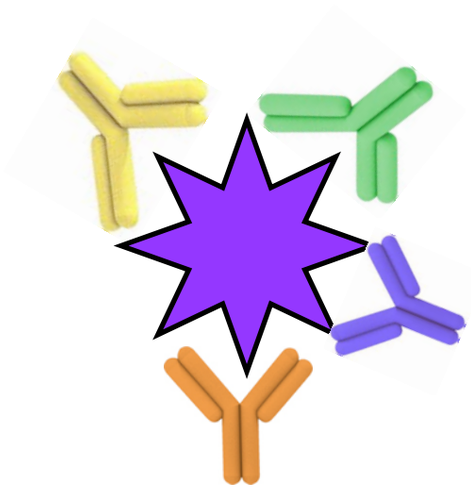
Many antibodies against your target.

Antibody-based methods for phosphorylation detection: WB, ELISA, Luminex

How do you make antibodies?

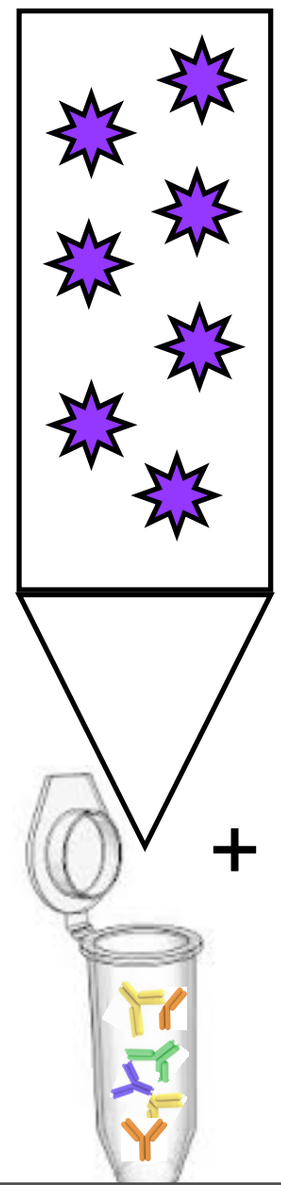
Polyclonal Antibodies





Many antibodies against your target.

+ purification



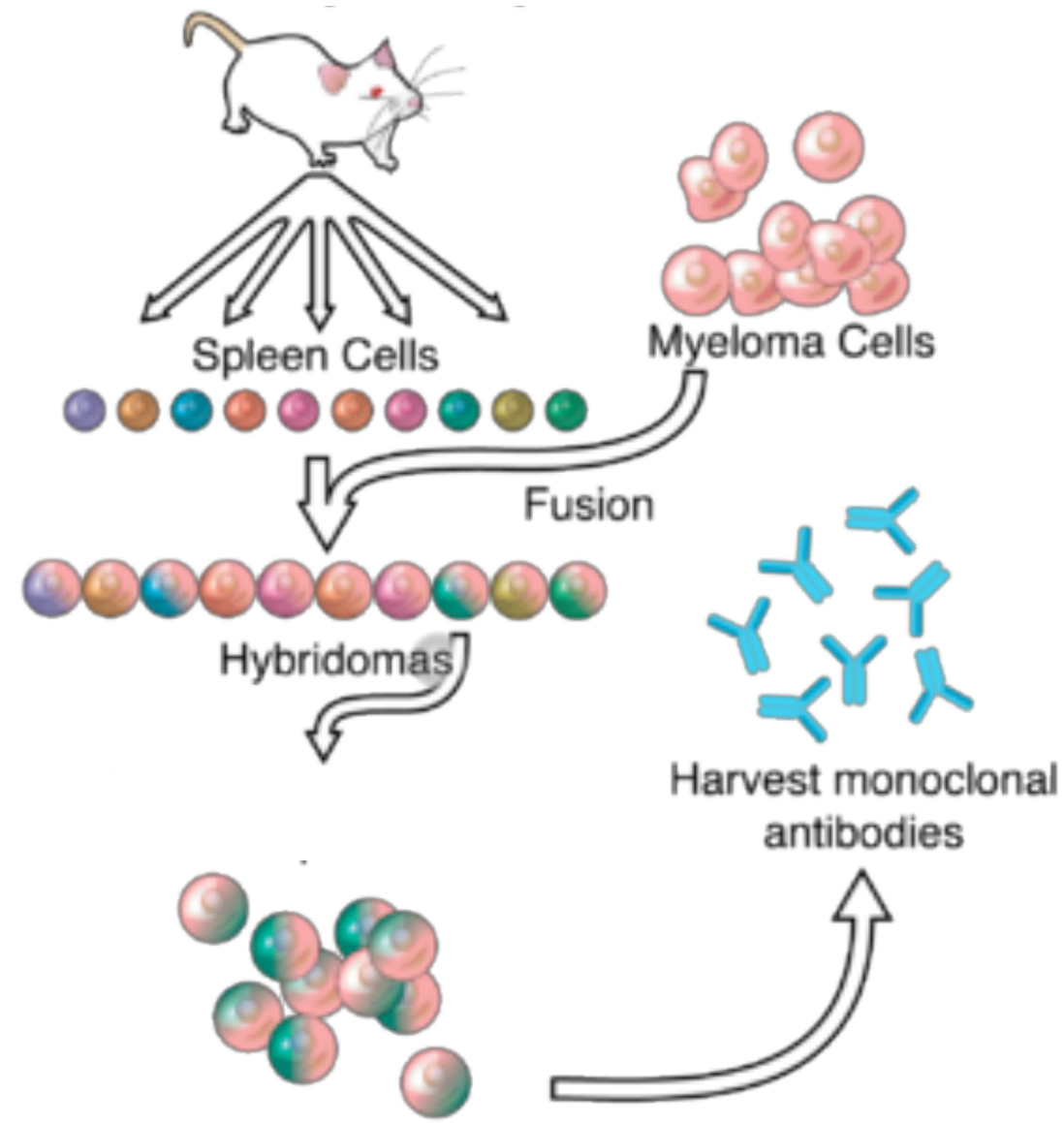
+ High Salt

Antibody-based methods for phosphorylation detection: WB, ELISA, Luminex

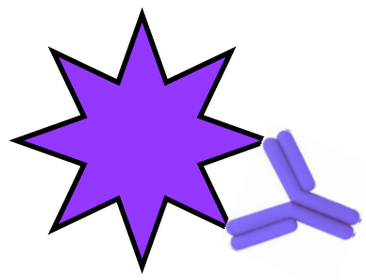
How do you make antibodies?

Monoclonal Antibodies

Mouse injected with antigen



One antibody against your target



Modified from Wikipedia Commons

Polyclonal vs. Monoclonal Antibodies

*cost to make your own

Polyclonal vs. Monoclonal Antibodies

Polyclonal	Monoclonal

*cost to make your own

Polyclonal vs. Monoclonal Antibodies

Polyclonal	Monoclonal
Cheap(er) ~ $1500*	Expensive ~$7,000*

*cost to make your own

Polyclonal vs. Monoclonal Antibodies

Polyclonal	Monoclonal
Cheap(er) ~ $1500*	Expensive ~$7,000*
Limited supply	Unlimited supply -- grow your own

*cost to make your own

Polyclonal vs. Monoclonal Antibodies

Polyclonal	Monoclonal
Cheap(er) ~ $1500*	Expensive ~$7,000*
Limited supply	Unlimited supply -- grow your own
Easy to purify (need antigen)	Easier to purify (need protein A)

*cost to make your own

Polyclonal vs. Monoclonal Antibodies

Polyclonal	Monoclonal
Cheap(er) ~ $1500*	Expensive ~$7,000*
Limited supply	Unlimited supply -- grow your own
Easy to purify (need antigen)	Easier to purify (need protein A)
Use to amplify signal	Often 1:1

*cost to make your own

Polyclonal vs. Monoclonal Antibodies

Polyclonal	Monoclonal
Cheap(er) ~ $1500*	Expensive ~$7,000*
Limited supply	Unlimited supply -- grow your own
Easy to purify (need antigen)	Easier to purify (need protein A)
Use to amplify signal	Often 1:1
Recognizes denatured protein	Recognize secondary structure

*cost to make your own

Polyclonal vs. Monoclonal Antibodies

Polyclonal	Monoclonal
Cheap(er) ~ $1500*	Expensive ~$7,000*
Limited supply	Unlimited supply -- grow your own
Easy to purify (need antigen)	Easier to purify (need protein A)
Use to amplify signal	Often 1:1
Recognizes denatured protein	Recognize secondary structure
Variable across animals	Consistent results

*cost to make your own

Polyclonal vs. Monoclonal Antibodies

Polyclonal	Monoclonal
Cheap(er) ~ $1500*	Expensive ~$7,000*
Limited supply	Unlimited supply -- grow your own
Easy to purify (need antigen)	Easier to purify (need protein A)
Use to amplify signal	Often 1:1
Recognizes denatured protein	Recognize secondary structure
Variable across animals	Consistent results
Some non-specific binding	Less non-specific binding

*cost to make your own

Polyclonal vs. Monoclonal Antibodies

Polyclonal	Monoclonal

*cost to make your own

So much data. Mistakes can be made!

http://www.cbsnews.com/video/watch/?id=7398476n

Retraction Watch

Tracking retractions as a window into the scientific process

The Anil Potti retraction record so far

with 16 comments

A 60 Minutes segment Sunday on Anil Potti has drawn national attention to the case, so we thought this would be a good time to compile all of the retractions and corrections in one place.

Duke has said that about a third of Potti's 40-some-odd papers would be retracted, and another third would have "a portion retracted with other components remaining intact," so this list will continue to grow. We'll update it as we hear about new changes.

Retractions:

1. "Gene-expression patterns predict phenotypes of immune-mediated thrombosis," in *Blood*
2. "Upregulated Oncogenic Pathways in Patients Exposed to Tobacco Smoke May Provide a Novel Approach to Lung Cancer Chemoprevention," in *CHEST*
3. "Characterizing the Clinical Relevance of an Embryonic Stem Cell Phenotype in Lung Adenocarcinoma," in *Clinical Cancer Research*
4. "An Integrated Genomic-Based Approach to Individualized Treatment of Patients With Advanced-Stage Ovarian Cancer" in the *Journal of Clinical Oncology* (JCO)
5. "Pharmacogenomic Strategies Provide a Rational Approach to the Treatment of Cisplatin-Resistant Patients With Advanced Cancer" also in the JCO
6. "Gene Expression Signatures, Clinicopathological Features, and Individualized Therapy in Breast Cancer" in the *Journal of the American Medical Association* (JAMA)
7. "Validation of gene signatures that predict the response of breast cancer to neoadjuvant chemotherapy: a substudy of the EORTC 10994/BIG 00-01 clinical trial," in *The Lancet Oncology*
8. "Genomic signatures to guide the use of chemotherapeutics," in *Nature Medicine*
9. "A Genomic Strategy to Refine Prognosis in Early-Stage Non-Small-Cell Lung Cancer," in the *New England Journal of Medicine* (NEJM)
10. "An Integrated Approach to the Prediction of Chemotherapeutic Response in Patients with Breast Cancer" in *PLoS ONE*
11. "A genomic approach to colon cancer risk stratification yields biologic insights into therapeutic opportunities" in the *Proceedings of the National Academy of Sciences* (PNAS)

Corrections:

1. "An integration of complementary strategies for gene-expression analysis to reveal novel therapeutic opportunities for breast cancer," in *Breast Cancer Research*
2. "Gene Expression Profiles of Tumor Biology Provide a Novel Approach to Prognosis and May Guide the Selection of Therapeutic Targets in Multiple Myeloma," in the JCO
3. "Age-Specific Differences in Oncogenic Pathway Dysregulation and Anthracycline Sensitivity in Patients With Acute Myeloid Leukemia," in the JCO

Pages

About Adam Marcus
About Ivan Oransky
The Retraction Watch FAQ, including comments policy
The Retraction Watch Store
The Retraction Watch Transparency Index
Upcoming Retraction Watch appearances
What people are saying about Retraction Watch

Search
RSS - Posts
RSS - Comments

Email Subscription

Enter your email address to subscribe to this blog and receive notifications of new posts by email.
Join 5,605 other followers

Sign me up!

Recent Comments

HC on Marc Hauser corrects a tiny pa...
david hardman on Italian cancer specialist faci...
pyshnov on Alirio Melendez categorically...

Retraction posts by author, country, journal, subject, and type

Select Category

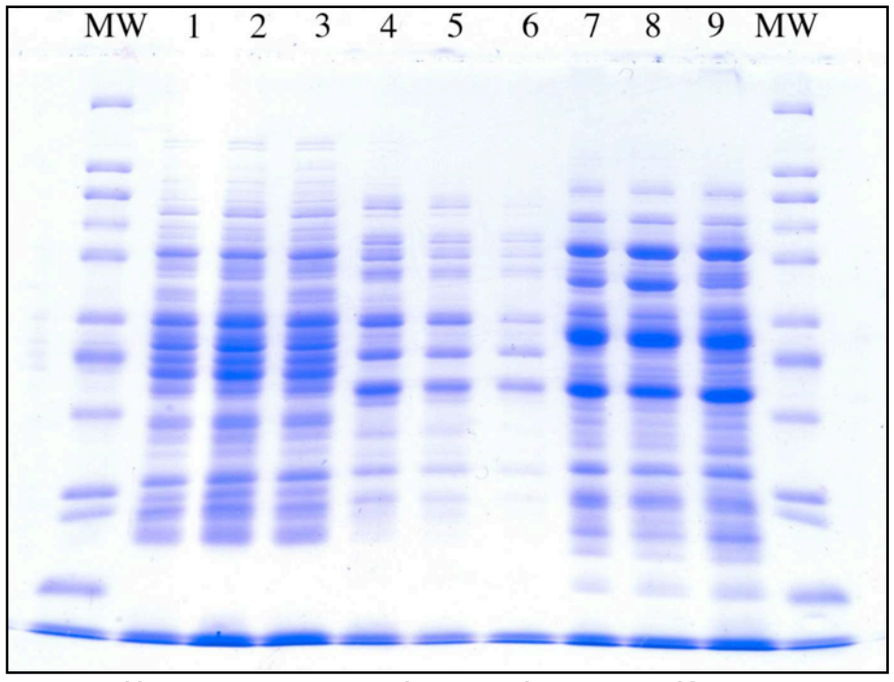
Follow Ivan on Twitter

is EWR>CHO to speak to @bobbiepops' class and

Semi-quantitative analysis: Western blot

1. Lyse cells

SDS-PAGE with whole cell lysate

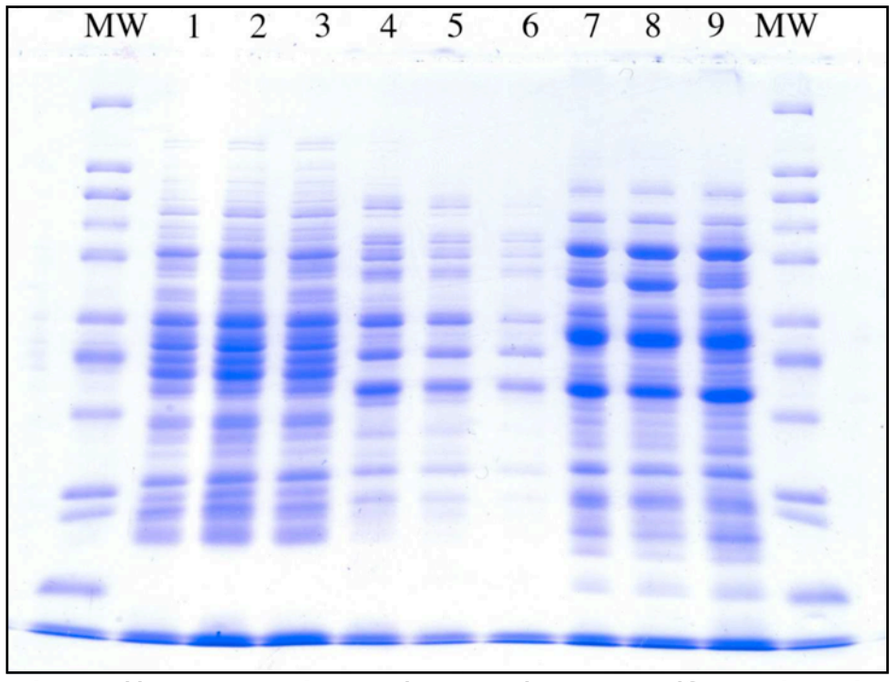


http://www.unibas.it/utenti/parente/fuzzy.html

Semi-quantitative analysis: Western blot

1. Lyse cells
2. Separate proteins using SDS-PAGE

SDS-PAGE with whole cell lysate

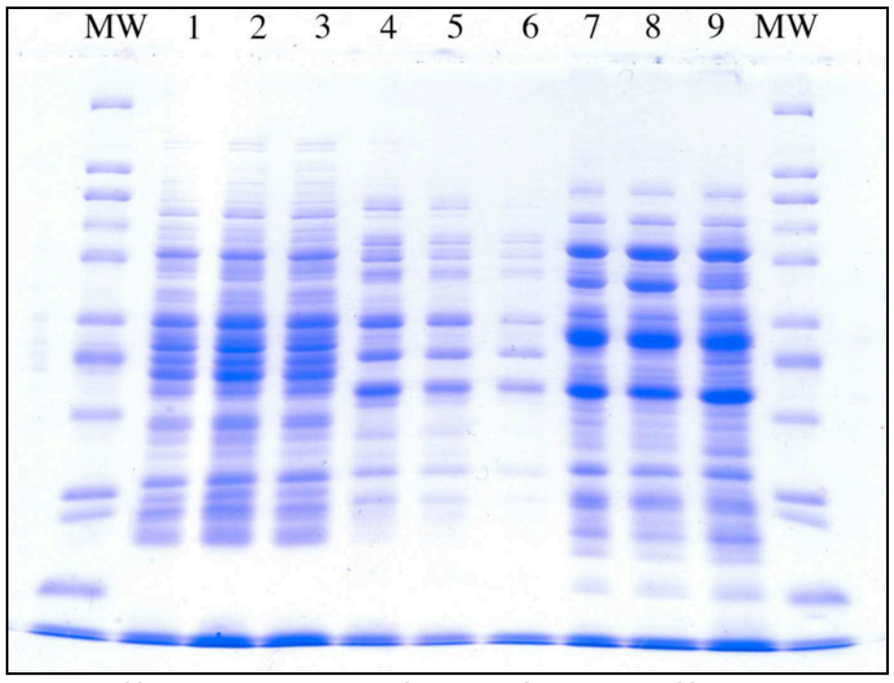


http://www.unibas.it/utenti/parente/fuzzy.html

Semi-quantitative analysis: Western blot

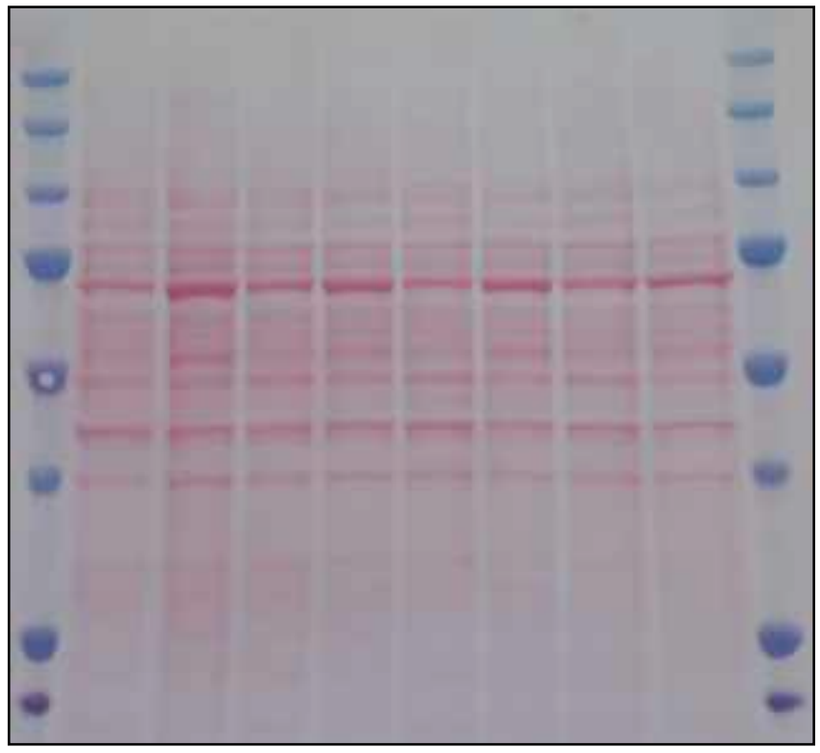
1. Lyse cells
2. Separate proteins using SDS-PAGE
3. Transfer proteins to a membrane (nitrocellulose)

SDS-PAGE with whole cell lysate



http://www.unibas.it/utenti/parente/fuzzy.html

Membrane with whole cell lysate

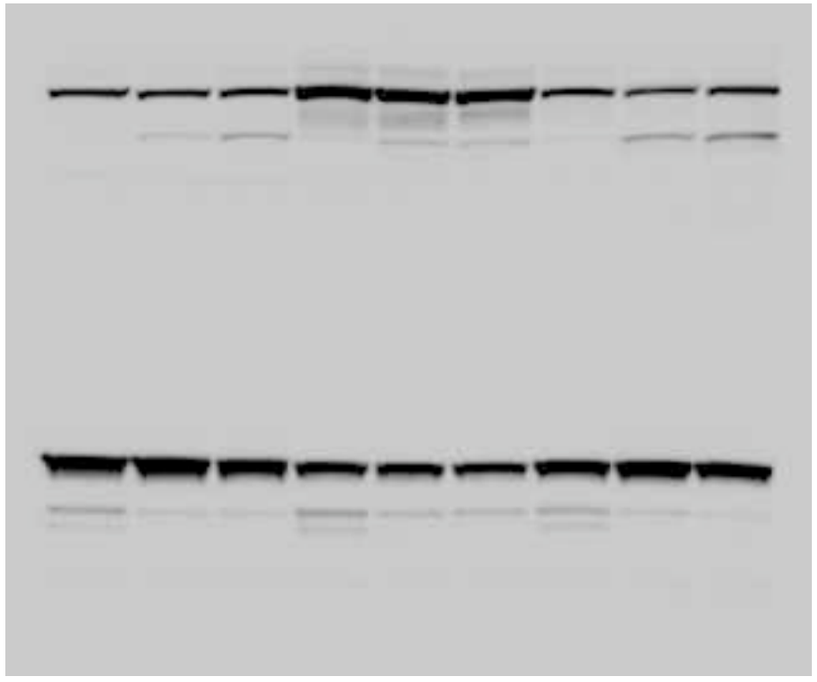


http://www.hollsberglab.dk/Methods/WB.php

Semi-quantitative analysis: Western blot

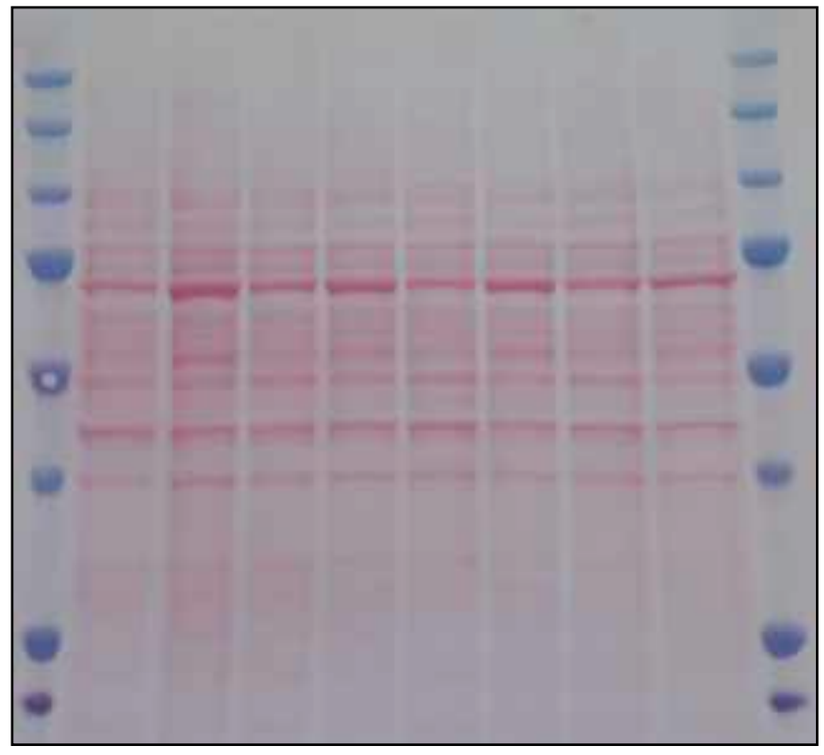
1. Lyse cells
2. Separate proteins using SDS-PAGE
3. Transfer proteins to a membrane (nitrocellulose)
4. Block all sites not containing protein
5. Probe with antibodies of interest

Membrane probed with antibodies



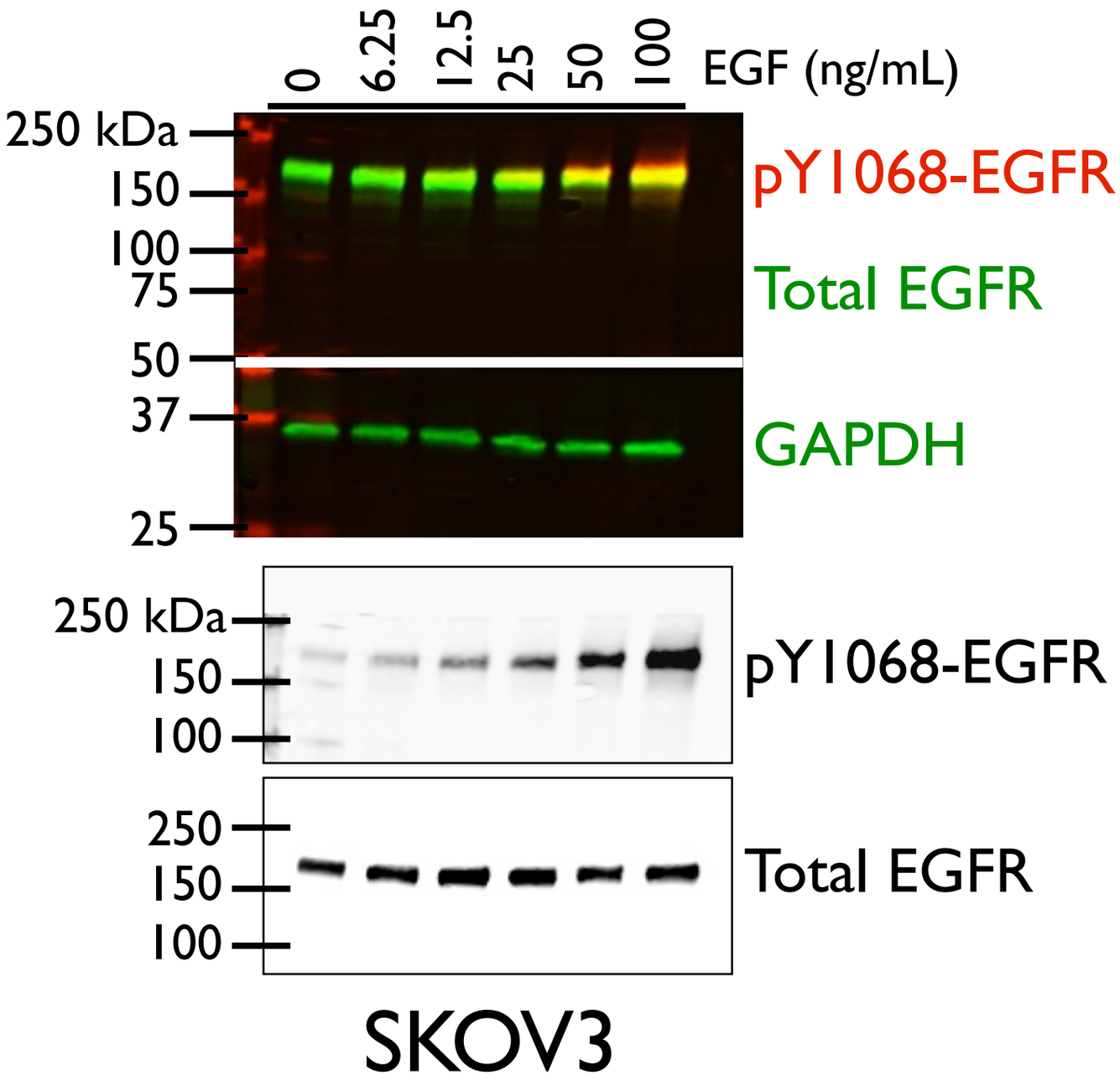
http://www.hollsberglab.dk/Methods/WB.php

Membrane with whole cell lysate



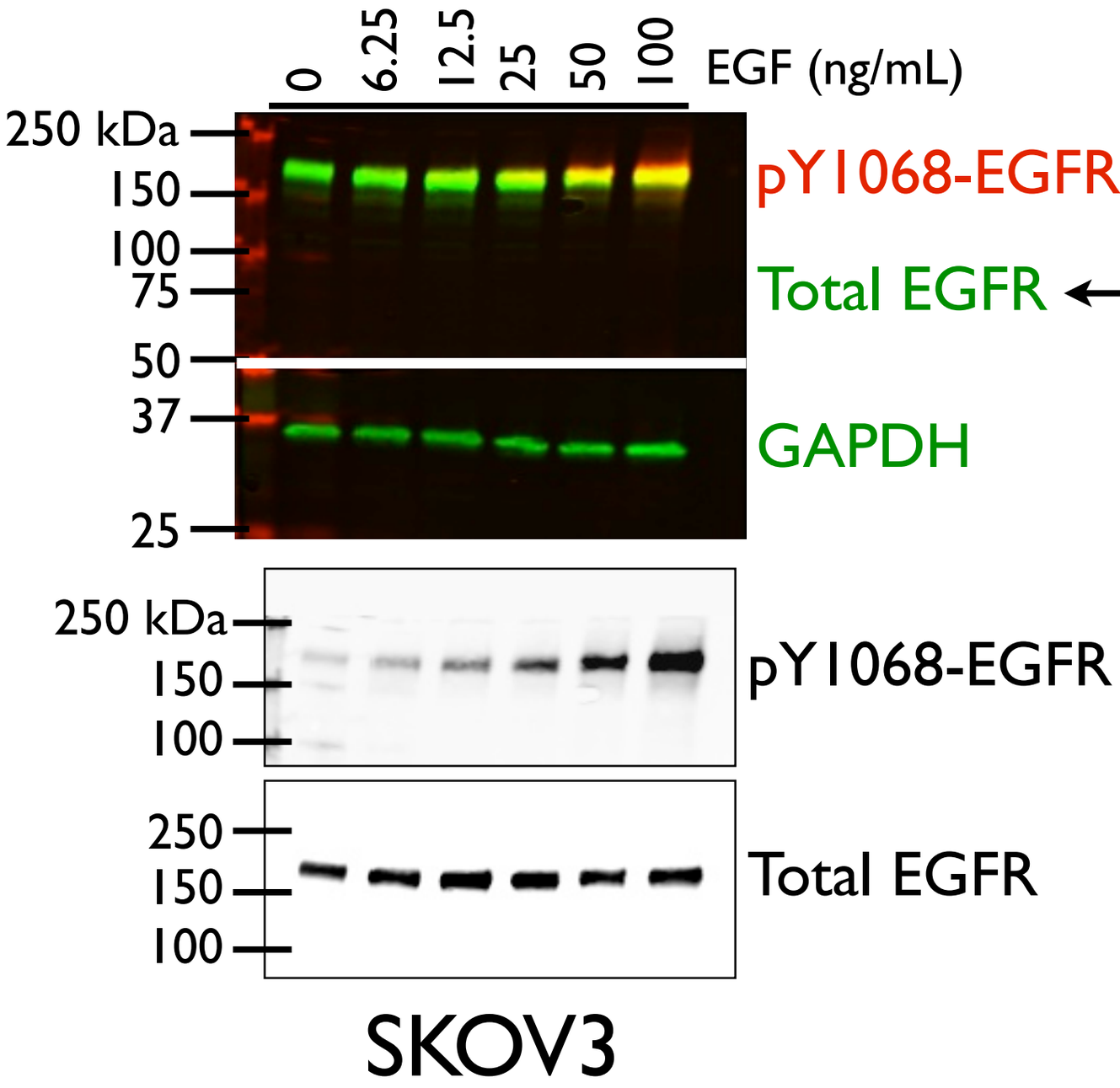
http://www.hollsberglab.dk/Methods/WB.php

Semi-quantitative analysis: Western blot



A few signals per lane -- up to ~17 lanes per mini gel.

Semi-quantitative analysis: Western blot



pY1068-EGFR ← Rabbit Ab

Total EGFR ← Goat Ab

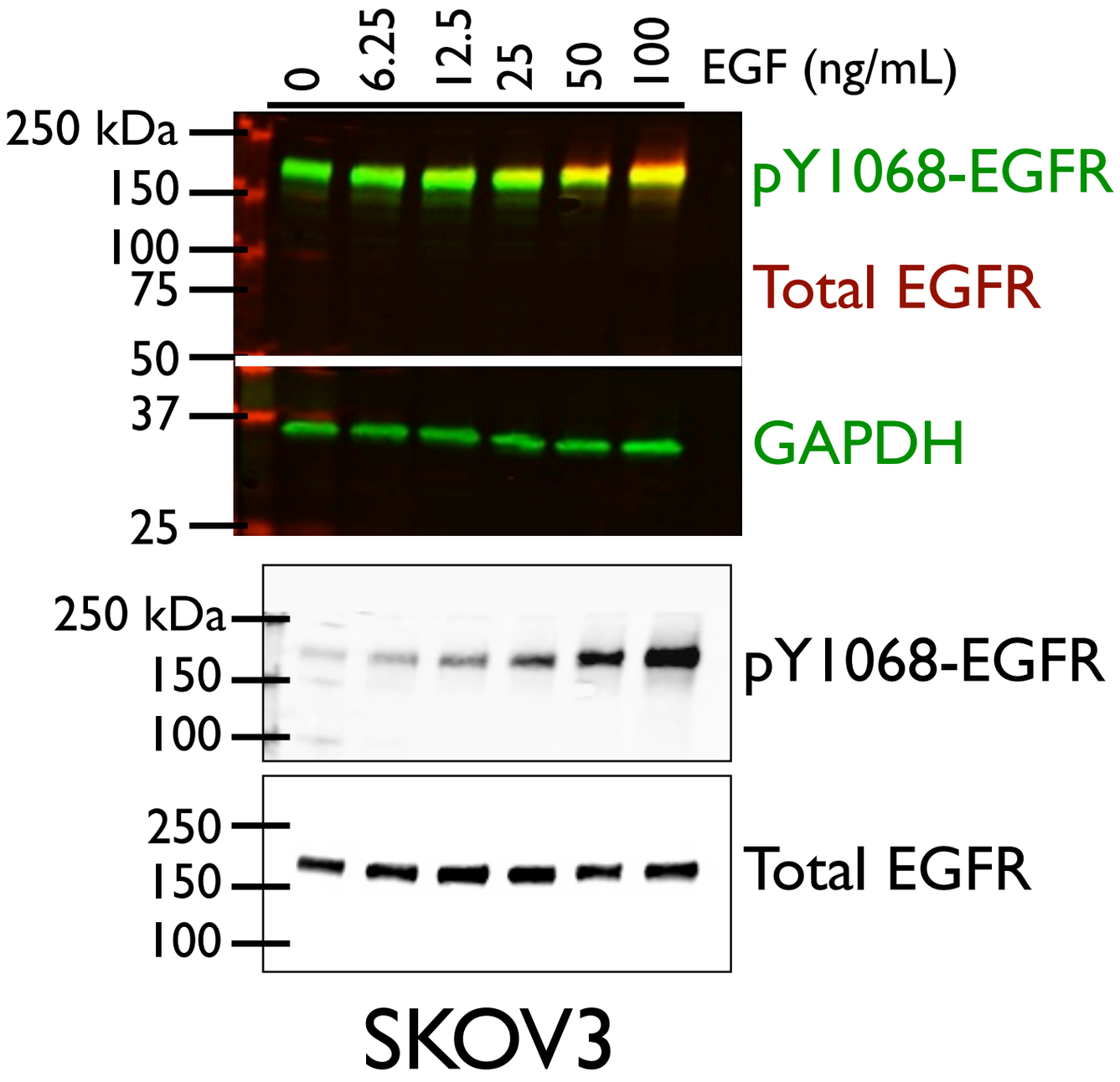
Infrared Secondary Abs:

anti-Rabbit IR680

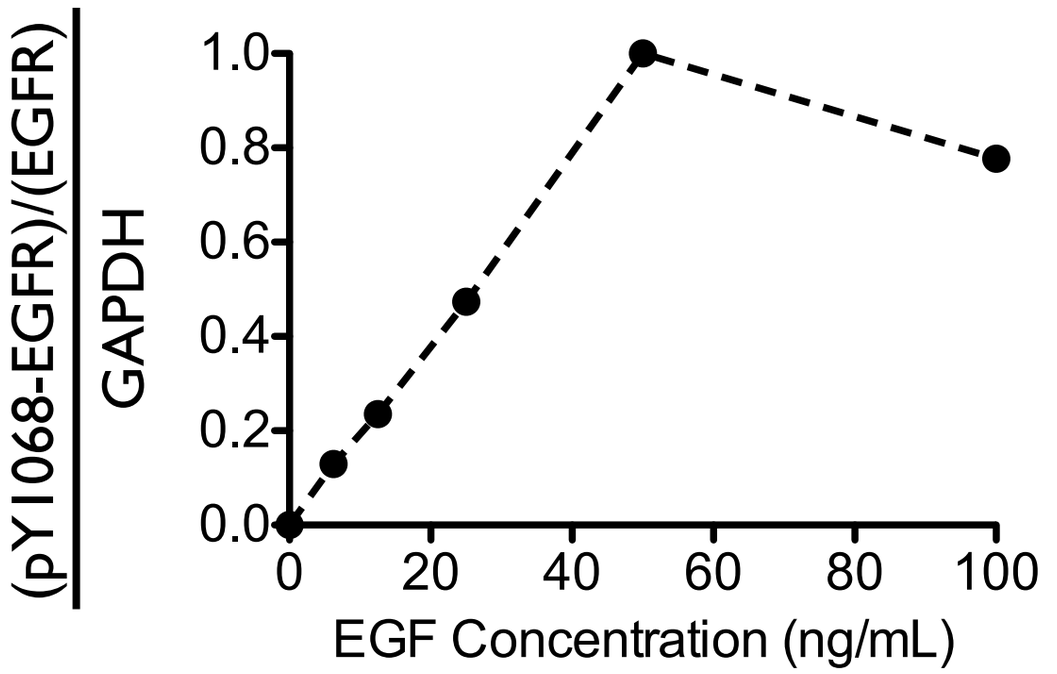
anti-Goat IR800

A few signals per lane -- up to ~17 lanes per mini gel.

Semi-quantitative analysis: Western blot



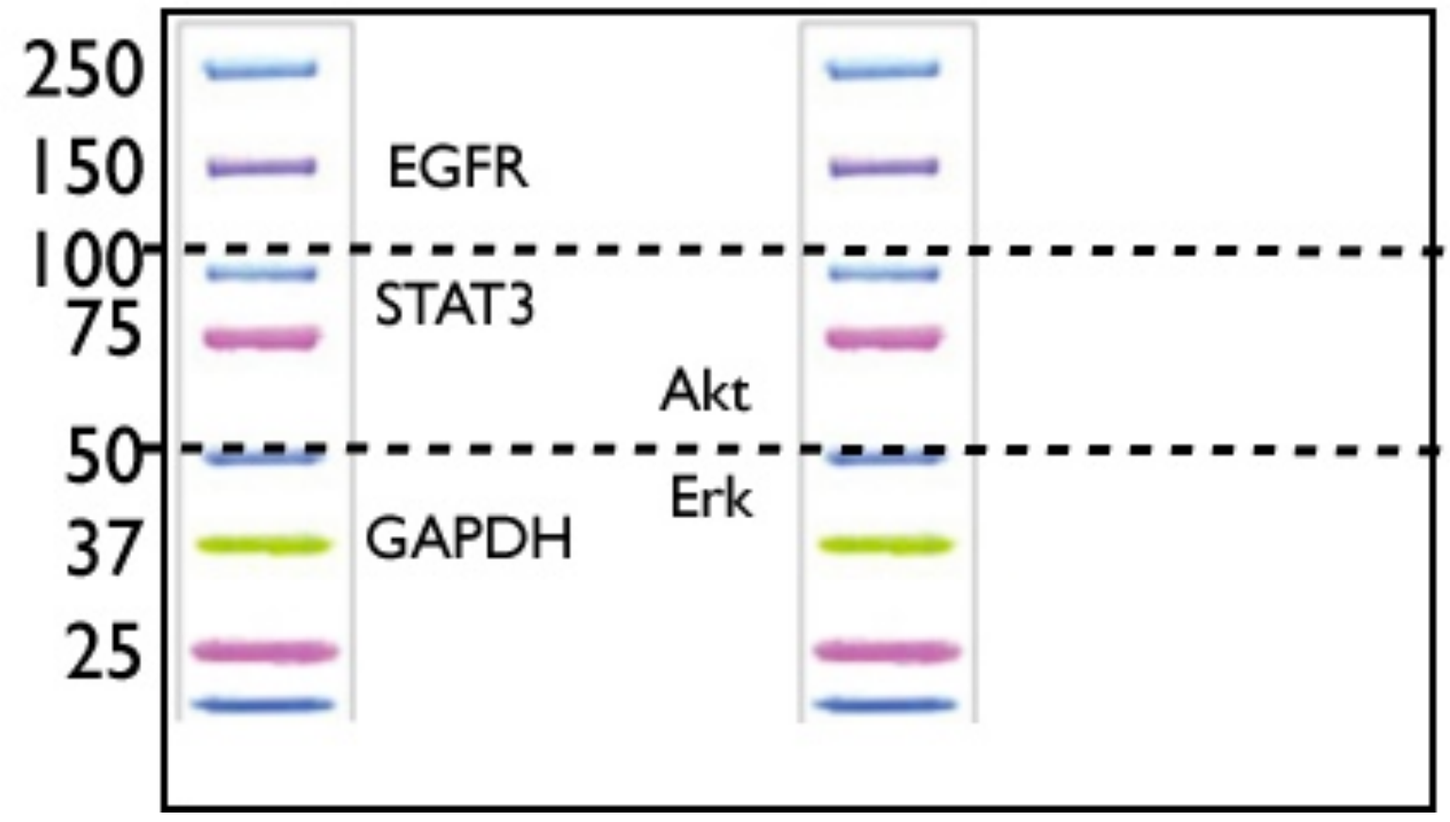
Densitometric Analysis



A few signals per lane -- up to ~17 lanes per mini gel.

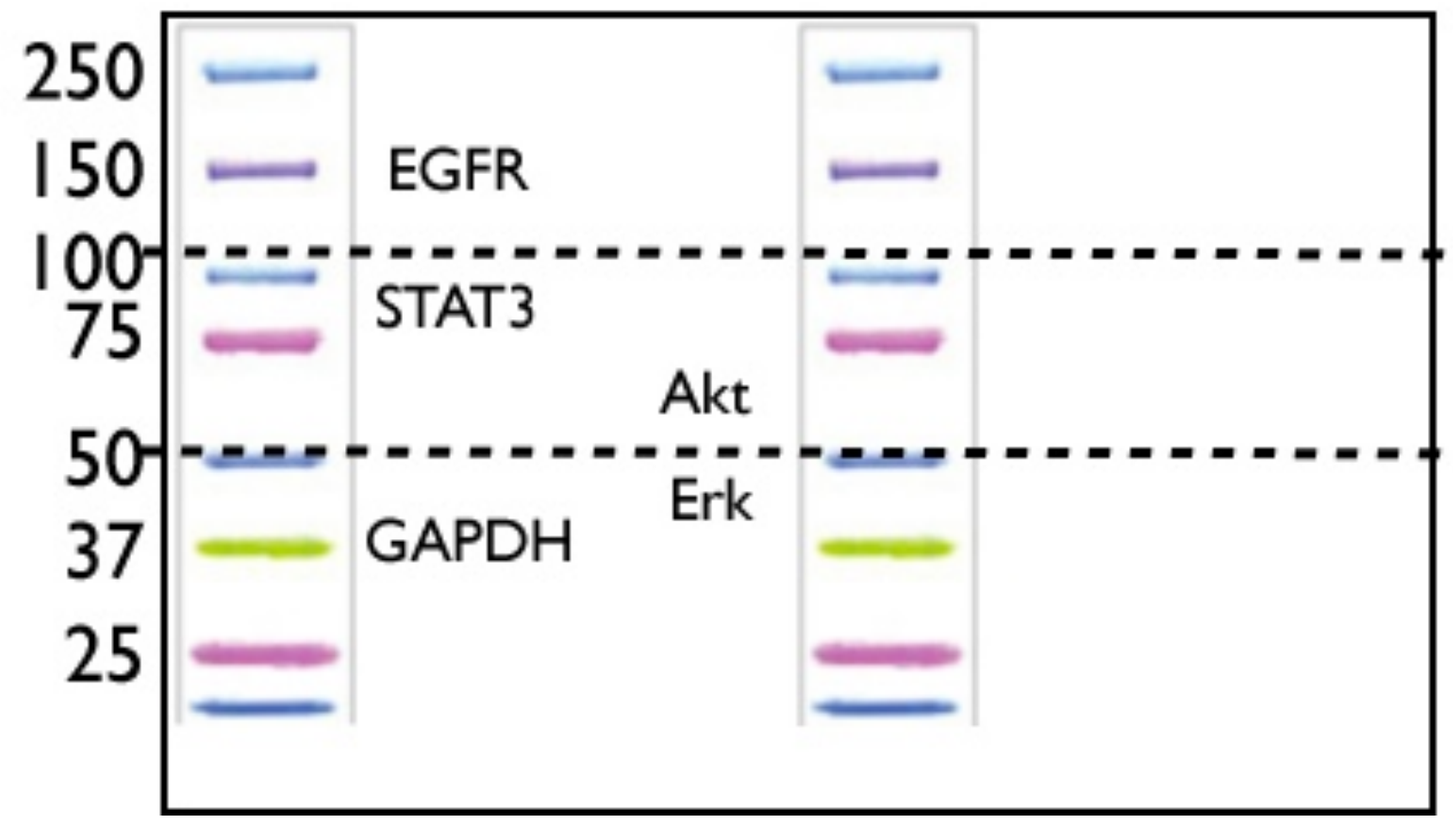
Semi-quantitative analysis: Your experiment

1. Stimulate cells with 8.4 nM (50 ng/mL) EGF ± Erlotinib



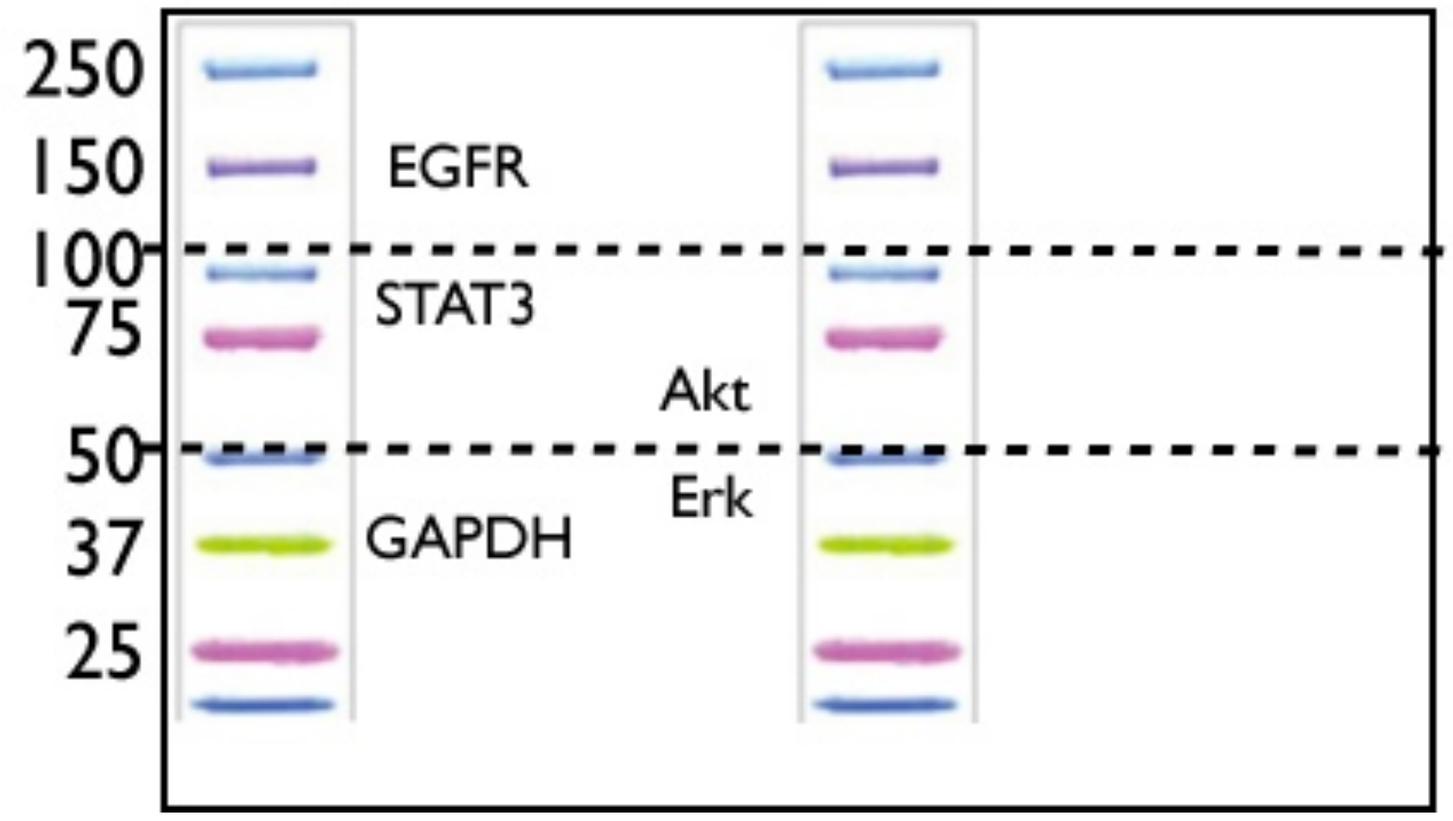
Semi-quantitative analysis: Your experiment

1. Stimulate cells with 8.4 nM (50 ng/mL) EGF ± Erlotinib
2. Lyse Cells -- KEEP EVERYTHING COLD



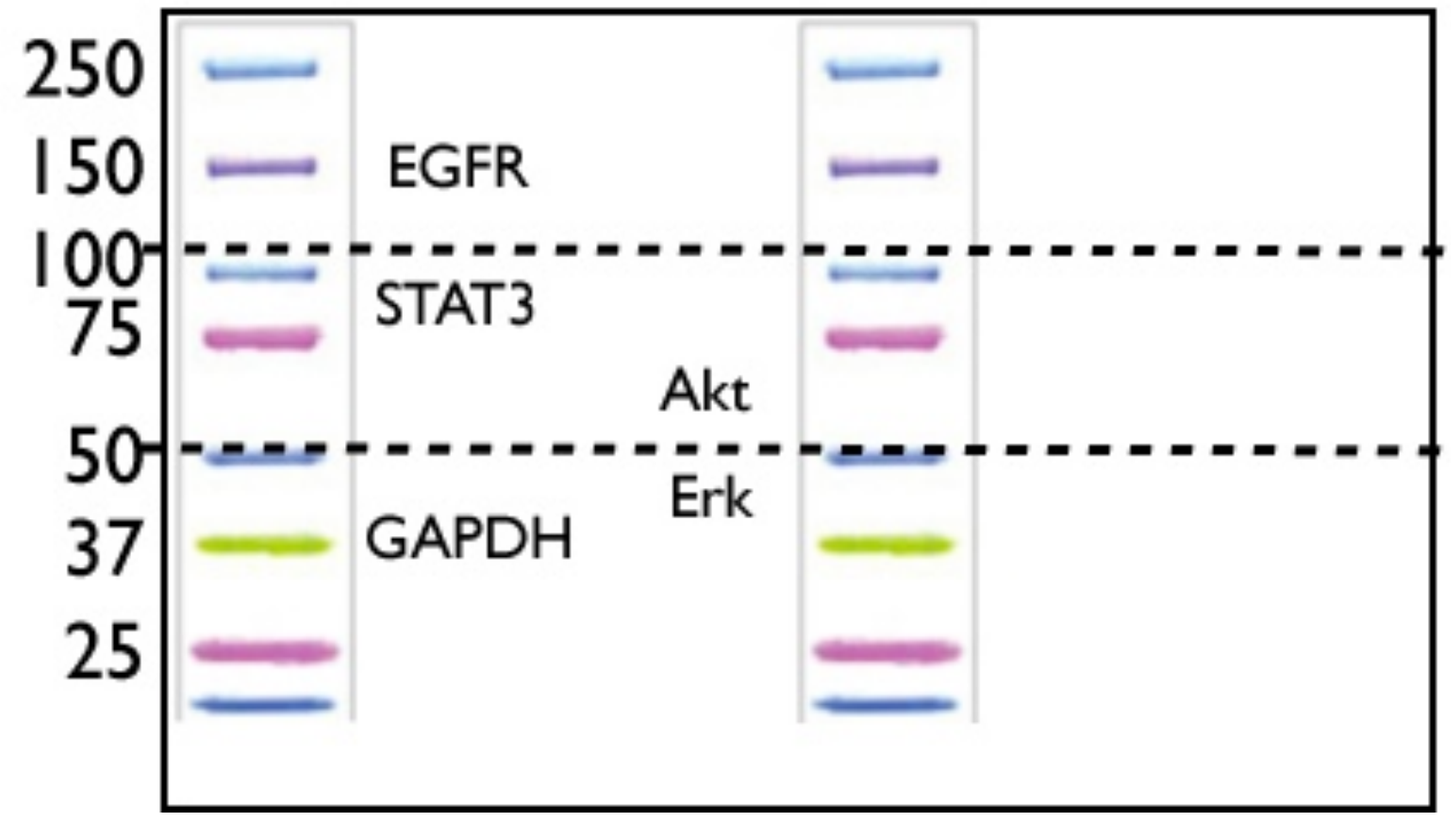
Semi-quantitative analysis: Your experiment

1. Stimulate cells with 8.4 nM (50 ng/mL) EGF ± Erlotinib
2. Lyse Cells -- KEEP EVERYTHING COLD
3. Measure protein concentration



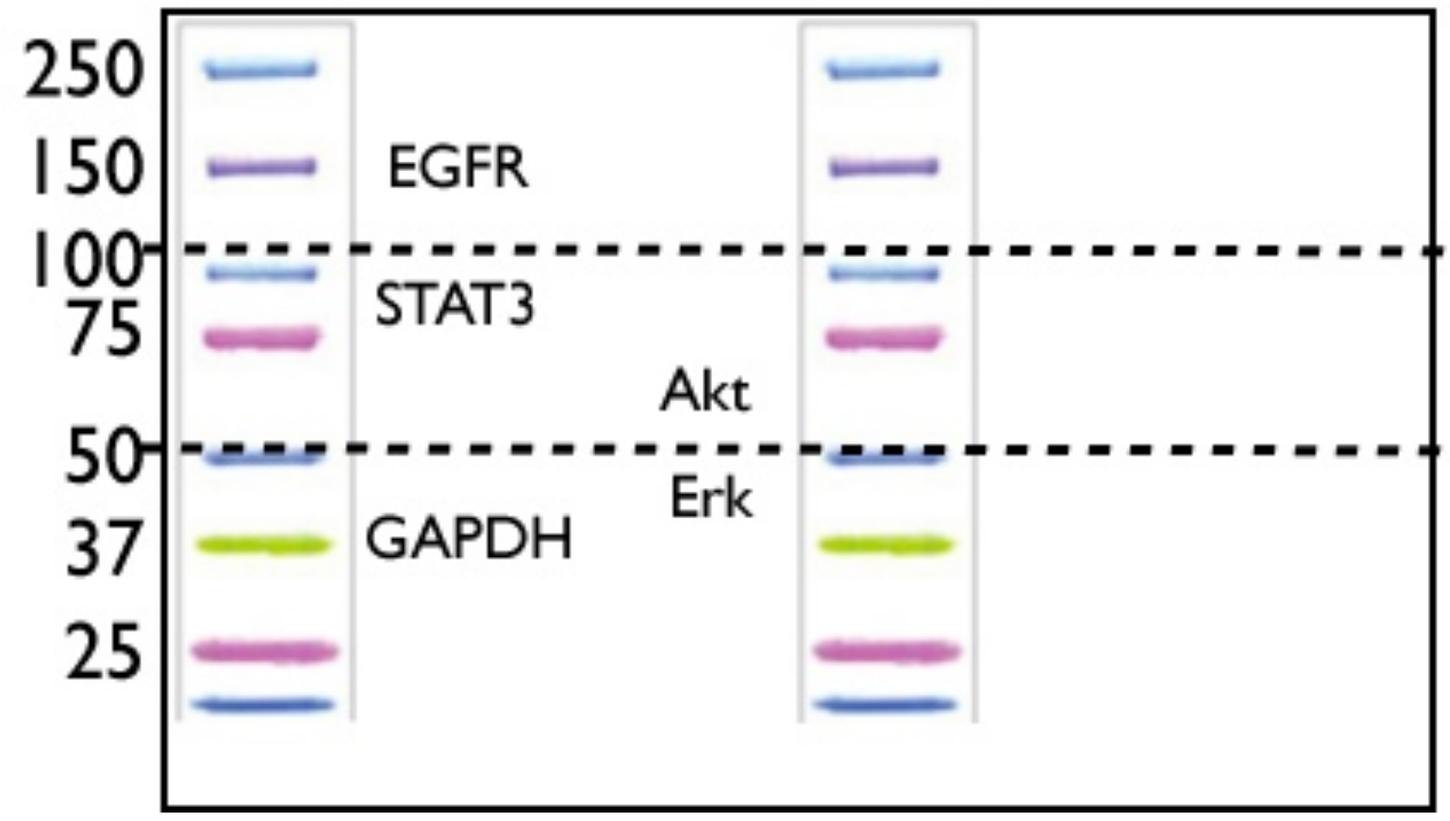
Semi-quantitative analysis: Your experiment

1. Stimulate cells with 8.4 nM (50 ng/mL) EGF ± Erlotinib
2. Lyse Cells -- KEEP EVERYTHING COLD
3. Measure protein concentration
4. SDS-PAGE



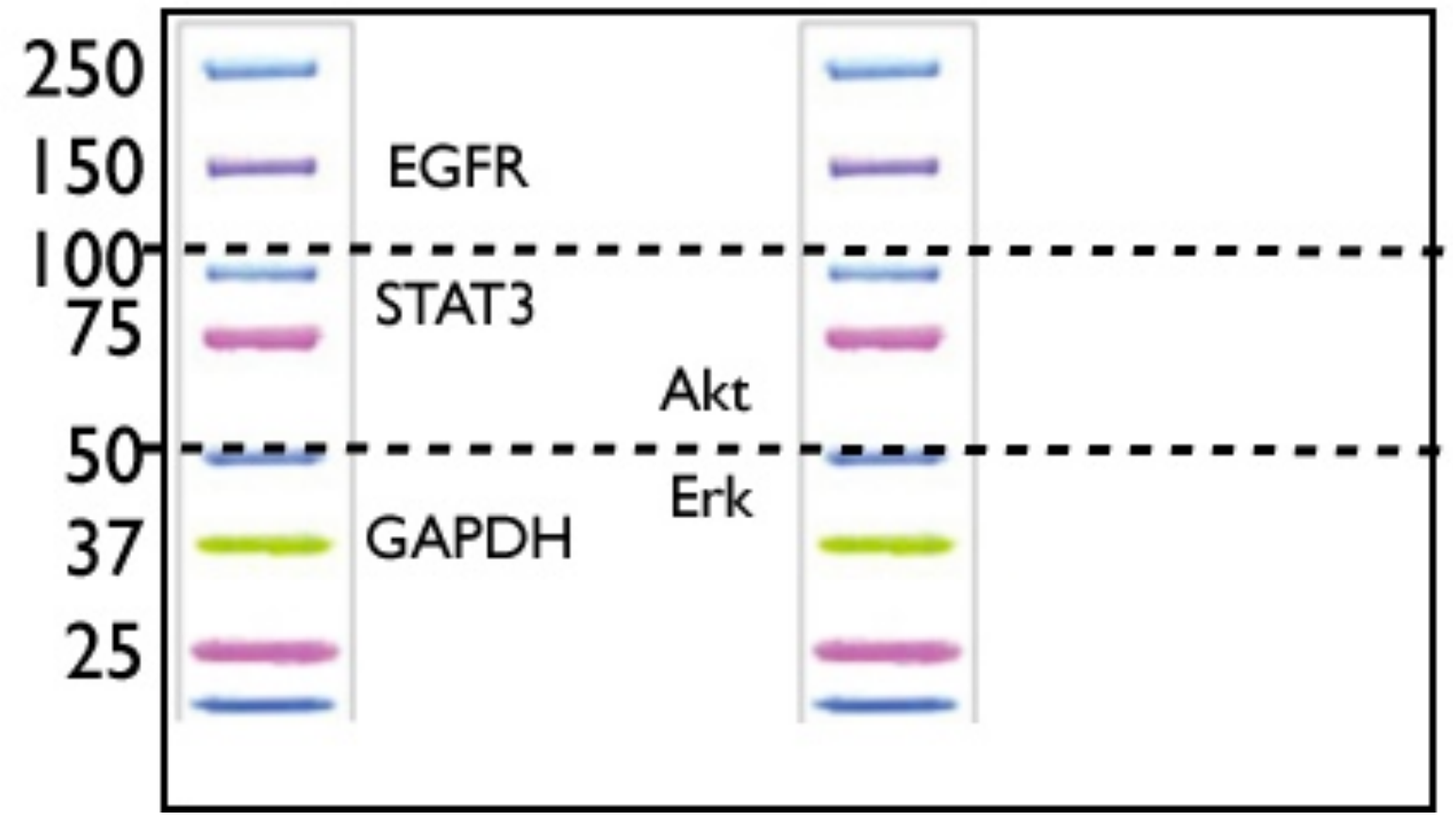
Semi-quantitative analysis: Your experiment

1. Stimulate cells with 8.4 nM (50 ng/mL) EGF ± Erlotinib
2. Lyse Cells -- KEEP EVERYTHING COLD
3. Measure protein concentration
4. SDS-PAGE
5. Western blot



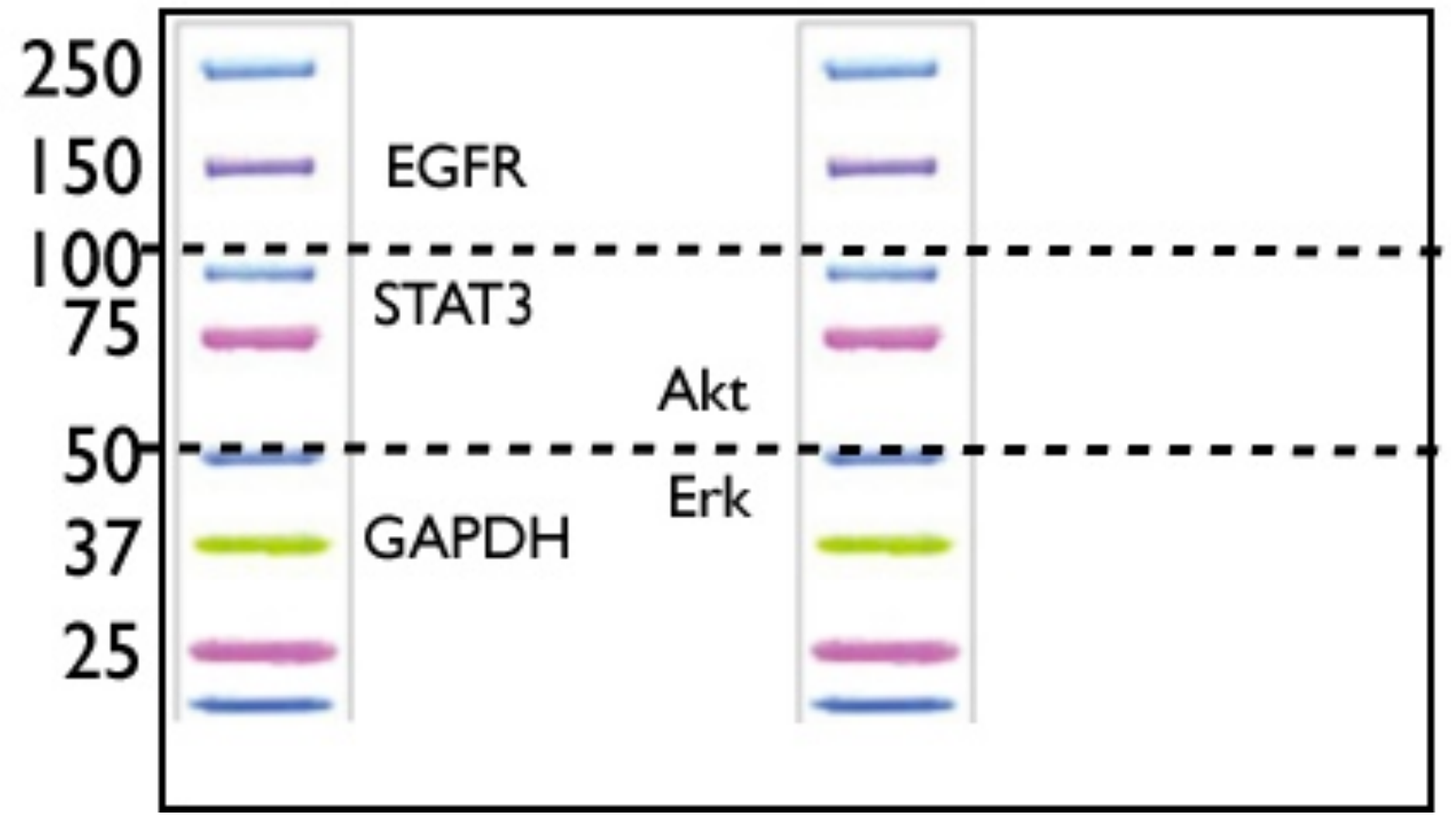
Semi-quantitative analysis: Your experiment

1. Stimulate cells with 8.4 nM (50 ng/mL) EGF ± Erlotinib
2. Lyse Cells -- KEEP EVERYTHING COLD
3. Measure protein concentration
4. SDS-PAGE
5. Western blot
6. All: pY1068-EGFR & Total EGFR



Semi-quantitative analysis: Your experiment

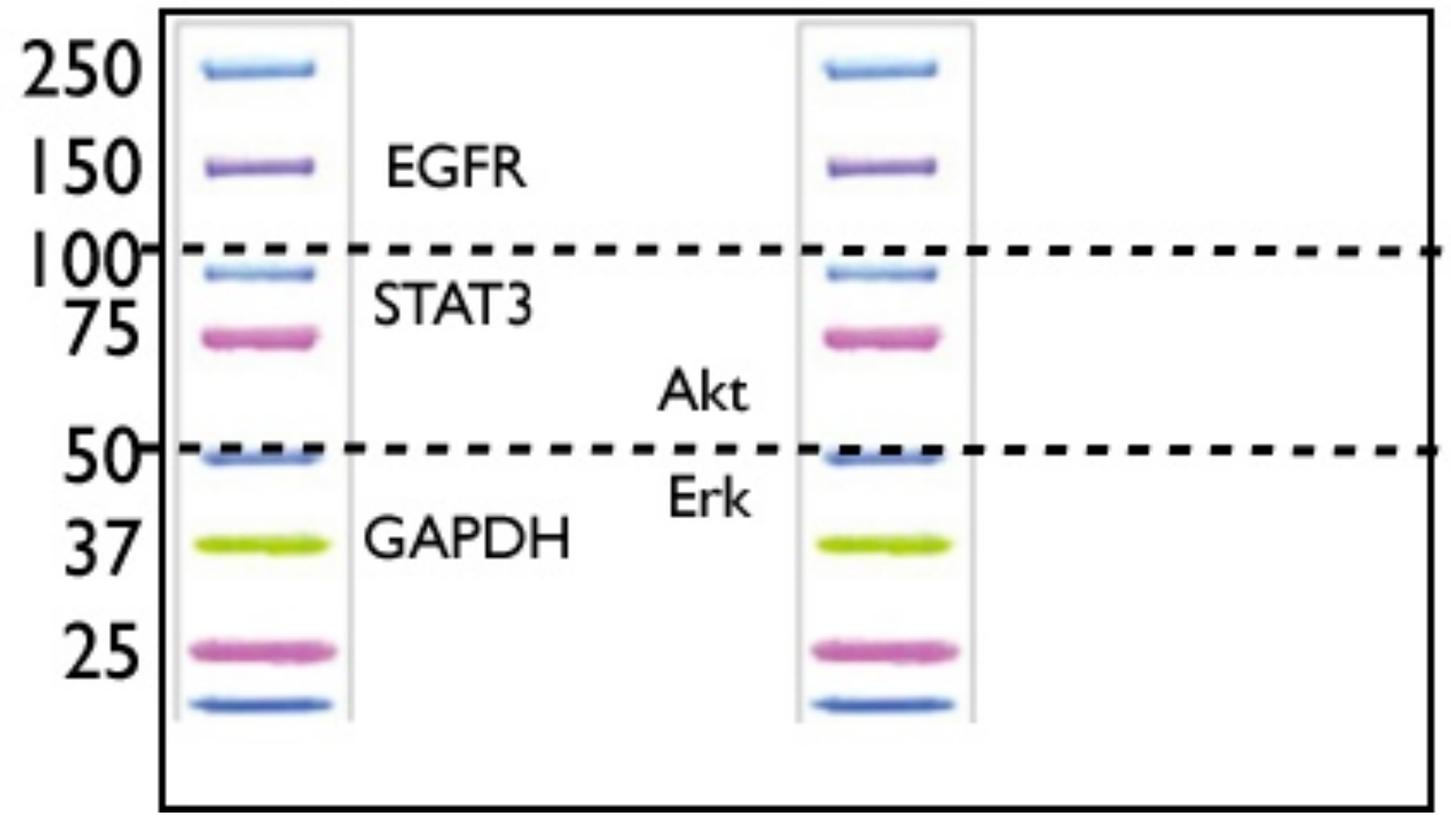
1. Stimulate cells with 8.4 nM (50 ng/mL) EGF ± Erlotinib
2. Lyse Cells -- KEEP EVERYTHING COLD
3. Measure protein concentration
4. SDS-PAGE
5. Western blot
6. All: pY1068-EGFR & Total EGFR
7. Pathway specific:



Semi-quantitative analysis: Your experiment

1. Stimulate cells with 8.4 nM (50 ng/mL) EGF ± Erlotinib
2. Lyse Cells -- KEEP EVERYTHING COLD
3. Measure protein concentration
4. SDS-PAGE
5. Western blot
6. All: pY1068-EGFR & Total EGFR
7. Pathway specific:

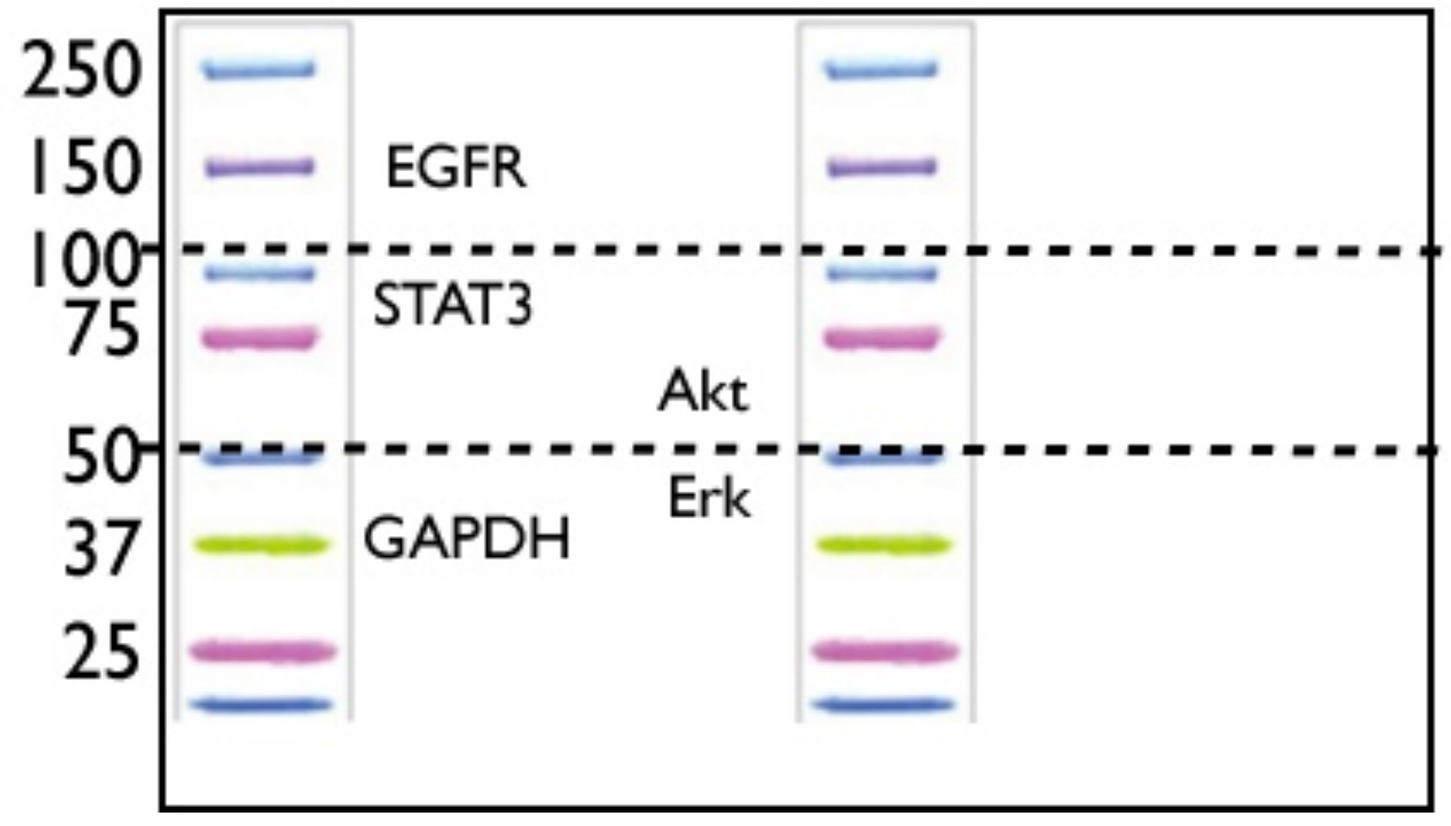
pERK & Total ERK



Semi-quantitative analysis: Your experiment

1. Stimulate cells with 8.4 nM (50 ng/mL) EGF ± Erlotinib
2. Lyse Cells -- KEEP EVERYTHING COLD
3. Measure protein concentration
4. SDS-PAGE
5. Western blot
6. All: pY1068-EGFR & Total EGFR
7. Pathway specific:

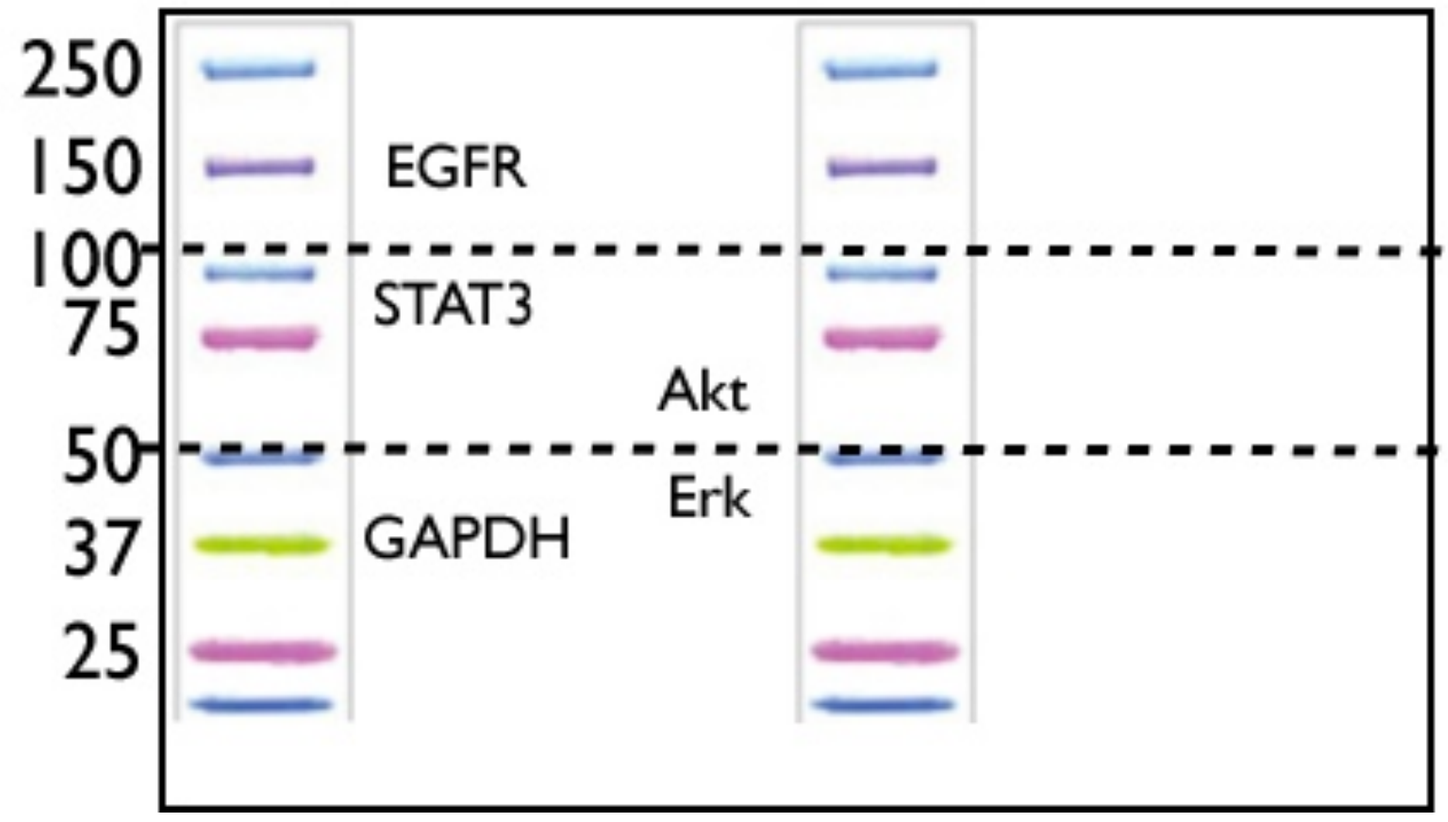
pERK & Total ERK
pAkt & Total Akt



Semi-quantitative analysis: Your experiment

1. Stimulate cells with 8.4 nM (50 ng/mL) EGF ± Erlotinib
2. Lyse Cells -- KEEP EVERYTHING COLD
3. Measure protein concentration
4. SDS-PAGE
5. Western blot
6. All: pY1068-EGFR & Total EGFR
7. Pathway specific:

pERK & Total ERK
pAkt & Total Akt
pSTAT3 & Total STAT3

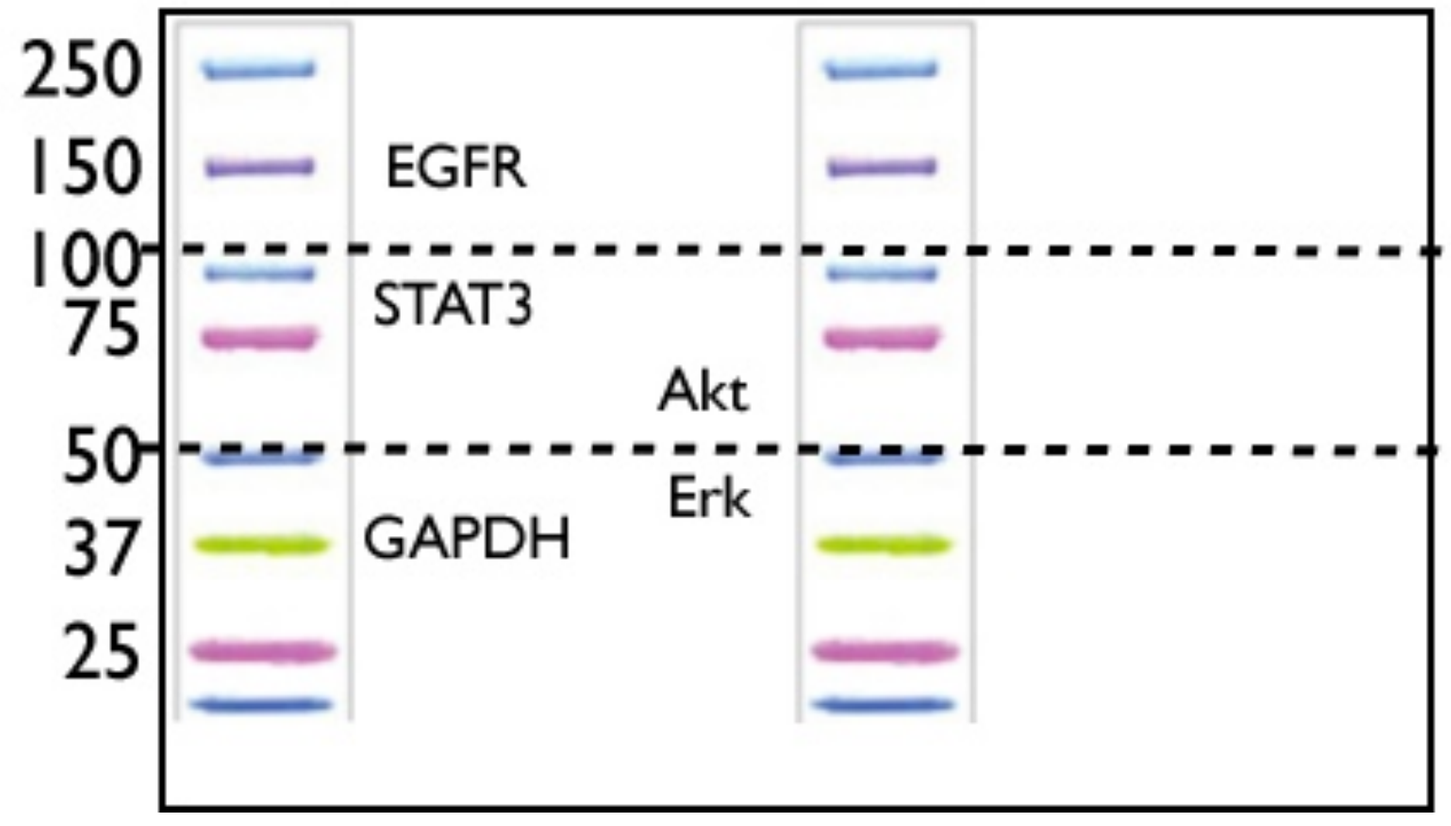


Semi-quantitative analysis: Your experiment

1. Stimulate cells with 8.4 nM (50 ng/mL) EGF ± Erlotinib
2. Lyse Cells -- KEEP EVERYTHING COLD
3. Measure protein concentration
4. SDS-PAGE
5. Western blot
6. All: pY1068-EGFR & Total EGFR
7. Pathway specific:

pERK & Total ERK
pAkt & Total Akt
pSTAT3 & Total STAT3

*Last two + GAPDH

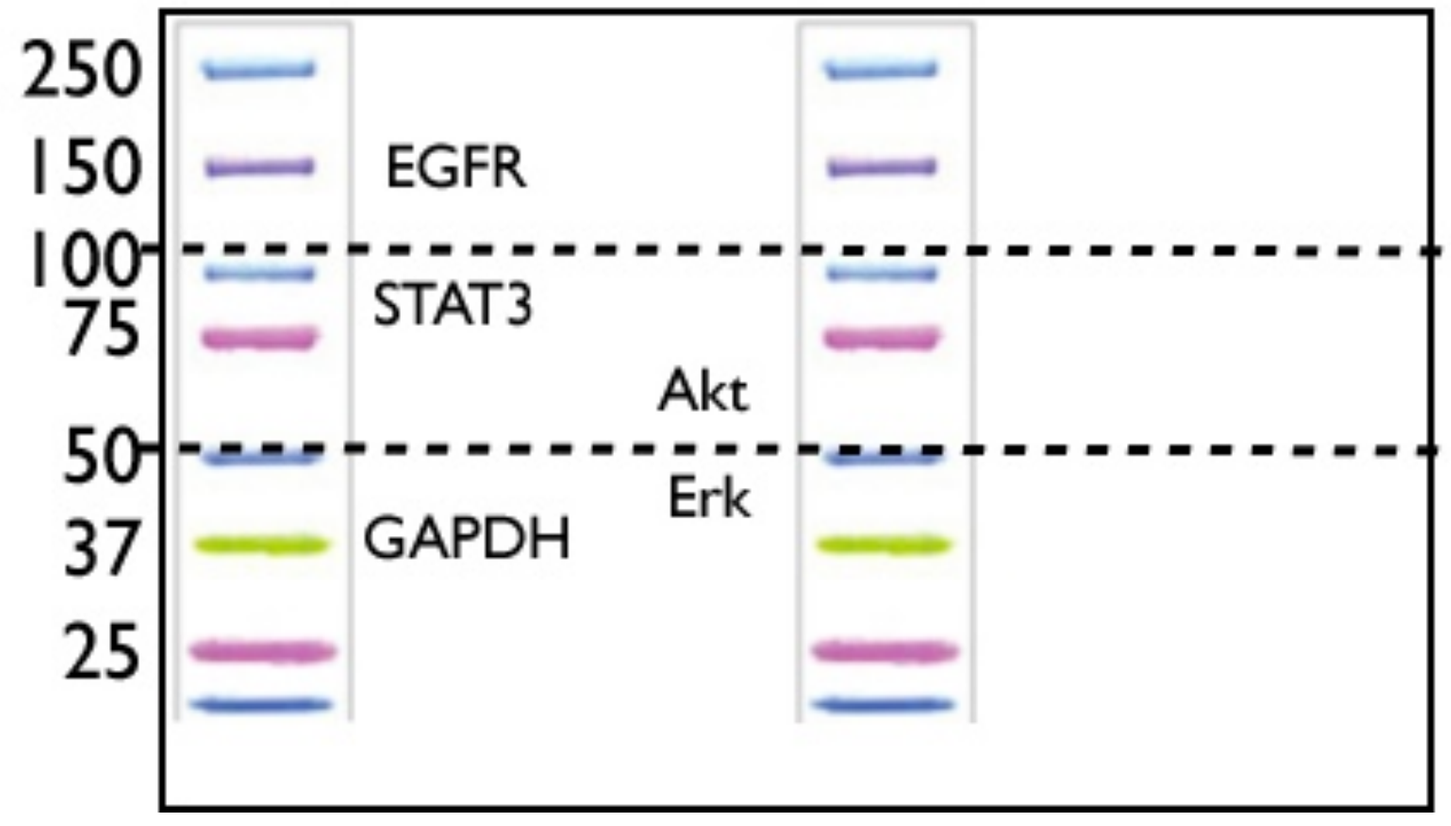


Semi-quantitative analysis: Your experiment

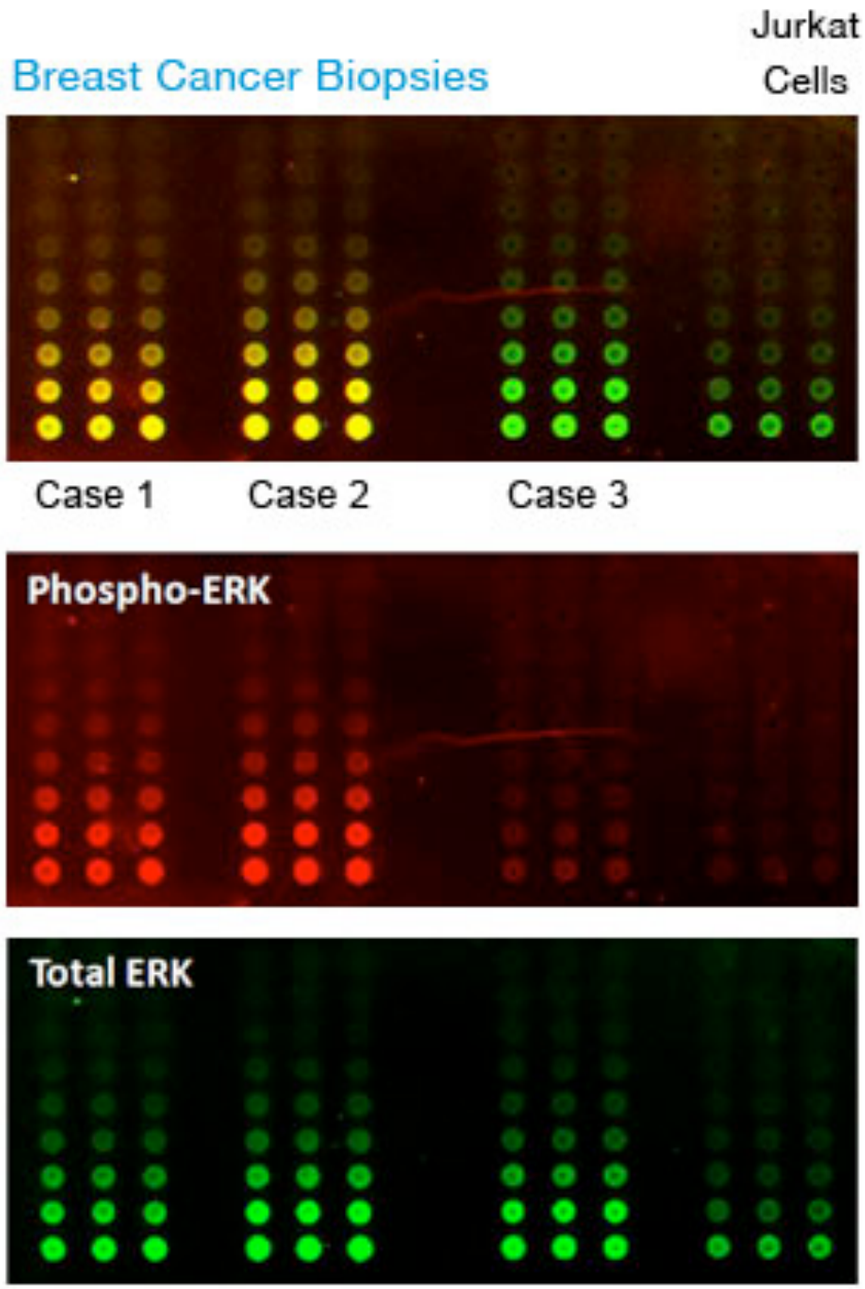
1. Stimulate cells with 8.4 nM (50 ng/mL) EGF ± Erlotinib
2. Lyse Cells -- KEEP EVERYTHING COLD
3. Measure protein concentration
4. SDS-PAGE
5. Western blot
6. All: pY1068-EGFR & Total EGFR
7. Pathway specific:

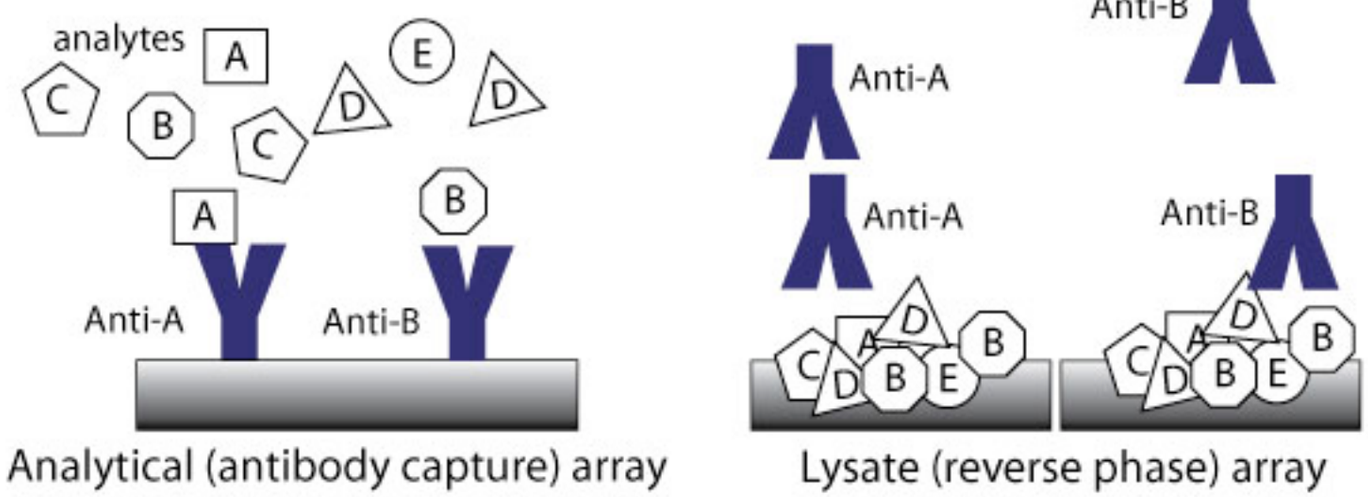
pERK & Total ERK
pAkt & Total Akt
pSTAT3 & Total STAT3

*Last two + GAPDH



Semi-quantitative analysis: Protein Microarray

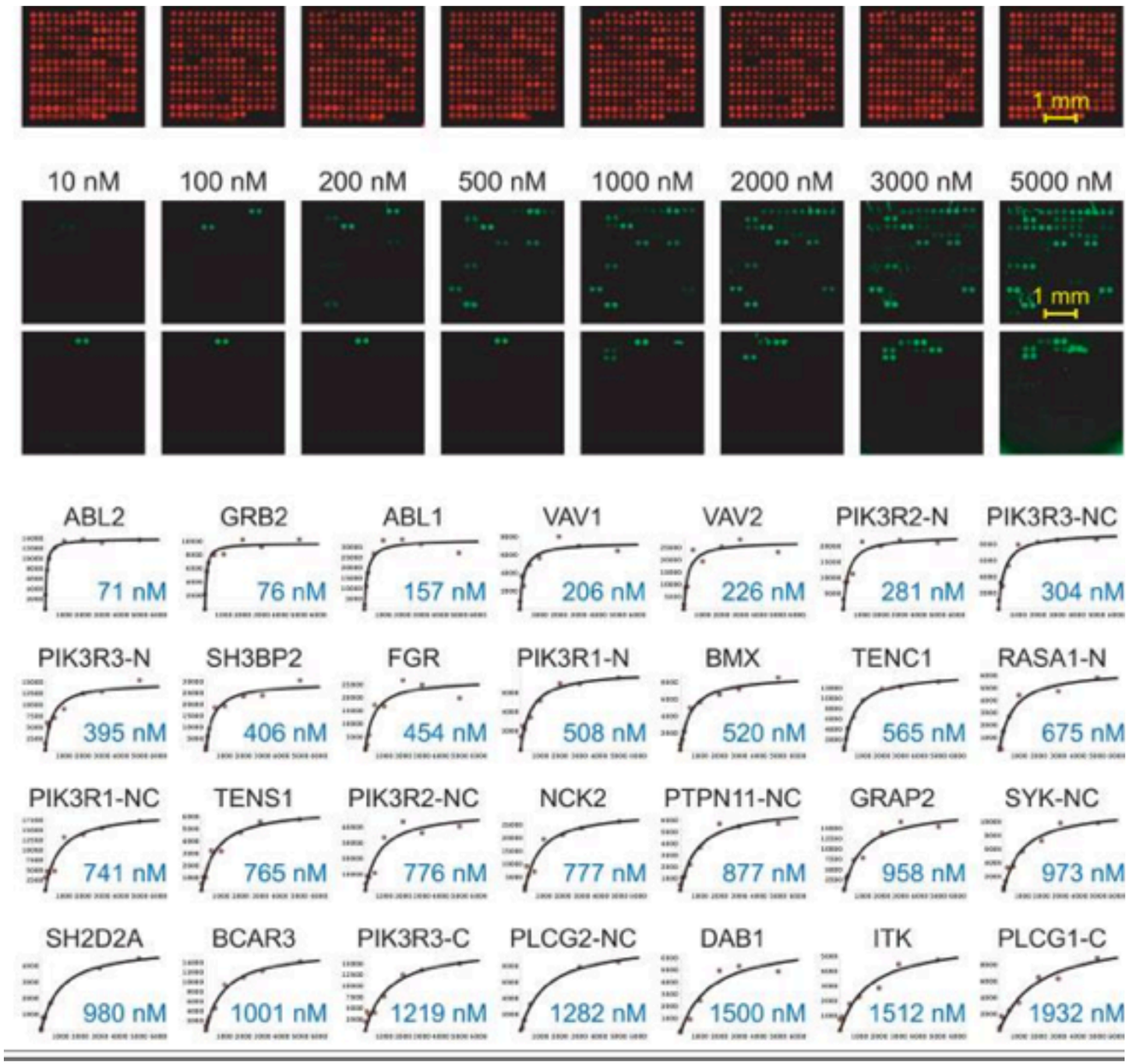




A few signals per spot -- up to hundreds of spots per slide.

http://www.licor.com/bio/applications/odyssey_applications/rpa.jsp

Semi-quantitative analysis: Protein Microarray



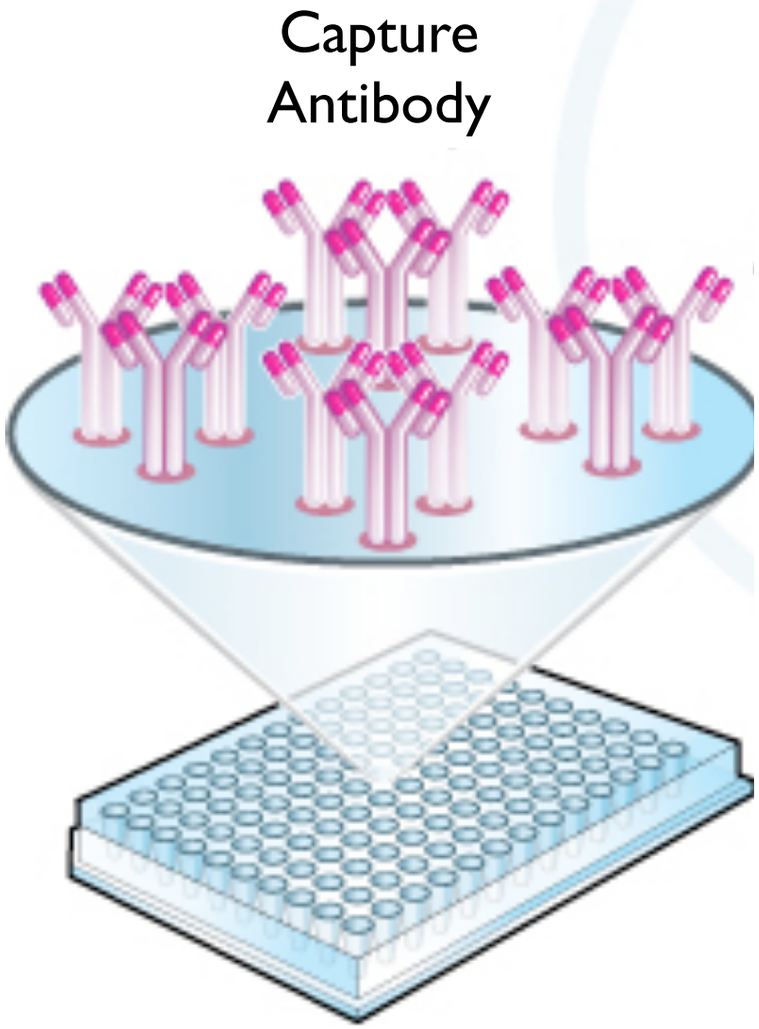
Strength:
Multiplex -- many conditions can be screened

Weakness:
Non-specific interactions (also huge problem in WB)

Dissecting Protein Function and Signaling Using Protein Microarrays
Curr Opin Chem Biol. 2009 October; 13(4): 398–405.

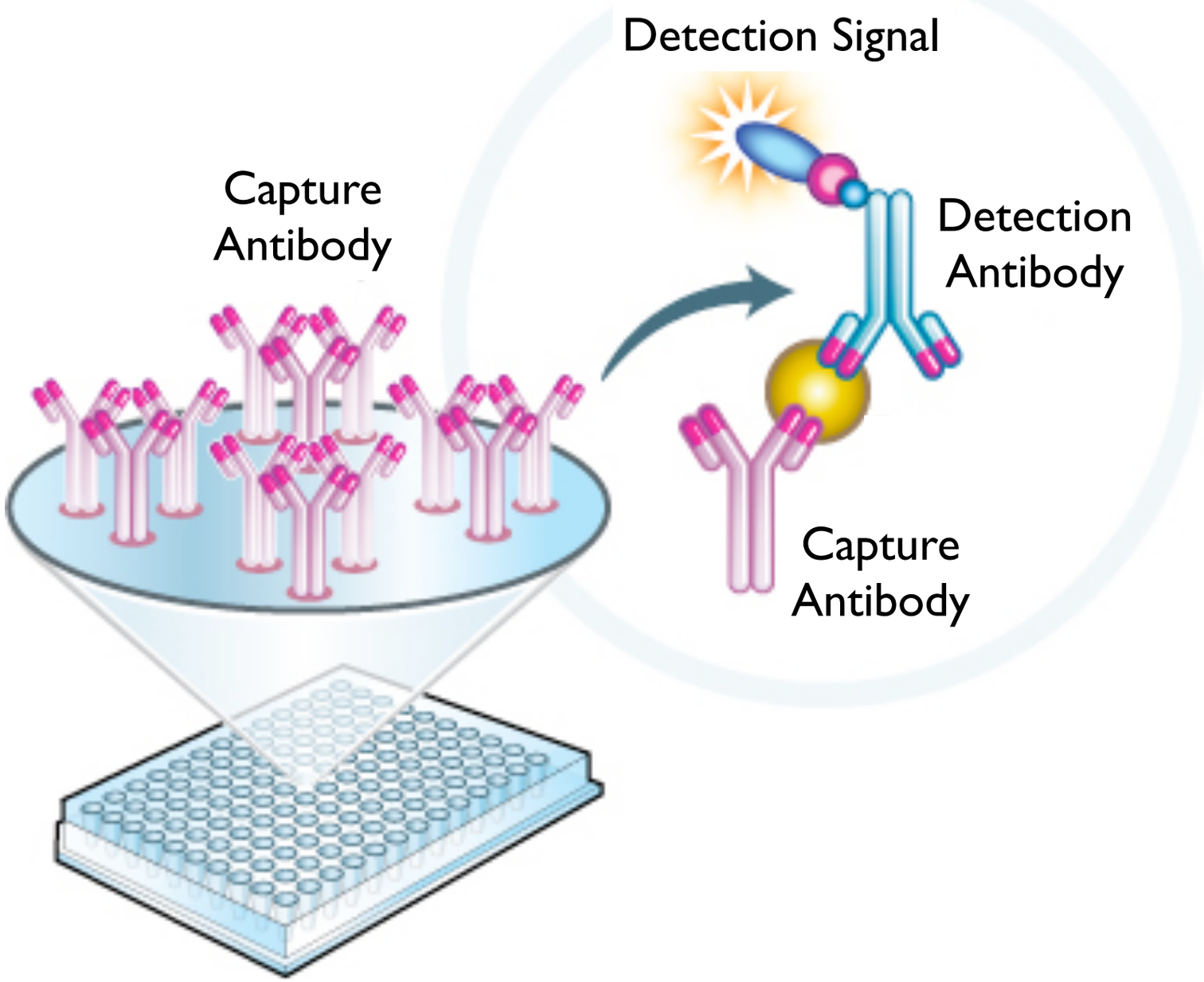
A few signals per spot -- up to hundreds of spots per slide.

Quantitative analysis: ELISA



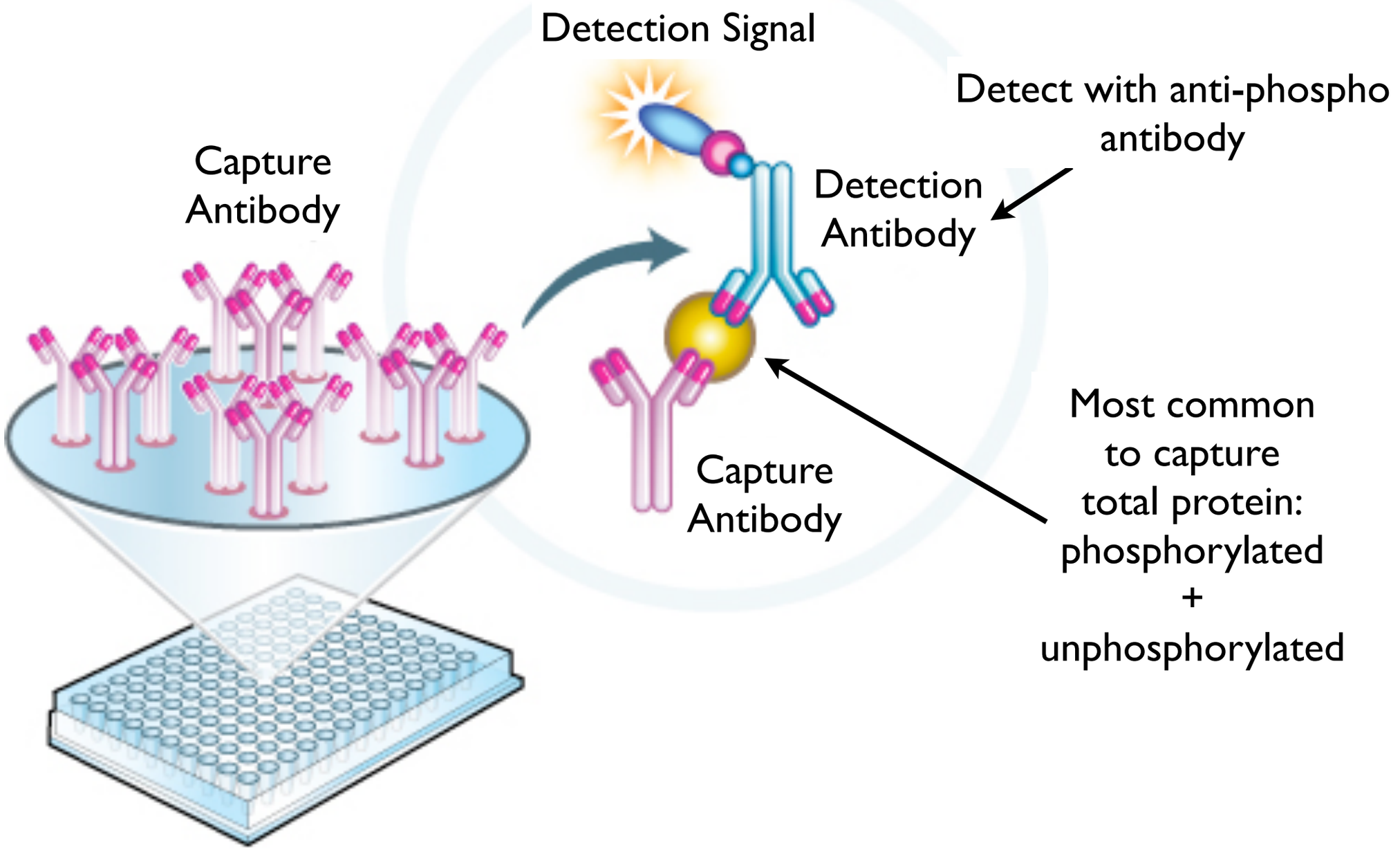
One signal per well - but up to 384 wells/experiment.

Quantitative analysis: ELISA



One signal per well - but up to 384 wells/experiment.

Quantitative analysis: ELISA

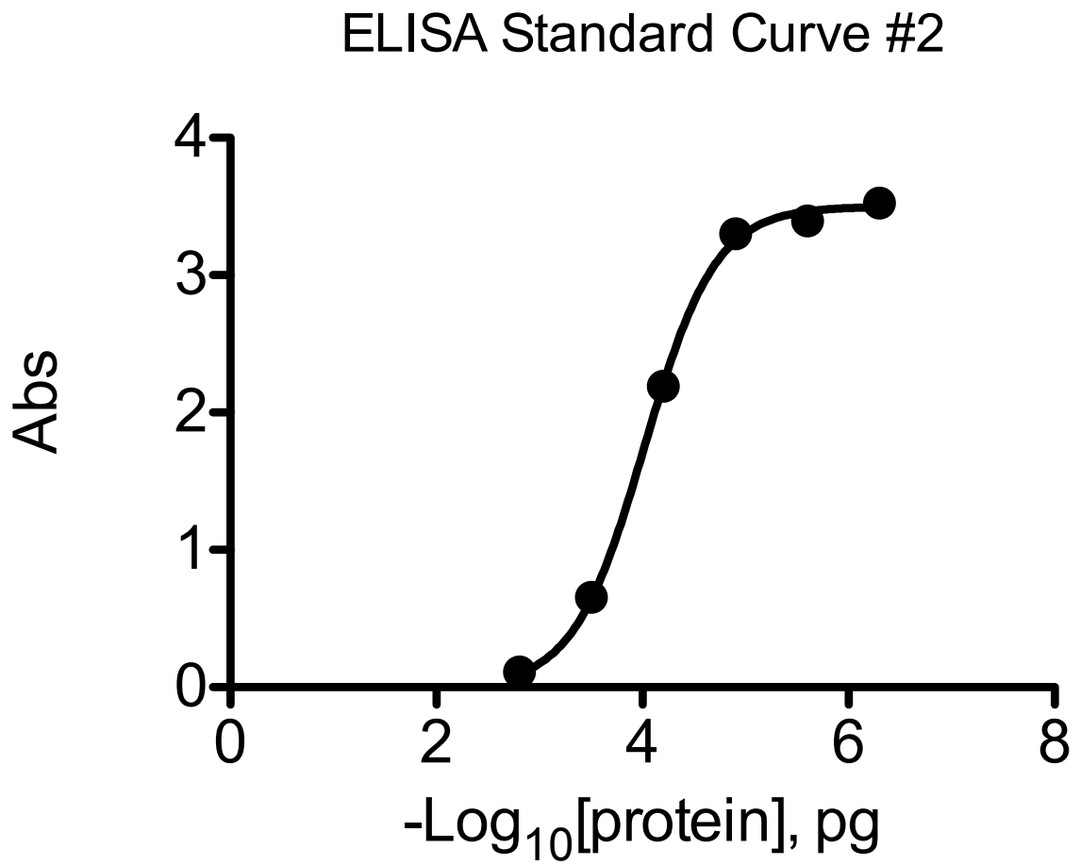


One signal per well - but up to 384 wells/experiment.

Quantitative analysis: ELISA

How to quantify:

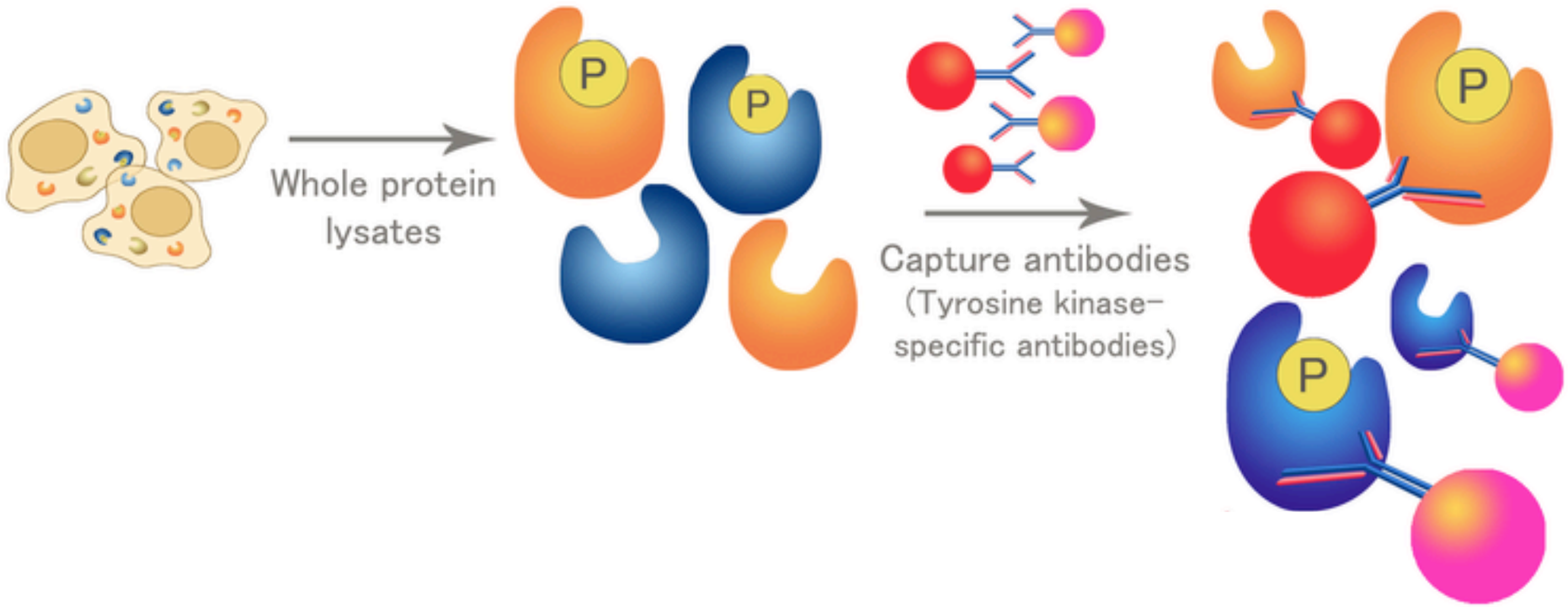
Very often not linear relationships




```
Y=Bottom + (Top-Bottom)/(1+10^((LogEC50-X)*HillSlope))
```

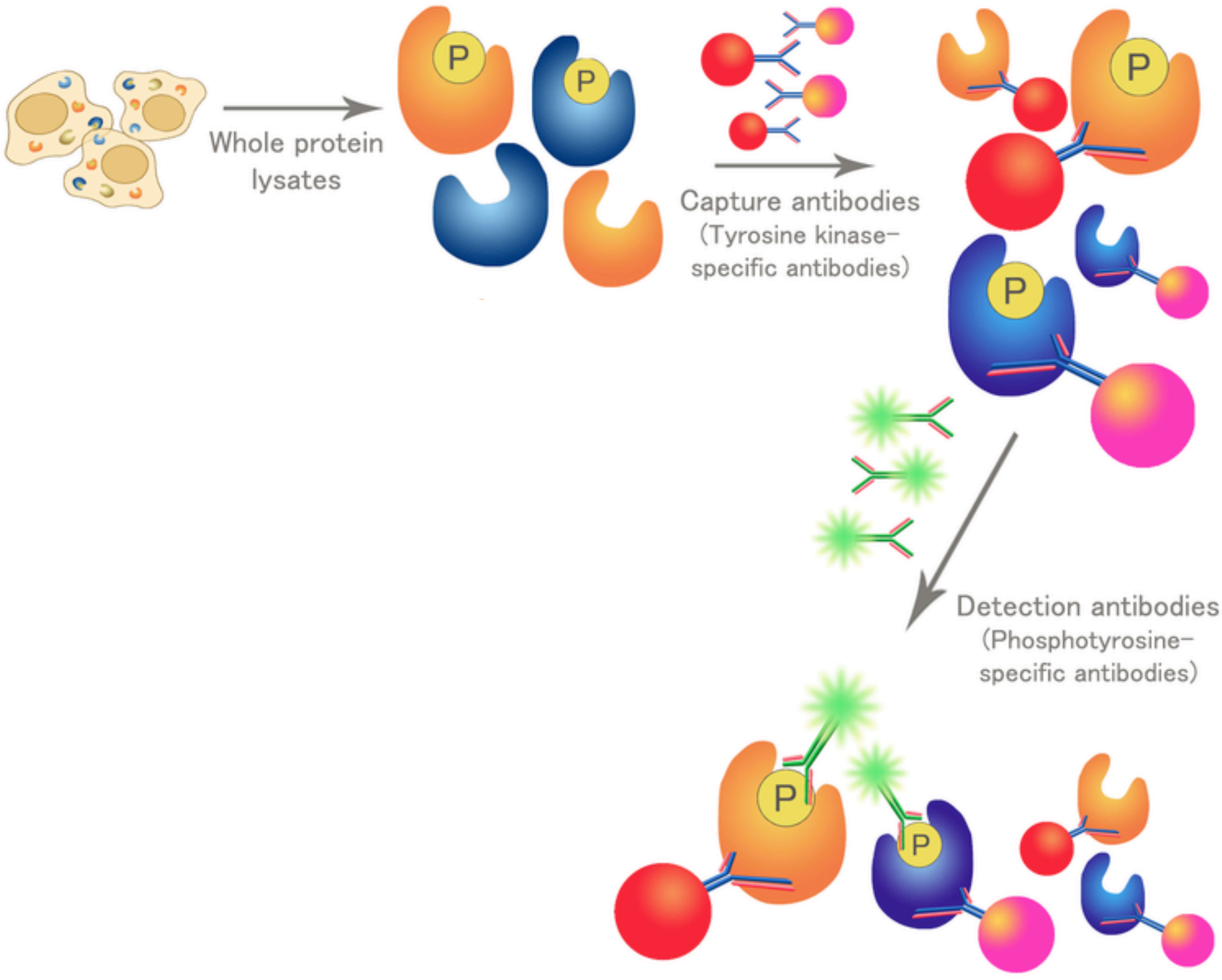
General protocol: Create a standard curve on each plate (usually supplied with kits or make your own)

Quantitative analysis: Multiplexed bead based ELISA



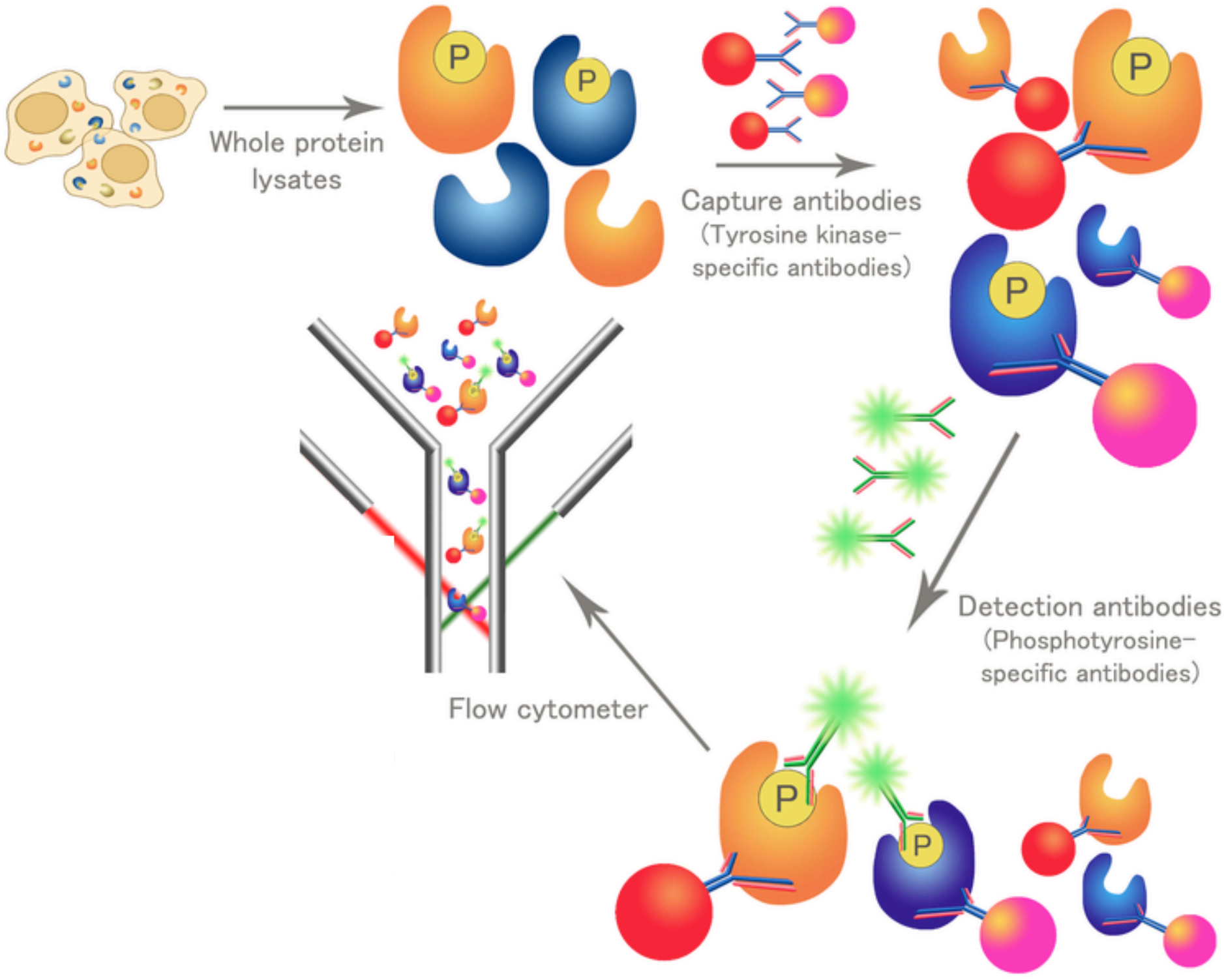
http://commons.wikimedia.org/wiki/File:Workflow_IA.png

Quantitative analysis: Multiplexed bead based ELISA



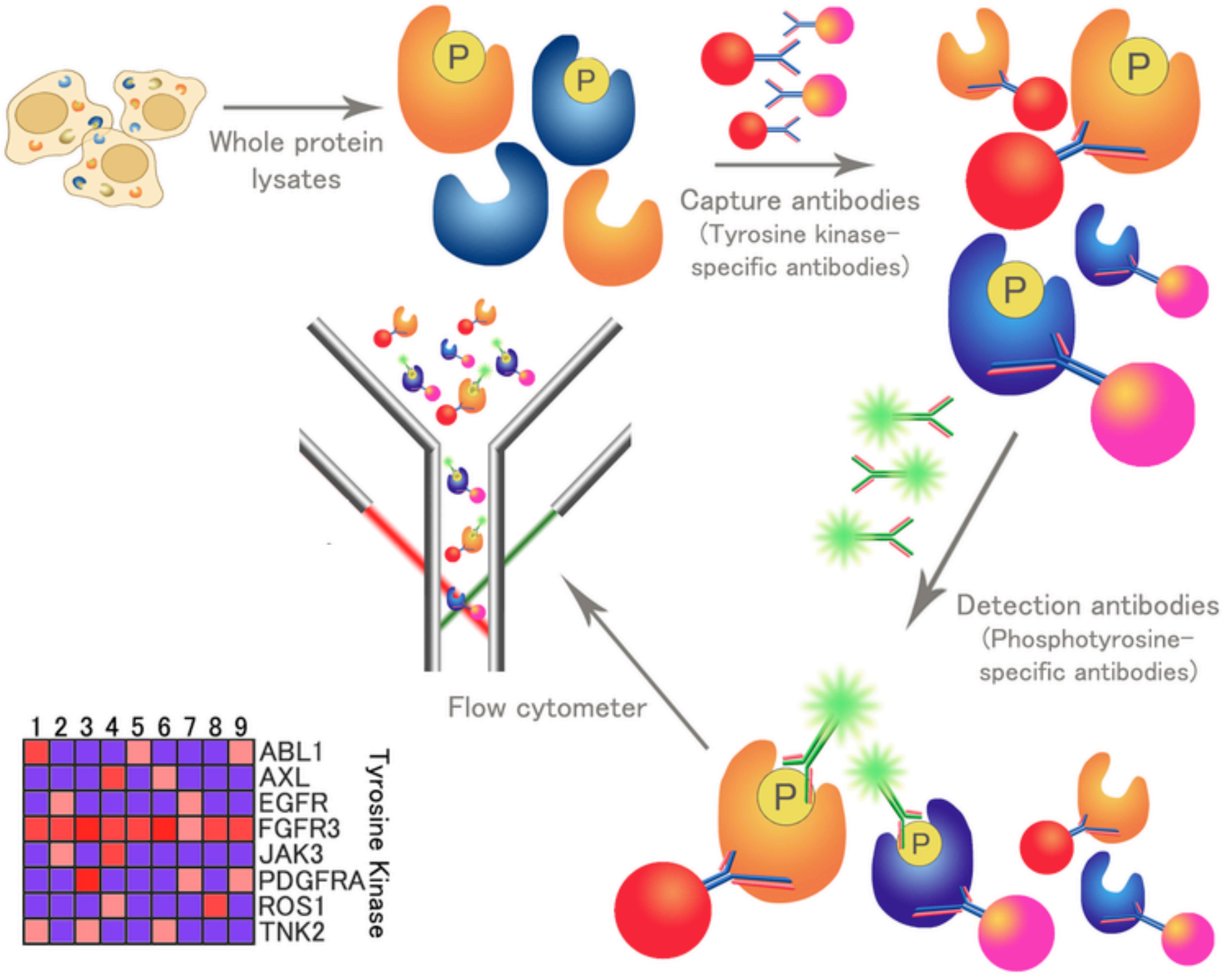
http://commons.wikimedia.org/wiki/File:Workflow_IA.png

Quantitative analysis: Multiplexed bead based ELISA



http://commons.wikimedia.org/wiki/File:Workflow_IA.png

Quantitative analysis: Multiplexed bead based ELISA

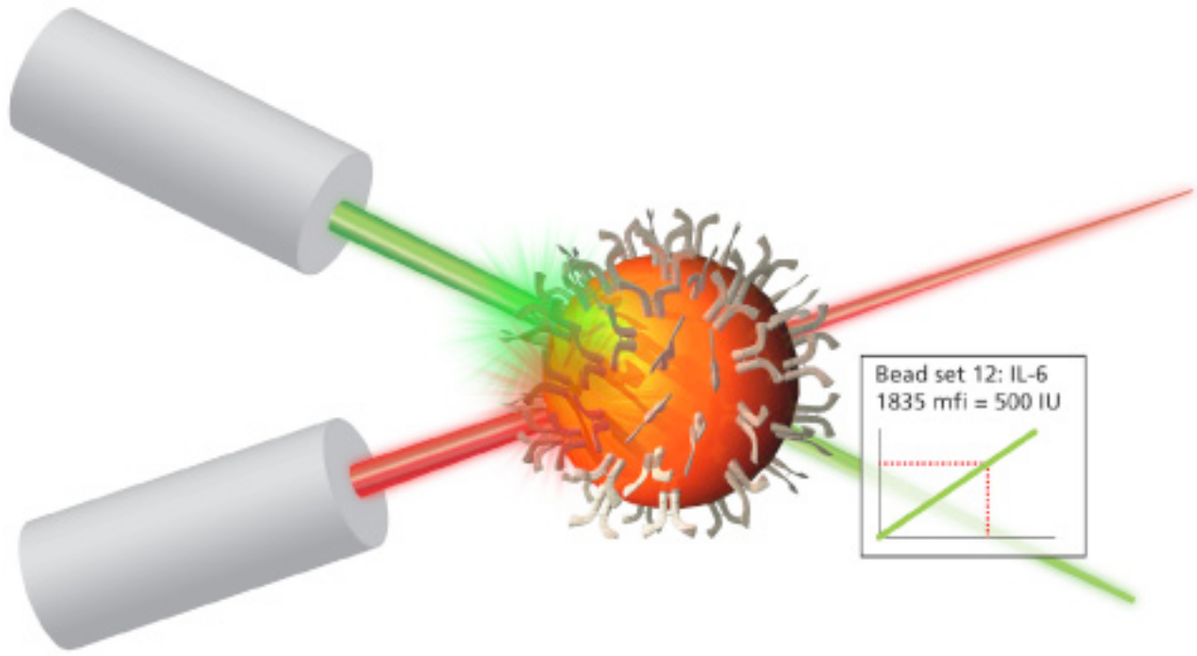


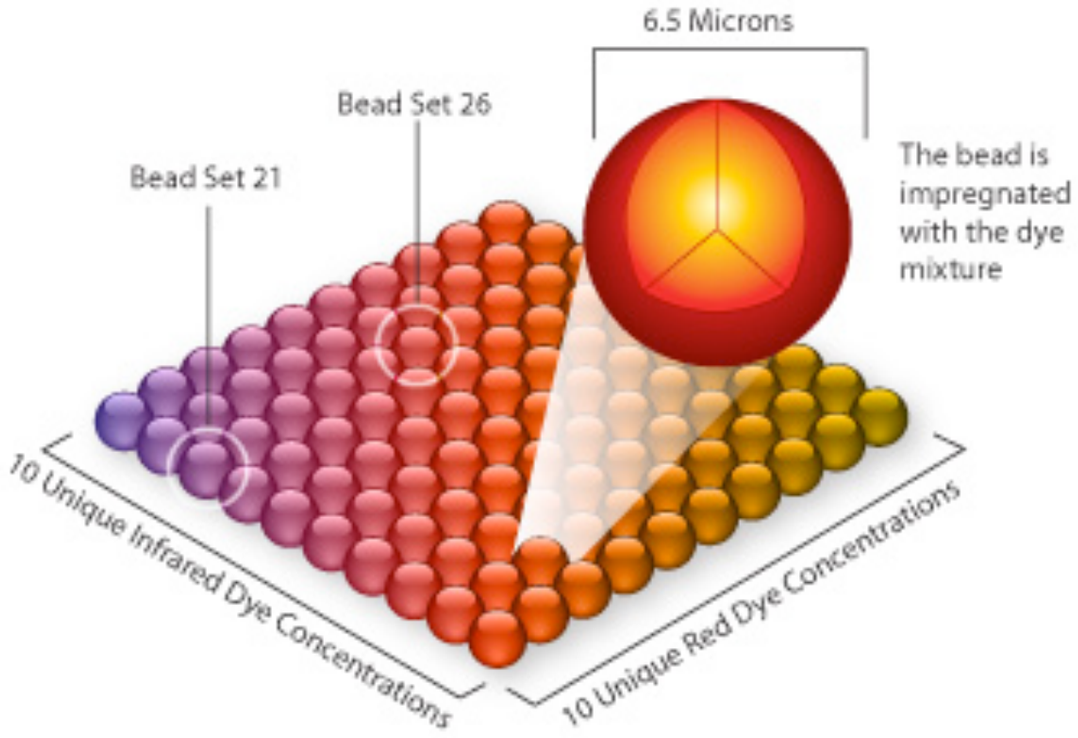
http://commons.wikimedia.org/wiki/File:Workflow_IA.png

Quantitative analysis: Multiplexed bead based ELISA

Theoretically up to hundreds of conditions per well -- 384 (sometimes >1500!) wells per experiment

In reality: 20-30 different phosphoproteins / well max





http://www.ebioscience.com/knowledge-center/product-line/procartaplex/technology.htm

Module 2: Systems Engineering

- A few announcements -- if you haven't watched my Oscar-worthy video
- Revisit sequencing analysis
- (Semi)Quantitative methods for measuring pathway activity
- A few hints for lab
- Scale it up!
- Journal club!