

- Announcements → HW, OH
- Quiz
- Review methods + goals
- Microarray analysis in the literature (w/Steve)

Methods review

- How do we measure luciferase activity?
 - i.e., what *are* LAR and Stop+Glo?
- Choosing a hybridization temperature for adding cDNAs and fluors to uarrays
 - Too high a T will...
 - Too low a T will...

Luciferase knockdown validation

Team Colour	validated		Team Colour	validated	
	Rep 1 ratio	Rep 2 ratio		Rep 1 ratio	Rep 2 ratio
Pink	2.73	1.92	Pink	2.87	2.91
Green	2.7	2.4	Orange	1.70	2.30
Orange	2.25	1.74	Red	0.385	1.636
Yellow	1.42	2.24	Blue	2.758433	8.679711
Grey	2.55	2.90	Purple	1.369	2.073
Red	1.31	1.51	Green	1.562	3.331
Blue	1.75	.51	Yellow	3.040	3.575
Purple	3.24	3.90	Grey	3.19	4.02

cf. pLuc ww/ or w/out scrambled siRNA ratios ~20-30

Luciferase experiment

Team Colour	experimental		Team Colour	experimental	
	Rep 1 ratio	Rep 2 ratio		Rep 1 ratio	Rep 2 ratio
Pink	14.7	17.0	Pink	18.10	18.08
Green	2.8	2.6	Orange	6.42	5.13
Orange	4.8	4.1	Red	1.96	1.72
Yellow	1.17	1.53	Blue	14.8144	12.87329
Grey	9.04	8.80	Purple	1.189	1.751
Red	1.53	1.61	Green	4.434	9.589
Blue	8.25	6.87	Yellow	1.989	2.203
Purple	7.55	8.33	Grey	3.25	8.35

uArray goals

- Today's notebook
 - Do you get knockdown of target?
 - Are housekeeping genes unaffected?
 - Is your data useable?
- Final report
 - Provide context: why are the targets interesting?
 - Can you make some hypotheses about other affected genes (indirect or off-target)?

uArray analysis

- MIAME – minimum *information* about a microarray experiment
- Not just one accepted *analysis* → document
- Making the choices: exciting? intimidating?

Minimum Information About a Microarray Experiment - MIAME

MIAME describes the **Minimum Information About a Microarray Experiment** that is needed to enable the interpretation of the results of the experiment unambiguously and potentially to reproduce the experiment. [[Brazma et al, Nature Genetics](#)]

The six most critical elements contributing towards MIAME are:

1. The raw data for each hybridisation (e.g., CEL or GPR files)
2. The final processed (normalised) data for the set of hybridisations in the experiment (study) (e.g., the gene expression data matrix used to draw the conclusions from the study)
3. The essential sample annotation including experimental factors and their values (e.g.,