Global Biological Standards Institute®

Enhancing Data Reproducibility through Cell Authentication Training

ASPET 2017
Chicago, IL
April 22, 2017

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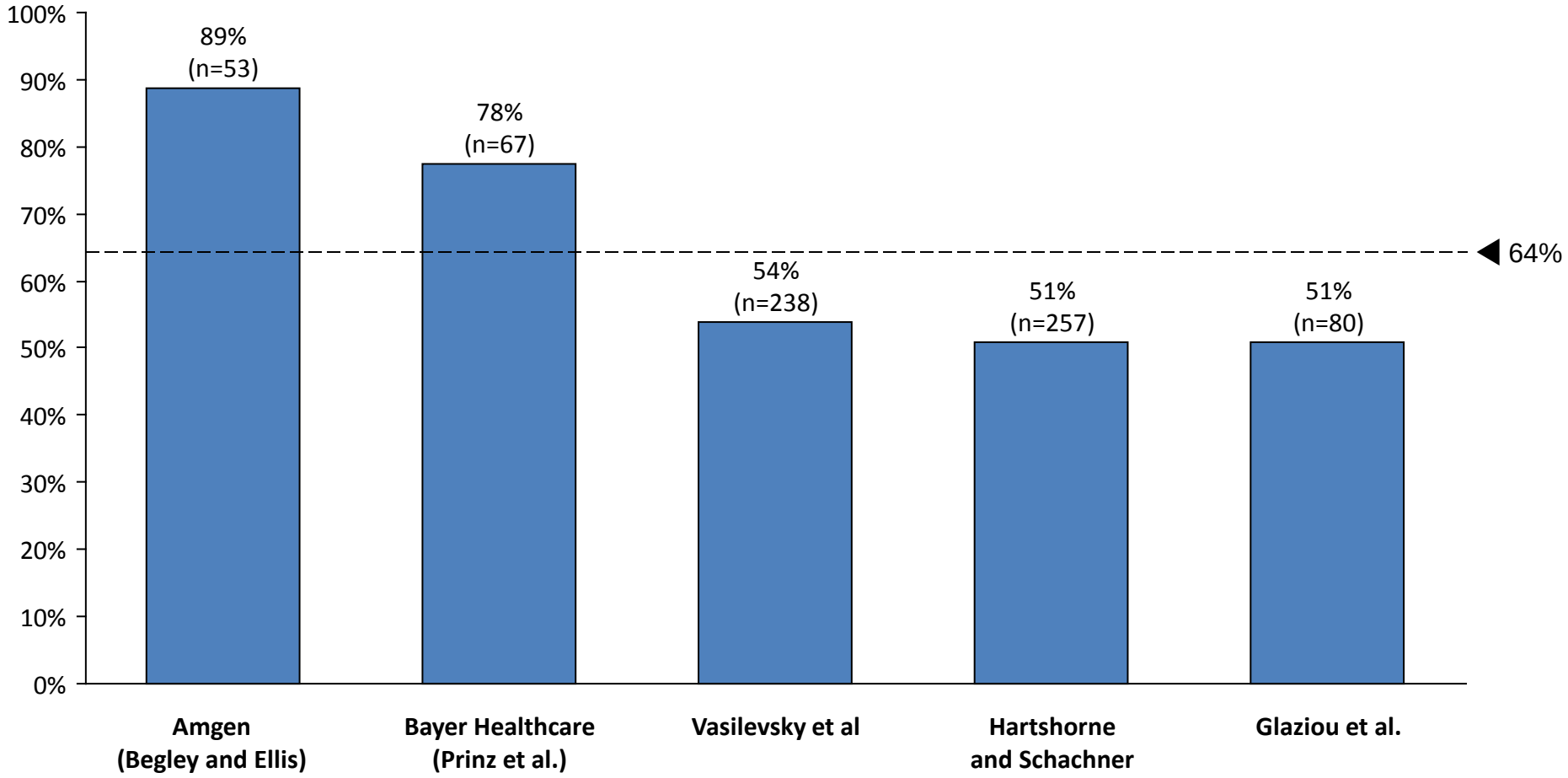
Data reproducibiity is a problem

Studies looking at the prevalence of irreproducibility estimate a rate of 50% or more

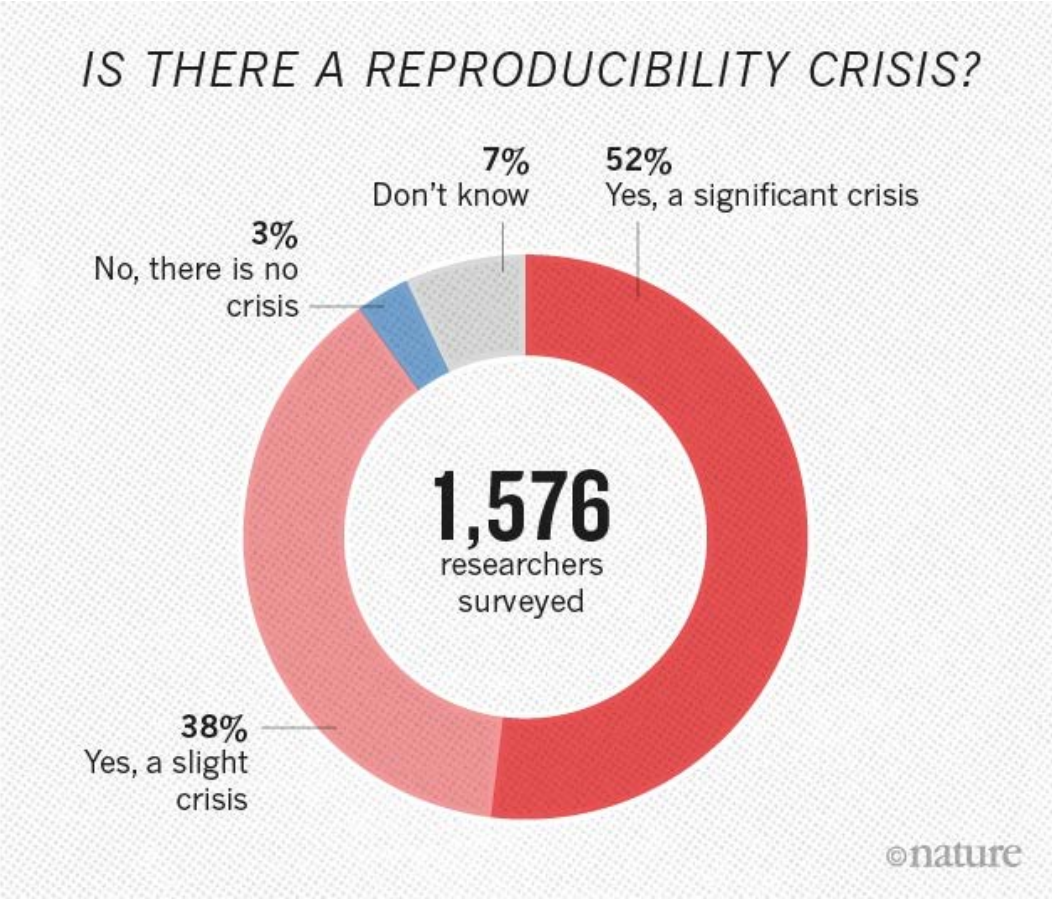


2016 Nature survey on reproducibility

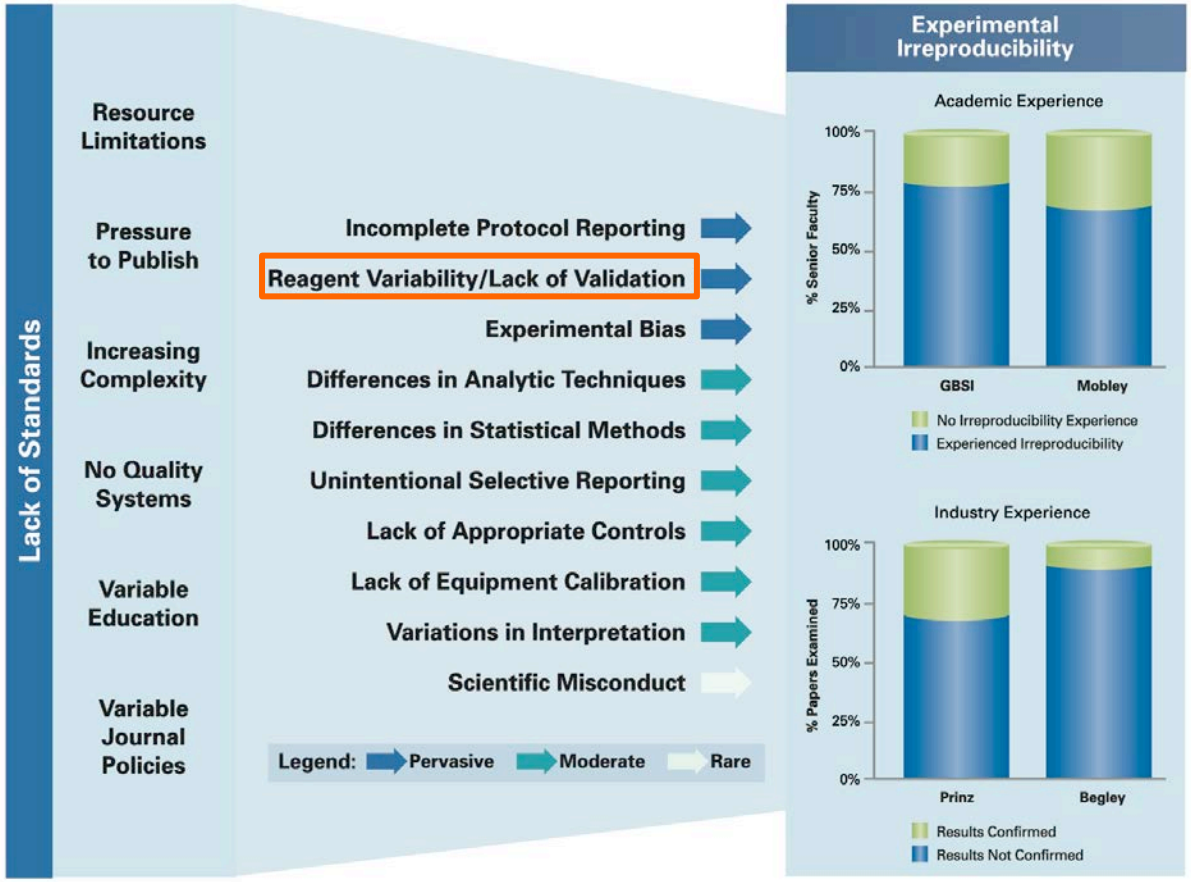
"1,500 scientists lift the lid on reproducibility"

"Survey sheds light on the 'crisis' rocking research."

- **Monya Baker**
- 25 May 2016



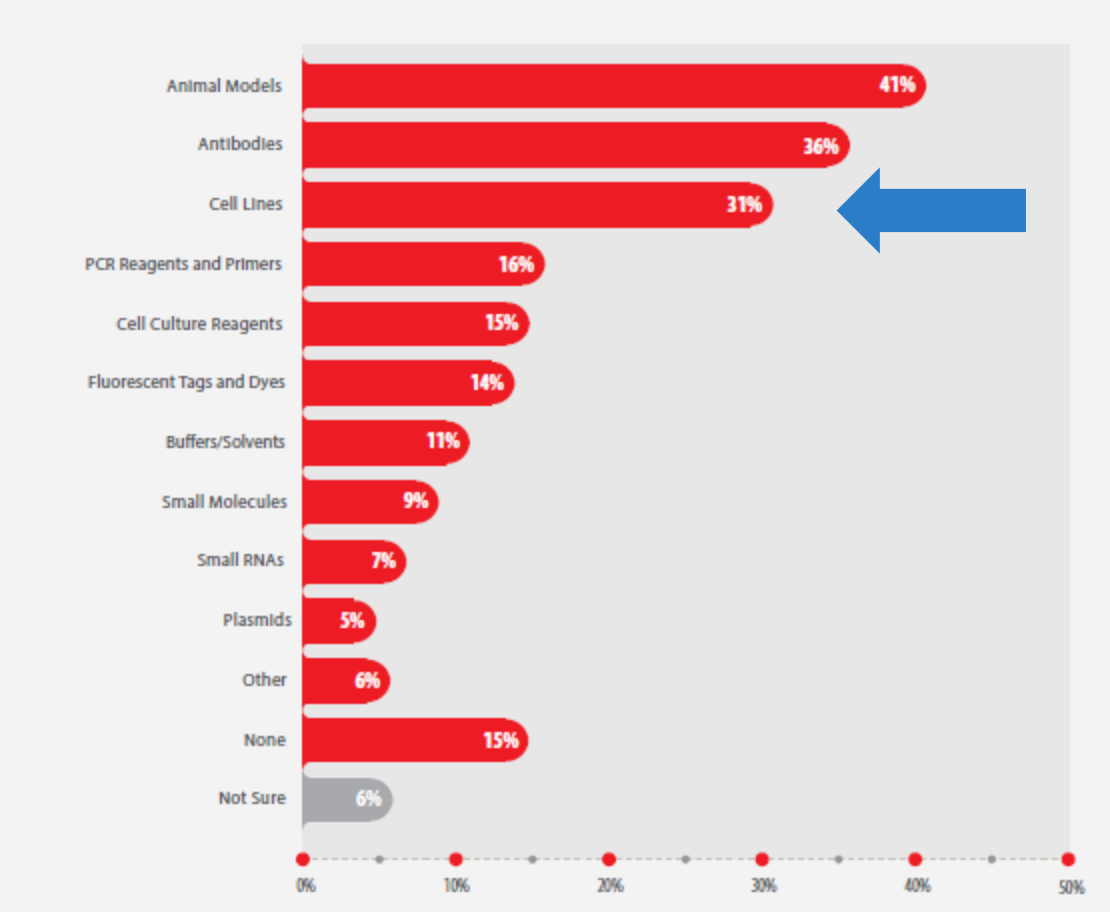
The causes of irreproducibility are complex



http://www.gbsi.org/publication/the-case-for-biological-standards/

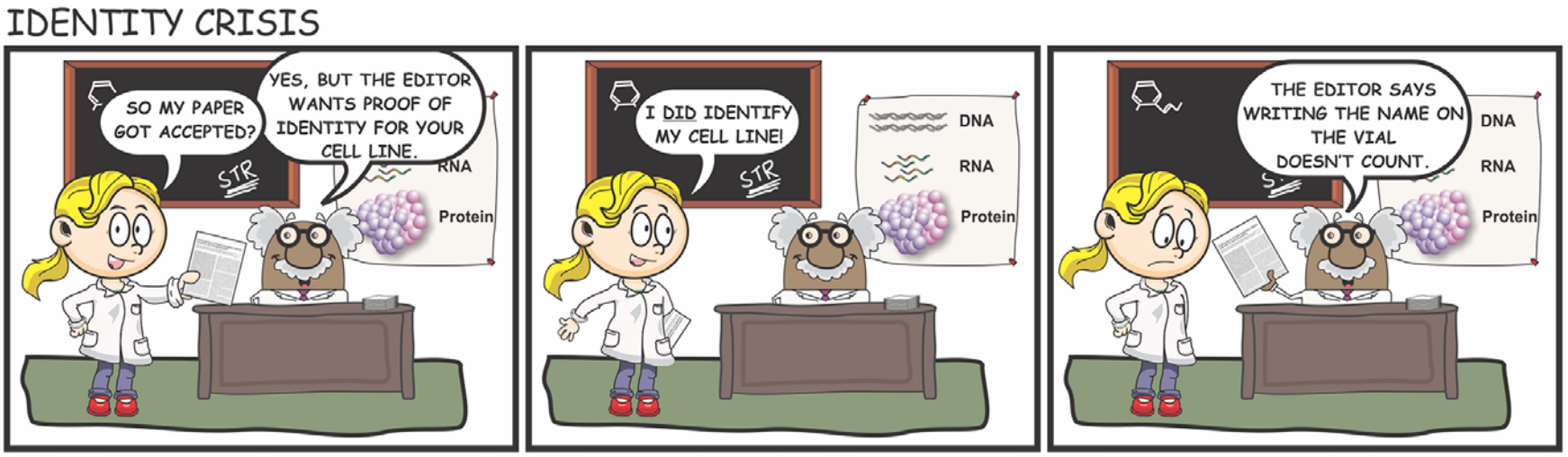
10: PROBLEMATIC PRODUCTS

Q: In the past year, what product groups caused you issues with reproducibility? (Check all that apply.)



The Scientist, 2016

Funding agencies and journals increasingly ask for information on how cell lines (and other key biological reagents) are validated



And for human cell lines, there's actually an approved standard for doing that…

STR cell authentication standard

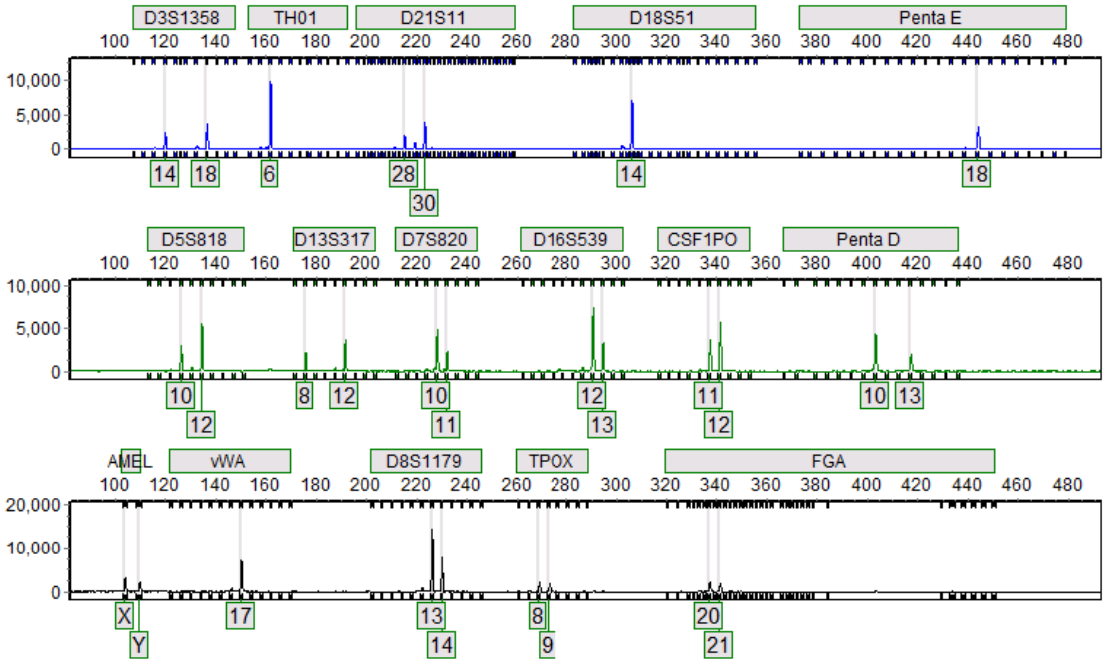
Multiple journals “strongly encourage” the use of cell line authentication

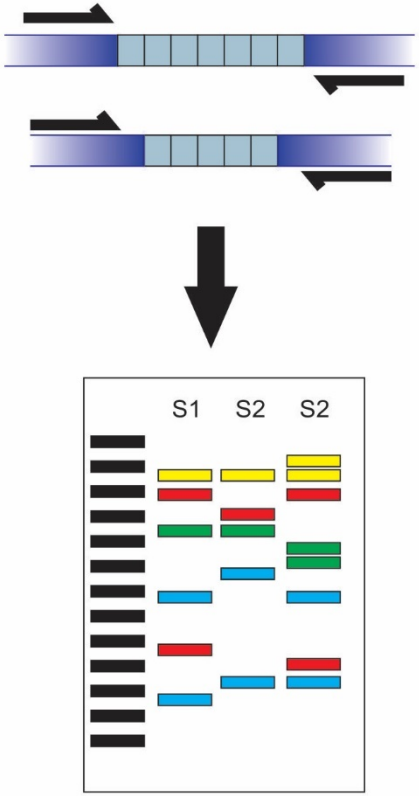
- Standard “ANSI/ATCC ASN-0002-2011: Describes human cell line authentication using STR
- Developed by an international group of experts from academia, major cell repositories, government agencies and industry
- Only one journal *requires* cell authentication

DNA profiling of cell lines: Short Tandem Repeats (STRs)

- Short, polymorphic sequences of DNA
- Often referred to as microsatellites
- 2-7bp
- Repeated multiple (~6-several dozen) times head-to-tail

  e.g. gtacgtacgtacgtacgtac





Species	Assays	Consensus Standard Method	Commercially Available Kit	Commercial Service	Comparative Data
Human	STR	ASN-0002	Yes	Yes	ATCC, DSMZ, JCRB, NCBI**
	SNP	No	Yes	Yes	[21], [32], NCBI
Mouse	STR*	No	No	Yes	Unpublished
	SNP	No	Yes	Yes	[19]
African green monkey	STR*	No	No	No	None
Chinese hamster ovary	STR*	No	No	No	None
Rat	STR*	No	No	No	None
Species-level identification	CO1 DNA barcode	ASN-0003	Yes	Yes	Barcode of Life Data System, NCBI**
	Species-specific primers	No	No	Yes	None needed

These methods are currently the most developed for this application. There are extensive data on human cell lines, but while there are some kits and services for some nonhuman species, there is little available data for nonhuman species, except for DNA barcoding, which only distinguishes cell lines on the basis of species, not individuals.

* STR markers have been identified [33,34]. Markers for rat and Chinese hamster ovary cells are still under development by NIST.

** These sources contain a significant amount of data from multiple sources. See text for URLs.

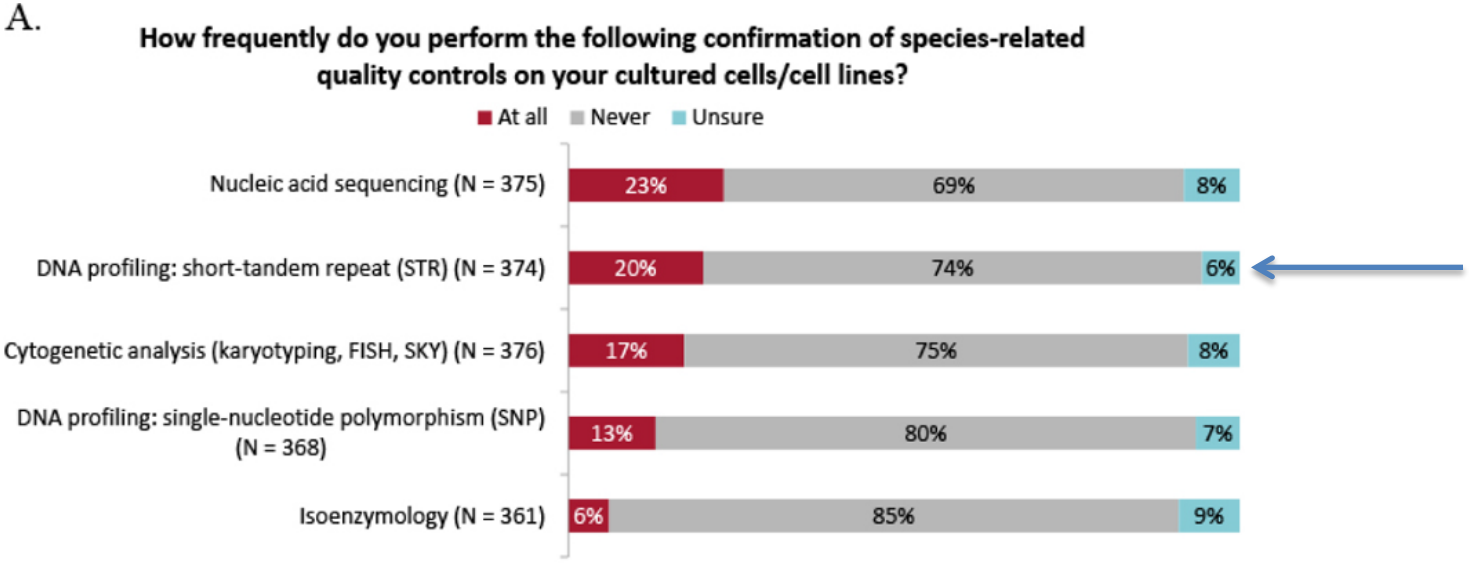
doi:10.1371/journal.pbio.1002476.t002

Do researchers standardly perform STR? Results from a 2015 Survey

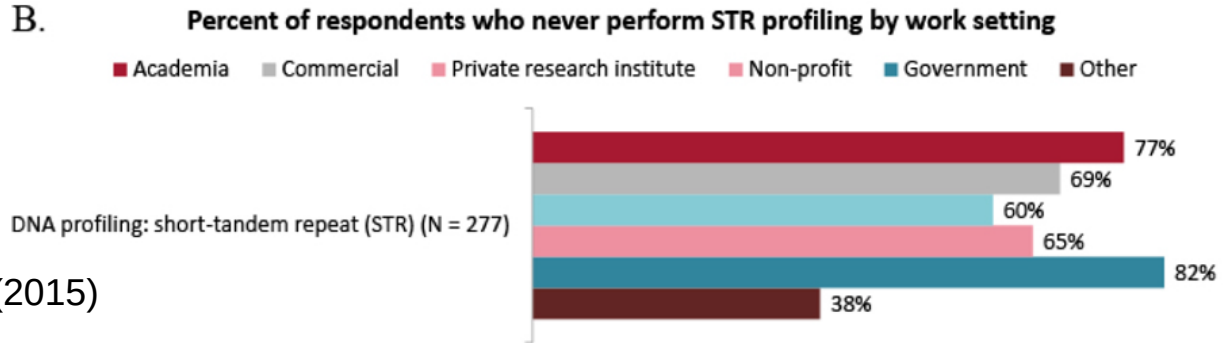
- *BioTechniques* 59: 189-192 (2015)
- Fielded for 30 days from May 6-June 5, 2015
- 446 total respondents
- Majority conducted basic research (91%) and worked in academia (79%), and used cultured cells on a daily basis (59%)

74% of surveyed researchers NEVER use STR profiling

A.

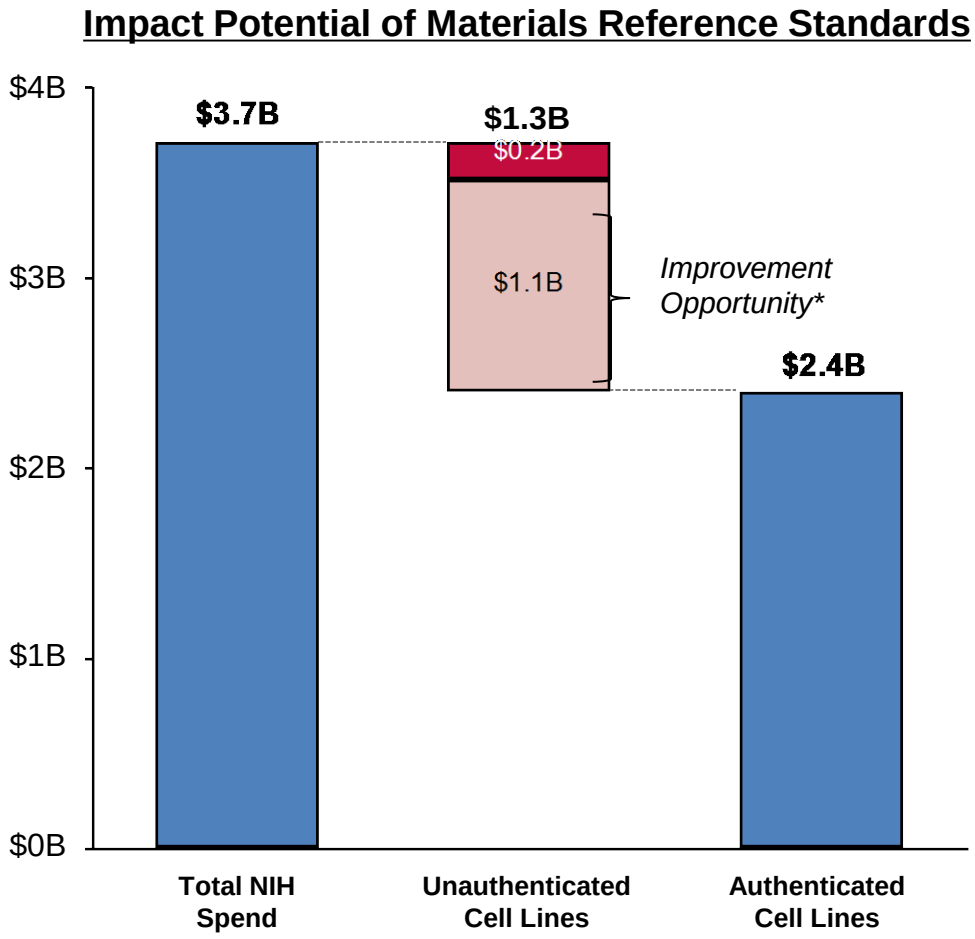


B.



BioTechniques 59: 189-192 (2015)

Potential $ impact on cell-based research



Financial Impact: Authenticated Cells

- NIH spends **$3.7B** on cell-related basic research
- **15%–36%** of cell lines misidentified **(cost ~$1.3B)**
- Standards Impact: would reduce use of misidentified cells to **5%–10% (cost lowered to ~$0.2B)**
- NIH would have more effective use of up to **$1.1B…**
- …And would **speed research progress** and development of new treatments

*Note: Assumes that effective cell line validation could reduce classification and contamination errors by 80%

So we decided to create a training module on cell authentication

“Enhancing data reproducibility through cell authentication training”

- Funded R25 training grant, with additional support from Susan G. Komen
- Goal: MOOC-style training module that can be used entirely online or in blended learning formats (within, for example, RCR training)
  - *Sends students back to the lab to perform cell authentication and assess lab practices*
- Year 1: pilot with live training
- Year 2: create online version and pilot
- End of Year 2: post, share, and disseminate online version (with teaching tools)

Year 1: Pilot with live training

- UCSF and UNM
- Two workshops with homework in between
  - 1st workshop: lecture on cell authentication and good cell culture practice in context of rigor and reproducibilty; work with sample data
  - Homework: back to lab to evaluate cell culture practice and prepare and send sample for cell authentication
  - Receive data from cell authentication for analysis prior to 2nd workshop
  - 2nd workshop: discuss data from cell authentication, and the need to change cell culture and authentication practices; role play negotiating with/persuading PI to change practice

Agenda for Session 1

Scientific reproducibility

History of misidentified cell lines (MI)

NIH Policies

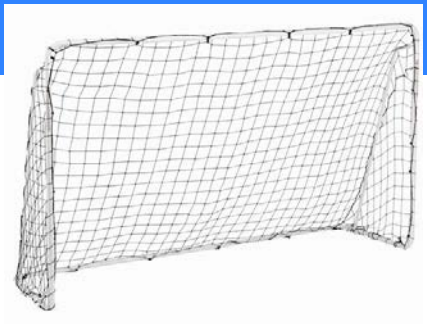
Causes and prevention of MI

Quality controls tests for cell lines (STR, SNP, mycoplasma)

Good Cell Culture Practice (GCCP)

Feedback / Discussion

Goals



- Cell authentication is important
- STR profiling know how
- Obtain your own STR data
- Encourage GCCP

Agenda for Session 2

Analyze STR profiling results

Discuss your lab CCPs

Enable you to encourage changes

IMPORTANTLY, get your feedback

Some takeaways from pilots

SUMMARY – including UCSF and UNM data

As a result of this training, knowledge of why and how to perform cell authentication improved. Participants also reported they feel sufficiently prepared to discuss these topics with colleagues, and their PI (See table).

	I feel knowledgeable about cell authentication techniques (N=41)	I feel sufficiently prepared to discuss these topics with colleagues (N=41)	I feel prepared to discuss these topics with my PI (N=25)
Pre-survey baseline	5%	41%	18%
Post-survey	83%	86%	100%

Application of material

Participants expressed the intention to include these tests in their personal cell culture practices:

- 36 of 40 (90%), incorporate DNA profiling (short tandem repeat) in their experimental design
- 32 of 40 (80%), include mycoplasma testing

Participants wrote in that they would incorporate better record keeping, and cell line testing or verification into their current personal cell line practices.

Workshop satisfaction

The workshop included a lecture, class activity (STR test kit), and difficult conversations portion.

- 35 of 40 (88%) participants found the lecture interesting and 62% thought the amount of time was appropriate (with about half saying the lecture was too long and half saying the lecture was too short)
- 22 of 26 (85%) participants found the activity interesting and 100% thought the amount of time was appropriate. One participant commented that they didn't realize how easy it was to perform STR.

Participants rated "performing STR" and "obtaining and sharing results from the STR" as the most useful parts of the workshop.

For the section on difficult conversations, 15 of 26 (58%) participants agreed that after attending the session, they were more comfortable having difficult conversations in general. The survey did not ask about the participants' interest in the material.

Some highlights

- Trying STR for themselves and learning how easy it is
- Faculty telling stories of their own problems with cell line misidentification or contamination
- Learning about the database of misidentified cell lines
- Case of misidentification during workshop – from an expert cell culturist
- Trainees teaching each other about good practices

6 months out…(yes, very small sample size)

- 6/7 respondents have recommended changes to lab practices related to cell line authentication
- 6/7 have discussed cell authentication with colleagues
- 7/7 interested in learning about new cell authentication techniques
- 7/7 would like to have access to an online version of the training

Year 2 (where we are now)

- Videos under production
  - Reproducibility in context
  - Stories of cell line misidentification
  - How to: Good cell culture practice
  - How to: Prepare samples for cell authentication
- Blended learning pilot
  - Rigor and reproducibility class inside RCR
- Quizzes and other interactive features
- Load full module onto LMS and pilot
- Disseminate with help of Society partners

National Institutes of Health

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susan G. komen.

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