

# NIST Genome Editing Consortium

## Overview

October 6, 2021

AEIC Fall meeting

# The value of Standards



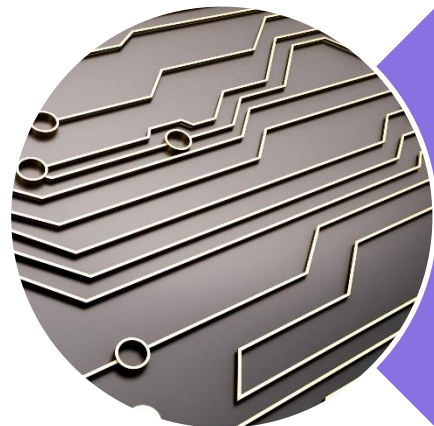
# Standards are Enabling and Empowering! **NIST**



Validity of data

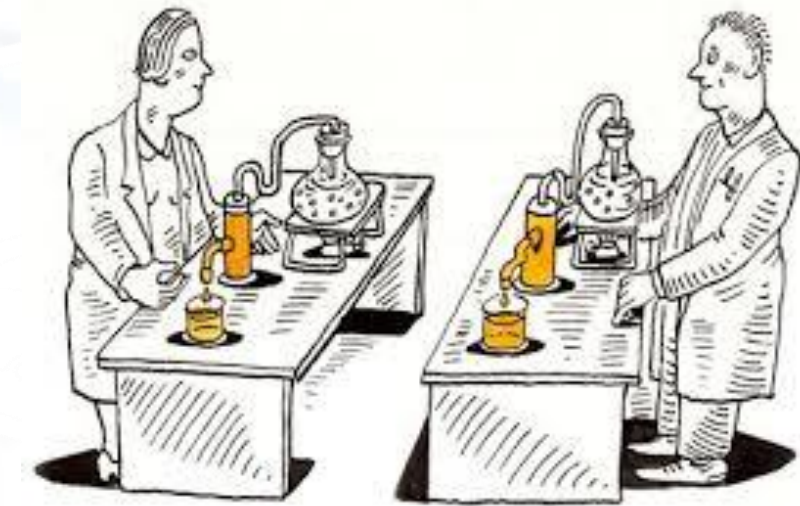


Confidence in and  
comparability of data



More rapid, integrated  
technology development

- ✓ Validity of data
- ✓ Confidence in and comparability of data
- ✓ More rapid, integrated technology development





# Reproducibility $\neq$ High Confidence

Just because a value can be reproduced does not mean you can be confident in the measurement



Sources of pre-analytical/analytical variability must be accounted for in order to appropriately tease out and attribute true biologic signal



How confident are you that the answer  
is correct?

What is the consequence if it is wrong?

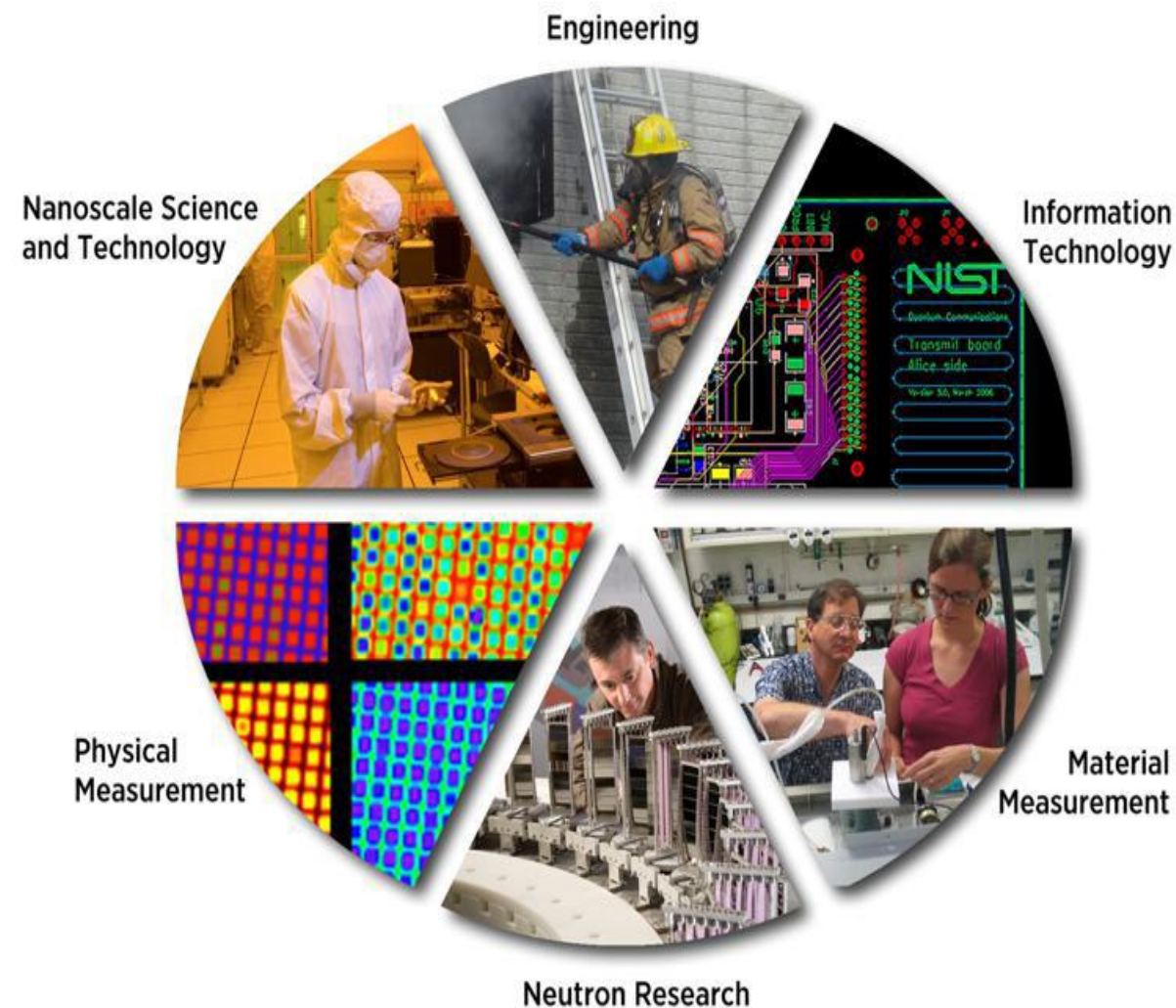
...What can be done to understand if the  
answer is correct?

# NIST – National Institute of Standards & Technology



## MISSION

To promote U.S. innovation and industrial competitiveness by advancing **measurement science**, **standards**, and **technology** in ways that enhance economic security and improve our quality of life



### The U.S. National Metrology Institute

Global harmonization of measurement and traceability to the SI

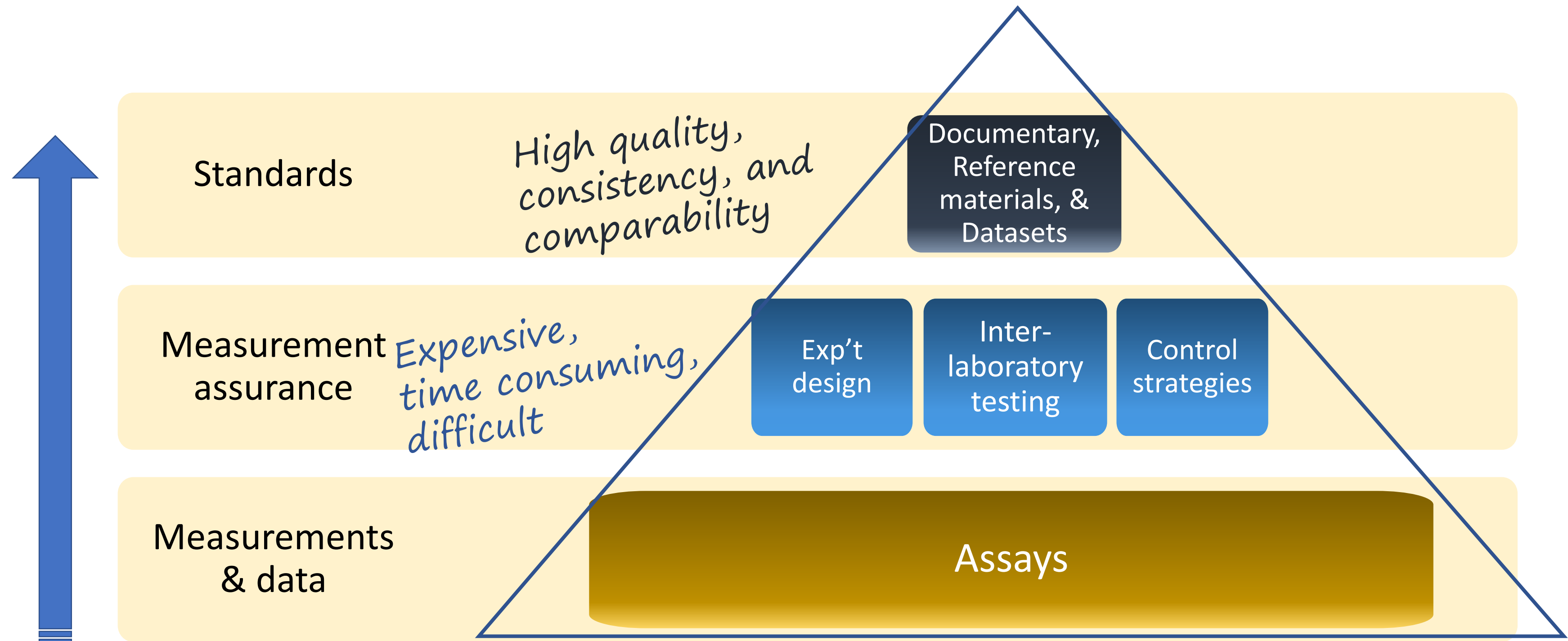
### “Industry’s National Laboratory”

Non-regulatory agency partnering/serving industry to help maintain US leadership in science and technology products

### Department of Commerce

Developing standards to support international trade and commerce

# Advanced Biometrology: Measurement Assurance



Demonstration of measurement assurance strategies:

<https://www.nist.gov/mml/bbd/measurement-assurance-strategies>

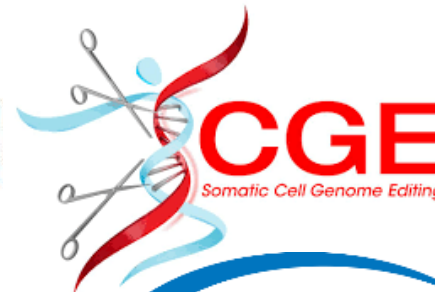


# How does NIST work with communities to meet needs?

- NIST one-on-one collaborations: Academic, Other gov., Industry
- NIST led consortia
- NIST coordination with international measurement institutes and organizations

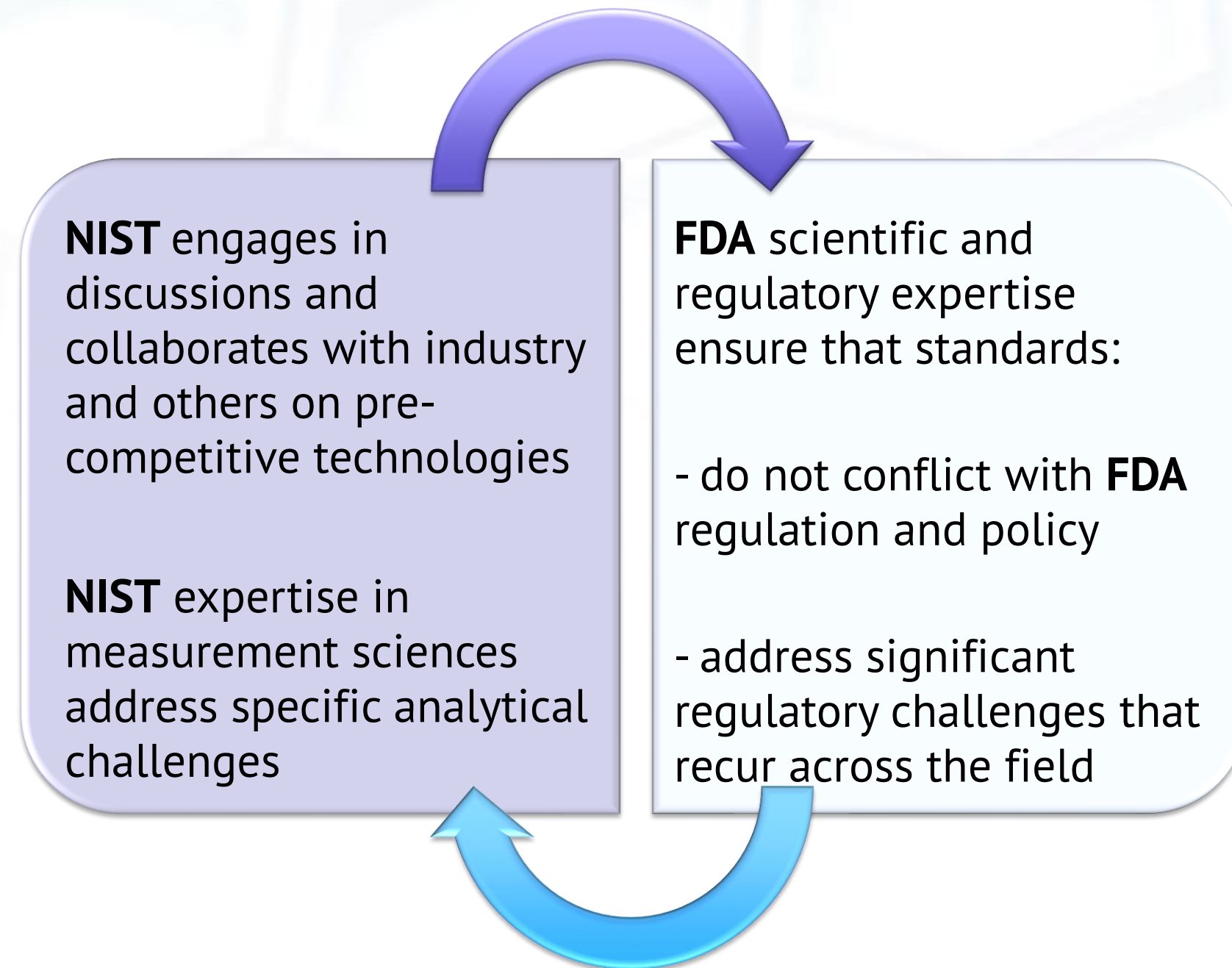


Technology Development  
Data/Measurement Quality  
Standards



# NIST-FDA Collaborations on Standards

Leveraging unique expertise



Cytotherapy

Volume 20, Issue 6, June 2018, Pages 779-784



Reports

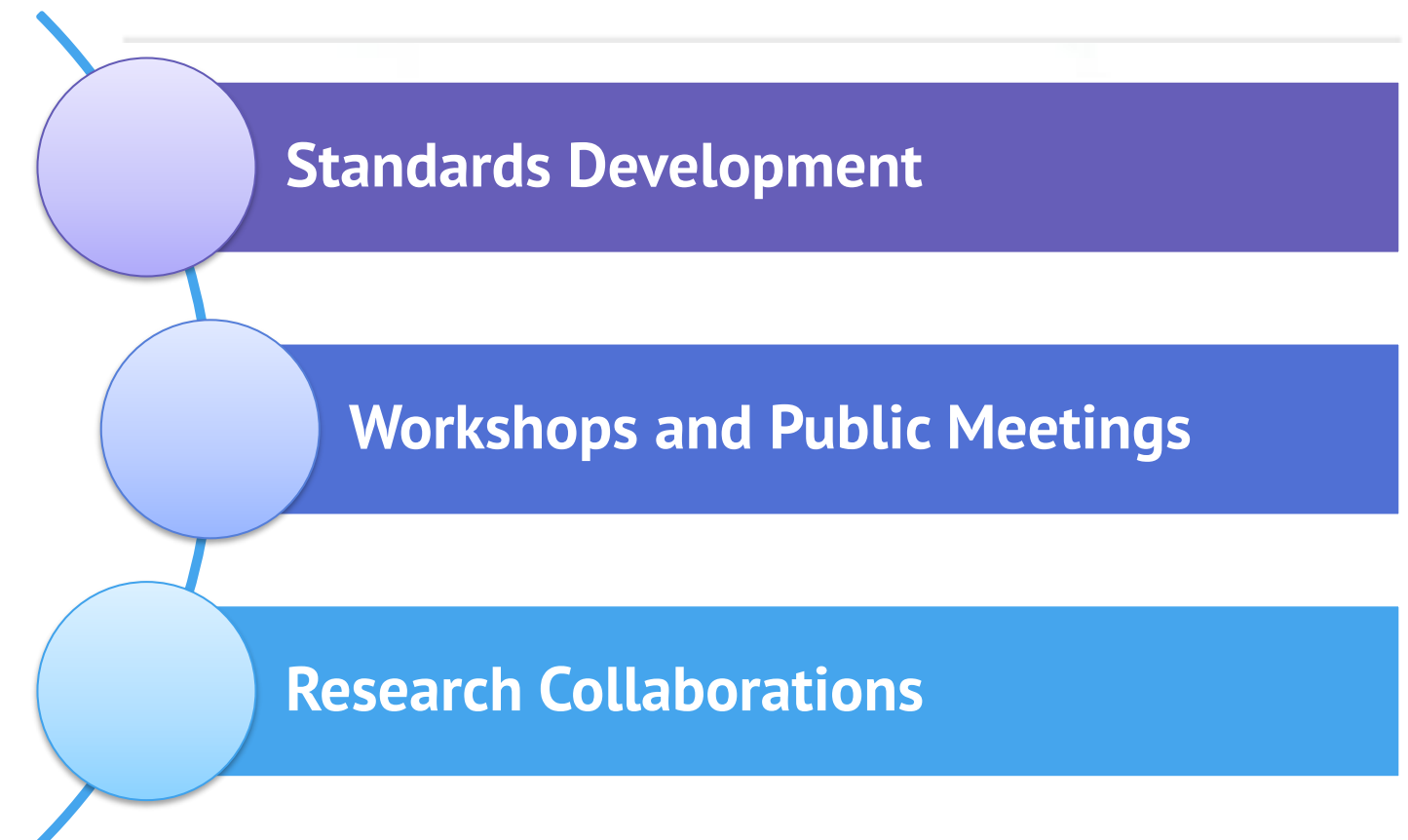
FDA and NIST collaboration on standards development activities supporting innovation and translation of regenerative medicine products

Judith A. Arcidiacono <sup>1</sup> ✉, Steven R. Bauer <sup>1</sup>, David S. Kaplan <sup>2</sup>, Clare M. Allocca <sup>3</sup>, Sumona Sarkar <sup>4</sup>, Sheng Lin-Gibson <sup>4</sup>

[Show more](#)

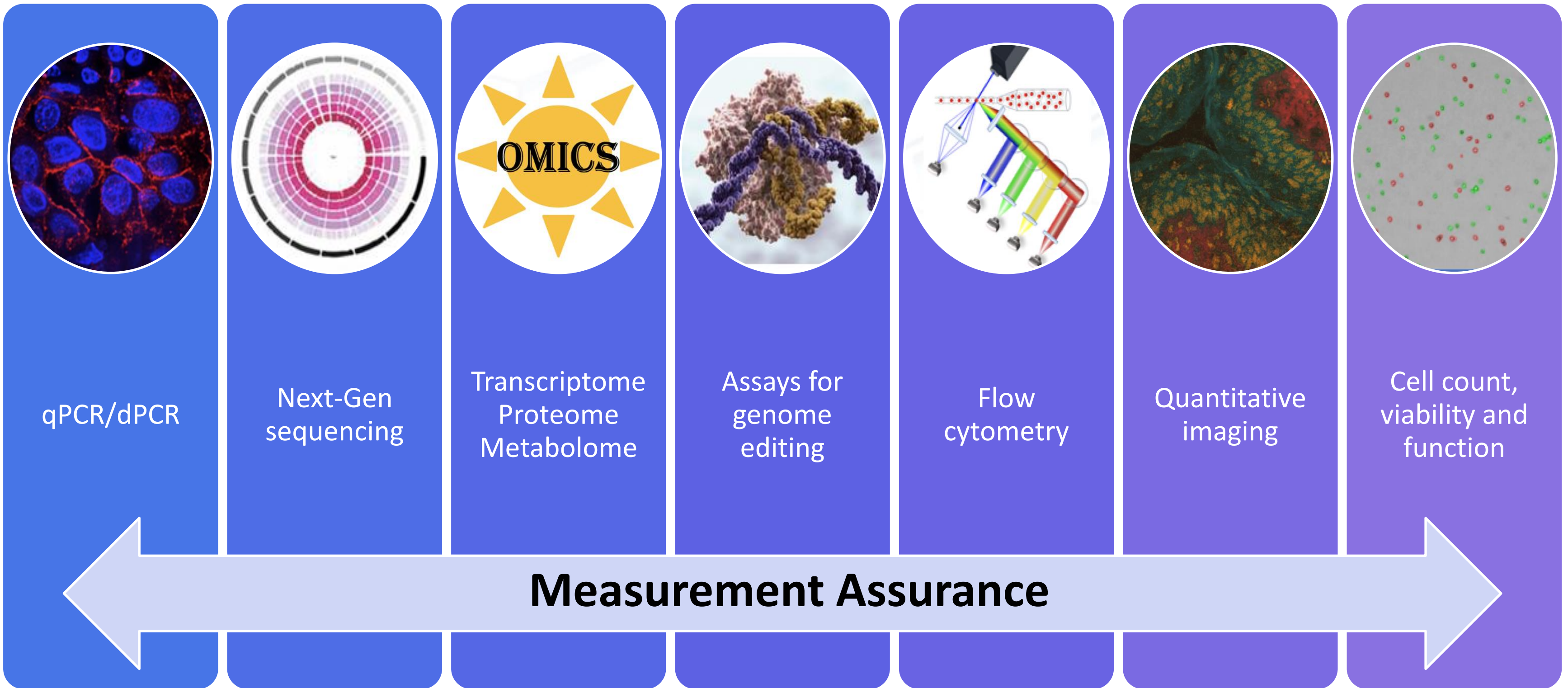
<https://doi.org/10.1016/j.jcyt.2018.03.039>

[Get rights and content](#)





# Select Measurement platforms





# Advanced biological reference materials



Genome in a  
Bottle (GIAB)  
DNA\*



Cancer Biomarker  
EGFR and MET\*



NISTmAB

\* Currently available  
\*\* High value material donation  
via MTA from an industry leader



Mixed  
pathogen DNA



Lentiviral  
vector\*\*

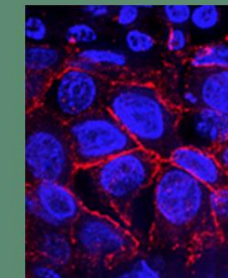


RMs for flow  
cytometry and  
imaging  
measurements

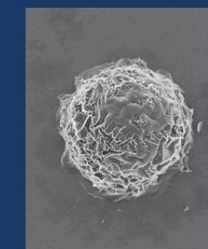
## Advanced “living” reference materials



Genome in  
a Bottle  
(GIAB)  
cells\*



Fully consented  
cancer & normal  
cells



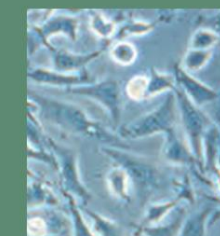
Jurkat cells  
with different  
VCNs\*\*



Genetically  
tagged strain  
of yeast



Microbial  
whole cell  
RMs



NIST\_CHO

# NIST Consortia



## GENOME IN A BOTTLE (GIAB) CONSORTIUM

Provides authoritative  
characterization of  
benchmark human  
genomes

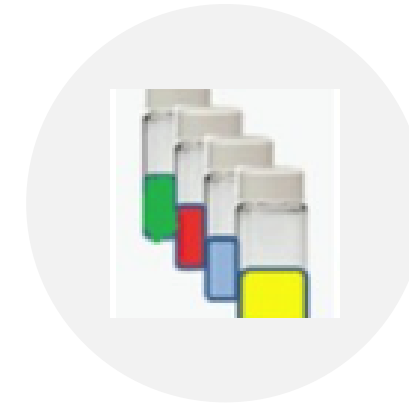
*POC: Justin Zook*



## GENOME EDITING CONSORTIUM

Addresses the  
measurements and  
standards needed to  
increase confidence and  
lower the risk

*POC: Samantha Maragh*



## FLOW CYTOMETRY STANDARDS CONSORTIUM

Addresses the  
measurements and  
standards needed for  
flow cytometry  
applications

*POC: Lili Wang*



## RAPID MICROBIAL TESTING METHODS CONSORTIUM

Addresses the  
measurements and  
standards needed to  
increase confidence and  
lower the risk

*POC: Nancy Lin*

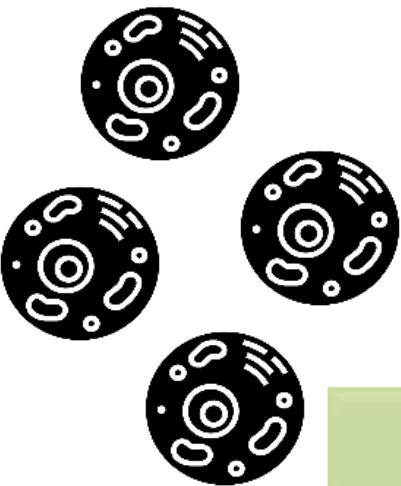
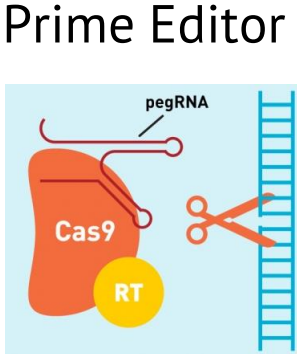
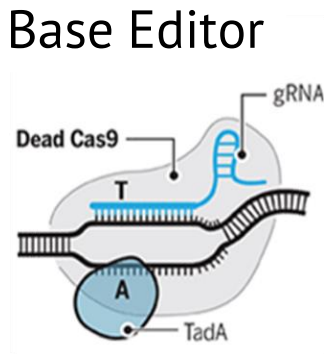
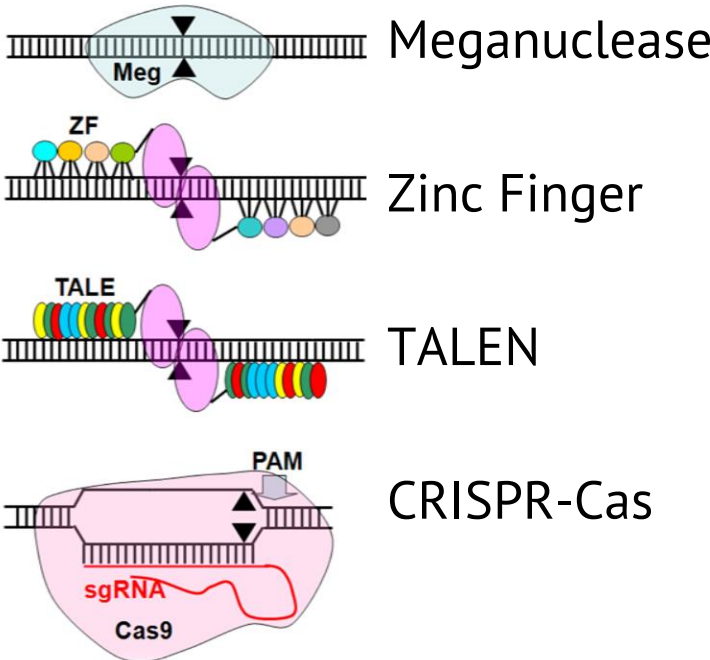
# Genome Editing overview



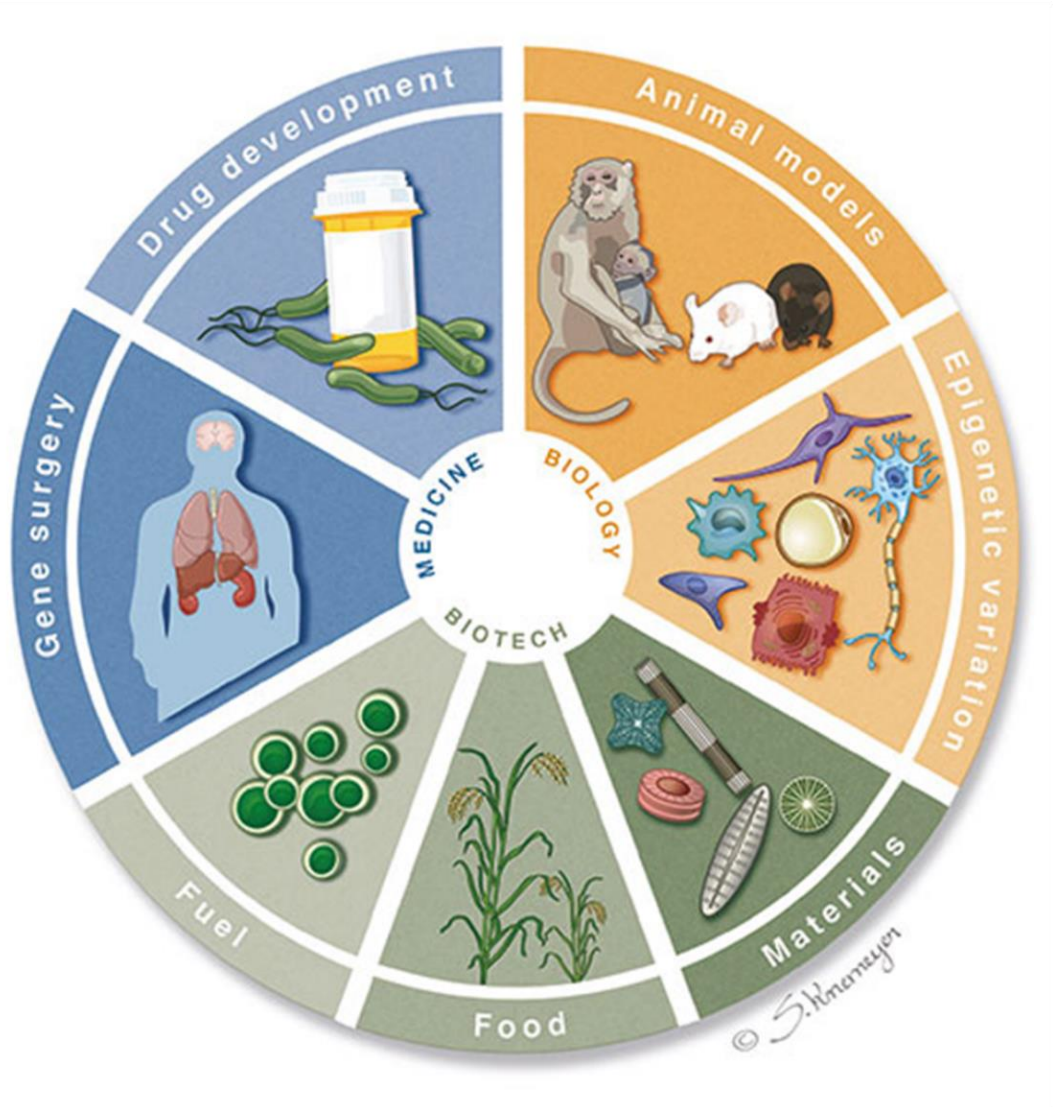
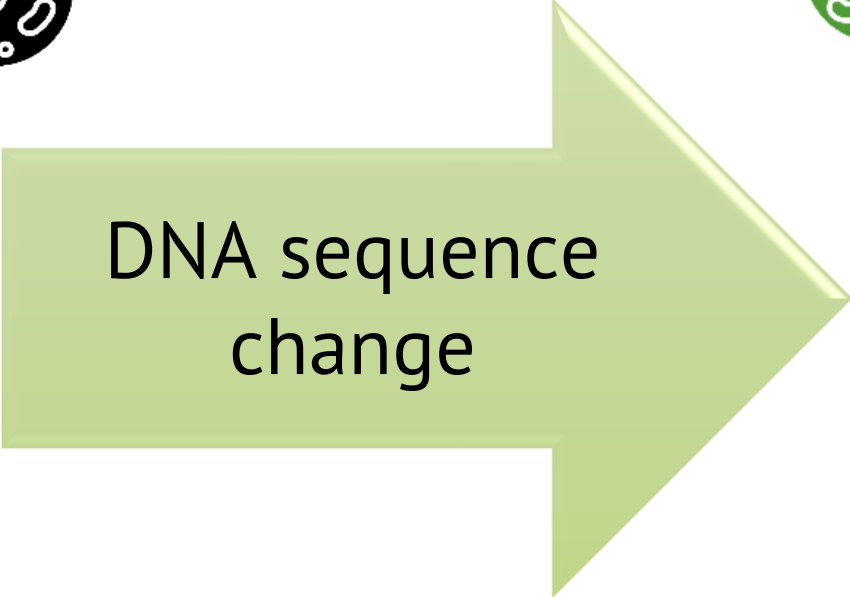
# Gene Therapy & Genome Editing



Viral vectors (various types)



+



Genome Editing/Gene Therapy Molecules

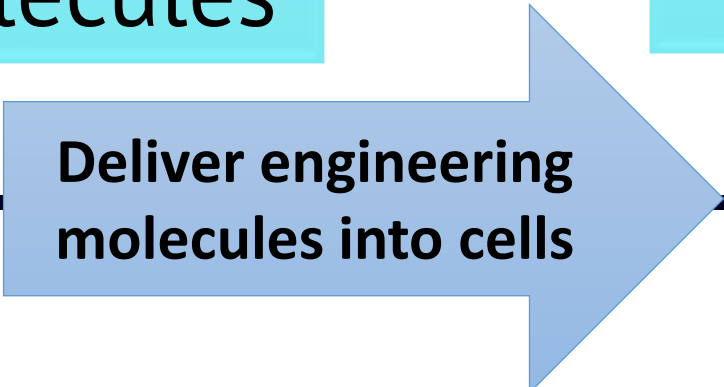
+

Cells of interest (*in vivo* or *ex vivo*)

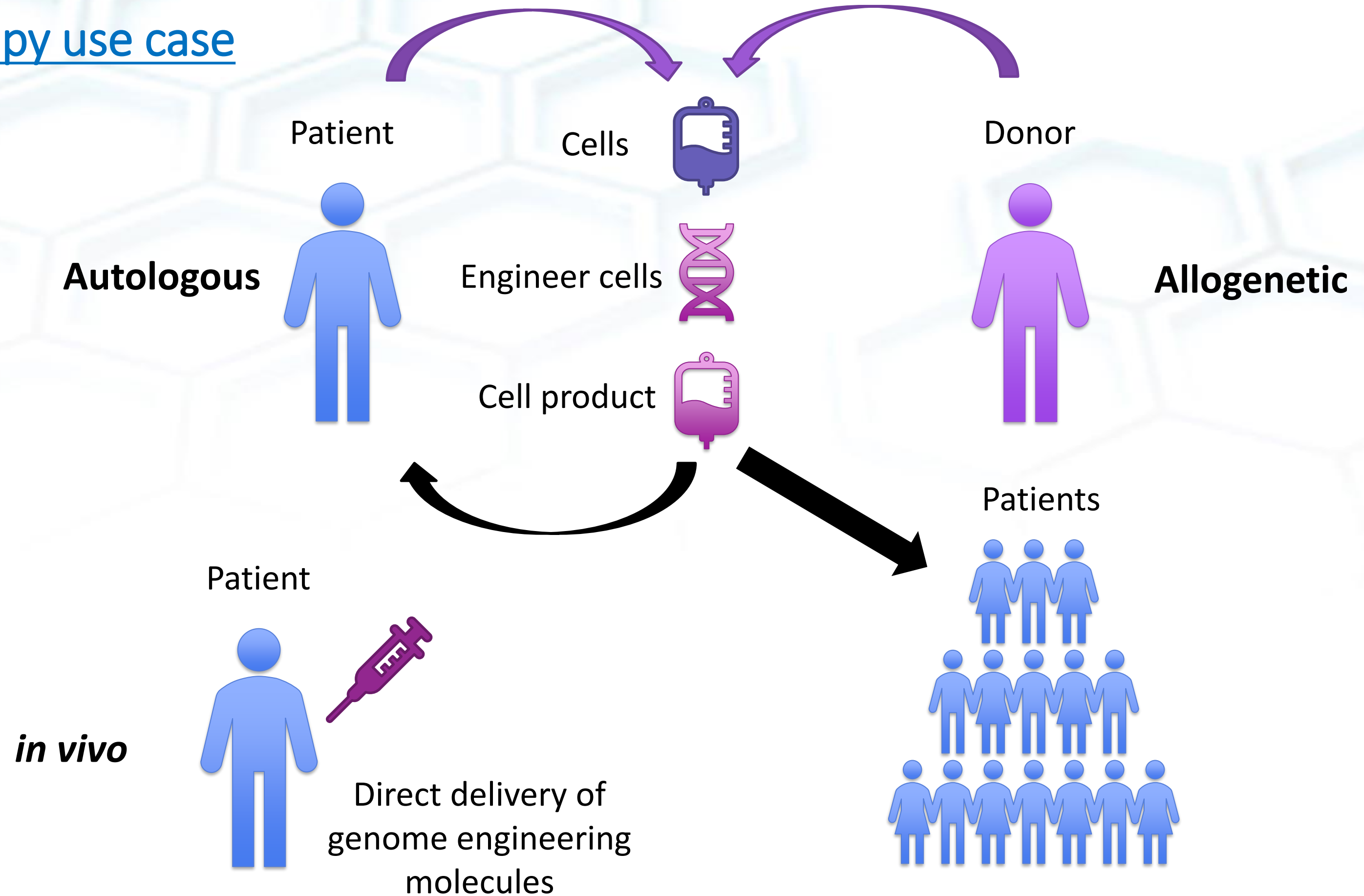
=

Engineered Cells

Fit for purpose?

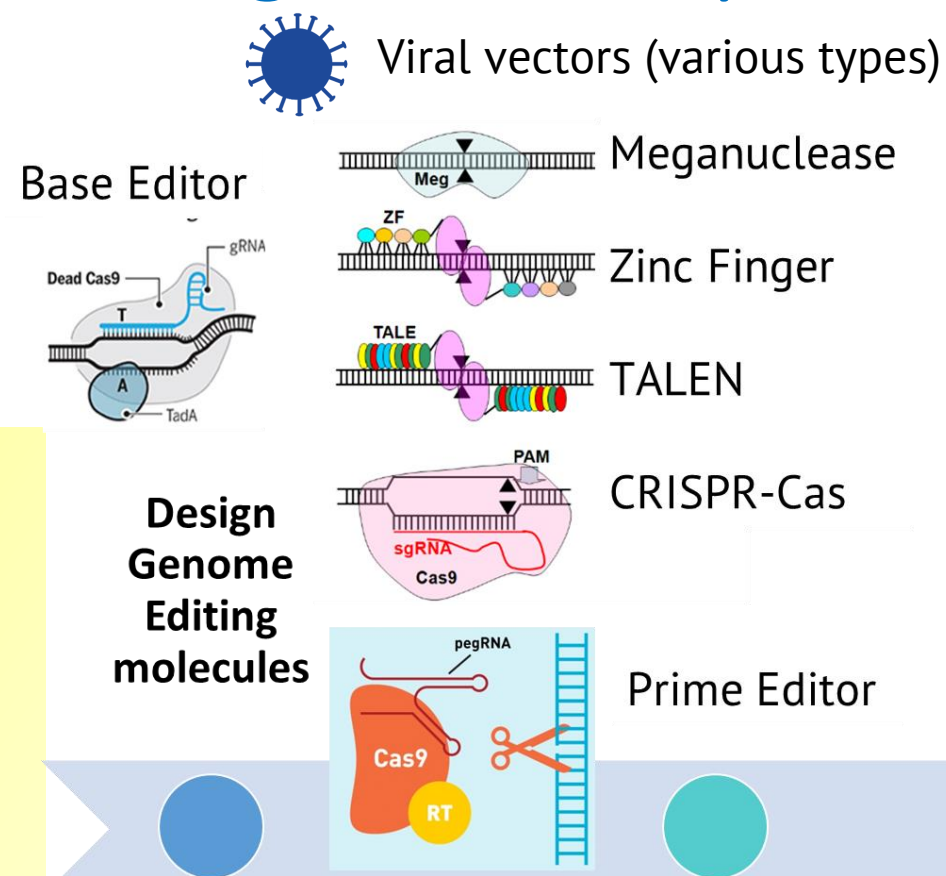


## Gene Therapy use case





# Standards needs identified by the Genome Editing Community



## Where may there be off-target activity?

- Assays to detect where editing molecules cut/nick DNA
- Demonstration these assays are reliable to report nominated off-target genomic positions

## Mechanism of Action

DNA double strand break  
Deamination + DNA single strand break/nick



## Confirm DNA alteration

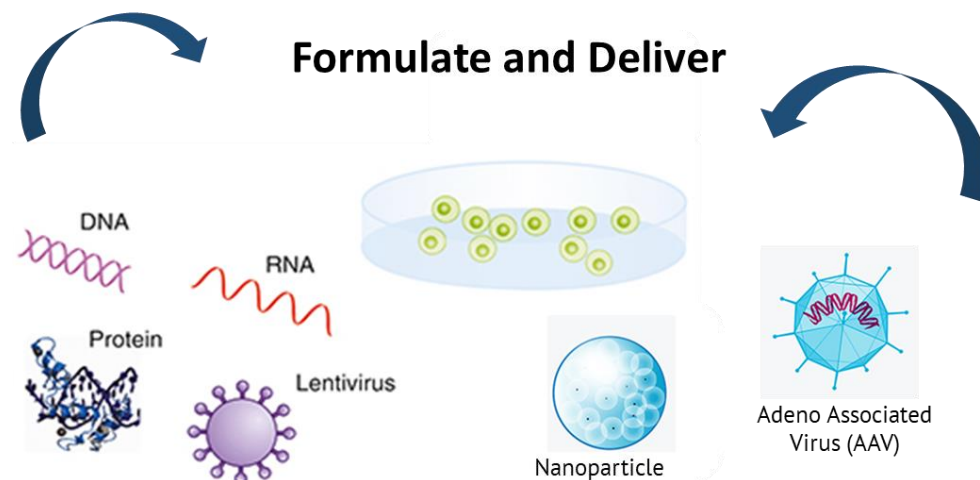


Determine fit for purpose

**Durability of edit(s), cell characterization, assessment of immune response**

## Genome editing components considered critical for manufacturing!

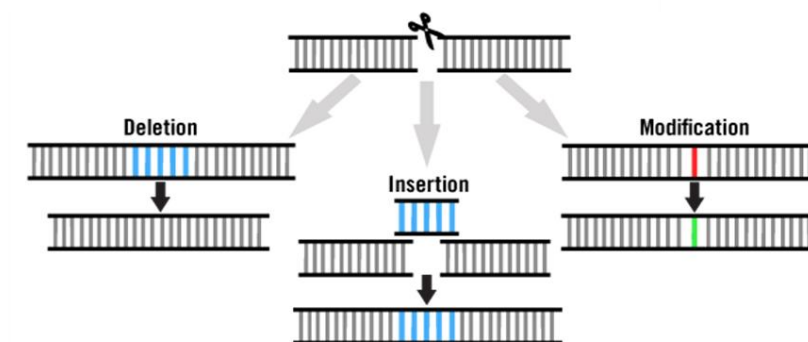
- cGMPs should be followed during manufacturing
- Components should be tested (identity, purity, activity)
- Specifications and controls needed for qualifying these starting materials



## How do we evaluate and compare delivery systems?

- Delivery systems are varied and expanding, may be part of an *ex vivo* cell engineering process or your final product, but there are no norms on how to evaluate them and compare

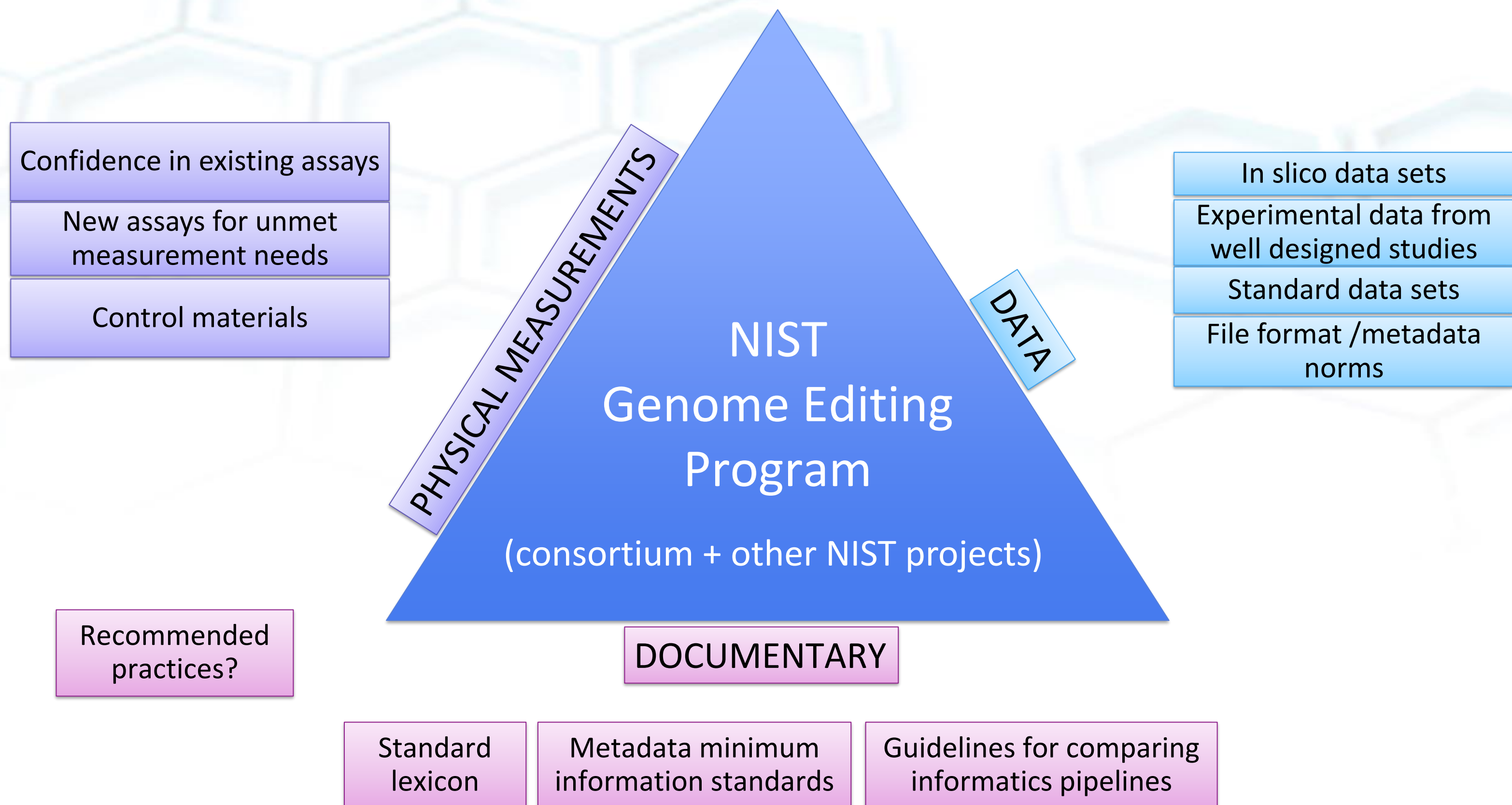
## Possible Edit Generated



## What are resources or practices to get most use out of data, understand if data is comparable, and understand bioinformatics performance?

- Metadata norms and infrastructure to capture and share metadata
- Standard datasets and interlab comparisons



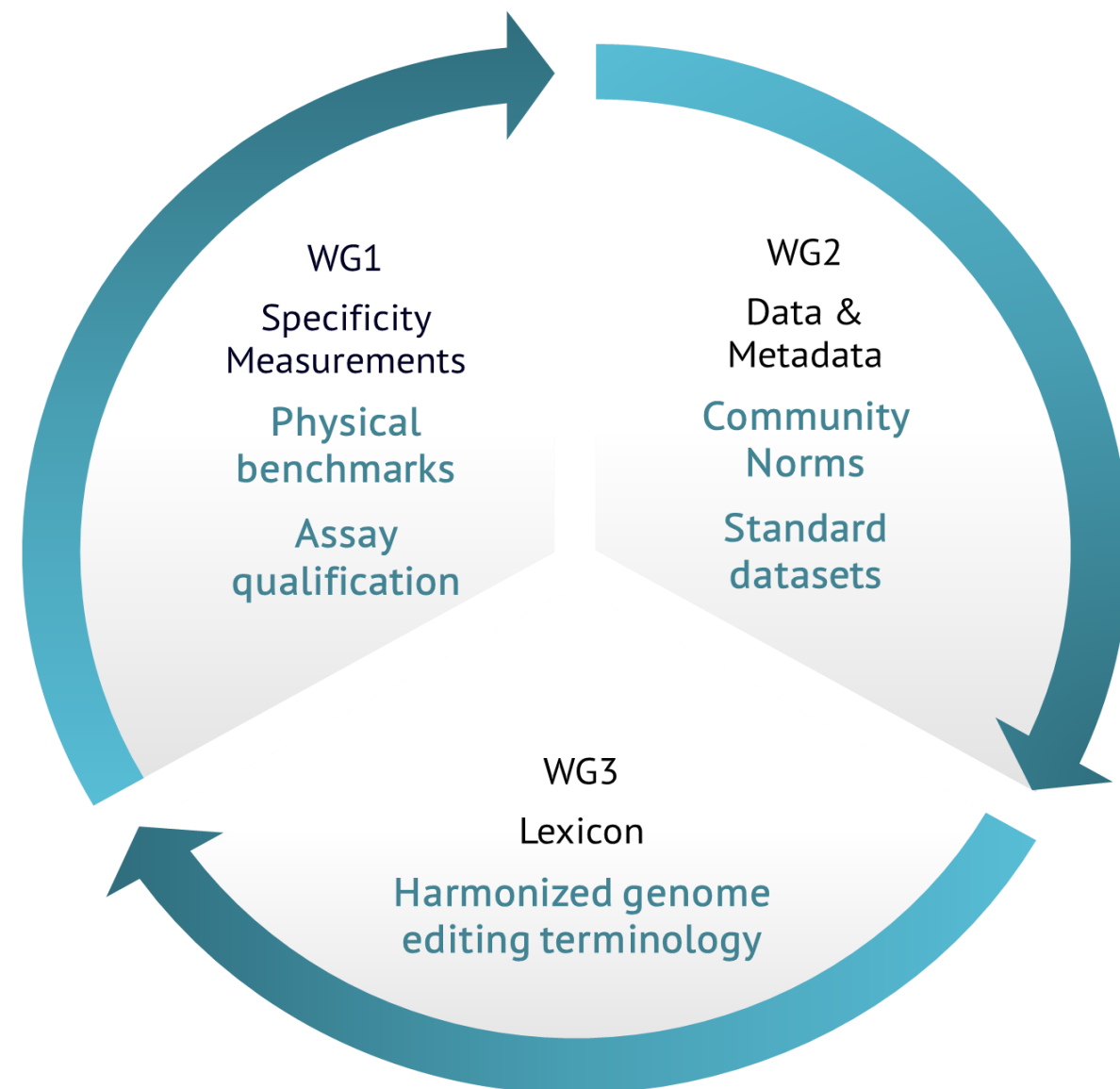


# Select NIST Genome Editing Program Projects



## NIST Genome Editing Consortium (launched October 2018)

Collaboration with genome editing stakeholders to define measurement challenges for utilizing existing measurement capabilities and develop shared solutions



Ongoing/extended through Dec, 1 2025

## NIST characterization of developing measurement capabilities (e.g. “off-target assays”)

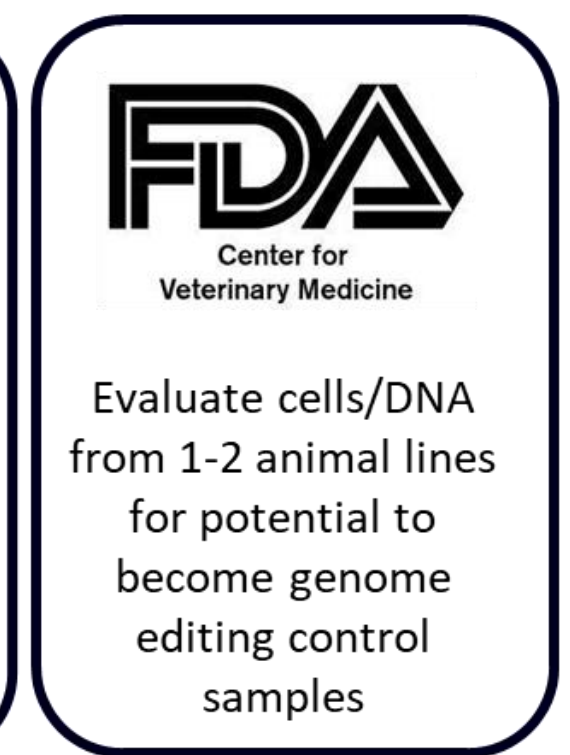
Collaboration with the assay developers and government to add measurement assurance (including bioinformatics) and SOPs for recently developed assays of broad interest for the genome editing field for evaluating precision of genome editing tools used to engineer cells



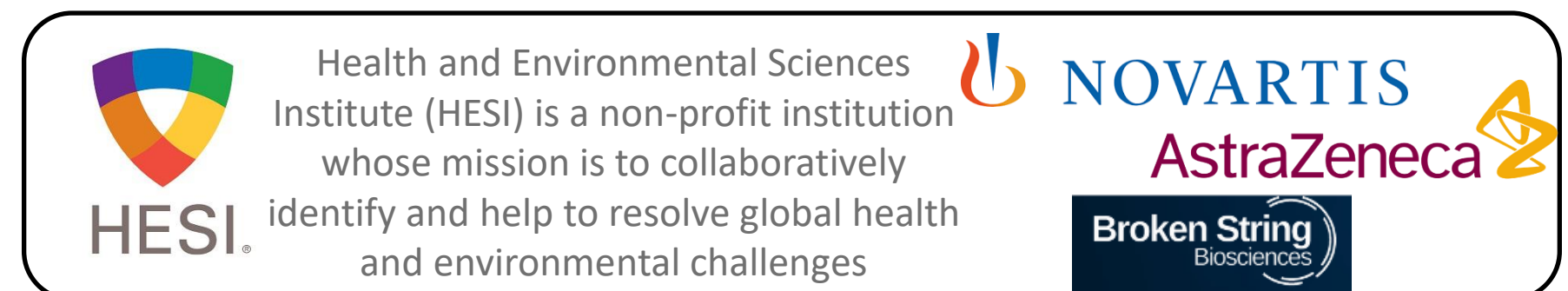
CHANGE-seq



ONE-seq



Cow/Pig cell lines



INDUCE-seq

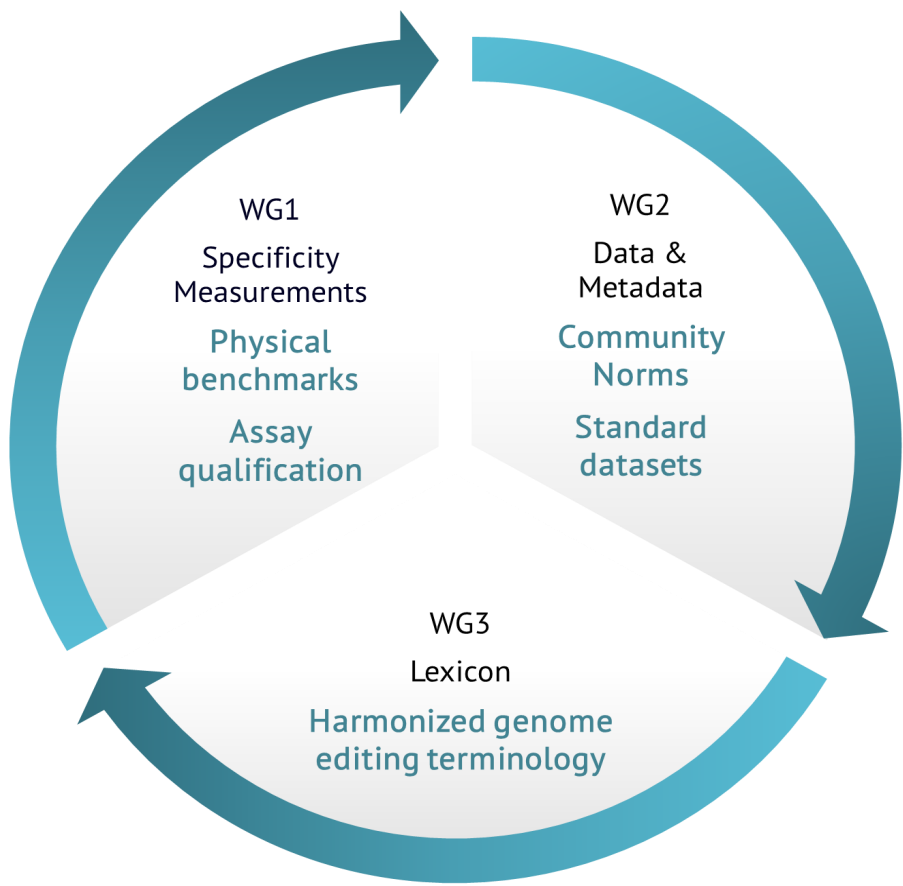
# NIST Genome Editing Consortium (launched October 2018)



## MISSION

Convene experts across academia, industry, non-profit & government to addresses the measurements and standards needed to increase confidence of utilizing genome editing technologies in research and commercial products

## ORGANIZATION



## MEMBER BENEFITS

- Access to a neutral forum for addressing pre-competitive needs
- Participation in the development of experimental benchmarks, guidelines and terminology
- Access to tools developed by the consortium ahead of public release

## MEMBERS

- Agilent
- Aldevron
- Applied StemCell
- AstraZeneca
- Bionano Genomics
- Bio-Rad
- Bluebird bio
- Caribou Biosciences
- Catalytic Data Science
- Cergentis
- COBO Technologies
- CRISPR Therapeutics
- DARPA
- DowDuPont Agrosience (Corteva)
- Editas Medicine
- EMBL-EBI
- **FDA CBER**
- Genomic Vision
- Horizon Discovery
- Illumina
- Inscripta
- Integrated DNA Technologies
- Intellia Therapeutics
- KromaTiD
- Lonza
- Macrogen
- Mass General Hospital
- Mission Bio
- Novartis
- New England Biolabs
- NIH/NINDS
- NIH SCG
- Precision Biosciences
- Sangamo Therapeutics
- SeQure Dx
- St. Jude Children's Research Hospital
- Synthego
- ThermoFisher Scientific
- Twinstrand Biosciences
- UCSC
- WhiteLab Genomics

NIST coordinates with FDA and Center for Veterinary Medicine (CVM)



Cost sharing model. All members contribute \$20,000 annually or *in-kind*



# Genome Editing Challenges of note

# Outcomes of Genome Editing

Your genome editing tool acted  
**at your intended target** DNA site

**on-target**



Intended  
edits

Unintended  
edits

**Meaningful to you**

No edit

Edits at the correct site, but  
may not intended

Your genome editing tool acted  
**at a DNA site different from your target**

**off-target**



Unintended  
edits

- off-target genome editing can occur
- It is difficult to measure/quantify with confidence

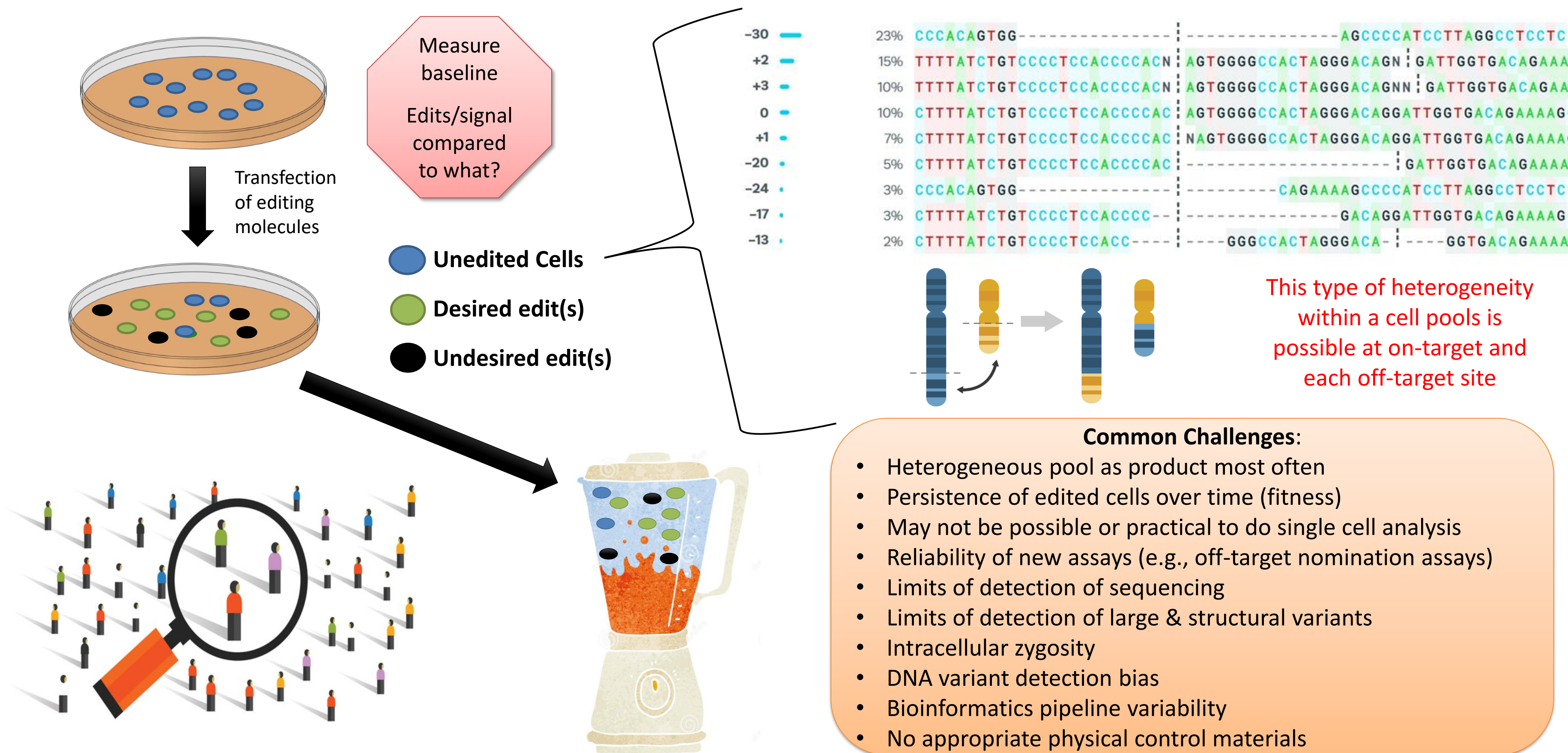
**FDA requires reporting including:**

- off-target genomic positions,
- on-target and off-target sequence change
- relative frequency of occurrence

Need to survey the entire  
genome but how when  
whole genome  
sequencing (for large  
genomes) doesn't give  
sufficient limit of  
detection?

**New Assays are Needed!**  
**How do you know new  
assays are working  
correctly???**

# Genome Editing Outcomes are Complex!





How confident are you that the  
answer is correct?

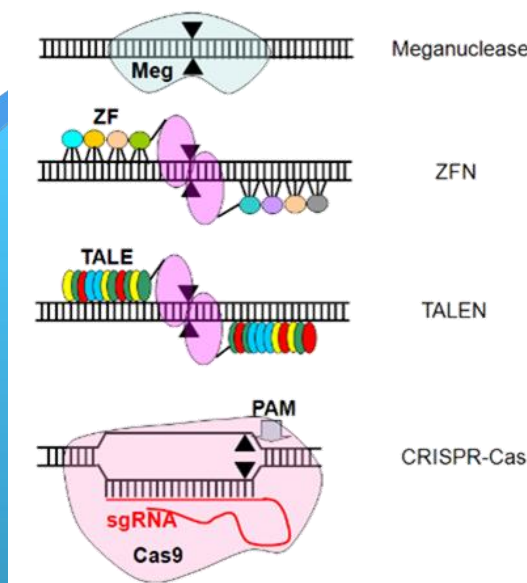
What is the consequence if it is  
wrong?

...What can be done to understand if  
the answer is correct?

# Genome Editing Process

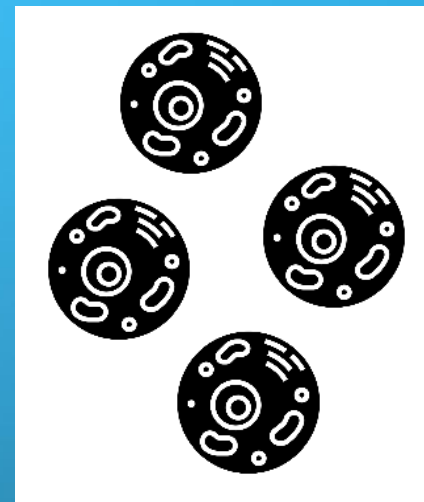
NIST

I N N O V A T I O N



Genome Editing  
Molecules

+



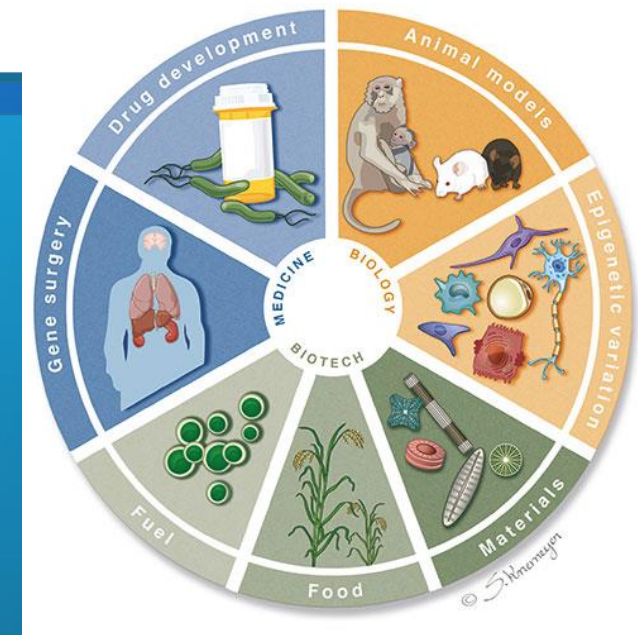
Cells of interest  
(*in vivo* or *ex vivo*)

=

DNA sequence  
change



Edited Cells



Fit for purpose?

Measurements

Data

Metadata

# Specificity working group

1. Assay Qualification
2. Physical control materials



**National Institute of  
Standards and Technology**  
U.S. Department of Commerce



# Condensed Genome Editing Process

NIST

## Design & Execute

Source sample  
Editing Target  
Editing molecule  
Editing molecule sequence  
Editing molecule formulation  
**Delivery**

## Look editing molecule activity

### Targeted

(e.g., T7 Endonuclease, TIDE, IDAA, ICE...)

### Genome Wide off-target assays:

(e.g., BLESS/BLISS, GUIDE-seq, Digenome-seq, CHANGE-seq, SITE-seq, Discover-seq, END-seq, INDUCE-seq...)

## Confirm sequence change

Targeted (sequencing, dPCR, hybridization)

Whole exome sequencing

Whole genome sequencing

Cell based structural  
variant detection

Which assay(s) is/are critical to qualify?  
Is the assay generalizable?  
Will there be something new?  
What could be generalizable control material?

New Assays  
+  
Assay Qualification  
+  
Control Materials

Assay  
Qualification  
+  
Control  
Materials

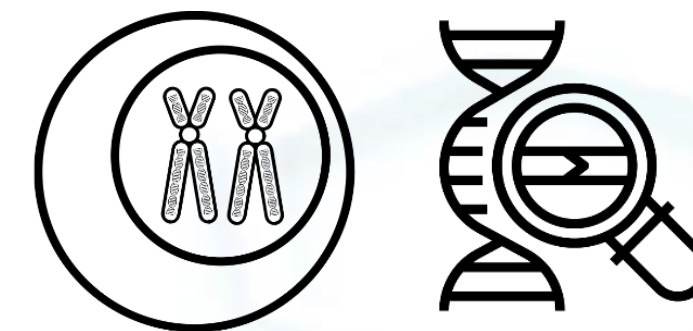
**Start  
Here!**

# SPECIFICITY MEASUREMENTS: DNA CONTROLS AND INTERLAB STUDIES

Is your process, instrument, reagents, operator (ect.) working? Controls that mimic genome edited cells/DNA and interlab studies



Characterize baseline



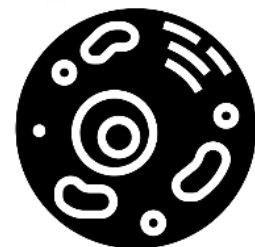
Documented  
process, Baseline  
data, & Mixture  
Studies

DNA control  
material  
design

Develop targeted assays

Control spike-  
ins

Control assays  
(e.g., ddPCR,  
NGS, DNA  
hybridization)



Engineered clonal controls



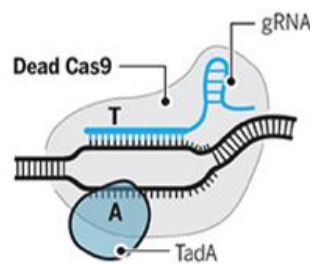
DNA and cells representing a variety of DNA sequence  
benchmarks “looks like a genome editing output”  
Deeply characterized at benchmark locations

## Uses

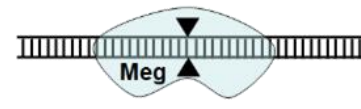
- Shared benchmark DNA and or cells that can be blended to achieve various allele and allele frequency combinations
- Purified DNA for sequence analysis benchmarks
- Cells for sequence analysis benchmarks
- Purified DNA for manipulation (e.g. *in vitro* cleavage)
- Cells for manipulation

# Genome Editing Overview:

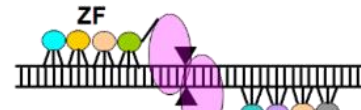
Base Editor



Design  
Genome  
Editing  
molecules



Meganuclease



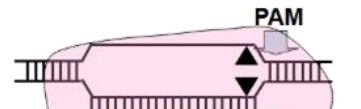
Zinc Finger



TALEN



CRISPR-Cas



Prime Editor

**Mechanism of Action**

DNA double strand break  
Deamination + DNA single  
strand break/nick

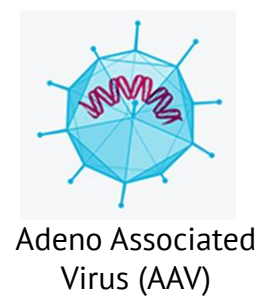
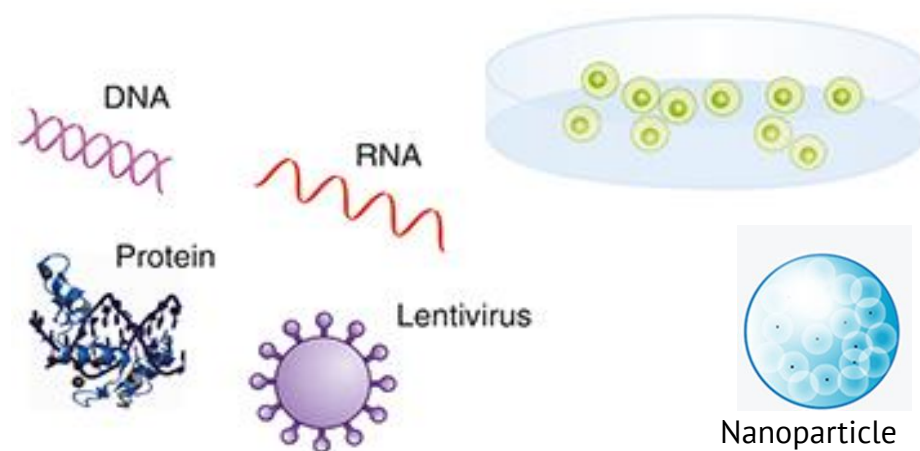
**WG1  
Control  
Material**



**Confirm DNA  
alteration**

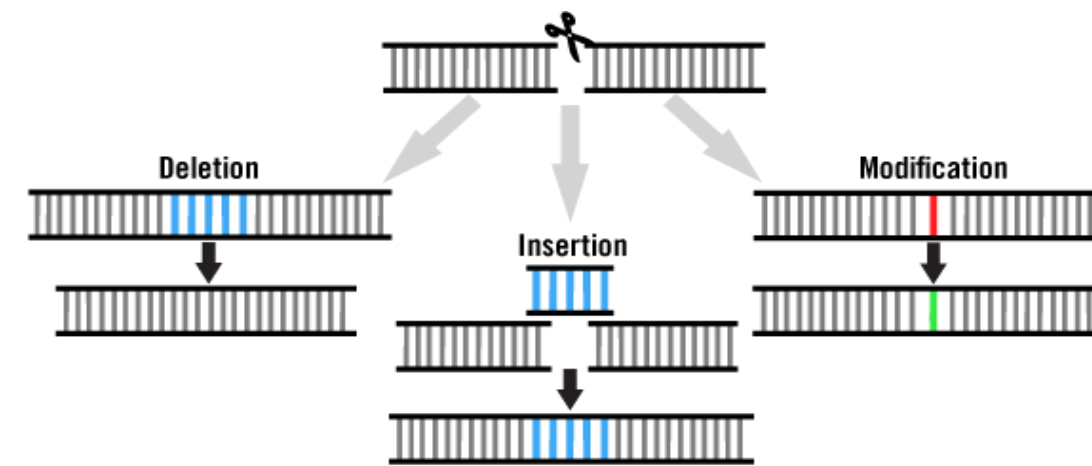


**Determine  
fit for purpose**



Adeno Associated  
Virus (AAV)

**Possible Edit  
Generated**





# Data & metadata working group

1. Identify community norms for **data formats** and **tools** for **benchmarking data analysis** including in silico and experimental data sets.
2. Determine the type of **metadata** that would be needed to be shared, housed, and interrogated from genome editing experiments.



- ✓ **Transfer of Knowledge**
- ✓ **Reproducibility**

**What** are the data we are generating?

**When** do we need to collect the metadata?

**How** do we store data and metadata?

**Where** do we store data and metadata

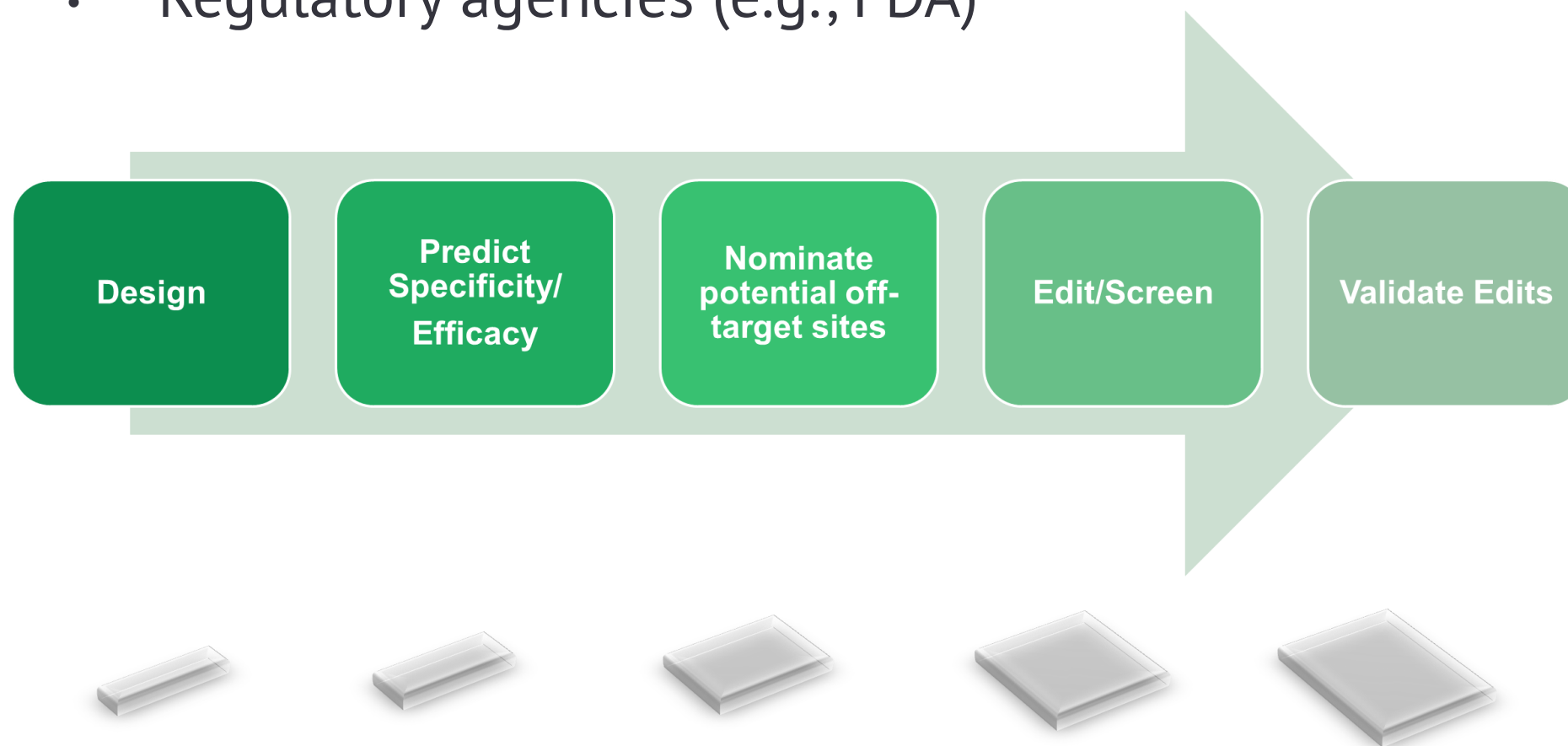
metadata from consortium studies & benchmark datasets as shared resources for the community

# Data & Metadata use cases

## Transfer/Document experimental process and results

- Within an organization (private)
- Between collaborators (limited access)
- Public databases (wide access)
- Regulatory agencies (e.g., FDA)

- Streamline integration of products with a well-established genome editing process
- Learning from and leveraging information within datasets (both public and private data)
- Benchmark datasets to compare bioinformatics pipelines and tools
- Exposure to potential customers/collaborators





# Data & Metadata

- ✓ Transfer of Knowledge
- ✓ Reproducibility

**What** are the data we are generating?

**When** do we need to collect the metadata?

**How** do we store data and metadata?

**Where** do we store data and metadata

metadata from consortium studies & benchmark datasets as shared resources for the community

## Metadata entry curation:

(how can this be normalized and low burden for a user)

	Metadata entry	Metadata ID	Metadata Type	Metadata example
Title	Study / publication title	study_title	string	Genome engineering using the CRISPR-Cas9 system
Target	Species	target_species	string	Homo sapiens
Target	Strain	target_strain	string	C57BL/6
Target	Substrain	target_substrain	string	C57BL/6N
Target	Biosample (Cell line/primary cell type)	target_biosample	Ontology	Ontology based e.g. EFO_0001185
Target	Biosample modifications	target_biosample_modifications	string	Plasmid GFP introduction
Target	Genome assembly	target_genome_assembly	string	hg19
Target	Target (gene vs. other genomic region)	target_region	Controlled vocabulary	{gene body,enhancer,intergenic}

## Metadata file format (GEM)

JSON format

1. Human readable
2. Database ready
3. Can be validated (JSON Schema)
4. Already used by NIST
5. Easy to extend

## Metadata access

Design and feasibility of a database with easy user interface

### Target sequences

(may have multiple target sequences) (region of the genome targeted).

Target sequence 1 \*

✕ Target sequence

↓

Target sequence 2 \*

✕ Target sequence

↑

+ Target sequence

✕ Last Target sequence

✕ All

Targeting Strand

Target start

0

## Datasets as control data and linked to metadata

# Lexicon working group

## Identify terms and related definitions to form a common genome editing community lexicon

- enable clear communication of scientific results
- facilitate effective communication with regulators (e.g., FDA, USDA)
- have the potential for international acceptance

# Lexicon contributing drafting organizations and expert commenters

## Industry and commerce – large industry

- Novartis
- AstraZeneca
- Thermo Fisher Scientific
- New England Biolabs
- Illumina
- Lonza
- Johnson & Johnson

## Industry and commerce – SMEs

- Bluebird bio
- Caribou Biosciences
- Corteva Agrosience
- CRISPR Therapeutics
- Editas Medicine
- Horizon Discovery
- Integrated DNA Technologies
- Intellia Therapeutics
- Precision Biosciences
- Sangamo Therapeutics
- Synthego
- Casebia Bio

## Government

- FDA
- USDA
- NIH

## Academic and research bodies

- Harvard University
- St Jude Children's research hospital
- The Broad Institute
- MIT
- Stanford University
- University of California Berkeley
- The Jackson Labs
- The University of Copenhagen
- The CRISPR Journal
- NC State University
- The University of Massachusetts Medical School
- UCSF
- UCSC

## Non-governmental organizations

- EMBL-EBI
- The World Health Organization
- Alliance for Regenerative Medicine
- USP



## Genome Editing Concepts

Gene editing  
Genome editing  
Genome engineering  
Specificity  
Target  
Off-target

## Genome Editing Tools

### General

Site-directed nuclease  
Site-directed DNA modification enzyme  
Repair template

### CRISPR-Specific

Guide RNA (gRNA)  
CRISPR RNA (crRNA)  
tracrRNA (trans-activating CRISPR RNA)  
sgRNA (single-guide RNA)  
PAM (protospacer adjacent motif)  
RNP (ribonucleoprotein)  
Cas nuclease target site  
Target strand

## Genome Editing Tools

### Meganuclease - Specific

Meganuclease  
Meganuclease single chain  
Meganuclease linker  
Meganuclease target site

### TALEN - Specific

TALEN  
TALEN linker  
Repeat variable diresidue (RVDs)  
TALEN target site

### megaTAL - Specific

megaTAL  
megaTAL linker  
megaTAL target site

### ZFN - Specific

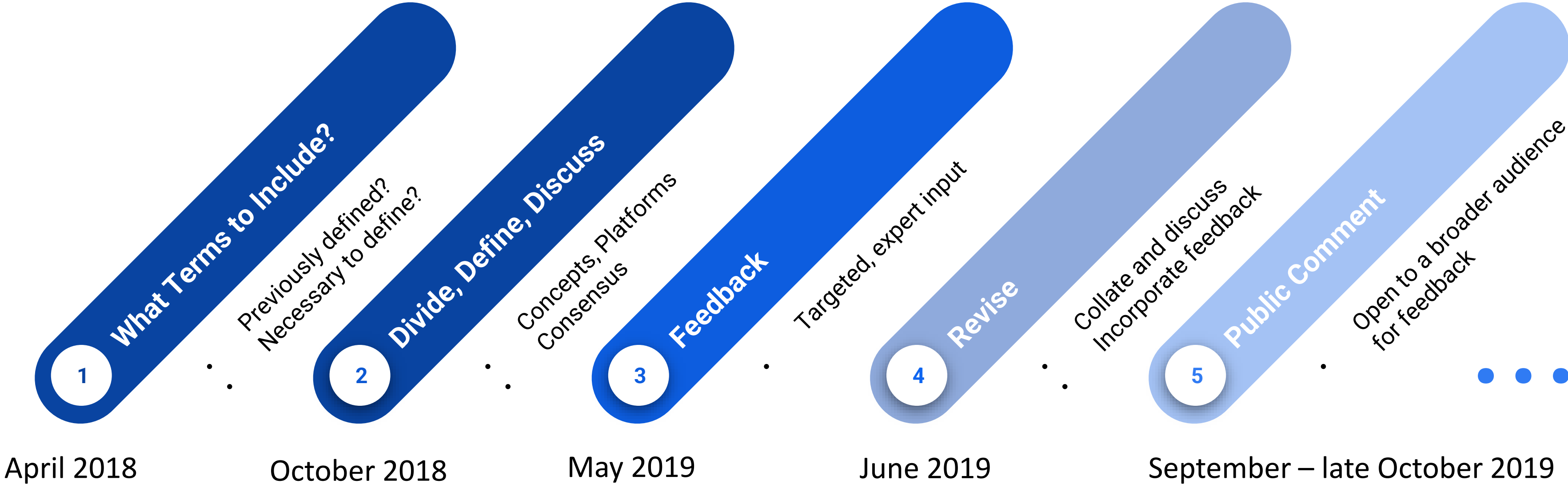
Zinc Finger Nuclease (ZFN)  
ZFN linker  
Zinc Finger Protein (ZFP)  
Zinc Finger  
Recognition helix  
ZFN target site

## Genome Editing Outcomes

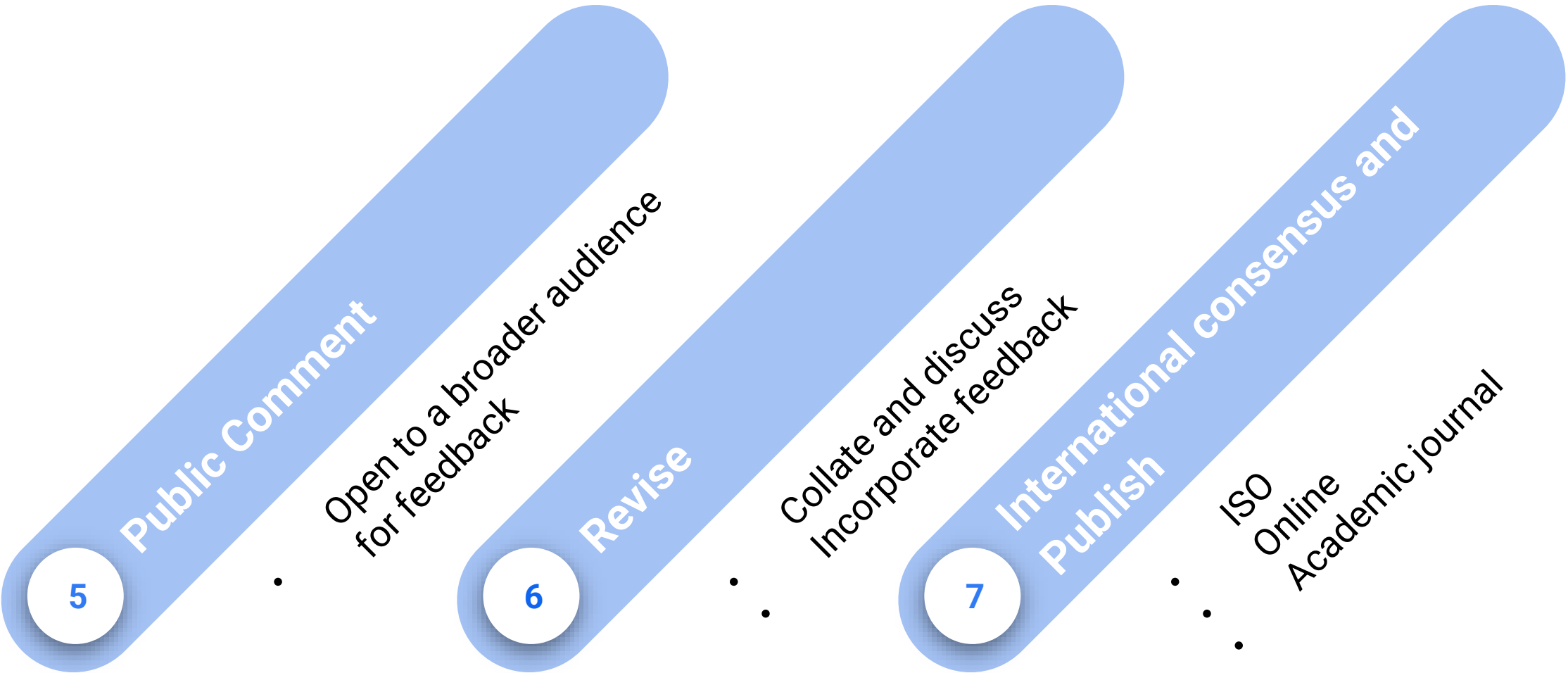
Edit  
Unintended edit  
Intended edit  
HDR (homology-directed repair)  
NHEJ (non-homologous end-joining)  
Microhomology-Mediated End Joining  
Repair (MMEJ)  
InDel mutation

ISO Standard *Genome Editing*  
*Part 1: Vocabulary* is in the last  
stage of approval

# Process prior to ISO submission



# Process prior to ISO submission continued



September – late October 2019

Dec 2019 – proposed as new project at ISO meeting  
May 2019 New Project ISO standard:

Genome Editing Terminology initiated....  
Estimated ISO standard publication Q4 2021/Q1 2022

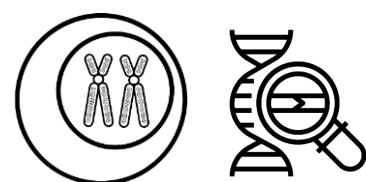


# NIST Genome Editing Consortium Progress



## WG1: Specificity Measurements

Develop cell and DNA based control materials and test via interlab analysis (Genome in a Bottle and Human iPSCs)



Documented process, baseline data, mixture studies, interlab studies, benchmark data



DNA and cells representing a variety of DNA sequence benchmarks “looks like a genome editing output” Deeply characterized at benchmark locations

## WG2: Data & Metadata

- Community norms for data formats and tools for benchmarking data analysis (*in silico* and experimental data sets)
- Identify metadata that would be needed to be shared, housed, and interrogated from genome editing experiments and develop tools to accelerate metadata sharing

✓ **Transfer of Knowledge**  
✓ **Reproducibility**

**What** are the data we are generating?  
**When** do we need to collect the metadata?  
**How** do we store data and metadata?  
**Where** do we store data and metadata

metadata from consortium studies and benchmark datasets as shared resources for the community

## WG3: Lexicon

Identify terms and related definitions to form a common genome editing community lexicon

## WG1 – Progress:

- A set of Phase 1 DNA and cell based control materials have been generated and an interlab study has been launched
- Samples have been qualified by NIST and sent to members for interlab comparison of DNA sequences detected and limit of detection
- Additional clonal engineered cell controls are in progress with some lines completed

## WG2 – Progress:

- Phase 1 metadata entries and template completed
- Testing use cases and user interfaces, and interoperability of a metadata standard format and database(s) to house records.

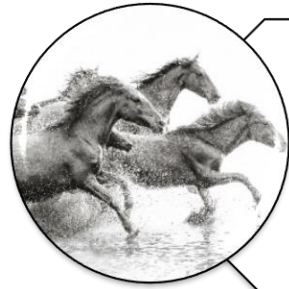
## WG3 – Progress:

- A first version lexicon has been generated that has gone through targeted expert review and public comment

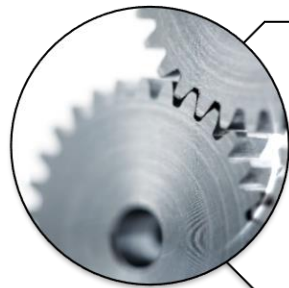
<https://www.nist.gov/programs-projects/nist-genome-editing-lexicon>

- An updated internationally harmonized version is in the last stages of approval to be released as an ISO Standard for *Genome Editing Vocabulary*

# Keeping pace with technology



Gene Therapy, Genome Editing Technologies and related capabilities (e.g. delivery systems) are rapidly growing and an intentional effort must be made to keep up with advances in knowledge and technology



It is important that standards are not limited to only the potential of current technology, but ‘future proofed’ to help with comparability and qualification of new technology advancements and comparability between current and future technologies that may be beneficial to take advantage even during the development lifetime of a product.



The NIST Genome Consortium is currently active through Dec 2025, to address standards needs identified by members and expert community. Additional members are welcome.



Additional engagement and expert community collaborative activities are critical for identifying standards needs and working towards consensus solutions and developing needed data and products



For commercial products, regulation will be different across countries, and it is important to appreciate key policies and positions from regulatory bodies particularly regarding safety



# Thank You NIST Colleagues!

Funding Collaborators



Natasha  
Kolmakova



Alex Tona



Tara  
Eskandari

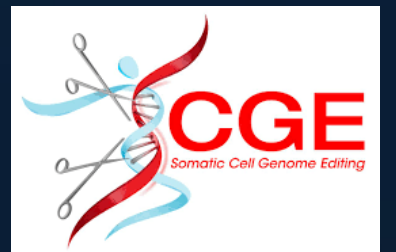


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