***Exercise 3 Coding***

Experimental Setup

Strains:

* WT-EGFR
* EGFR Null
* EGFR-M1
* EGFR-M2
* NoUB
* ConstActive
* His-EGFR-FLAG
* His-EGFR-M1-FLAG
* His-EGFR-M2-FLAG

Variables to use:

* Treatment Duration

Treatment details

|  |  |
| --- | --- |
| Treatment Name | Treatment Duration |
| Growth media only | 30 sec |
| 1 min |
| 6 hrs |
| Growth media + EGF | 30 sec |
| 1 min |
| 6 hrs |
| Growth media + buffer | 30 min |
| Growth Media + ProK | 30 min |

Use only the following samples for experiments (delete the other combinations):

|  |  |  |
| --- | --- | --- |
| Strain | Treatment | Treatment Duration |
| WT-EGFR | Growth media only | 30 sec |
| 1 min |
| 6 hrs |
| Growth media + EGF | 30 sec |
| 1 min |
| 6 hrs |
| Growth media + buffer | 30 min |
| Growth media + ProK | 30 min |
| EGFR Null | Growth media only | 30 sec |
| 1 min |
| 6 hrs |
| Growth media + EGF | 30 sec |
| 1 min |
| 6 hrs |
| Growth media + buffer | 30 min |
| Growth media + ProK | 30 min |
| EGFR-M1 | Growth media only | 30 sec |
| 1 min |
| 6 hrs |
| Growth media + EGF | 30 sec |
| 1 min |
| 6 hrs |
| EGFR-M2 | Growth media only | 30 sec |
| 1 min |
| 6 hrs |
| Growth media + EGF | 30 sec |
| 1 min |
| 6 hrs |
| NoUB | Growth media only | 30 sec |
| 1 min |
| 6 hrs |
| Growth media + EGF | 30 sec |
| 1 min |
| 6 hrs |
| ConstActive | Growth media only | 30 sec |
| 1 min |
| 6 hrs |
| Growth media + EGF | 30 sec |
| 1 min |
| 6 hrs |
| His-EGFR-FLAG | Growth media + buffer | 30 min |
| Growth media + ProK | 30 min |
| His-EGFR-M1-FLAG | Growth media + buffer | 30 min |
| Growth media + ProK | 30 min |
| His-EGFR-M2-FLAG | Growth media + buffer | 30 min |
| Growth media + ProK | 30 min |

Group by: Strains

Then by Treatment, Then by duration (this is default)

Western Blotting

What samples are available?

* Whole cell lysate

What percentage acrylamide?

* 10%
* 12%
* 15%

Antibodies

|  |  |
| --- | --- |
| Primary | Secondary |
| Mouse anti-EGFR | Rabbit anti-mouse HRP |
| Mouse anti-pEGFR | Rabbit anti-mouse HRP |
| Mouse anti-pMEK | Rabbit anti-mouse HRP |
| Mouse anti-pRAF | Rabbit anti-mouse HRP |
| Mouse anti-PGK1 | Rabbit anti-mouse HRP |
| Rabbit anti-6xHis | Goat anti-rabbit HRP |
| Rabbit anti-FLAG | Goat anti-rabbit HRP |

For sample and sizes/intensities see excel document:

Lisa’s Exercises -> Exercise 3 -> SCB coding Exercise 3\_Ver5

Background Bands?

* NO

Flow Cytometry

Sample Prep:

|  |  |  |
| --- | --- | --- |
| Cell Treatments | Analysis | Conditions |
| Fixed Cells | Antibody-labeling | EGFR A488 |

Analyze:

See SCB coding Exercise 3\_Ver5 for the possible histograms, the list of samples assigned to each histogram, and details about axes labels

Microscopy

* Analysis: Antibody-labeling IF (immunofluorescence) for all samples listed below
* 5 different conditions, described below in the table
* The first condition listed below, EGFR A488, is available for the following strains & treatments:
* WT-EGFR: Growth media only (all timepoints)
* EGFR Null: Growth media only (all timepoints)
* EGFR-M1: Growth media only (all timepoints)
* EGFR-M2: Growth media only (all timepoints)
* The other conditions are available for ONLY the WT-EGFR strain, and exclusively the “Growth media only” condition (all time points).

|  |  |
| --- | --- |
| Condition | Folder of images to use (Curriculum -> Lisa’s Exercises -> Exercise 3 -> images -> microscopy images -> \_\_\_\_\_\_\_\_\_\_\_) |
| EGFR A488 | pm final for WT-EGFR, EGFR-M1 and EGFR-M2 strains. For EGFR Null sample, use ‘negative control’ image (black square). |
| PM A488 | pm final |
| Cyto A488 | cyto final |
| Nuc A488 | nucleus final |
| ER A488 | er final |
| NM A488 | nm final |

\*all images seen only in GREEN FILTER, (objective N/A)

Randomize images within folders.

Note for Anna: All other strains and treatment combinations do not have available images for microscopy analysis. In the past, we have listed these samples, but in grayed out font. They also don’t have checkboxes on the left side.