AccuGenomics

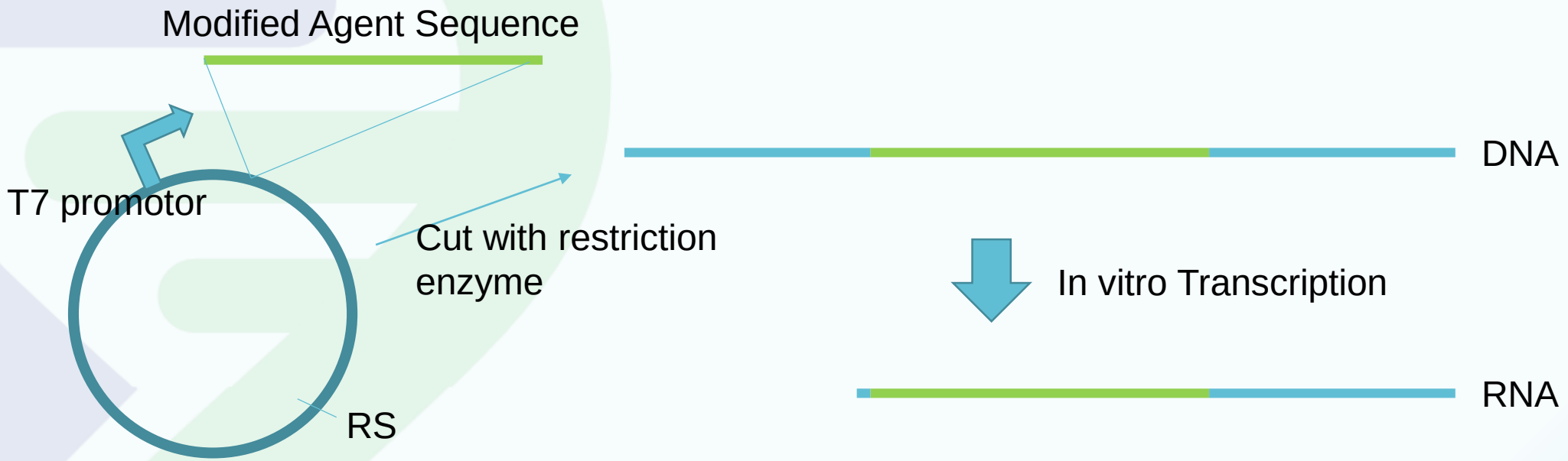
Next Generation Standards

Challenges of AA Molecular Testing

- Molecular based adventitious agents testing is complicated by the complexity of the method.
- Advanced automation and rigorous process controls can reduce procedural errors that lead to inaccurate results
- Reference materials provide process controls but don't ensure adequate per sample QC.
- Internal reference controls or traditional xeno spike-in sequences can provide an indication of yield, but not a per sample LOD

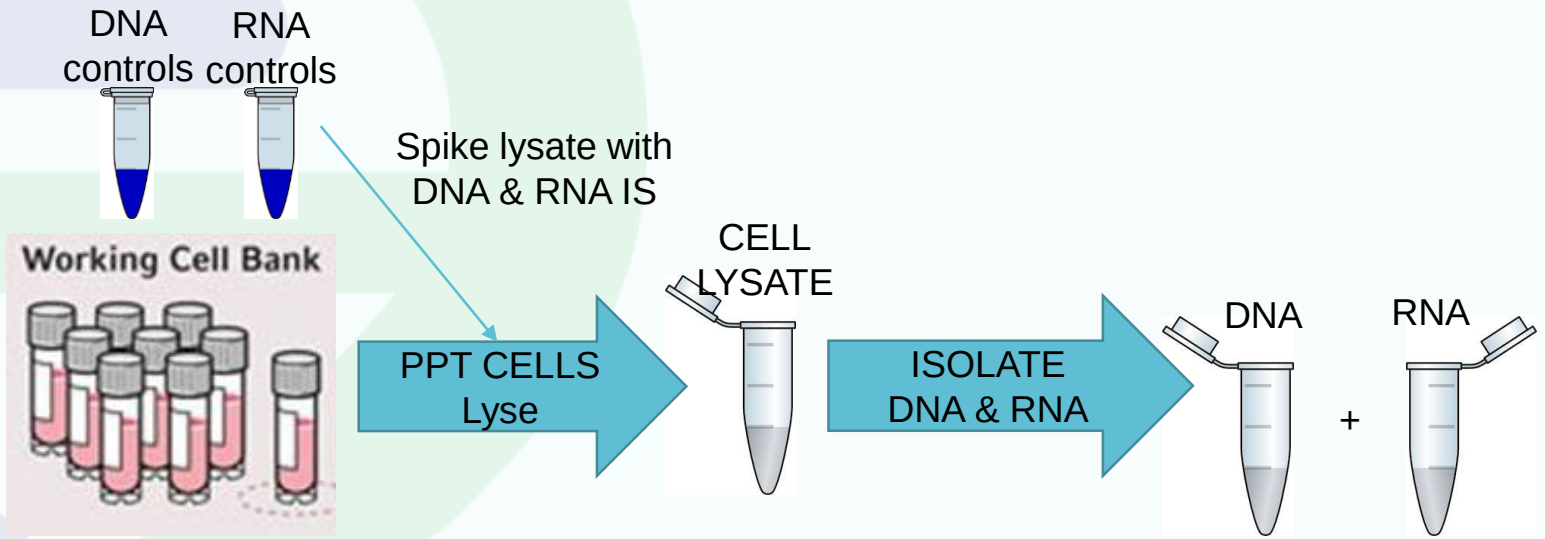
- Received a NIIMBL grant to create a new type of internal control
  - A mixture of controls to measure the LOD test performance of each AA in every sample.

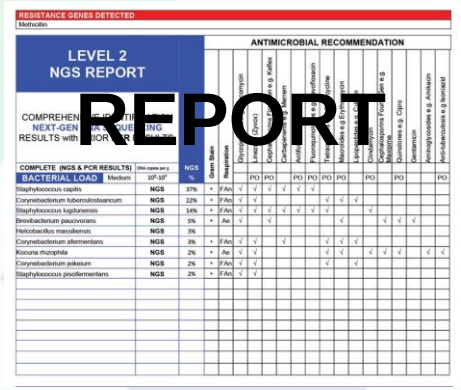
Control Manufacture



- Create vector with synthetic AA control sequence
- Sequence modified to meet library prep/detection needs
- Produce either dsDNA and ssRNA controls
- Create separate RNA and DNA mixtures at desired copies/ul
- dsDNA stable for >15 years, RNA controls stable 3 years.

AA Control Workflow





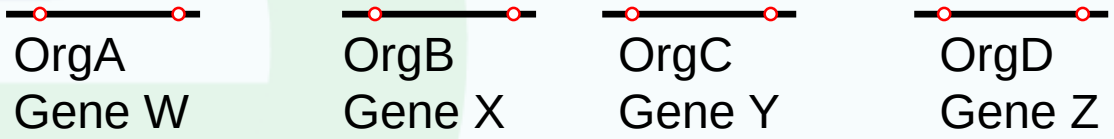
Control & Native Counts

Library Prep

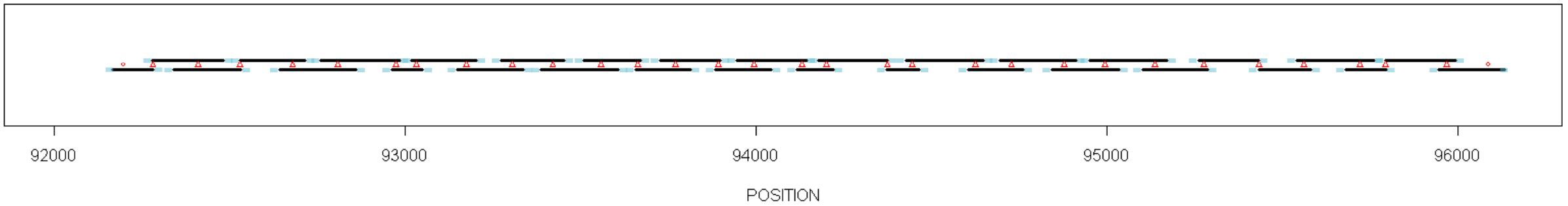
- Adjust control spike-in levels to achieve LOD at library preparation stage
- Good Result: Positive for all agent controls, negative for AA
- **A positive control for negative results**

Internal Control Types

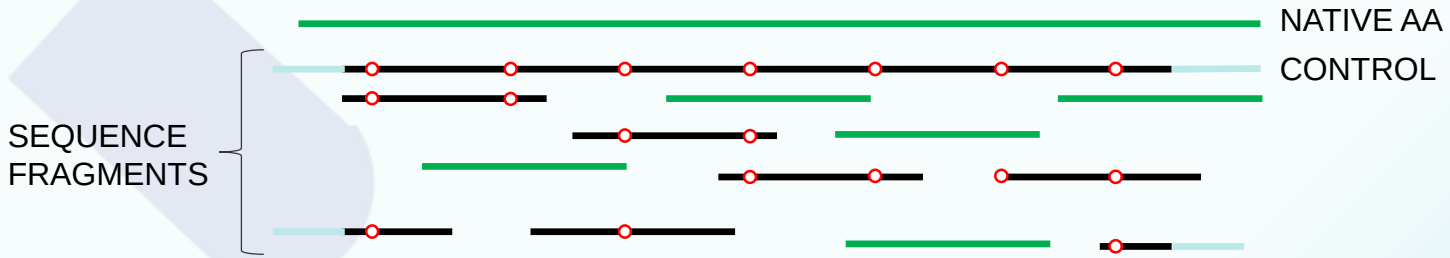
Control for Different Agents for Amplicon Libraries



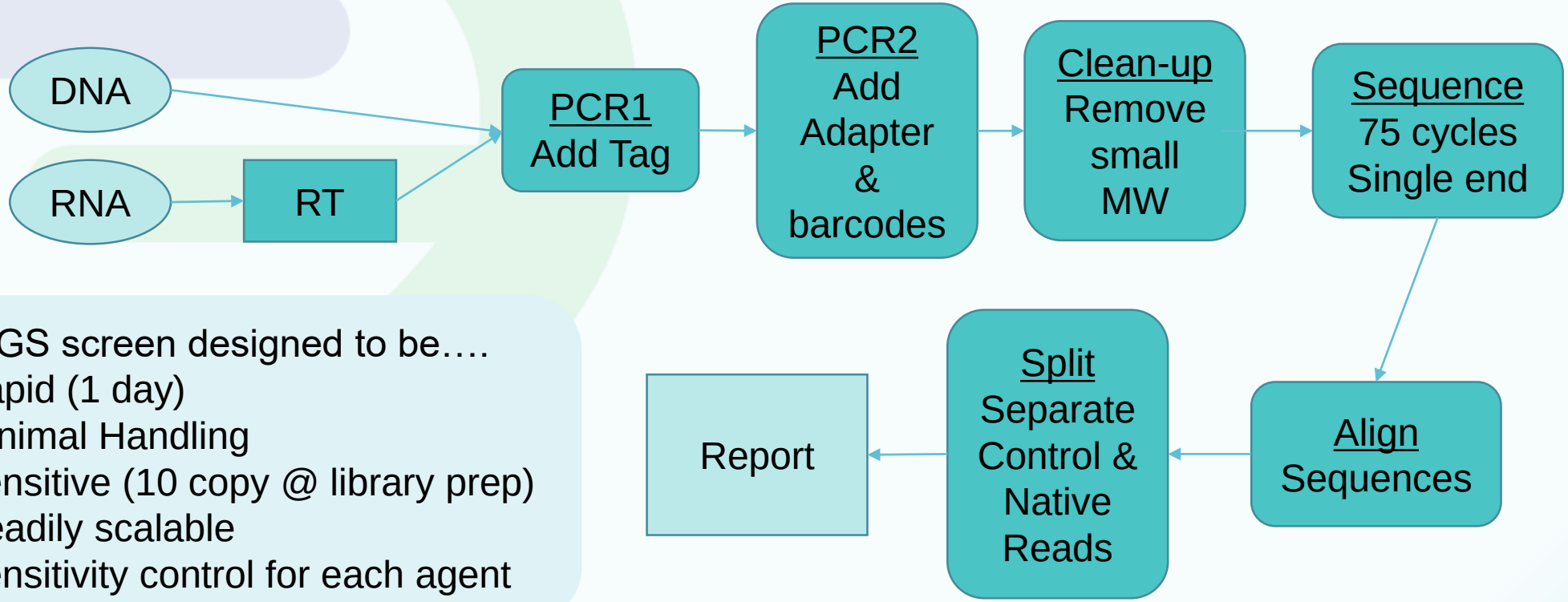
Control for Large Contiguous Regions for Tiled Amplicon Libraries



Control for Short Fragment Libraries



NGS Library Preparation



AA NGS screen designed to be….

- Rapid (1 day)
- Minimal Handling
- Sensitive (10 copy @ library prep)
- Readily scalable
- Sensitivity control for each agent

- Simple steps allow sample to answer in 20 hours (including purification)
- Ten copy LOD (internal controls), <5% sequence crosstalk, 5 hours hands-on
- Samples are run in three replicates
  - A positive control result from 1 of 3 replicates has counts above LOB
  - A positive AA result results from 2 of 3 replicates with counts above LOB

Results thus far…

- Demo panel designed to detect 8 DNA & 12 RNA agents.
- Panel created by screening literature validated primers for NGS yields at 10 copy input.
- Milestone was to demonstrate 32 of 32 agent negative 1 ug DNA/RNA samples were negative for agent but positive for each agent control.
- Partner validating performance with nucleic acid purification step.

- We've observed failures:
  - Pipetting error
  - New RT lot had lower yield
  - PCR well failure
  - "Negative" cell matrix
  - Gremlins

Milestone 2: Internal Standard Detection

	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30	S31	S32
BADV5	1	3	2	1	3	3	3	3	3	2	2	3	3	3	2	3	2	2	3	2	3	3	3	2	3	2	3	2	3	2	2	2
BCOV2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3
BEV1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
BPAV1	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
BPIV3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
BPV5	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3
BRSV2	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
BTV1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
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CVV11	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
EHDV1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
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MVM5	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
PCOV23	3	3	3	3	2	3	3	3	2	3	3	2	3	3	3	3	3	3	3	2	3	3	3	3	3	3	3	2	3	3	3	3
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PTGV2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
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Milestone 2: Native Template Detection

	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30	S31	S32
BADV5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BCOV2*	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BEV1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BPAV1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BPIV3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BPV5	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0
BRSV2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BTV1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BVDV2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CVV11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EHDV1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HADV1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MVM5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCOV23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCV2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PHEV2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PTGV2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RAB2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SVV3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VV7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Next up…

- Submit grant to…
- Replace HAP, MAP & RAP with targeted NGS
  - In vivo tests that detect specific agents
- Create >1 target per agent for redundancy
- Work with FDA upfront to design testing process that meets regulatory requirements

NIIMBL Grant Team

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- NCSU
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