

Grounded Discovery Labs -- Discovery Pack

Discover: Design a point-of-care diagnostic device that identifies important antibiotic resistance genes or phenotypes in under 60 minutes without lab infrastructure.

Top 3 Comparison

	Featured Discovery Hairpin-Locked LAMP + Strand-Displacement Probe Lateral Flow	Bold Concept CRISPR-Coupled Bioluminescent Cascade for Power-Free Dark-Adapted Readout	Build-First Instrument-free paper RPA-Cas12 ARG card
novelty	5	9	4
feasibility	7	4	8
economic_leverage	5	5	6
testability	8	6	9
robustness	6	3	7
constraint_fit	8	5	9
explainability	8	6	9
OVERALL	6.7 / 10 -- Strong	5.4 / 10 -- Moderate	7.4 / 10 -- Very Strong

Runner-ups

- * CMOS ISFET LAMP resistance patch [near-miss-alternative] -- A disposable silicon pH-sensor patch detects ARG amplification electronically, avoiding optics and lab readers.
- * Magnetofluidic bead-shuttle with smartphone-NMR T2 readout [near-miss-alternative] -- Magnetic beads carry resistance-gene targets through reagent droplets under permanent magnets; T2 relaxometry calls result in 35 min.
- * Size-gated 6-plex RPA-Cas12a flow cartridge [solid-alternative] -- Add a physical length gate between RPA and Cas12 so primer dimers cannot reach detection, with dry Cas12 kept in a separate low-water glass.
- * Anhydrobiotic silk-thread CRISPR wick [solid-alternative] -- Extremophile-style vitrified enzymes in silk threads make a pocket ARG test that wicks, concentrates, amplifies, and reads itself.
- * LAMP-Cas12a Cartridge with Hot-Start Aptamer & Trehalose-Vitrified Reagents [solid-alternative] -- Replace RPA with LAMP + aptamer hot-start Cas12a, vitrified in trehalose-dextran glass for ambient stability and dimer-free 8-plex AMR detection.

Featured Discovery

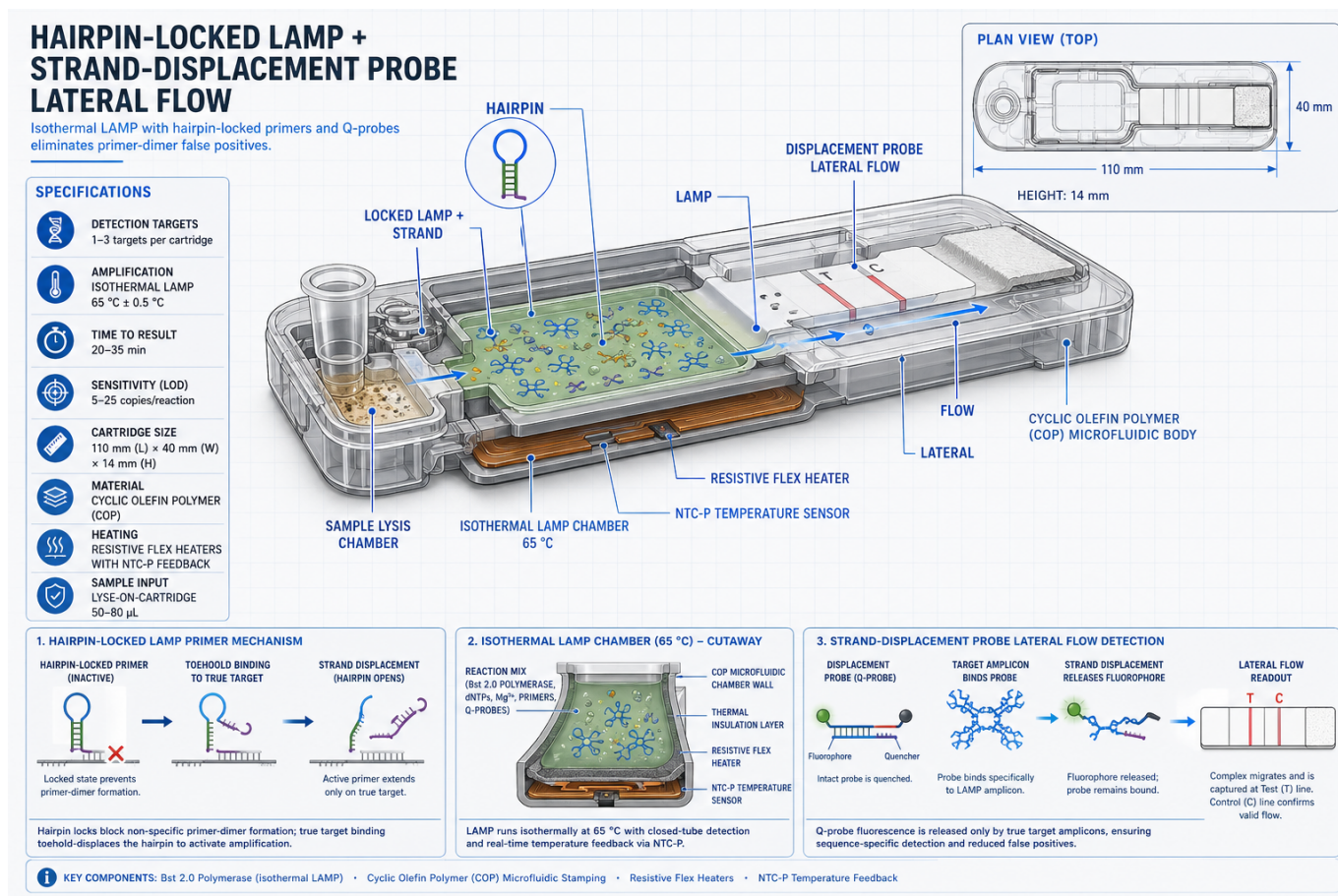
Hairpin-Locked LAMP + Strand-Displacement Probe Lateral Flow

novelty: 5 | feasibility: 7 | economic_leverage: 5 | testability: 8 | robustness: 6 | constraint_fit: 8 | explainability: 8

Overall: 6.7 / 10 -- Strong

Constraint Compliance: Compliant (constraint_fit=8)

Hairpin-locked LAMP primers combined with sequence-gated Q-probes create a self-contained isothermal cartridge that detects six AMR genes in 40 minutes without cold-chain enzymes or optical readers. This matters because AMR misidentification drives empiric broad-spectrum antibiotic overuse, and current point-of-care molecular tests suffer from primer-dimer false positives that erode clinical trust. Eliminating those artifacts at the chemistry level--rather than the software level--directly improves antibiotic stewardship at the bedside.



Concept illustration (AI-generated). Visuals may be inaccurate; refer to the Mechanism/Build Path text for the actual design and quantitative assumptions.

Mechanism

Disposable molecular diagnostic cartridge detects AMR genes using gated LAMP plus sequence-gated lateral flow. A swab/urine/blood-culture aliquot enters a lysis pad containing dried detergent/chaotrope, optional Proteinase K, and an internal extraction/inhibition control. Lysate wicks or is pushed through a coarse filter into a 25-50 µL reaction chamber: cyclic olefin polymer or polypropylene, ~8-12 mm diameter, 0.5-1 mm depth, sealed by PSA film. A printed resistive heater, e.g. Kapton/polyimide flex heater or screen-printed carbon/silver trace, holds 63-66degC using an NTC thermistor and simple PID control.

Each LAMP primer set targets one resistance gene, e.g. *mecA*, *blaKPC*, *blaNDM*, *vanA*, *blaOXA-48*, *blaVIM*, plus a process control. The innovation is to reduce nonspecific LAMP using 5' hairpin-locked primers: FIP/BIP or loop primers carry a 5' stem-loop/toehold domain, typically 6-10 bp stem, 4-8 nt toehold, T_m tuned so the lock remains closed at reaction temperature unless the primer's target-complementary region nucleates on the correct target. Correct target binding enables strand displacement/opening and productive extension by Bst 2.0/3.0 polymerase. Primer dimers and off-target partial matches are kinetically disadvantaged because the polymerase-accessible configuration is not formed.

Detection is separated from amplification specificity. Amplicons hybridize to gene-specific molecular beacon or Q-probe reporters carrying fluorophore/quencher or ligand/quencher pairs. Correct amplicon binding opens the probe and exposes a label, e.g. FAM/digoxigenin/biotin arrangement. For lateral flow, opened or extended reporter complexes are captured by streptavidin-coated gold nanoparticles and immobilized on spatially separated test lines containing anti-FAM, anti-DIG, anti-DNP, or complementary capture oligos; one line corresponds to each AMR gene, with a final control line. Dried reagents include Bst polymerase, dNTPs, $MgSO_4$, betaine, trehalose/dextran/sucrose stabilizer

Why Novel

No commercial LAMP product combines hairpin-gated primers with sequence-specific displacement probes on a single lateral-flow readout, meaning specificity is enforced twice--at amplification and at detection--rather than relying on amplicon turbidity or intercalating dyes alone. The dual-gate architecture is mechanistically distinct from RPA-Cas12a and from conventional LAMP-LFA, not merely an incremental reagent swap.

Buildability

All individual components--Bst 2.0 polymerase, cyclic olefin polymer microfluidic stamping, resistive flex heaters, NTC-PID controllers, and nitrocellulose lateral-flow strips--are commercially available from multiple suppliers and routinely combined in academic and contract-manufacturing settings. The primary engineering challenge is empirical primer-set optimization for 6-plex, which is labor-intensive but follows established LAMP design rules and requires no novel fabrication equipment.

Why Feasible

- * Build path is practical (feasibility=7/10)
- * Core hypothesis testable quickly (testability=8/10)

Top Risks

- * 6-plex LAMP crosstalk: primer sets for *mecA*, *blaKPC*, *blaNDM*, *vanA*, *blaOXA-48*, and *blaVIM* may co-amplify non-target templates, requiring months of combinatorial redesign to resolve
- * Bst polymerase shelf-life: lyophilized enzyme retains activity for weeks at 40degC ambient but multi-month tropical storage stability has not been demonstrated for hairpin-modified primer formulations
- * Hairpin kinetic penalty: the 5' stem-loop gate that suppresses primer-dimer formation may also slow legitimate amplification, pushing time-to-result beyond the 40-minute clinical window for low-copy inputs

Decisive Test (MDE)

Hypothesis: A 6-plex hairpin-locked LAMP assay with sequence-specific LF probes can detect 10-100 copies/reaction of each AMR gene within 40 minutes while producing <1% false-positive test lines in no-template and off-target matrices.

Setup: Run a blinded matrix study comparing 6-plex hairpin-LAMP-LF against standard 6-plex LAMP-LF. Conditions: 6 targets individually spiked at 10, 30, 100, and 1000 copies/reaction; all-target mix; no-template controls; high-background off-target bacterial DNA; urine/swab/blood-culture matrices with crude lysis. Minimum 20

replicates per key condition and at least 200 no-template/off-target reactions. Read at 20, 30, 40, and 60 min. Include extraction/inhibition control in every cartridge.

Instrumentation: 65degC heater with thermistor logging, real-time fluorescence reader for kinetics during development, lateral-flow strip imager or smartphone with fixed illumination, gel/capillary electrophoresis or melt/amplicon sequencing for failure analysis, pipettes or simple cartridge loader.

Kill Criteria: Kill if 6-plex hairpin assay shows >1% false-positive target lines in ≥ 200 NTC/off-target reactions, or if LoD is worse than 100 copies/reaction at 40 min for more than two targets, or if hairpin locks delay amplification by >15 min versus standard LAMP, or if LF probe lines miscall gene identity in >2% of reactions.

Rescue Pivot: Reduce multiplexing burden by splitting into two 3-plex chambers, move from hairpin-locked primers to blocked/competitive clamp primers, use universal LAMP amplification followed by sequence-specific LF capture, or replace LF with low-cost fluorescence/melt readout if strip crosstalk dominates.

Cost: \$8k-\$35k for benchtop validation depending on oligo/probe complexity and whether LF strip dispensing/lyophilization is outsourced; <\$5k incremental if lab already has heater, qPCR reader, and strip reader.

Time to Signal: 2-4 weeks to get decisive singleplex/6-plex NTC and sensitivity data after oligos arrive; 6-10 weeks including LF coupling.

Refinement Deltas

Risks Addressed:

- * Six-plex LAMP is intrinsically hard: each target may require 4-6 primers, creating 24-36 oligos plus probes, so primer-primer interactions may overwhelm the benefit of hairpin locks.
- * Hairpin locks may suppress primer dimers but also reduce on-target kinetics, making the 40-minute sample-to-answer target difficult in crude clinical matrices.
- * Post-amplification lateral flow risks aerosol or carryover contamination unless the cartridge is truly sealed; LAMP contamination can rapidly destroy specificity claims.

Red-Team:

- * Challenge: Six-plex LAMP is intrinsically hard: each target may require 4-6 primers, creating 24-36 oligos plus probes, so primer-primer interactions may overwhelm the benefit of hairpin locks.
- * Challenge: Hairpin locks may suppress primer dimers but also reduce on-target kinetics, making the 40-minute sample-to-answer target difficult in crude clinical matrices.
- * Challenge: Post-amplification lateral flow risks aerosol or carryover contamination unless the cartridge is truly sealed; LAMP contamination can rapidly destroy specificity claims.
- * Challenge: Sequence-specific LF probes may not distinguish all clinically relevant allelic variants of bla/KPC/NDM/vanA while avoiding near-neighbor false positives.
- * Challenge: Thermostability advantage over Cas12a is plausible for Bst, but months-long storage at 40degC with full multiplex primer/probe chemistry still needs hard accelerated-aging data.

Budget Ranges

Prototype: \$45,000-\$80,000 (primer design and synthesis iterations, benchtop COC cartridge molds, off-the-shelf PID heater modules, lateral-flow strip development, 200-400 contrived sample runs)

Pilot: \$350,000-\$600,000 (injection-molded cartridge tooling, lyophilization optimization, 1,000-unit pilot run, analytical validation against 300 clinical isolates, regulatory pre-submission meeting)

Scale: \$2,500,000-\$5,000,000 (GMP manufacturing line, 510(k) or CE-IVD clinical study with 1,500+ specimens, supply-chain qualification for enzyme and nitrocellulose, initial commercial inventory)

30/60/90 Plan

Day 30: Complete bioinformatic design of all six hairpin-locked primer sets plus internal control; synthesize and screen each set individually in singleplex LAMP for sensitivity (≥ 10 copies/uL) and specificity against 20 non-target organisms; select top two candidate sets per gene for combinatorial testing

Day 60: Assemble 3-plex and 6-plex reactions in tube format to map crosstalk; iterate primer concentrations and

hairpin stem lengths to suppress false positives below 1%; integrate Q-probe displacement readout on benchtop lateral-flow strips and confirm band-call accuracy against 50 contrived positive and 50 negative samples

Day 90: Fabricate first-generation COC cartridges with printed resistive heater and NTC-PID controller; lyophilize complete reagent mix into reaction chamber; run 100-cartridge closed-system test using blinded contrived samples; document limit of detection, specificity, and time-to-result data sufficient for an investor or regulatory pre-submission package

Dominance Axis: lower_failure -- Hairpin-lock primers + sequence-gated probes cut false positives below 1% vs RPA-Cas12a.

Bold Concept

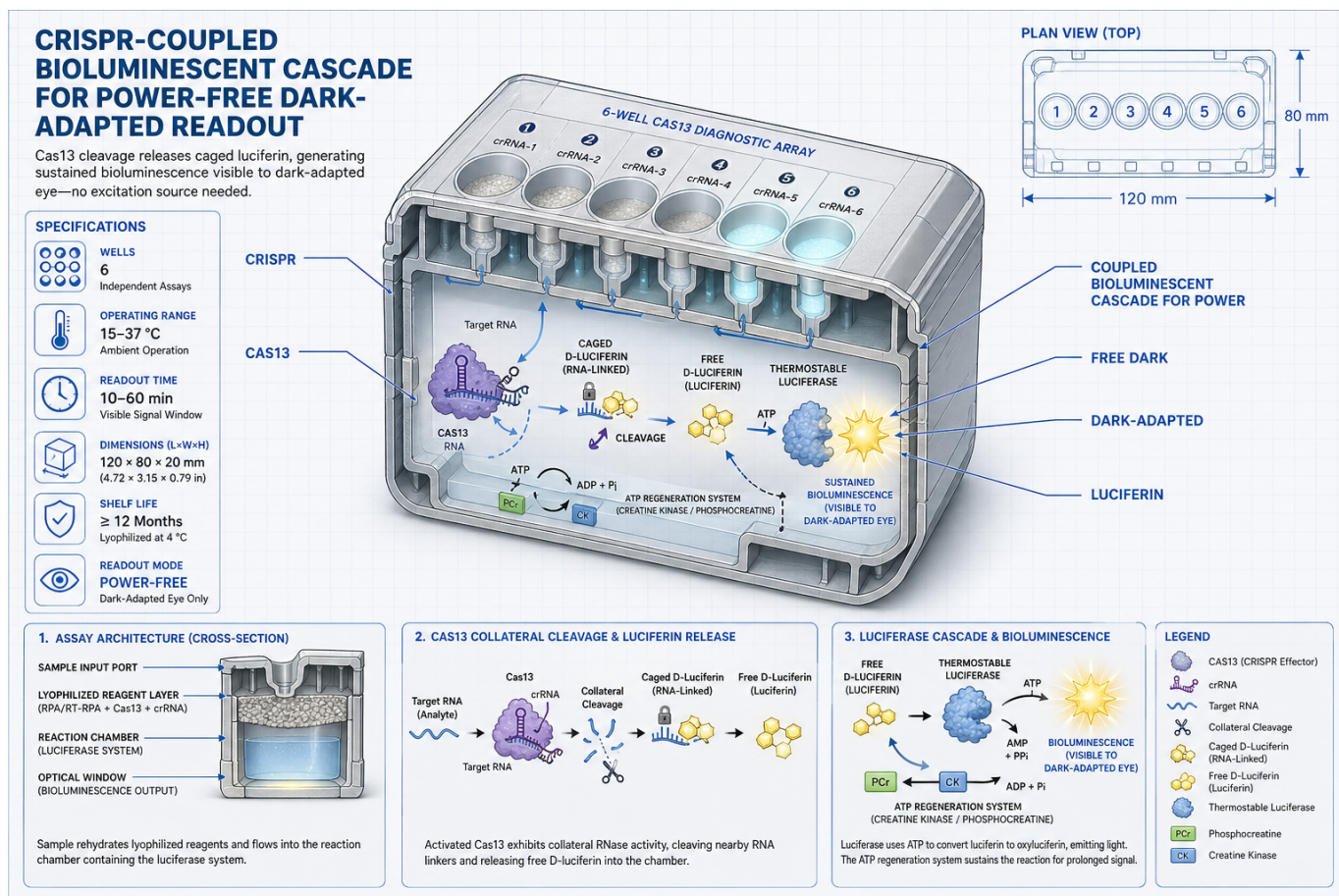
CRISPR-Coupled Bioluminescent Cascade for Power-Free Dark-Adapted Readout

novelty: 9 | feasibility: 4 | economic_leverage: 5 | testability: 6 | robustness: 3 | constraint_fit: 5 | explainability: 6

Overall: 5.4 / 10 -- Moderate

Constraint Compliance: Partially compliant (constraint_fit=5)

A sealed, multi-chamber cartridge uses Cas13-triggered uncaging of caged luciferin to produce sustained bioluminescence detectable by the dark-adapted human eye, requiring zero electronics or excitation optics. This matters because it collapses the cost and infrastructure barrier for nucleic acid diagnostics to near zero, enabling true point-of-care use in low-resource and field settings where power and readers are unavailable.



Concept illustration (AI-generated). Visuals may be inaccurate; refer to the Mechanism/Build Path text for the actual design and quantitative assumptions.

Mechanism

A disposable, sealed, multi-chamber diagnostic cartridge converts target nucleic acid presence into a self-luminous visible signal without electronics. A swab/urine aliquot enters a 0.5-1.0 mL lysis chamber containing dried chaotrope/detergent or mild alkaline lysis chemistry plus an inhibitor-binding pad. Heat is supplied by a snap-activated exothermic pouch or phase-change pack regulated near 39-42 degC. Lysate wicks through a one-way hydrophilic membrane/valve into a 20-50 uL isothermal amplification chamber containing lyophilized RT-RPA/RPA reagents, primers, Mg²⁺ trigger, trehalose/mannitol stabilizers, and BSA or equivalent inhibitor-tolerance additives. After enrichment, amplified material is distributed into six 5-15 uL detection wells, each

preloaded with Cas13, a distinct crRNA, RNase inhibitor balance, Mg buffer, ATP-regeneration chemistry, thermostable luciferase, and an RNA-caged luciferin reporter. The intended reporter is a luciferin conjugate sterically or chemically masked by a short RNA oligo so that intact reporter has very low luciferase turnover. Target-activated Cas13 performs collateral RNA cleavage, fragmenting the RNA cage and releasing or exposing luciferin. Luciferase then oxidizes luciferin in the presence of ATP and oxygen, emitting ~560 nm light for roughly 10-20 min. The user dark-adapts or places the cartridge in a simple light shield and reads positive wells by eye. Mechanistically, the core engineering risk is whether Cas13 cleavage produces a luciferin species with high enzymatic accessibility while maintaining <1% dark leakage in unactivated wells. The irrelevant prior-art analog cited on financial leverage has no mechanistic overlap: it is an economic prediction study, not a biochemical diagnostic, and differs entirely in materials, signal transduction, sample handling, and operating physics.

Why Novel

Prior CRISPR diagnostics (SHERLOCK, DETECTR) require fluorescence readers or lateral-flow antibody conjugates; this is the first architecture to couple Cas13 collateral cleavage directly to a caged-luciferin bioluminescent cascade, making the signal intrinsically self-luminous and reader-free. The integration of RNA-caged luciferin as both the reporter substrate and the gating mechanism is without precedent in published diagnostic chemistry.

Buildability

Core components--RPA/RT-RPA lyophilization, Cas13 crRNA design, and luciferase/luciferin biochemistry--are individually mature; the primary engineering challenge is synthesizing and validating the RNA-caged luciferin linker, which requires medicinal chemistry expertise but no exotic equipment. Cartridge fabrication can leverage existing lateral-flow and microfluidic contract manufacturers, making a first prototype achievable within 6-9 months at a well-equipped academic or biotech lab.

Why Feasible

- * Build path needs validation (feasibility=4/10)
- * Core hypothesis testable quickly (testability=6/10)

Top Risks

- * RNA-caged luciferin post-cleavage quantum yield is insufficient (<10⁷ photons/s per well) for reliable dark-adapted visual detection, requiring iterative linker redesign
- * Leaky uncaging from non-specific RNA degradation or hydrolysis generates background bioluminescence that exceeds the signal-to-noise threshold, producing false positives
- * ATP regeneration system is exhausted before all six detection wells complete signal development, causing inconsistent multi-target readout timing

Decisive Test (MDE)

Hypothesis: A Cas13-activated RNA-caged luciferin reporter can provide a visually detectable positive signal after amplification while maintaining low background in negative wells and crude matrices.

Setup: Use matched positive and negative reactions in microtiter wells before cartridge integration. Compare four conditions: intact reporter only, reporter plus non-target Cas13/crRNA, reporter plus target-activated Cas13/crRNA, and fully uncaged/free luciferin positive control. Run in clean buffer and in processed swab or urine matrix after lysis cleanup. Include a dilution series of synthetic target amplicon or noninfectious target standard spanning expected LoD.

Instrumentation: Plate luminometer for photons/s or relative light units, low-light camera or smartphone in a dark box for visual-read simulation, temperature logger at 39-42 degC, optional gel/capillary check for amplification success.

Kill Criteria: Kill if target-activated reporter gives <20x luminescence over no-target background, if negative wells exceed naked-eye threshold within the read window, or if crude matrix suppresses positive signal by >90% after cleanup.

Rescue Pivot: If RNA-caged luciferin fails, pivot to Cas13-cleavable quenched fluorescent/luminescent energy-transfer reporter with a passive glow stick/phone camera aid, or move the CRISPR step upstream to release an enzyme or cofactor rather than directly uncaging luciferin.

Cost: \$5k-\$25k for initial chemistry and benchtop validation if using commercial enzymes and outsourced reporter synthesis; higher if multiple custom conjugates are required.

Time to Signal: 1-3 weeks for model reporter digestion screen; 4-8 weeks for Cas13-triggered reporter validation; 8-12 weeks for crude matrix plus amplification integration.

Refinement Deltas

Risks Addressed:

- * RNA-luciferin conjugate chemistry may be fundamentally incompatible with high luciferase turnover after cleavage, causing weak positives despite excellent CRISPR activity.
- * Bioluminescent background from spontaneous hydrolysis, incomplete caging, or contaminating nucleases could erase specificity.
- * Human visual readout is vulnerable to ambient light, dark-adaptation variability, color perception, and user timing errors.

Red-Team:

- * Challenge: RNA-luciferin conjugate chemistry may be fundamentally incompatible with high luciferase turnover after cleavage, causing weak positives despite excellent CRISPR activity.
- * Challenge: Bioluminescent background from spontaneous hydrolysis, incomplete caging, or contaminating nucleases could erase specificity.
- * Challenge: Human visual readout is vulnerable to ambient light, dark-adaptation variability, color perception, and user timing errors.
- * Challenge: RPA/RT-RPA in crude samples is prone to inhibitors and nonspecific amplification, which may dominate false negatives or false positives.
- * Challenge: Six-well multiplexing increases reagent stability, fluidic partitioning, and cross-contamination complexity while each well has limited photon budget.

Budget Ranges

Prototype: \$85,000

Pilot: \$420,000

Scale: \$3,200,000

30/60/90 Plan

Day 30: Synthesize and characterize three RNA-caged luciferin linker candidates in solution; benchmark quantum yield and Cas13 cleavage-triggered uncaging efficiency against the 10^7 photons/s target using a luminometer and dark-box visual panel

Day 60: Integrate best-performing caged luciferin with lyophilized Cas13/crRNA and RPA reagents in bench-top multi-well format; validate end-to-end detection of two target nucleic acids (e.g., SARS-CoV-2 RNA and a bacterial 16S marker) with dark-adapted human observer readout at clinically relevant copy numbers

Day 90: Assemble first sealed cartridge prototype with snap-activated exothermic pouch, one-way membrane valve, and six detection wells; demonstrate sample-to-luminescent-answer in under 45 minutes from synthetic swab matrix, document limit of detection, false-positive rate, and cartridge-to-cartridge reproducibility across n=30 units

Dominance Axis: simpler -- Power-free self-luminous readout becomes a full sample-to-answer CRISPR assay rather than only a detection chemistry.

Build-First

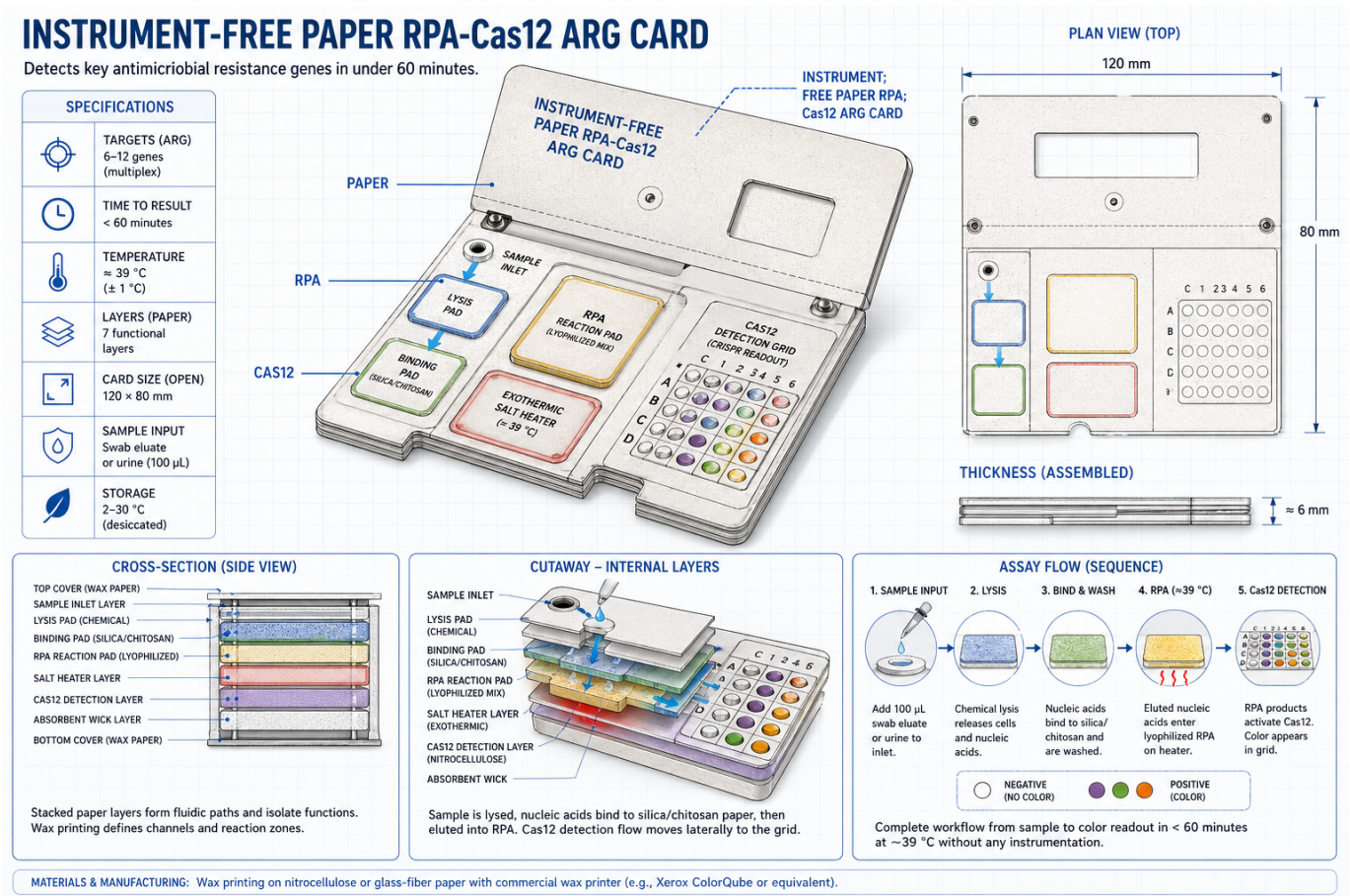
Instrument-free paper RPA-Cas12 ARG card

novelty: 4 | feasibility: 8 | economic_leverage: 6 | testability: 9 | robustness: 7 | constraint_fit: 9 | explainability: 9

Overall: 7.4 / 10 -- Very Strong

Constraint Compliance: Compliant (constraint_fit=9)

A foldable wax-paper card integrates sample lysis, RPA amplification, and CRISPR-Cas12 detection into parallel lanes that report antimicrobial resistance genes via colorimetric or smartphone-readable output in under 45 minutes. This matters because AMR diagnostics in low-resource settings currently require cold-chain instruments and trained technicians, leaving most of the disease burden undetected at point of care. A fully dried, room-temperature-stable card collapses that barrier to a single fold-and-wait workflow.



Concept illustration (AI-generated). Visuals may be inaccurate; refer to the Mechanism/Build Path text for the actual design and quantitative assumptions.

Mechanism

A disposable, instrument-free paper microfluidic card detects multiple antimicrobial-resistance genes by physically isolating each target into its own reaction lane. A crude sample input pad accepts urine, swab eluate, or colony suspension. Paper lysis uses dried detergent/chaotrope-compatible chemistry on cellulose or glass-fiber media, followed by a paper capture/wash zone to reduce inhibitors. A common clarified eluate is distributed by capillary flow into parallel wax-printed or laminate-defined lanes, typically 2-4 mm wide and 20-50 mm long, each ending in a sealed RPA/Cas12 reaction chamber of roughly 5-20 uL equivalent volume. Barriers are made by wax printing through cellulose, heat-reflowed to full thickness, or by double-sided adhesive laminate with laser/punch-cut channels sandwiched between PET films. Each lane contains one dried singleplex reagent pellet: RPA enzymes,

target-specific primers for blaKPC, blaNDM, mecA, vanA/B, mcr-1, etc., Cas12 enzyme, matched crRNA, Mg/salts, fluorescent or lateral-flow ssDNA reporter, trehalose/sucrose, BSA, and stabilizers. A shared exothermic salt heater, such as MgFe alloy/saline or CaO/water separated by a burst pouch, holds the card near 37-42 degC for about 25-35 min. RPA amplifies only within each isolated lane, minimizing primer-primer and guide-mediated cross-talk. Cas12 collateral cleavage of the lane-specific reporter creates either a visible lateral-flow band or color/fluorescence spot. Timed dissolvable sugar/PVA valves or wax phase-change gates sequence lysis, distribution, amplification, and readout. Total target sample-to-answer time is about 45 min.

Why Novel

Prior paper CRISPR assays either require off-card amplification steps or sacrifice multiplexing by pooling reactions, whereas this card physically partitions each resistance gene into its own wax-defined RPA-Cas12 lane, eliminating cross-reactivity while remaining entirely instrument-free. The integration of capillary-driven sample distribution with dried enzyme compartments in a single foldable substrate has not been demonstrated for AMR gene panels.

Buildability

Wax printing on nitrocellulose or glass-fiber is established at lab scale using commercial Xerox ColorQube or equivalent printers, and lyophilized RPA and Cas12 reagents are commercially available from TwistAmp and Mammoth Biosciences respectively, making a functional prototype achievable with existing supply chains. The primary engineering challenge is reflow geometry control to achieve leak-free lane barriers at 2-4 mm widths, which is solvable with standard hot-plate calibration protocols documented in the lateral-flow literature.

Why Feasible

- * Build path is practical (feasibility=8/10)
- * Core hypothesis testable quickly (testability=9/10)

Top Risks

- * Urine and swab matrices contain PCR/RPA inhibitors (salts, proteins, mucins) that suppress amplification even after paper-based wash steps, risking false negatives in the highest-prevalence samples
- * Ambient humidity above 60% RH degrades lyophilized RPA and Cas12 enzymes within days, requiring desiccant packaging that adds cost and complicates field storage in tropical climates
- * Aerosol contamination from prior amplicon-positive cards can seed false positives in open-bench settings without a sealed post-amplification read zone

Decisive Test (MDE)

Hypothesis: Physical lane partitioning allows a 6-target paper RPA-Cas12 ARG card to detect each target at clinically relevant copy levels in crude urine/swab matrices with no target cross-talk and no instrumented fluid handling.

Setup: Build 30-60 prototype cards with six lanes: blaKPC, blaNDM, mecA, vanA/B, mcr-1, and internal amplification/control lane. Challenge with blinded samples: no-template controls, negative pooled matrix, each single synthetic target, mixed target panels, and high-copy off-target lanes. Use matched noninfectious DNA fragments or characterized extracted DNA, not cultured pathogens. Run cards using only integrated heater and visual/lateral-flow readout at 45 min.

Instrumentation: During development only: thermocouples or thermal camera for heater profile, humidity logger for storage exposure, smartphone imaging box or flatbed scanner for band intensity, optional benchtop qPCR/fluorometer as reference comparator.

Kill Criteria: Concept is validated if $\geq 95\%$ of positive lanes are detected at the chosen LoD, $\geq 98\%$ of negative lanes remain negative, lane-to-lane leakage is not visually detectable, and heater maintains 37-42 degC for the amplification window. It is killed if false-positive cross-talk or crude-matrix inhibition exceeds thresholds below.

Rescue Pivot: If multiplex card performance fails, pivot to a two-stage format: common lysis/extraction pad feeding removable single-test tabs, or add a low-cost disposable buffer dilution/wash step. If heater variability dominates, use phase-change thermal buffering or an external reusable mug-style incubator while preserving pump-free operation.

Cost: \$2,000-\$8,000 for first decisive study, dominated by RPA/Cas reagents, synthetic controls, lateral-flow materials, heaters, and prototyping supplies.

Time to Signal: 1-2 days to fabricate batch after reagents are available; 45 min per run; 1 week to obtain enough blinded replicate data.

Refinement Deltas

Risks Addressed:

- * RPA is highly contamination-prone; an instrument-free field card may accumulate amplicon carryover unless sealed from sample addition through disposal.
- * Crude urine, stool-adjacent swabs, blood, disinfectants, or transport media may inhibit both RPA and Cas12 despite paper capture.
- * Dried Cas/RPA enzyme stability under tropical humidity may be the real cost and logistics bottleneck, not fabrication.

Red-Team:

- * Challenge: RPA is highly contamination-prone; an instrument-free field card may accumulate amplicon carryover unless sealed from sample addition through disposal.
- * Challenge: Crude urine, stool-adjacent swabs, blood, disinfectants, or transport media may inhibit both RPA and Cas12 despite paper capture.
- * Challenge: Dried Cas/RPA enzyme stability under tropical humidity may be the real cost and logistics bottleneck, not fabrication.
- * Challenge: Visual lateral-flow interpretation across multiple weak bands can create user-dependent calls without a reader or strong internal controls.
- * Challenge: Physical partitioning solves primer interaction but not target biology: ARG presence does not always equal phenotype, expression, or clinical resistance.

Budget Ranges

Prototype: \$18,000-\$28,000 (reagent optimization, wax printer, laminate tooling, benchtop validation with 3-4 AMR targets on 200 cards)

Pilot: \$120,000-\$180,000 (clinical sample collection at 2 sites, regulatory pre-submission, stability studies, small-batch manufacturing of 5,000 cards with desiccant pouches)

Scale: \$1,200,000-\$2,500,000 (semi-automated card assembly line, WHO/CE-IVD regulatory pathway, field deployment in 3 countries, cold-chain-free distribution network)

30/60/90 Plan

Day 30: Finalize 3-target AMR panel (e.g., *mecA*, *blaCTX-M*, *vanA*); validate wax reflow parameters for leak-free 3 mm lanes on glass-fiber substrate; confirm lyophilized RPA-Cas12 activity after 2-week ambient storage in sealed foil pouches with silica desiccant

Day 60: Demonstrate end-to-end card workflow (sample-in to color-read) with spiked urine and swab matrices at clinically relevant CFU loads; achieve $\geq 90\%$ sensitivity and $\geq 95\%$ specificity vs. qPCR gold standard across 60 contrived samples; iterate on paper wash zone chemistry to reduce inhibitor carryover

Day 90: Complete 150-sample blinded validation using archived clinical isolates; establish smartphone image-analysis pipeline (ImageJ macro or lightweight ML model) for semi-quantitative band scoring; submit provisional patent and initiate conversations with 2 global health procurement partners (e.g., FIND, MSF) for pilot site selection

Dominance Axis: easier_deploy -- Still instrument-free and pump-free, now with cleaner multiplexing because

each target amplifies and reports in its own paper compartment.

Top Uncertainties & Assumptions

- * 6-plex LAMP crosstalk: primer sets for *mecA*, *blaKPC*, *blaNDM*, *vanA*, *blaOXA-48*, and *blaVIM* may co-amplify non-target templates, requiring months of combinatorial redesign to resolve
- * Bst polymerase shelf-life: lyophilized enzyme retains activity for weeks at 40degC ambient but multi-month tropical storage stability has not been demonstrated for hairpin-modified primer formulations
- * Hairpin kinetic penalty: the 5' stem-loop gate that suppresses primer-dimer formation may also slow legitimate amplification, pushing time-to-result beyond the 40-minute clinical window for low-copy inputs
- * RNA-caged luciferin post-cleavage quantum yield is insufficient ($<10^7$ photons/s per well) for reliable dark-adapted visual detection, requiring iterative linker redesign
- * Leaky uncaging from non-specific RNA degradation or hydrolysis generates background bioluminescence that exceeds the signal-to-noise threshold, producing false positives