

Amplicon-contained lateral flow test that enables sequential delivery: a demonstration on HIV drug resistance detection

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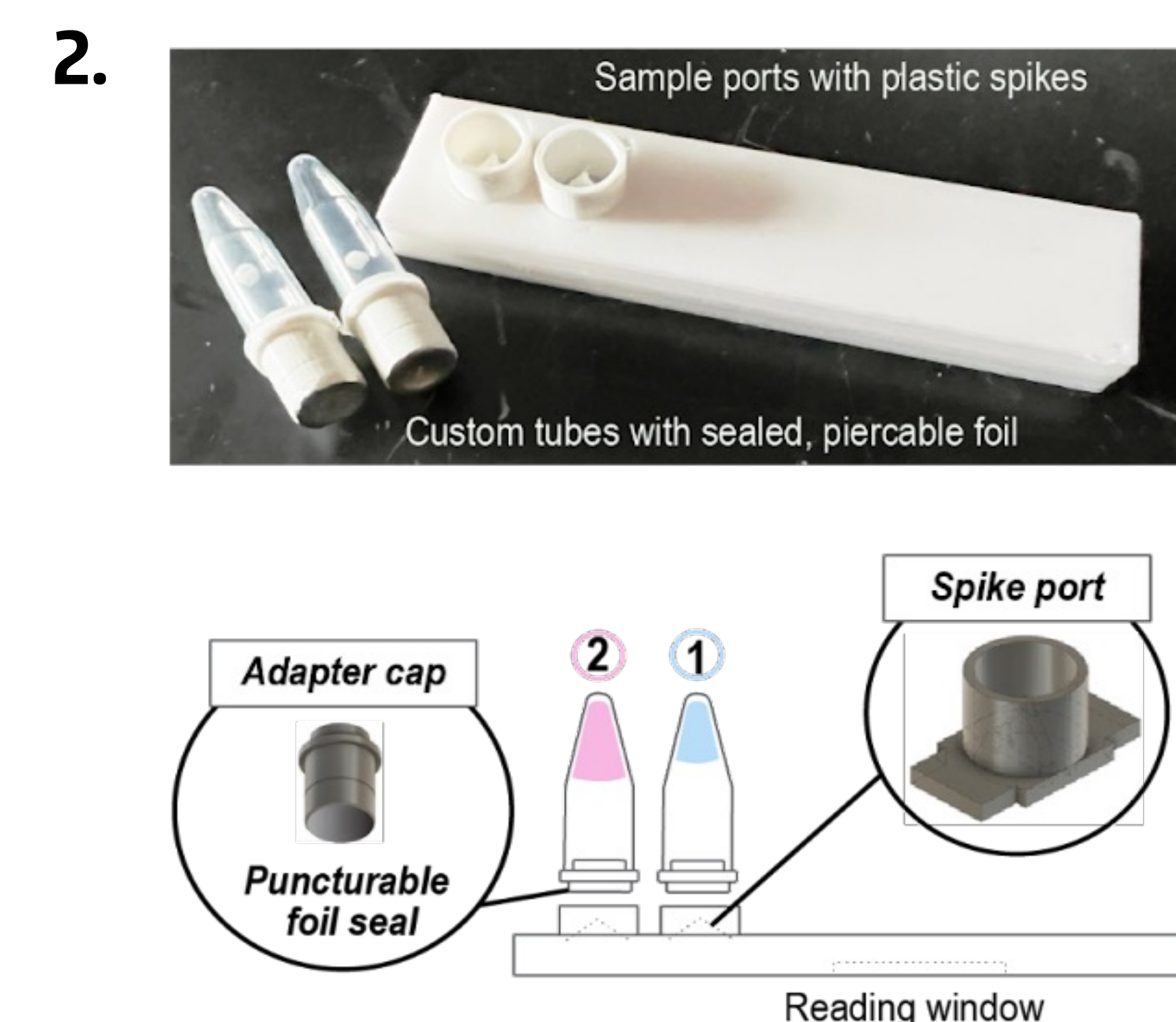
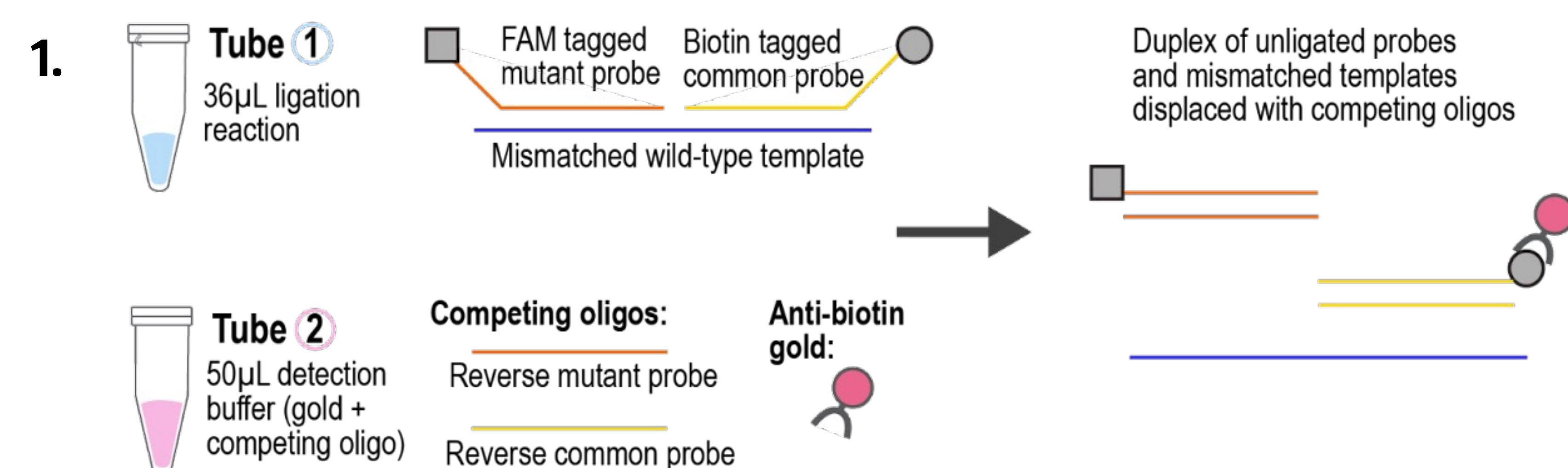
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Background

- HIV drug resistance (**HIVDR**) testing is important for treatment management
 - The oligonucleotide ligation assay (**OLA**) kit, "OLA-Simple" uses allele-specific probe ligation to detect HIVDR-associated mutations. [1,2]
- Lateral flow tests (LFTs)** used in molecular assays involve opening tubes containing amplicon and several transfer steps. Detection of OLA products requires:
 - Displacement of unligated probes** from their mismatched HIV templates to eliminate false mutant signal **Fig. 1**
 - Sequential delivery** of ligation product and detection buffer on the LFT
- These steps expose substantial risks for amplicon contamination and increase the complexity of LFTs, especially with inexperienced users
 - Need: a functional self-contained LFT that enables sequential delivery**

Materials and Methods

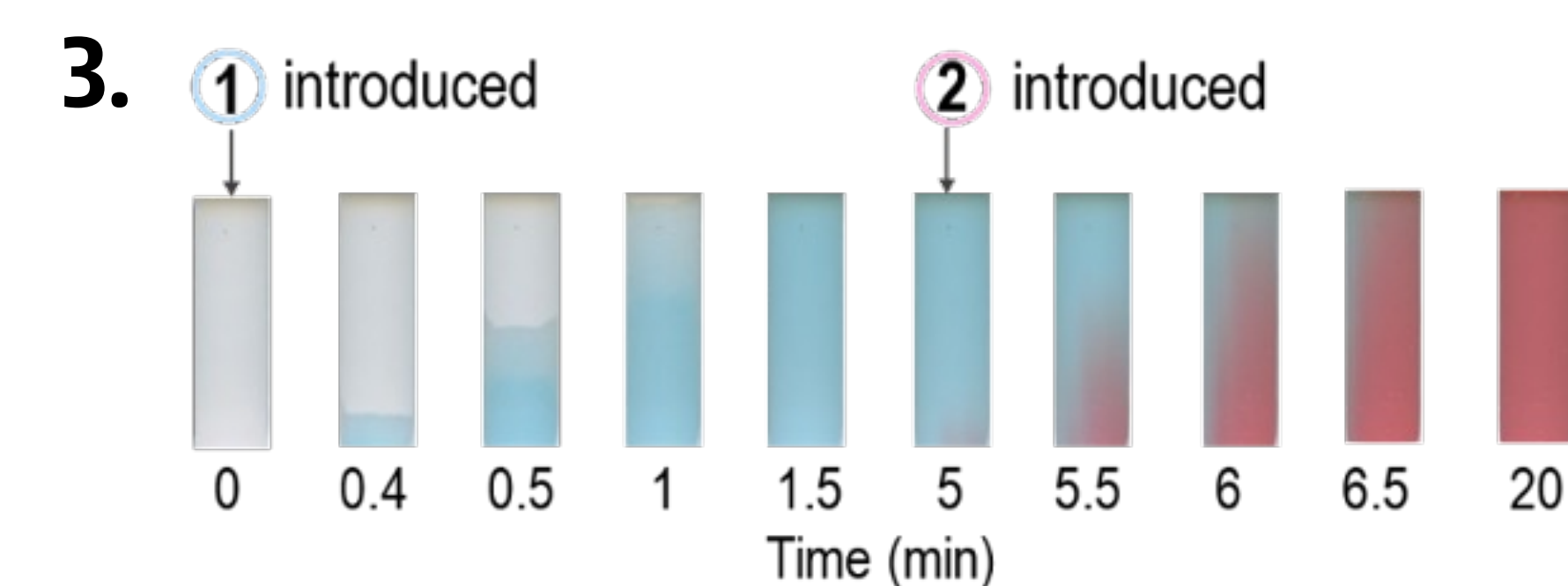


Components:

- Tube 1** contained HIV wild-type (WT) templates annealed to mismatched mutant (MUT) and common probes → false mutant signals **Fig. 1**
- Tube 2** contained gold solution with or without **competing oligonucleotides (CO)** → **CO should displace the unligated probes to eliminate false mutant signal**
- Custom LFT device** prototype **Fig. 2** has:
 - Two sample ports with **plastic spikes**
 - Reaction tubes with **custom foil-sealed caps**
 - Reading window at the bottom

User steps: (i) Sample tubes are loaded into the sample ports. (ii) The foil-sealed caps are punctured sequentially to release the two fluids.

Results and Discussion



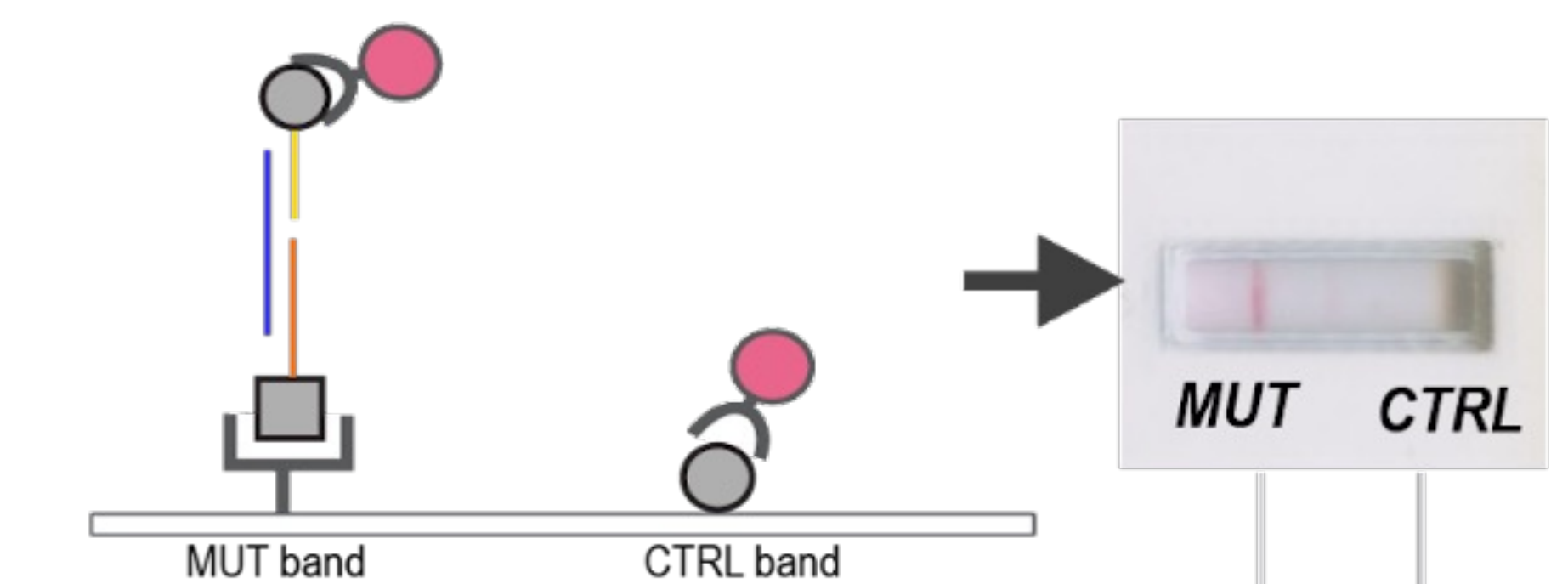
Flow

- The flow experiment showed **successful sequential delivery of two different colored fluids** **Fig. 3**, from blue (first tube) to red (second tube)

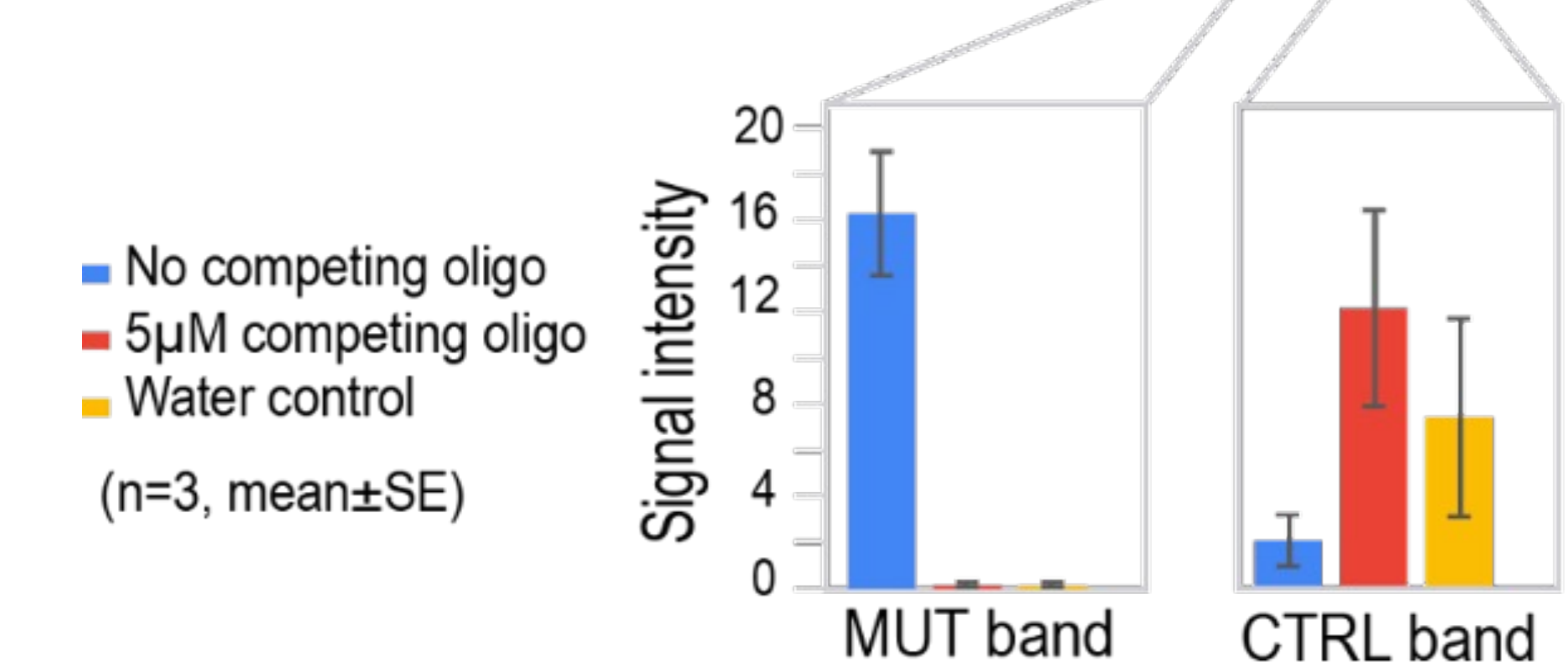
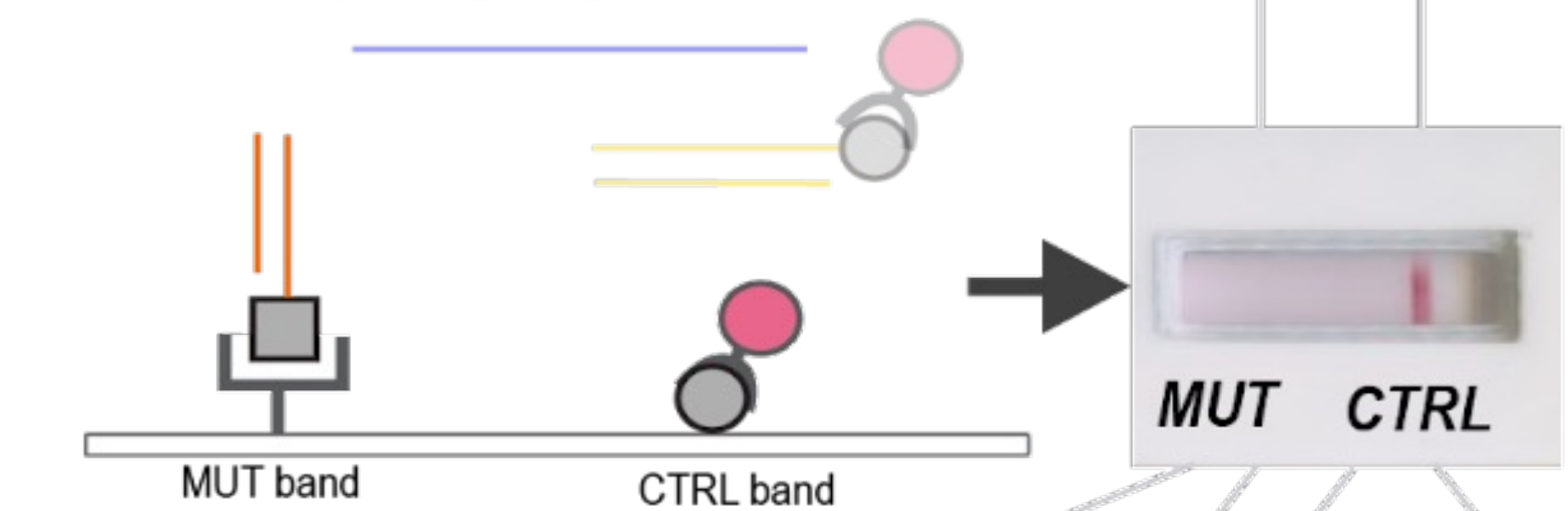
Sequential delivery

- When the **detection fluid did not contain CO**, strong mutant bands could be distinguished visually **Fig. 4**:
 - Output intensities (n=3, mean ± SE) at the mutant band **without CO** were **16.3 ± 2.7**
- When the **detection fluid contained CO**, signal at the mutant bands was not visible and was comparable to the signal of the negative controls:
 - Output intensities at the mutant band **with CO** were **0.17 ± 0.11**

4. Without competing oligos in detection mix:



With competing oligos in detection mix:



Conclusions

- Demonstrated a proof-of-concept custom LFT:
 - Self-contained/streamlined** workflow
 - Sequential delivery** of ligation product and detection buffer without "opening" amplicon-containing tubes
- Potential to improve the implementation of OLA-Simple for HIVDR testing in low-resource settings:
 - Reduces amplicon contamination in laboratories
 - Costs **\$0.25 USD per unit** vs. \$15 USD per unit for commercial amplicon-contained LFTs
 - Uses **biodegradable plastics with safe plastic pins** vs. metal blades which require thicker plastic to ensure user safety [3]
- Some inconsistent flow across replicates will require further 3D printer quality optimization

Acknowledgments

Collaborators: Dr. David McIntyre for prior contributions to earlier designs of the custom LFT device; Dr. Annie Wong-On-Wing, Annapurni Sriram, and Dr. Amy Oreskovic for their contributions to assay chemistries; Parker Ruth and Brian Tran for software analysis; Dr. Lisa Frenkel and her group at Seattle Children's Research Institute for their expertise in OLA for HIVDR.

Funds: NIH R01 (R01AI145486). The funder has no role in the study design or interpretation of this work.

References

