

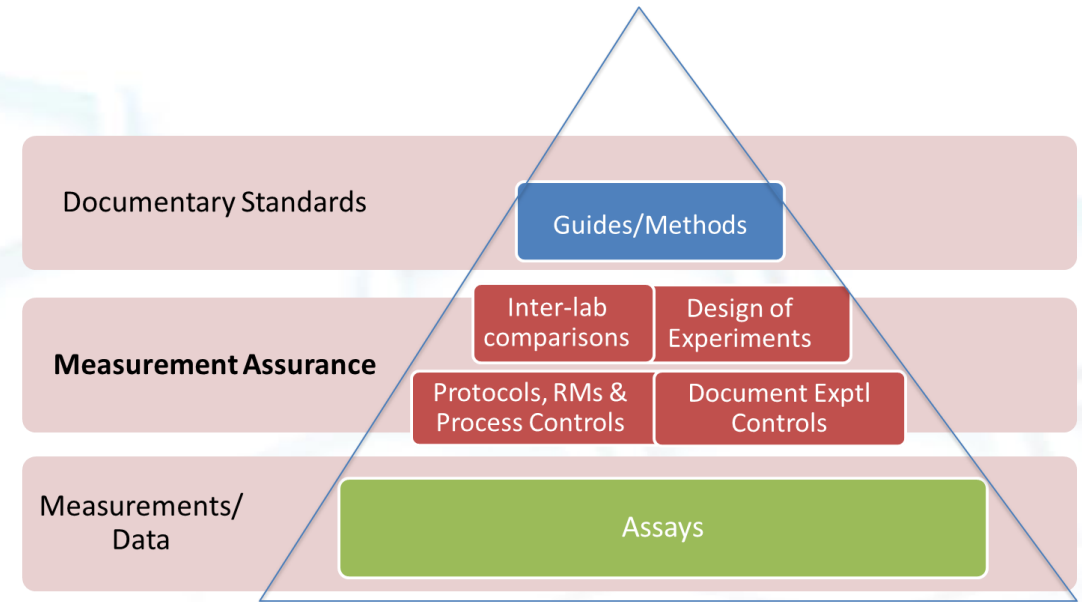


Assay Qualification

Recall...

Assay Qualification:

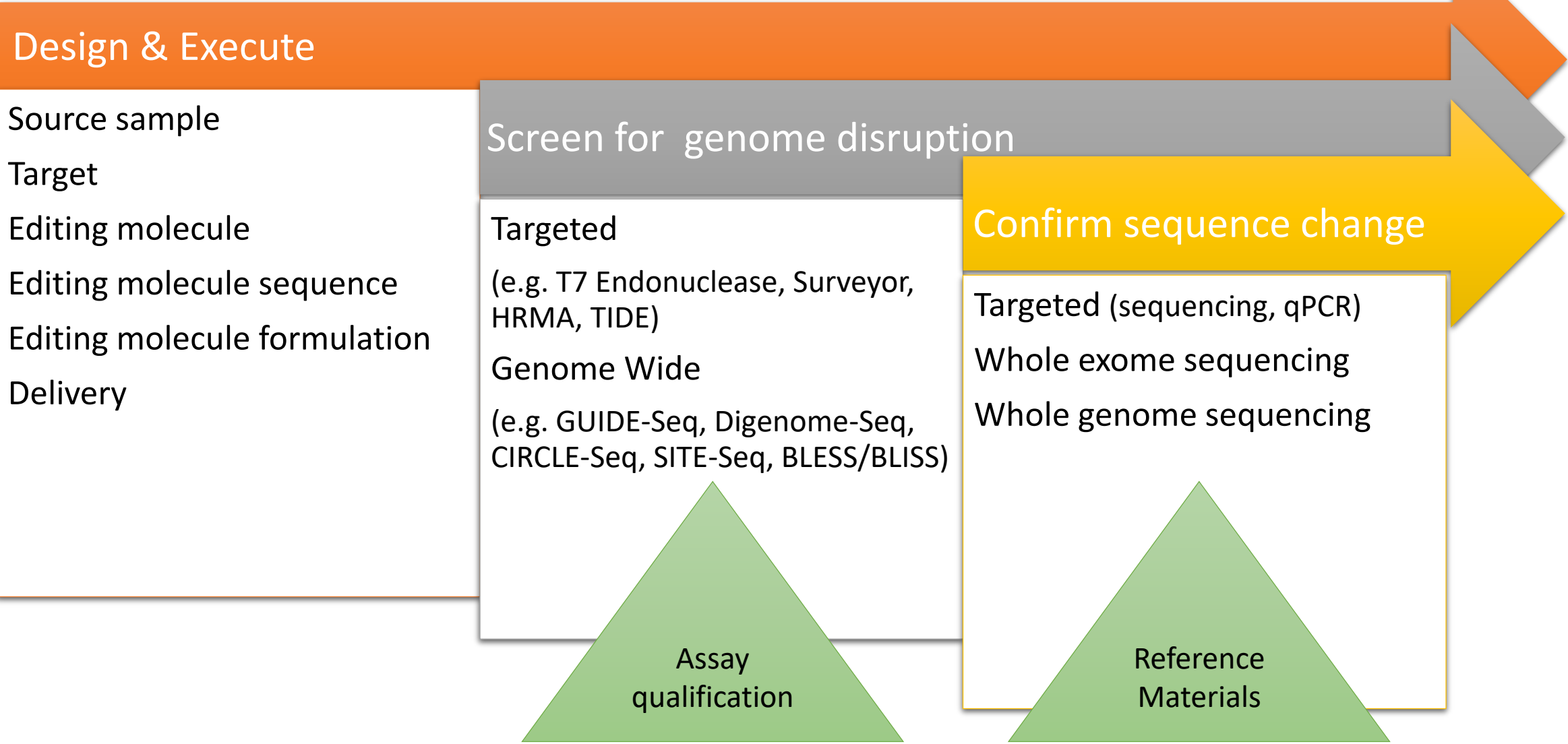
- **ACCURACY:** Orthogonal method
- **PRECISION:** Reproducibility: same day replicates, day to day, different operators
- **ROBUSTNESS:** sensitivity to assay parameters
- **SPECIFICITY:** sensitivity to matrix effects
- **DYNAMIC RANGE AND RESPONSE FUNCTION:** Instrument benchmarking. +/- controls. Calibration curve. Limit of detection



Goal

Establish confidence in genomic assays used to identify on-target and off-target genome editing genomic locations and sequence variants.

Simplified Genome Editing Process



Activities and outputs:

1. Qualify genomic assays used to identify on-target and off-target genome editing genomic locations and sequence variants.
2. Develop & qualify reference materials
 - designed to mimic genome edited DNA complexity for use in benchmarking sequence verification assay limits of detection and sensitivity.

Assay Qualification Process

Identify assay(s) of primary importance (community consensus)

Assess assay precision (initially within NIST laboratories)

Assess sources of variability and improve assay protocols and robustness (DoE) (NIST)

Refined assay protocols will be evaluated and optimized in collaboration with other labs

Establish (pre-edited) baseline data appropriate to each assay (NIST)

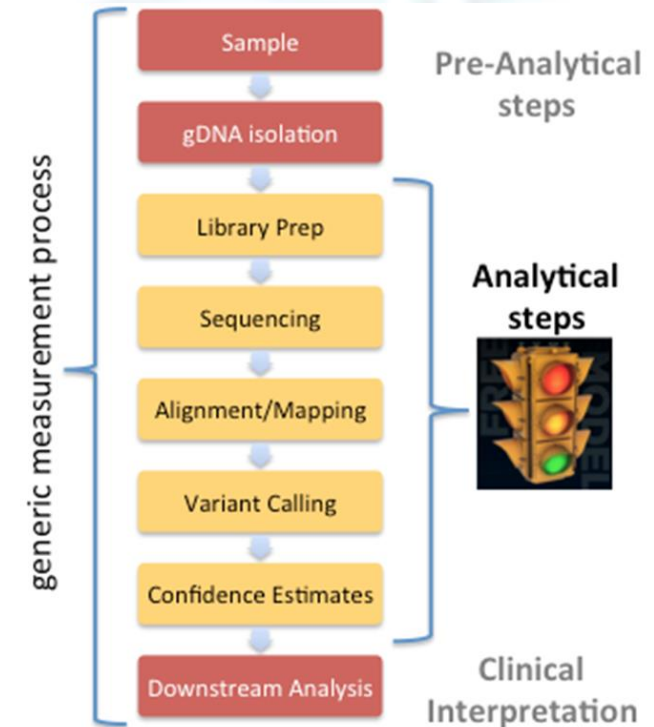
Determine where and what in-process control materials are needed. (NIST + collaborators)

Reference Materials

- Specific reference materials can be configured as controls for specific parts of a gene editing workflow
- A genomic DNA reference material for genome editing can be used to ensure process quality for the detection and analysis steps of the workflow
- A DNA reference material DOES NOT control for upstream steps such as the quality of the gene editing reagents, the transfection efficiency into the cells or the DNA extraction procedure
- Additional upstream in-process controls or reference materials can be used to control for these steps

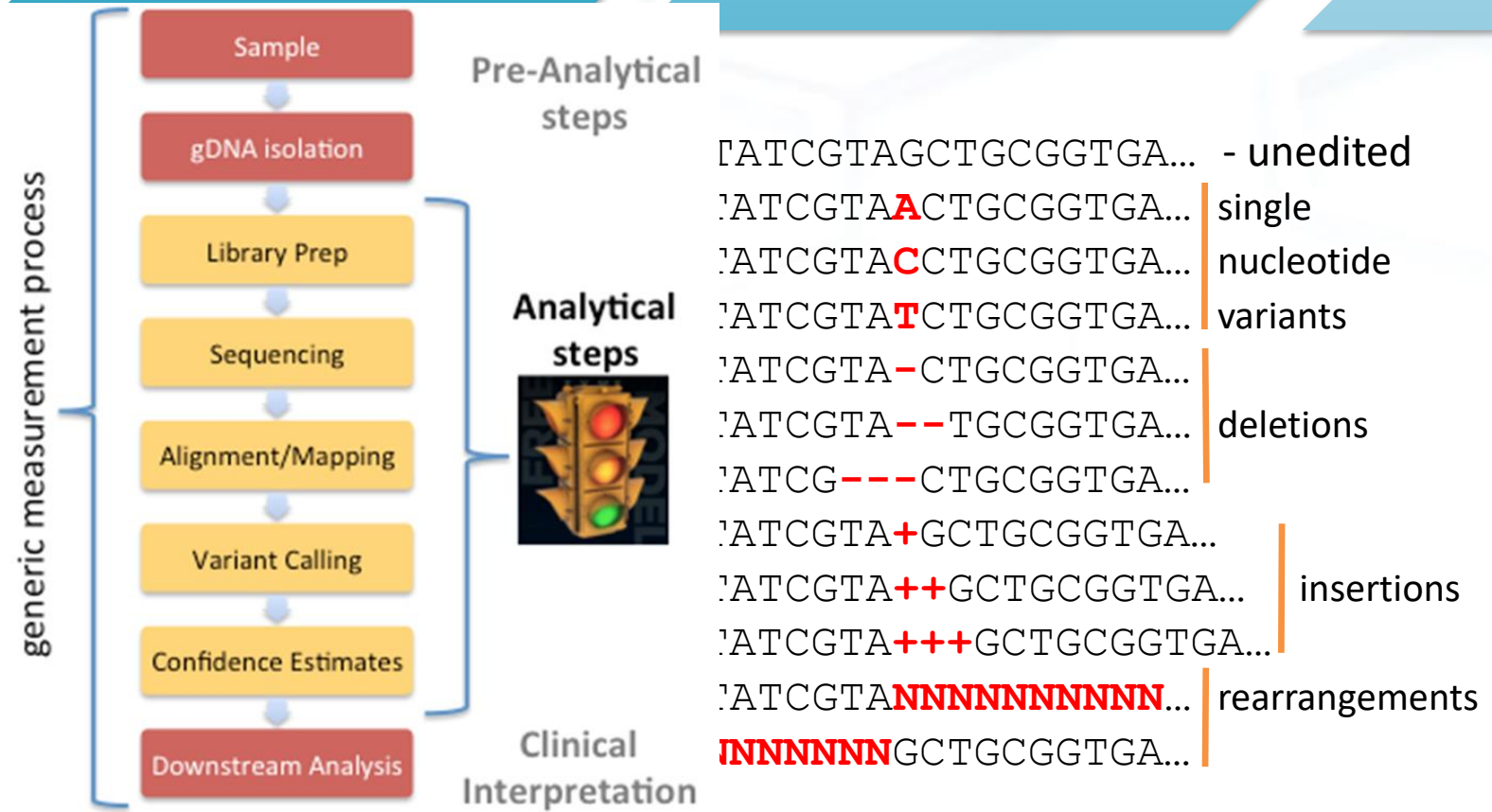
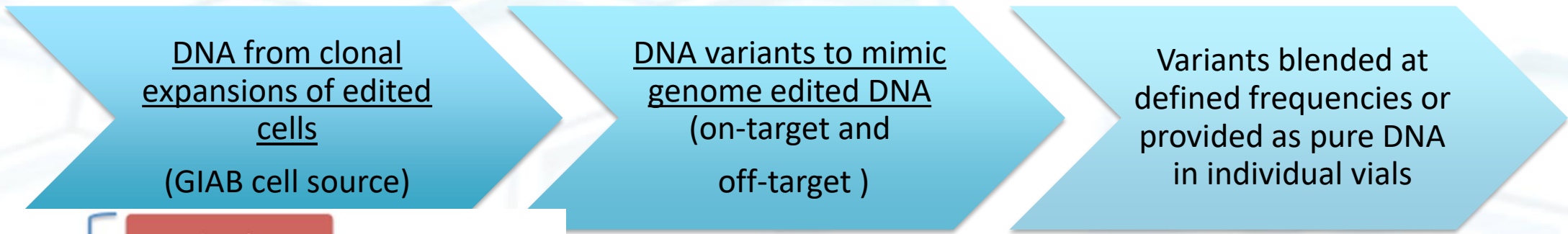
Develop & qualify reference materials

- Define purpose of the reference material. What process step does this control for?
 - Reference materials for sequence variant detection
 - Understanding limits of detection and sensitivity of sequence verification
- GIAB reference material for baseline NGS fidelity in sequence calling exists as a valuable resource (platform, sequence variants, bioinformatics)



Genome in a Bottle
Consortium

Genome Editing relevant DNA Reference Material– pilot design



Impact of Outputs

1. Reproducibility of results in different laboratories. Understanding the unique benefits of each tested assay.
2. Confidence in the results of assays relative to benchmark, known, materials.
3. Ability to compare results from different editing methods and when performed under different conditions with different cellular and genomic (patient) materials.
4. Data that are evaluated and benchmarked for sharing among the community of experts.

Path for Success

- Continue signing up consortium participants and identify cash and in-kind contributions.
- Identify experts to contribute expertise to design reference materials.
- Identify expert labs that can assist in refining and testing assay protocols.
- Develop a plan for coordinating between laboratory activities, bioinformatics and terminology experts.



Assay Qualification Breakout Session

Qualifying Existing (on-target or off-target) Genomic Assays

1. Which assays are most relevant to the field and in need of qualification (provide rank order beginning with most important)?
2. Are certain variables known to create challenges for getting reliable data from the priority assays you listed above?

Reference materials for benchmarking sequence variant detection (allelic series)

1. Is the presented paradigm fit for purpose for generating reference materials suited for assessing assay limits of detection and assay sensitivity?
2. In the presented reference material design, is the complexity of variants sufficient? If not provide design needs.