



# 2016 Interlab Study

Organized by:

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**Raytheon**  
BBN Technologies



SYNTHACE

Imperial College  
London



# Why do an interlaboratory study?



## Measurement in Synthetic Biology:

- How precisely can the behavior of a part be characterized?
- How much do *de facto* protocols for measurement vary?
- What are the dominant causes of variation in measurement?

***Measurement is fundamental to everything we do.***



# What we achieved last year



RESEARCH ARTICLE

## Reproducibility of Fluorescent Expression from Engineered Biological Constructs in *E. coli*

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<sup>¶</sup> Membership list can be found in the Acknowledgments section.

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# What we asked teams to do

- Culture supplied plasmids
  - Positive, negative controls
  - Strong, medium, weak
- Measure fluorescence
- Plate reader:
  - Measure OD for LUDOX
  - Fluorescein dilution series
- Flow cytometer:
  - Calibrate to beads
- Report protocol, data

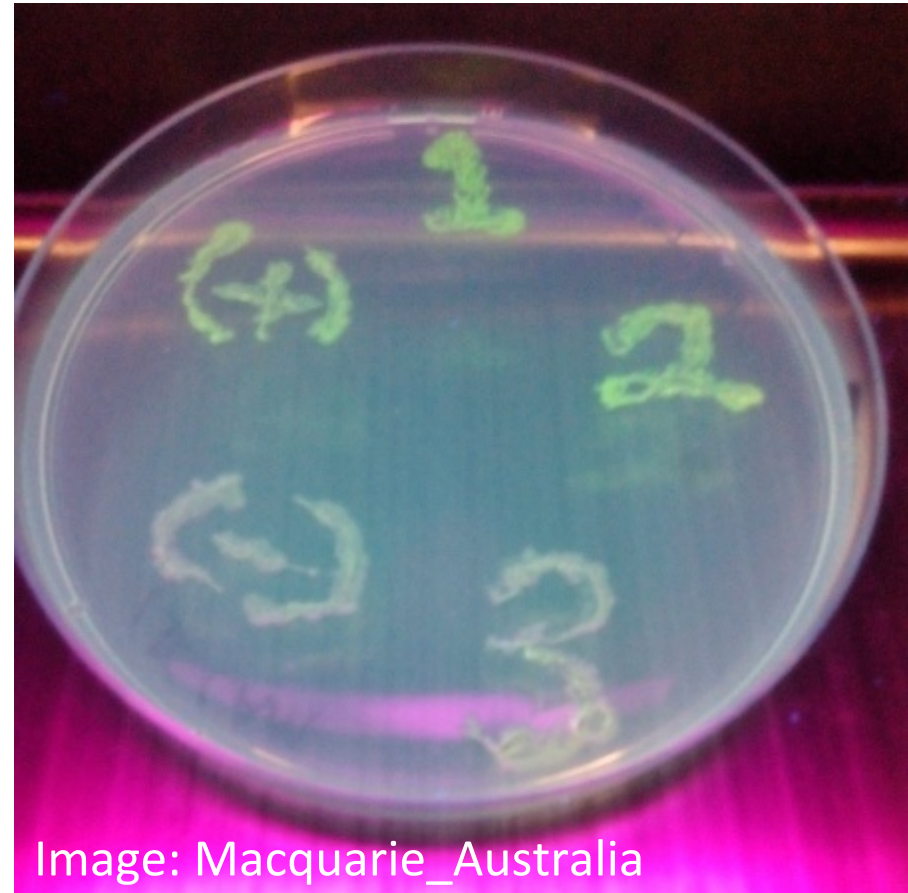
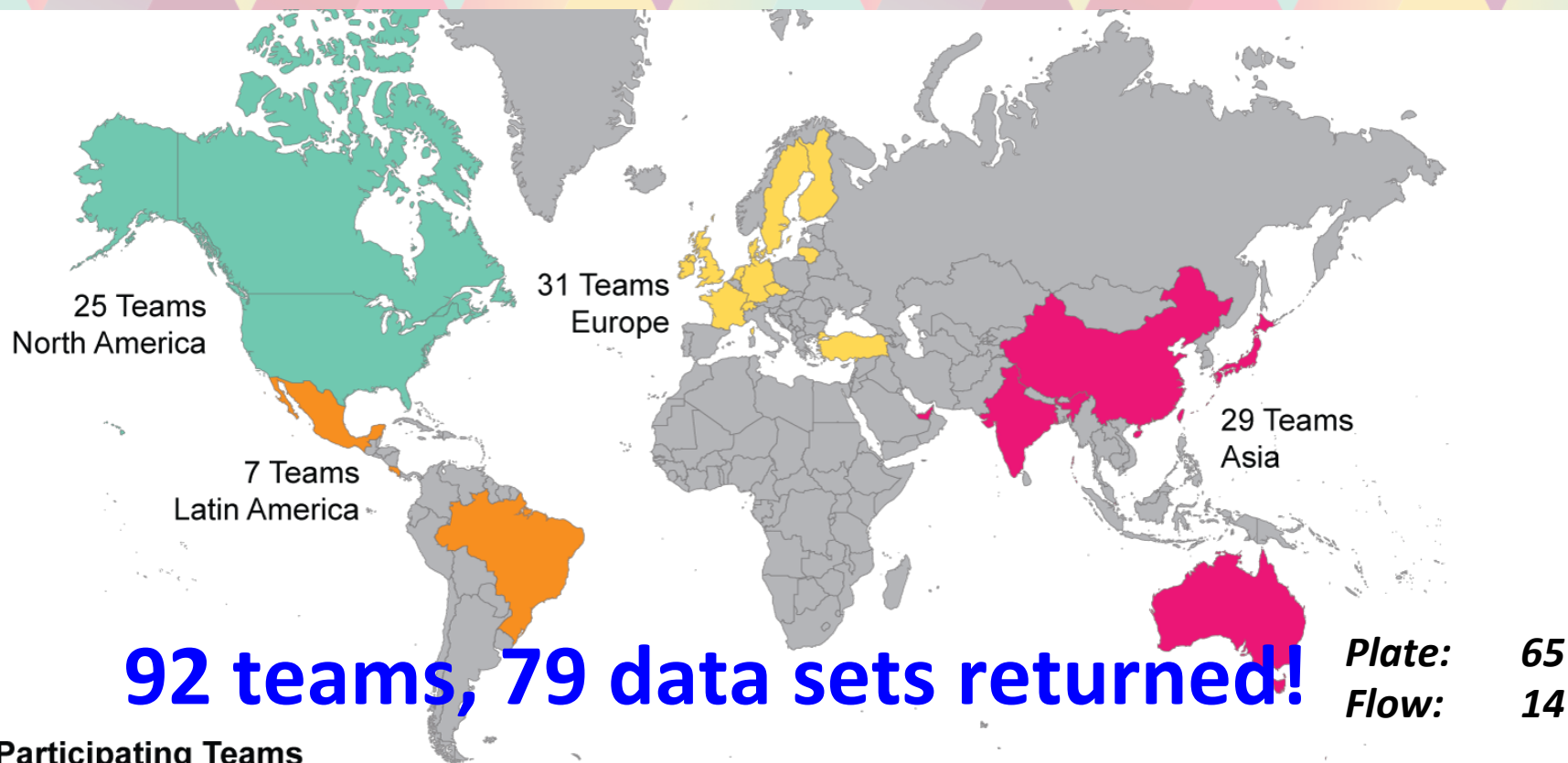


Image: Macquarie\_Australia



# Worldwide Participation: biggest yet!



## Participating Teams

Aachen	Denver Biolabs	Gifu	INSA de Lyon	Northeastern	SCSU-New Haven	TEC-Costa_Rica	UMaryland	William_and_Mary
Aalto-Helsinki	DTU_Denmark	Glasgow	Istanbul_Tech	Northwestern	SDSZ_China	TecCEM	UNebraska-Lincoln	WPI-Worcester
Aix-Marseille	Duesseldorf	Guanajuato_Mx	Jilin_China	NTU-Singapore	ShanghaiTechChina	TecCEM_HS	Leicester	XJTLU-CHINA
Alverno_CA	Duke	Hannover	Leiden	NYU-AD	Stanford-Brown	TU Delft	Melbourne	XMU-CHINA
ATOMS_Turkiye	Edinburgh_UG	Hong_Kong_HKUST	LMU-TUM Munich	Oxford	Stony_Brook	TU-Eindhoven	UNSW_Australia	
AUC_TURKEY	Edinburgh_OG	HUST_China	Macquarie_Australia	Paris_Bettencourt	SUSTech_Shenzhen	Tuebingen	Uppsala	
Austin_UTexas	EEL-USP Brazil	HZAU-China	McMasterU	Paris_Saclay	SVCE_CHENNAI	UCC_Ireland	USP_UNIFESP-Brazil	
BIT	ETH_Zurich	iGEMKyoto	MIT	Pasteur_Paris	Sydney_Australia	UChicago	Vanderbilt	
BostonU	Evry	IISc_Bangalore	NAU-China	Peking	SYSU-CHINA	UCLA	Vilnius-Lithuania	
CGU_Taiwan	Exeter	IIT Kharagpur	Newcastle	Pittsburgh	SYSU-Software	UESTC-China	Warwick	
CSU Fort Collins	Georgia_State	IIT Madras	NKU_China	Purdue	Tec-Chihuahua	UIUC_Illinois	WashU_StLouis	



# Returning Alumni & Extra Credit

- 44 of the teams participated in 2014 or 2015
- 9 teams have participated all three years!

**Aalto-Helsinki, ATOMS\_Turkiye, Austin\_UTexas, BIT, BostonU, Gifu, Oxford, SYSU-Software, WPI-Worcester**

## Extra credit to:

Aachen

CGU\_Taiwan

CSU Fort Collins

Edinburgh\_UG

ETH\_Zurich

Evry

Exeter

Georgia State University

Glasgow

IISc\_Bangalore

LMU-TUM Munich

NKU\_China

Oxford

Paris Bettencourt

Pittsburgh

Purdue

ShanghaitechChina

Sydney\_Australia

TU-Eindhoven

Tuebingen

UESTC-China

USP\_UNIFESP-Brazil

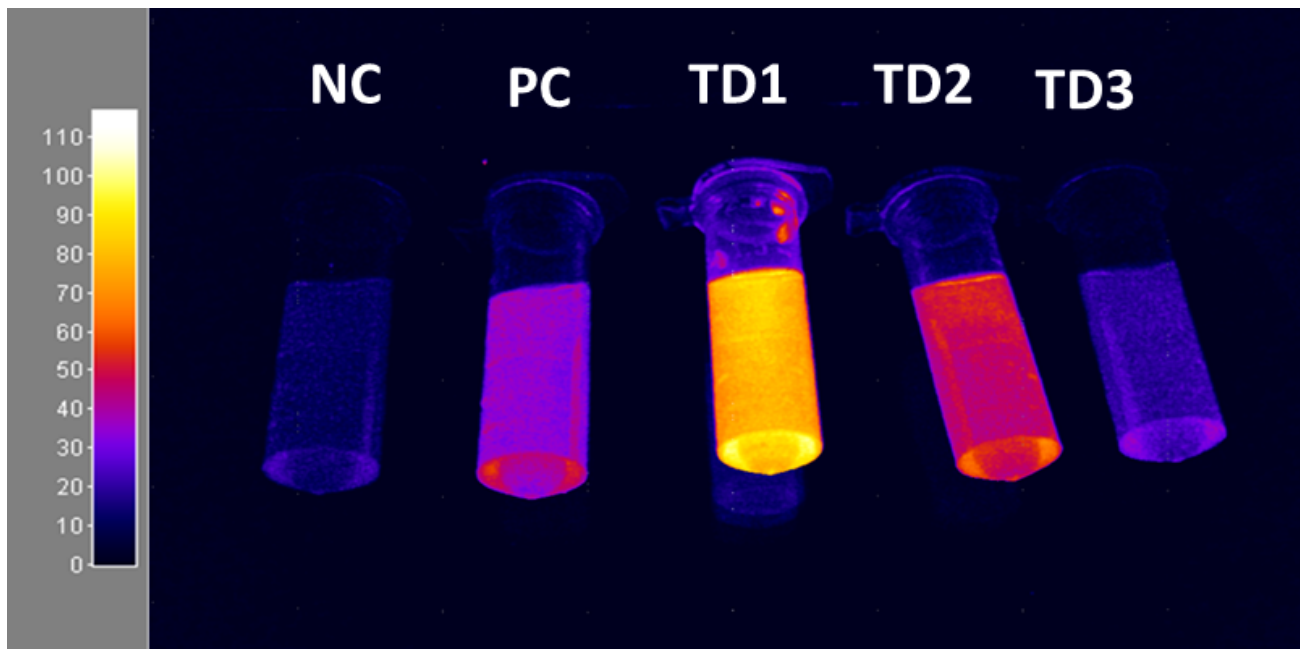
WashU\_StLouis





# Going above and beyond

- IISc Bangalore: corrected 100x error in our protocol
- LMU-TUM Munich: Comparison of 8 strains  
DH5 $\alpha$ , W3110, KS272, XL-1 blue, JM83, OriB, 10 $\beta$  NEB turbo
- USP\_UNIFESP-Brazil : DIY cellphone fluorimetry





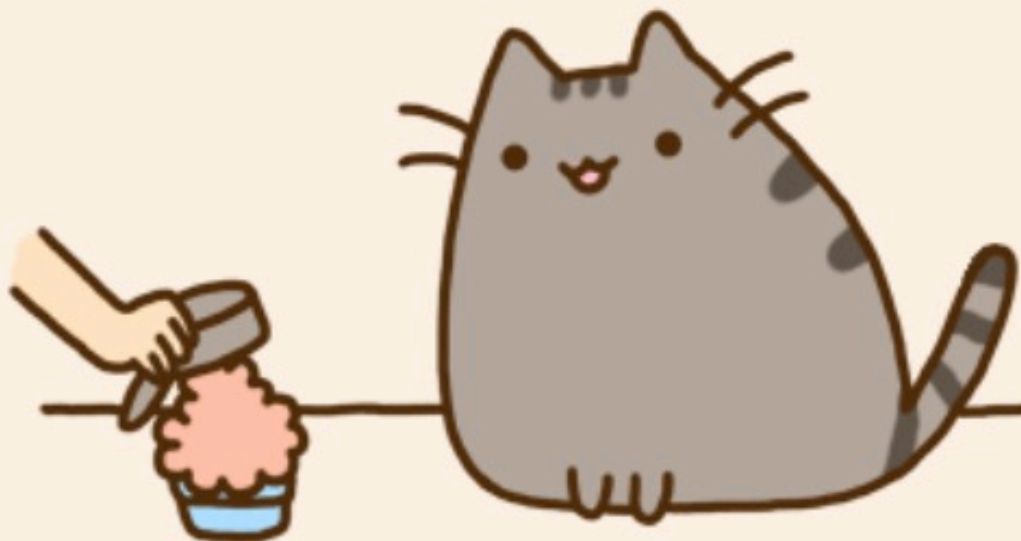
# Lessons Learned at HQ





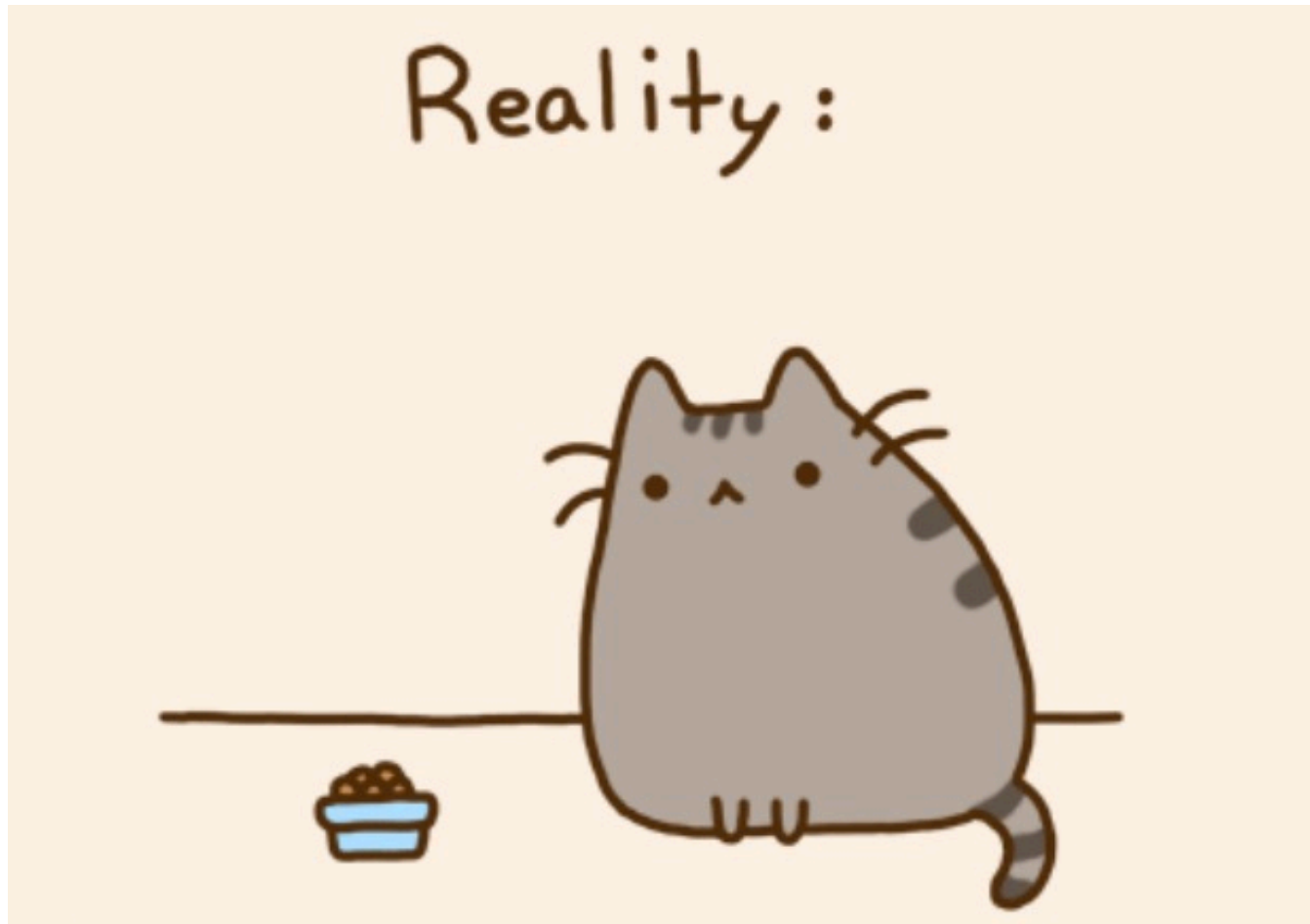
# Started off with high expectations...

Expectation:





...ended up with some frustrations





# The Expectation: Everything Will Be Easier and Work Out of the Box!





# The Reality

- Many teams reported evaporation / no liquid in their tubes
  - Cryovials are not so air tight after all...
- Many teams froze their kit and thus the LUDOX
  - Found out in June this causes precipitation!
- A handful of teams requested more FITC
  - We only had enough for 1 tube / team
- Protocol problems with the FITC concentration
  - Fixed it but it was late in the process



# The Reality

- Many teams reported evaporation / no liquid in their tubes

- Cry

- Many

- Fou

- A hand

- We

- Proto

- Fixe

The end result for HQ:

- 46 teams requested and received new materials

- Protocol confusion and lack of clarity for teams

UDOX

ration



# Moving Forward

- Send dried down DNA instead of liquid
- Prepare plenty of extra materials for mistakes and problems
- Fully test the protocols before releasing them “into to the wild”
- Have everything finalized by February (rather than May/June...)

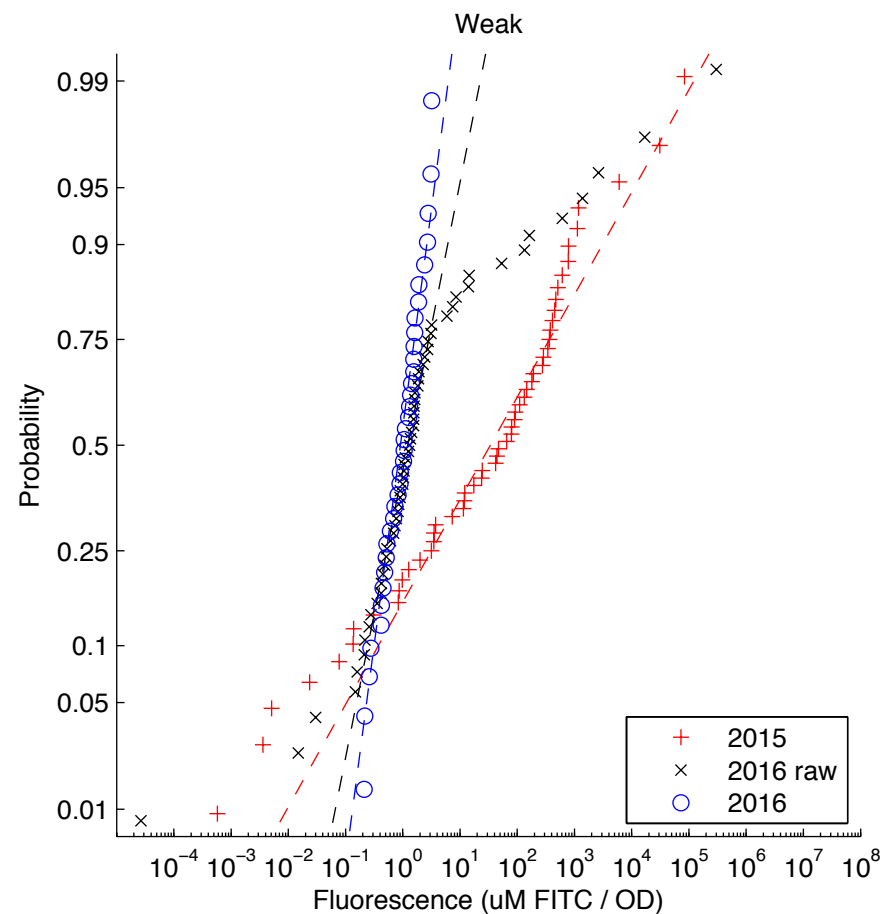
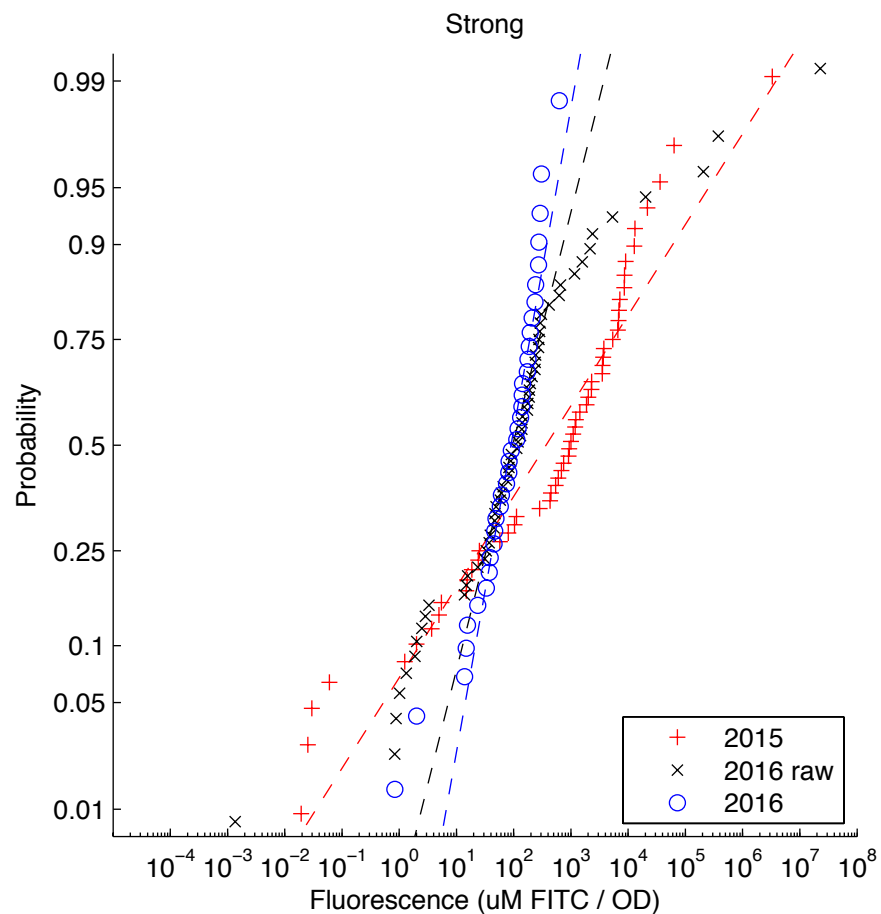




# Results!



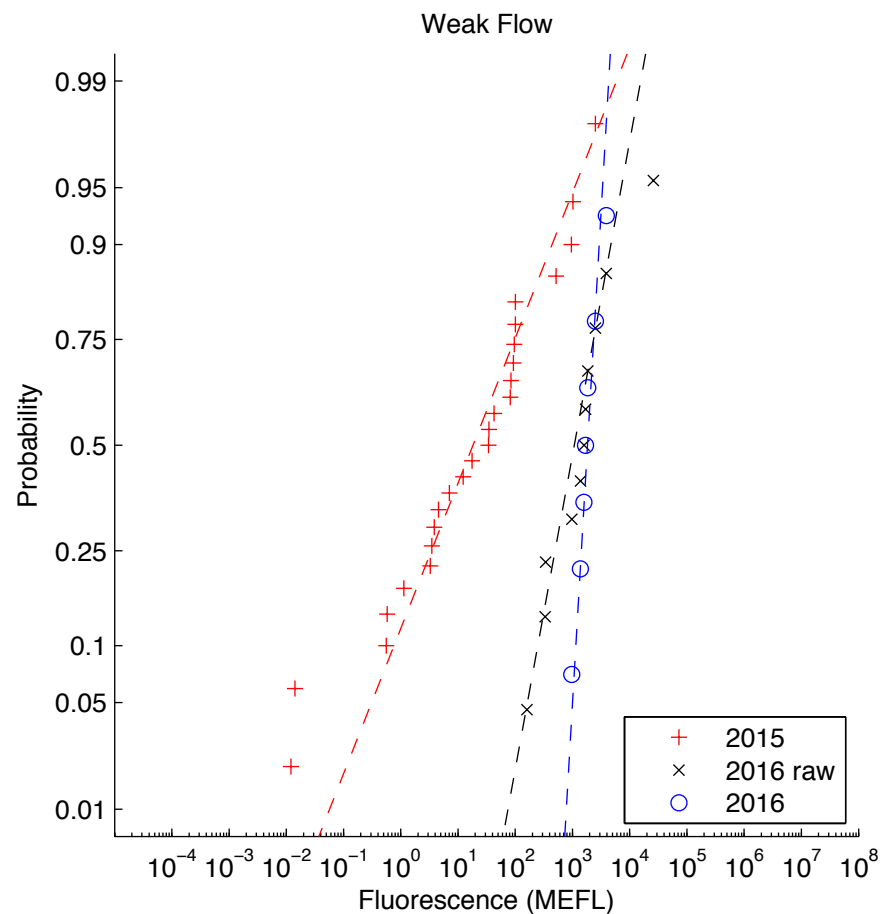
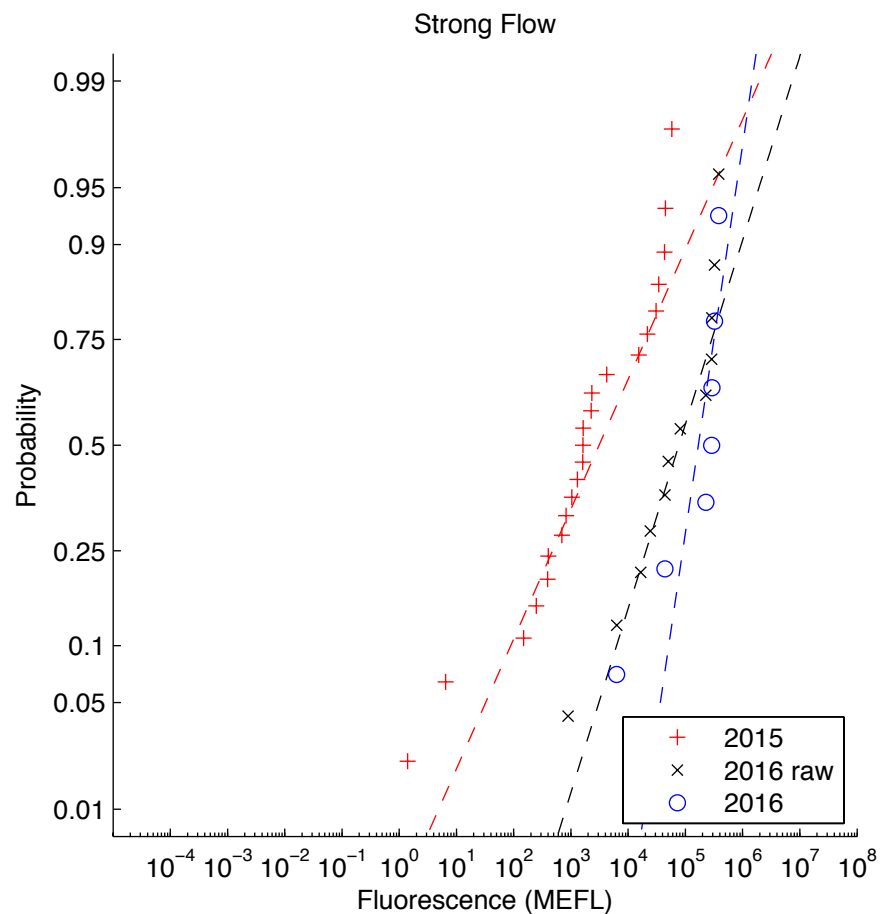
# Results: Measurement Comparison



***Calibration & controls help plate reader***



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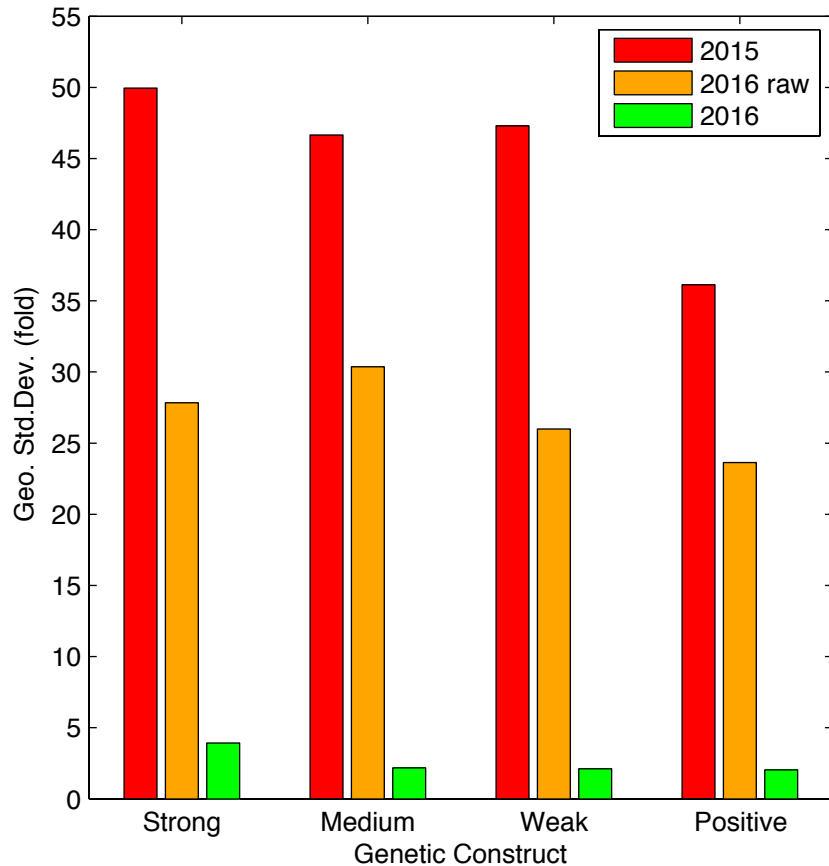
***Calibration & controls also help flow cytometer***



# UNITS MATTER

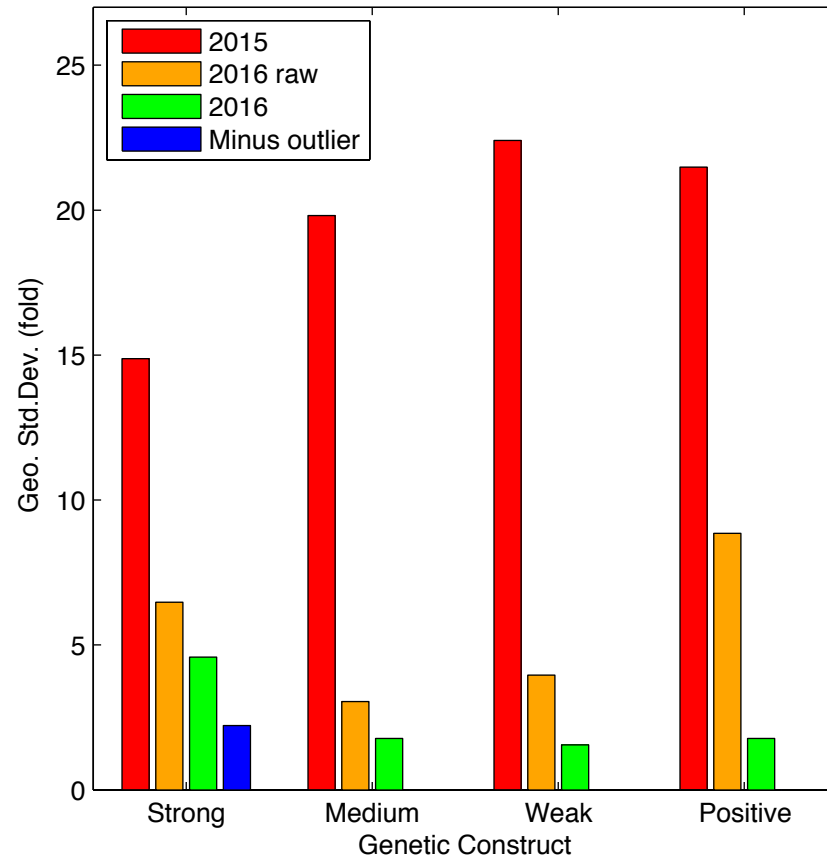
Plate Reader

Observed Variation



Flow Cytometer

Observed Variation

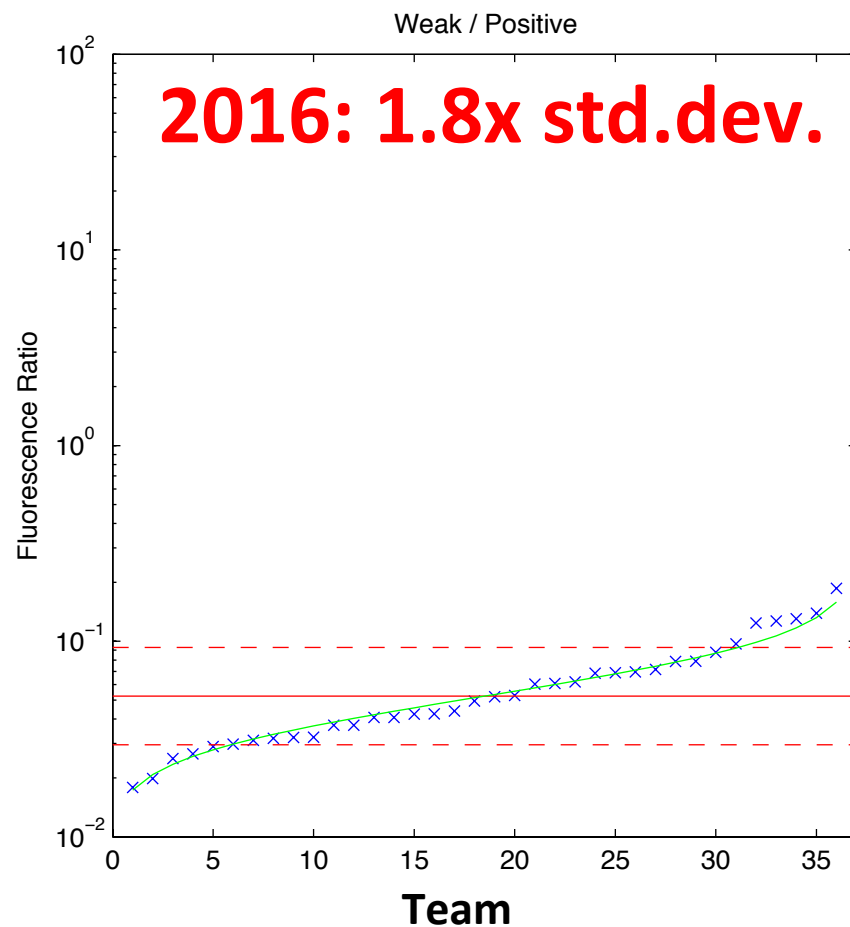
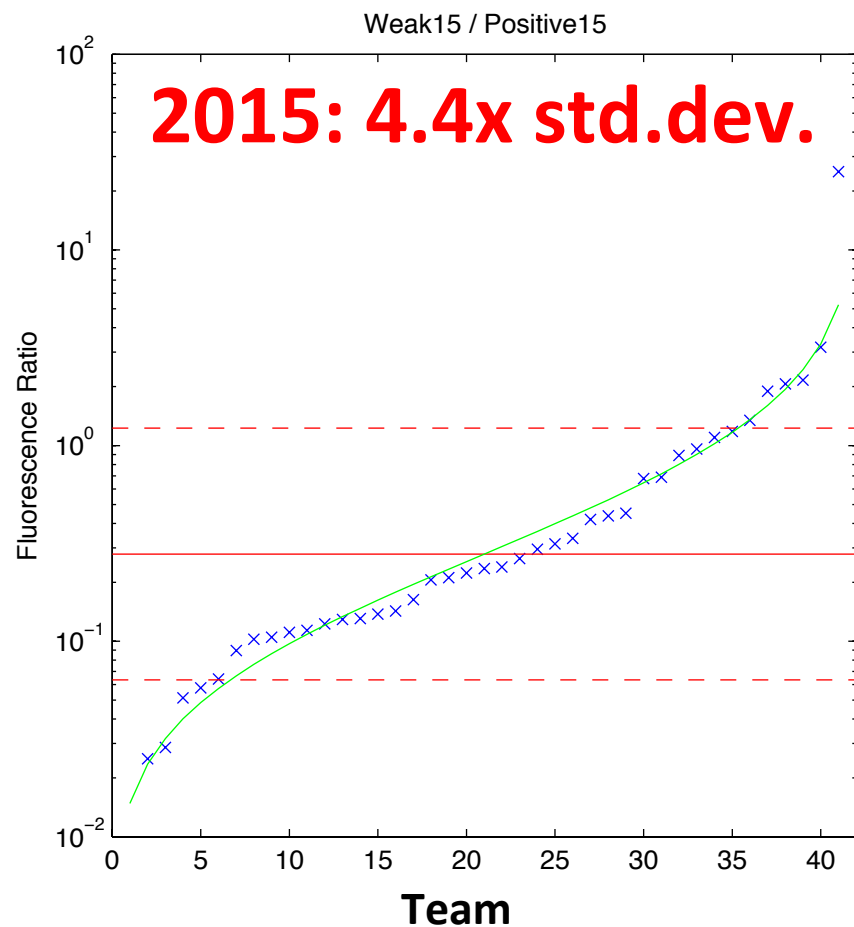


***Four to five orders of magnitude smaller range!***



# Results: Ratios in 2016 vs. 2015

## Plate Reader



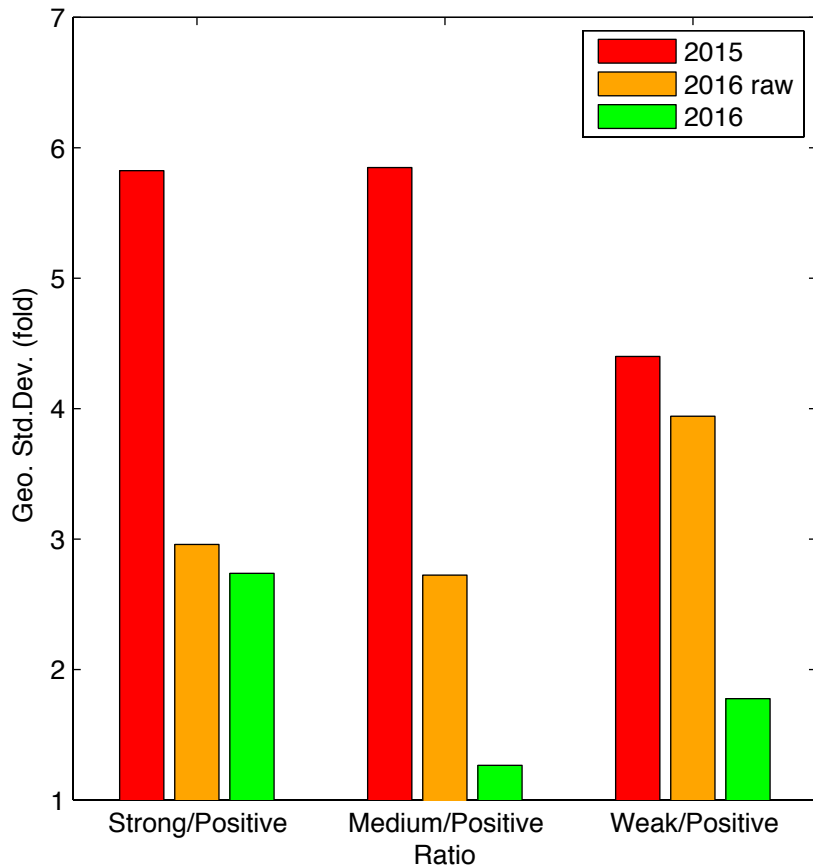
*Units really matter!*



# Without units, ratios cannot save you!

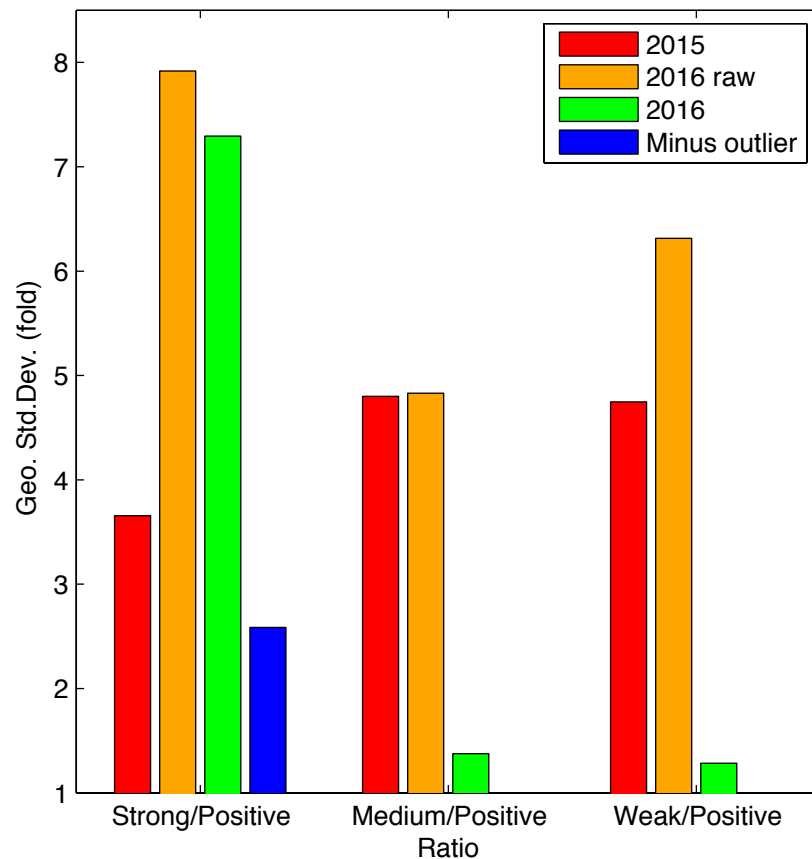
Plate Reader

Observed Variation



Flow Cytometer

Observed Variation



***Still one to two orders of magnitude better range!***





# Time for Feedback!

*What worked? What didn't work?*



*How can it be bigger and better next year?*