

DESIGN BRIEF

Low-cost visual diagnostic for colorectal cancer via bacterial detection of *kRAS*, *TP53*, and *APC* mutations

Ibrahim B. Abumohsen, Luka Ascencio, Havish Bathina, Madeline Boram-Rella, Brayden Carty, Alyssa Gay, Isaac J. Pare, and Priyansh Shah ■ Athens Drive Magnet High School, Raleigh, North Carolina, United States of America

Reviewed on 9 May 2026; Accepted on 22 June 2026; Published on 26 October 2026

Colorectal cancer (CRC) is the second most commonly diagnosed cancer, with an estimated 154,270 individuals diagnosed in 2025 alone, and 52,900 deaths in the US. Yet of all Americans, only 60% of the at-risk population will be tested. This hesitancy is in part due to current diagnostic methods (*e.g.*, fecal immunochemical testing, colonoscopy, computed tomography colonography), which are either expensive, invasive, or require both specialized medical equipment and operational expertise. Live biologic-based diagnostic technologies show great promise for closing the diagnostic gap in CRC. In particular, a sensor capable of detecting genetic mutants that employs the chassis organism *Acinetobacter baylyi* (*A. baylyi*). This bacterium acquires DNA from its environment, such as the gastrointestinal tract. When combined with a modified CRISPR counterselection system, this organism can detect critical mutations in the *kRAS* oncogene with extraordinary specificity, capable of distinguishing DNA base pair alterations. Here, we developed a design for a novel diagnostic technology, which detects three of the most prevalent CRC-associated gene mutations (*APC*, *TP53*, and *kRAS*) while fixed within a calcium alginate hydrogel applied on a nondigestible pill. This results in the expression of an *AmilCP* reporter, allowing for a color change in the presence of genetic mutants. This biologic system aims to minimize the diagnostic gap of CRC by facilitating easy at-home detection and providing patient-interpretable results.

Keywords: Biosensor pill, CRISPR-Cas3 detection, colorectal cancer screening, *Acinetobacter baylyi*, cell-free DNA, cfDNA, CRISPR



The authors were mentored by Kaitlin M. Dailey, PhD; University of Rhode Island, College of Pharmacy, Kingston, Rhode Island, United States of America; Legorreta Cancer Center at Brown University, The Warren Alpert Medical School, Brown University, Providence, Rhode Island, United States of America; Rhode Island IDeA Network of Biomedical Research Excellence, University of Rhode Island, Kingston, Rhode Island, United States of America. Please direct correspondence to: Kaitlin Dailey (kaitlin.dailey@uri.edu). This is an Open Access article, which was copyrighted by the authors and published by BioTreks in 2026. It is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited.

CANCER is one of the most prevalent causes of death worldwide, accounting for 613,349 recorded deaths in the US alone during 2023 (Cancer Data and Statistics | Cancer, 2026). Colorectal cancer, or CRC, is the second most common cause of cancer death - largely due to the lack of early diagnosis (Colorectal Cancer Statistics, 2023, n.d.). During the early stages of CRC, it is very easily treatable, and can have up to a 91% 5-year survival rate when the cancer is still localized, which is why it is best for the cancer to be detected as soon as possible (Colorectal Cancer Survival Rates | Colorectal Cancer Prognosis, 2026). However, when the cancer spreads to other parts of the body in later stages, the 5-year survival rate drops

to approximately 8% (**Table 1**). Unfortunately, only 1 in 3 cases are diagnosed during the initial stages (I and II), when the cancer is still localized (About Colorectal Cancer, n.d.). This is largely attributed to lack of patient compliance with recommended CRC screening processes. In fact, the CDC reports that approximately 33% of adults aged 50-64 have an outdated CRC screening (Use of Colorectal Cancer Screening Tests | Colorectal Cancer, 2025). This lack of regular screening is at least in part due to current CRC screening methods' complexity and invasiveness. In 2005, a multi-institution surveyed 660 patients with open ended questions regarding CRC screening barriers (Devers et al., 2010). 74% responded that the

TABLE 1
RELATIVE FIVE YEAR SURVIVAL RATE FOR CRC PATIENTS
BY STAGE (ADAPTED FROM (O'CONNELL ET AL., 2004))

Stage	5 Year Survival Rate
1	>90%
2	82%
3	60%
4	8%

most important barrier to CRC screening was fear or anxiety surrounding the process. This design aims to encourage patients to get screened by helping them evaluate their risk factor for acquiring this disease.

Here, we describe a live pill (**Figure 1**) able to detect biomarkers of CRC, which is meant to provide a cost effective, user-friendly, at-home test meant to help patients analyze their risk factor. Although this device is not intended to replace the current clinical standard care, it will detect common signs of CRC or precancerous lesions - thereby providing prescreening, addressing both primary care provider case load and patient financial burdens. Based on whether these biomarkers are detected, the patient can make an informed decision about the need for further medical attention. A key feature of this pill is the ability to detect CRC and precancerous lesions during their early stages when the cancer is still localized and therefore easier to treat (*e.g.*, polypectomy). CRC is a very treatable disease if detected and treated early enough, thus this novel intervention will directly have a positive patient impact.

Briefly, to detect CRC, the pill is ingested orally and subsequently moves through the digestive tract into the large intestine. During transport, the pill repeatedly incorporates exogenous DNA from its environment (the colorectal organs) and detects sequence variants indicative of CRC. The system uses *A. baylyi* as a bacterial chassis, genetically engineered to serve as a living biosensor. *A. baylyi* has demonstrated a natural capacity to acquire DNA from its environment. When this ability is harnessed and connected to an *amilCP* reporter, it can thus be engineered to express *amilCP* upon exposure to certain genetic mutations that often serve as CRC biomarkers. When these biomarkers are detected, the pill will turn a dark blue color. After defecation, the user will be able to determine if the pill has detected indicators of CRC through this visible color change. The biomarkers chosen (*APC*, *TP53*, and *KRAS*) are all genes that are typically mutated in the early stages of CRC. *APC* and *TP53* are both tumor suppressor genes that, when mutated, fail to stop the uncontrolled proliferation caused by *KRAS*. These genetic mutations are present in over 90% of CRC cases, making them a valuable biomarker (Su *et al.*, 2024). This

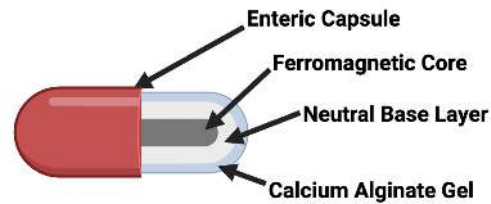


Figure 1. Showcasing the structure and layers of the pill.

design aims to overcome the challenges faced by at-risk populations in screening for CRC and thus reduce CRC mortality rates.

Systems level

Here, a conceptual architecture for detecting colorectal cancer-associated DNA mutations and generating a visible colorimetric output is presented. This system combines the natural competency of *A. baylyi* to take up DNA with a CRISPR-Cas3-based counterselection system to reduce potential false positives as well as with a genetic 'landing pad' within the cell to detect the presence of the mutated sequence via homologous recombination (Figure 2). We selected the Type I-F Cas3 nuclease over canonical Cas9 for its dual advantages in delivery and mechanism. Because Cas3 is endogenous to the host cell, it bypasses the requirement for complex heterologous delivery systems. Furthermore, unlike the localized double-strand breaks characteristic of Cas9 or Cas12, Cas3 drives processive DNA degradation. Although less suited for high-precision editing, this robust degradation is ideal for our application, as it eliminates wild-type genomic DNA and prevents unintended homologous recombination, effectively lowering false-positive rates. A second, transcriptionally controlled reporter sequence then colorimetrically indicates the presence of a mutation. Together, these components enable a non-invasive, macroscopic indication of disease-associated mutations within the gastrointestinal tract.

Following oral ingestion, the capsule passes through the stomach and into the intestinal tract, where environmental conditions enable the enteric coating to dissolve. CfDNA fragments of colorectal cancer including those associated mutations are shed from intestinal adenomas and can diffuse into the pill to interact

with the engineered *A. baylyi* sensors. Wild-type target gene sequences are eliminated via CRISPR-Cas3 counterselection mechanism, and only mutated genes are allowed to interact with the detection system. Upon target sequence recognition, the detection module activates gene expression pathways that drive pigment production. This ultimately results in a visible color change within the pill, serving as the system's output signal.

DNA uptake

The intake of nucleic acid sequences represents the first step of the design. *A. baylyi* is naturally competent, meaning it can take up DNA from its environment. When this capacity is paired with the diffusion of cell-free DNA (cfDNA) shed from the intestinal wall, DNA is taken up as short, digested 100–200 bp double-stranded sequences (Matov, 2024). Once the DNA is inside the cell, it can proceed to the next step. However, this poses a challenge: the availability of high-quality DNA impacts sensor effectiveness. Given the detection system's reliance on homologous recombination, insufficient long homologous regions (*e.g.*, from poor fragment length or DNA integrity) hinder the ability to verify gene identity and thus directly contribute to avoidance of both false negatives and false positives.

Selectivity via CRISPR counterselection

Following *A. baylyi* DNA uptake, a degradation step is utilized. Here, a CRISPR counter selection system digests wild-type genes to prevent false detection of wild-type sequences. Guide RNAs (gRNAs) are designed to specifically target and initiate digestion of the wild-type gene of interest and to place the targeted mutant gene site of interest in direct proximity to the Protospacer Adjacent Motif (PAM) site - enabling highly specific Cas3 digestion. When *A. baylyi* takes up a mutated version of the gene, Cas3 is unable to degrade it due to an inherent lack of sequence homology, thereby facilitating detection. By leveraging a CRISPR/Cas3 system to degrade wild-type DNA collected from the gastrointestinal tract, these sequences are prevented from undergoing homologous recombination, thereby enhancing sequence specificity and limiting potential false positives. By selecting Cas3 as our degradation pathway over the more common Cas9 or Cas12, we are able to take advantage of existing CRISPR systems within the cell. Also, Cas3 digests DNA not through a double-strand break, but by shredding DNA, whereby the Cas complex reels up one of the DNA strands, and Cas3 digests the other strand into short 10-nucleotide segments (Künne *et al.*, 2016). The size of these fragments entirely inhibits the opportunity for homologous recombination, unlike the double strand

breaks of Cas9 which leave long sections of homologous DNA.

Recombination based detection

Once the biomarker DNA sequence (*KRAS*, *APC*, *TP53*) has passed through the counterselection system, it interacts with prepared genetic landing pads at a neutral cellular loci. These sites consist of homology arms specific to the target biomarker gene, allowing for recombination-sensitive control of downstream expression via a TetR repressor. The TetR repressor suppresses the bacterial reporter site. When the mutated gene is present, it can undergo homologous recombination with the corresponding homology arms subsequently resulting in the repressor sequence removal.

Reporter activation

The reporter sequence is activated upon removal of the repressor sequence. The reporter is located at a second neutral locus in the genome consisting of a *PLtetO-1* promoter - notably repressed by TetR in baseline conditions. Upon repressor sequence removal from a successful recombination event, the promoter becomes transcriptionally active leading to the production of a visible pigment.

Delivery

The engineered *A. baylyi* are encapsulated within an alginate matrix, which serves as immobilization while still facilitating exposure to cfDNA fragments via diffusion. When the detection system is activated and the reporter is expressed, a visible color change can be detected on the surface of the capsule. The alginate gel is covered in an enteric coating selected to withstand the gastric environment and dissolve in the higher pH environment of the small intestine, where the target nucleic acids are more likely to be encountered.

Device level

The physical architecture of the pill is engineered to facilitate a 'lab-on-a-chip' - effectively integrating complex diagnostic workflows into a miniaturized, autonomous platform capable of operating within the complex environment of the human gastrointestinal tract. The primary engineering challenge for the device is therefore maintaining high permeability for environmental DNA, while ensuring robust biocontainment of the genetically modified *A. baylyi*.

Chassis bacteria

In order for the user to identify a positive result and thus an indication of predisposition for CRC disease, we selected the chassis bacteria *A. baylyi* engineered

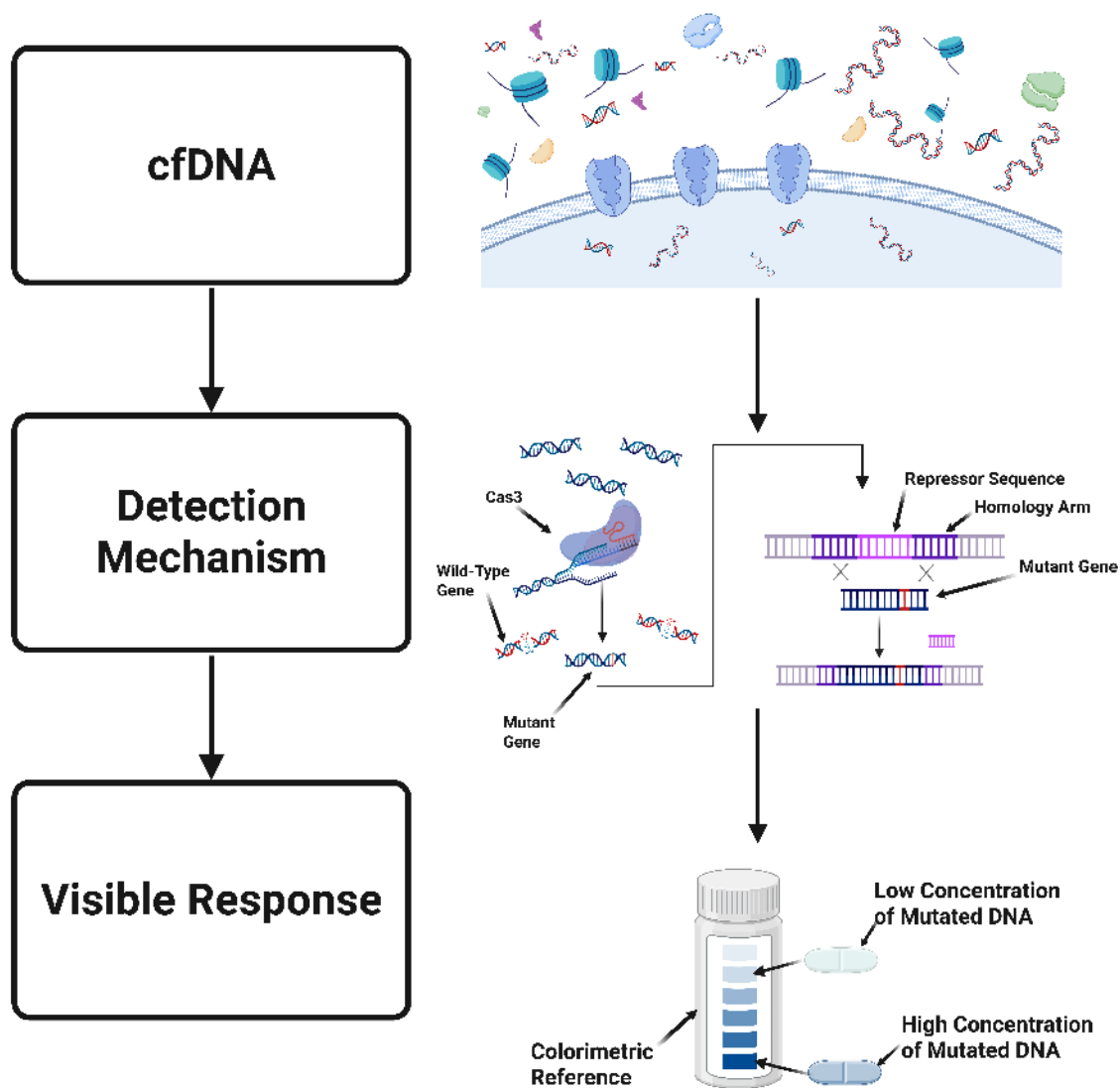


Figure 2. Schematic overview of mechanical processes behind the proposed detection method.

to emit an indigo color after exposure to a biomarker (e.g., genetic mutation of choice). *A. baylyi* was selected because of its natural competency, meaning it is able to take in DNA from its environment without any artificial catalysis or intervention. Here, we have genetically engineered *A. baylyi* to act as a living biosensor connected to an *amilCP* reporter and a detection mechanism. This schematic facilitates straightforward expression of *amilCP* when biomarkers are present.

The immobilization matrix

A calcium alginate hydrogel is utilized as the primary immobilization scaffold. Alginate, a polysaccharide derived from brown algae, undergoes rapid gelation when exposed to divalent cations like Ca^{2+} . This matrix creates a fibrous network with pores ranging from 5 to 100 nm. Because the DNA fragments shed by colorectal tumors are roughly 50-60 nm in length (typically 150-200 base pairs), the hydrogel remains permeable to the target biomarkers while physically trapping *A. baylyi*, which are 1 to 1.5 μm in length. This size-exclusion principle ensures that bacteria cannot enter the gut microbiome, minimizing the risk of horizontal gene transfer.

Protective coatings and pH stability

To address the extreme stomach acidity ($\text{pH}=1.5\text{-}3.5$), which can lead to alginate shrinkage and degradation, the device incorporates a cellulose-acetate-based coating. Cellulose-acetate is a biocompatible, semipermeable plastic that is able to survive the acidity of the stomach and pass through the neutral pH of the intestines (Vidal-Romero *et al.*, 2021). Due to its semi-permeability, this coating allows nutrient diffusion, thus keeping the chassis bacteria alive while simultaneously facilitating the diffusion of cell-free DNA. This layer is crucial for maintaining the viability of *A. baylyi* while the pill travels towards the more neutral pH of the large intestine, where tumor DNA largely accumulates.

Surface area optimization

To maximize the probability of a 'capture event' between a bacterium and a rare mutant DNA fragment, this device is designed with a textured surface. As an alternative to a smooth coating, we propose using alginate beads packed into a semi-permeable capsule. This significantly increases the effective surface area for diffusion, ensuring that the limited transit time through the colon is sufficient for the bacteria to take up the DNA and trigger the CRISPR-Cas3 counterselection mechanism.

Parts level

Early and accurate detection of tumor-associated mutations at the base-pair level is critical for improving patient outcomes. However, distinguishing between wild-type and mutated sequences remains a significant challenge because of the inherent high level of similarity - most mutations consist of single letter polymorphisms (SLP) in critical genes (Giglia & Chu, 2016). To address this issue, the color-change mechanism of the pill employs a modular design consisting of distinct elimination, detection, and reporting components. By coordinating these elements, the system converts molecular recognition events into a detectable signal. To better understand the rationale behind these design choices and evaluate system performance, an in-depth analysis of each genetic component is provided below. A schematic representation of the proposed system is included in Figure 3.

CRISPR counterselection

CRISPR-based elimination is a critical step in the system, as it reduces false positives by selectively degrading wild-type sequences via Cas3 activity. Once degraded, these wild-type sequences are unable to interact with downstream components. To implement this system, genetic constructs containing the CRISPR elements were assembled and transformed into *A. baylyi*. Successful integration would be verified using colony PCR (Cooper *et al.*, 2023), ensuring that no selection sequences were incorporated that could interfere with pigment production.

Integration homology arms

To integrate the genetic construct into the *A. baylyi* genome, specialized homology arms were designed to ensure that genomic insertion did not disrupt cellular viability or essential functions. These arms (~1000 bp) were selected to target non-essential genomic regions. The CRISPR construct replaced segments of genes *ACIAD_2185*, *ACIAD_2186*, and *ACIAD_2187* (Cooper *et al.*, 2023).

Native promoter

A. baylyi already encodes the necessary machinery for a Type I-F CRISPR system, a native promoter is used to drive expression of the engineered CRISPR RNA (crRNA). This approach minimizes discrepancies between endogenous and introduced CRISPR components, avoiding issues associated with under- or over-transcription. Specifically, the 684 bp region upstream of the native CRISPR locus is then utilized and has been shown to function effectively (Cooper *et al.*, 2023).

Native repeat

A native repeat sequence is incorporated to ensure proper crRNA processing by host machinery. Upon transcription, these repeats form characteristic hair-pin loop structures required for recognition and cleavage by processing enzymes such as Cas6f to prepare the crRNA for use by Cas3.

crRNA spacer target

The CRISPR spacer sequence is derived directly from the target genes that are known biomarkers for CRC (Cooper *et al.*, 2023). These 33 bp sequences were designed to place mutation hotspots (*e.g.*, KRAS G12D) in close proximity to the protospacer-adjacent motif (PAM), thereby maximizing targeting specificity and degradation efficiency.

Detection module

Following elimination of wild-type sequences, mutated sequences (which are biomarkers of CRC) proceed to the detection stage. Using sequence-specific homology arms, the system enables recombination-mediated removal of an embedded repressor sequence. Loss of this repressor allows activation of a downstream promoter, which in turn drives expression of a reporter gene encoding a chromoprotein.

Integration arms into neutral site 1 (ntrl1)

The detection module was integrated into a known neutral genomic locus (ntrl1) in *A. baylyi*, where insertions do not compromise cellular viability. Homology arms (~1000 bp each) flanking the construct facilitated recombination into this site. The detection construct would replace gene *ACIAD_2826* (Cooper *et al.*, 2023).

Target gene homology arms

To ensure specificity, 1000 bp homology arms corresponding to regions flanking the target gene (*e.g.*, *KRAS*) were incorporated into the detection construct. These sequences guide homologous recombination events, reducing the likelihood of false positives and negatives by restricting recombination to the intended genomic target.

TetR repressor

A TetR repressor cassette will be positioned between the homology arms to regulate reporter expression. TetR binds its operator with high affinity ($K_d \approx 10^{-12}$) in the absence of anhydrotetracycline (aTc), effectively repressing transcription (Ramos *et al.*, 2005). However, experimental observations (Cooper *et al.*, 2023)

indicated that wild-type TetR was overly repressive, leading to false negatives. A modified TetR variant with reduced binding affinity, featuring an 8-amino-acid C-terminal extension and a W75R mutation, was isolated and experimentally shown to function (Cooper *et al.*, 2023).

Reporting module

Following successful recombination and removal of the TetR repressor, intracellular TetR levels rapidly decrease, permitting activation of the reporter construct. The system is optimized to maximize chromoprotein production by combining a strong inducible promoter, an efficient ribosome binding site (RBS), and a robust transcriptional terminator.

Integration into neutral site 2 (ntrl2)

A second set of homology arms was chosen to integrate the reporter construct into another neutral genomic locus (ntrl2), ensuring no detrimental effects on cell viability. This integration replaced regions of genes *ACIAD_1076* and *ACIAD_1077*. (Cooper *et al.*, 2023).

P_LTetO-1 promoter

The P_LTetO-1 promoter, a well-characterized TetR-repressible promoter, was selected for its strong transcriptional activity and compatibility with the modified TetR variant. Its performance in prokaryotic systems has been extensively characterized (Lutz & Bujard, 1997)

Ribosome binding site (RBS) (BBa_B0030)

While a strong promoter enhances transcription, efficient translation requires a high-performance Ribosomal Binding Site (RBS). The *BBa_B0030* RBS was selected due to its well-documented strength and reliability in promoting high levels of protein expression in *E. coli* and related systems.

AmilCP reporter

AmilCP, a blue-purple chromoprotein derived from *Acropora millepora*, was chosen as the reporter due to its distinct and easily distinguishable coloration. The gene was codon-optimized for expression in *A. baylyi* as *A. millepora* is a corals. Its blue color provides contrast against typical background pigmentation (*e.g.*, brown, yellow, or red tones) found in the intestinal tract, thus facilitating visual detection. Furthermore, AmilCP serves as an excellent candidate for spectrophotometric quantification. Its distinct absorbance maximum (λ_{Max}) at 588 nm enables the straightforward assessment of translational efficiency.

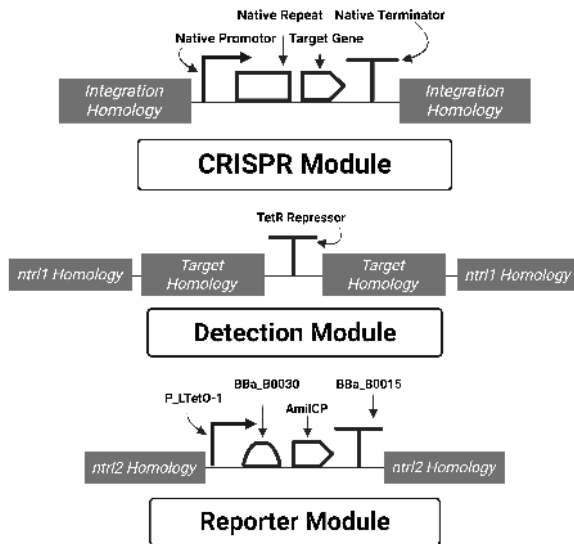


Figure 3. Schematic representation of genetic constructs for proposed sensor bacteria.

Rho-independent terminator (BBa_B0015)

To ensure efficient termination of transcription and prevent read-through into downstream regions, the *BBa_B0015* double terminator was chosen. This well-characterized rho-independent terminator enhances transcriptional efficiency and helps concentrate cellular resources on reporter production (Do, *et al.*, 2025).

Safety

When constructing biological designs, certain design risks are required. These risks must be carefully considered and, through careful design, mitigated and, whenever possible, eliminated. The capsule in this case comes with some inherent risks as all transgenic organisms pose a potential environmental contamination risk. This particular organism and system carries additional risk associated with the ferromagnetic core. However the benefits of the ferromagnetic core, being that one will not have to dissect their feces, heavily outweighs any concern (Figure 4). This is because the ferromagnetic core will attract magnetic material meaning a magnet can be passed over any waste and the pill can easily be found for a quick color analysis. Here, through intentional and careful design, we detail plans to minimize these inherent risks. Although these risks do warrant acknowledgment the potential benefit far outweighs them by offering an opportunity to significantly increase the proportion of high risk groups which undergo regular screening, in particular in resource-constrained environments.

Potential risks associated with *A. baylyi*

As an *Acinetobacter*, *A. baylyi* are largely considered harmless environmental organisms found in soil and water (Santala & Santala, 2021). The genus does however contain a few opportunistic pathogens, most notably *Acinetobacter baumannii*, which is known for involvement in antibiotic-resistant infections. However, *A. baylyi* is considered a soil pathogen and has been proven to be largely safe in most circumstances (Santala & Santala, 2021). It is worth noting, in a 2008 study six highly immunocompromised patients were reported to have *A. baylyi* in their system (Chen *et al.*, 2008). This was attributed to *Actinobacteria*'s general tendency to form biofilms within medical equipment and subsequently becoming an opportunistic pathogen. This intervention was designed with this context in mind to avoid infection by entrapping *A. baylyi* within an immobilizing alginate matrix. However, as a safety precaution this pill would not be clinically recommended for immunocompromised or high-risk patients.

Ferromagnetic concern: magnetic resonance imaging (MRI)

The minimal quality of the ferromagnetic material at the core of the pill, while small, could be sufficient to disrupt medical devices that depend on interactions with magnets or very high electromagnetic fields (*e.g.*, MRI). In these situations, the amount of material inside the pill may pose an elevated risk of physical harm to the recipient as a result of rapidly accelerating (Eshed *et al.*, 2010). As a result, it would be heavily contraindicated to have an MRI scan while the pill was still present in the GI tract. For additional safety, proximity to high electromagnetic fields would be against the suggested protocol with patients receiving thorough compliance counseling.

Allergen response

Ferromagnetic materials can elicit an immune response in individuals with metal-induced hypersensitivity (McKee & Fontenot, 2016). A condition similar to autoimmunity, metal-induced hypersensitivity occurs more frequently in immunocompromised people due to T-cell sensitization. In an attempt to avoid this, the core is encased in a variety of barrier materials such as polypropylene, chitosan, and finally a high density alginate, minimizing the majority of the risk of immune response (Küçük *et al.*, 2025) while also ensuring the viability of the sensor bacteria.

Genetic environmental contamination

Genetic Contamination is commonly a high concern for any synthetic biological design. The risk associated

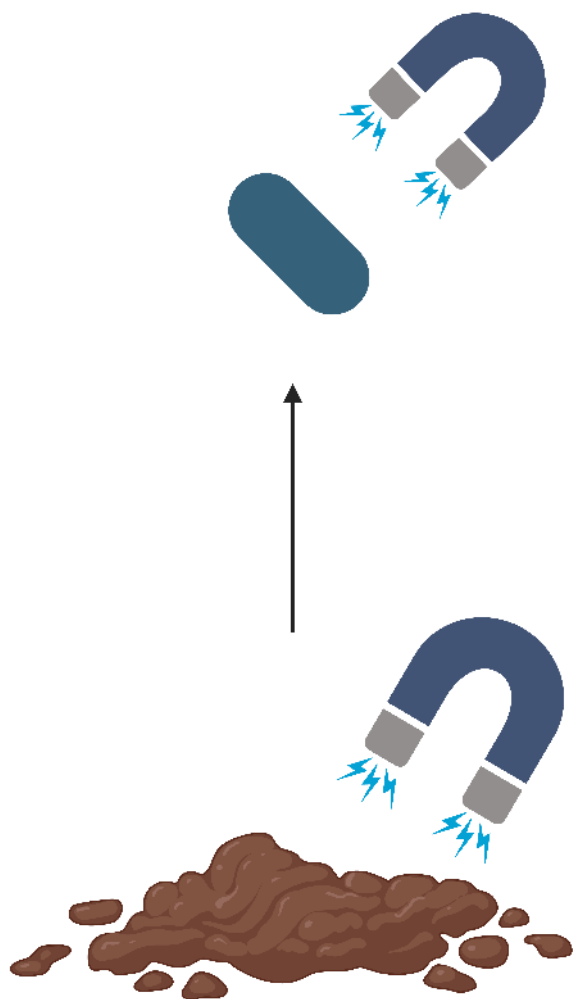


Figure 4. Schematic of the pill retrieval system, showcasing the ease and cleanliness of using a magnet to find the pill.

with the transfer of antibiotic resistance genes, unnatural expression, virulence material, and biosynthesis pathways, giving rise to atypical phenotypic traits pose an inherent risk. A solution to these problems would be to implement biocontainment with a 'kill' switch via auxotrophy or timed expression construct (Chan *et al.*, 2016). By neutralizing the bacteria naturally following activation of a specific genetic circuit for example triggered by an included activator subsequently limits the bacterial lifespan. Again, the use of an immobilization matrix aids in safety by ensuring the containment of any modified bacteria within the matrix where genetic information is less likely to migrate out from the cell. We further forgo the option of antibiotic resistance genes entirely, instead employing methods such as colony PCR we can screen for successful transformants without introducing antibiotic resistance genes

which could also pose a threat to the production of the chromoprotein.

Discussions

The transition of synthetic biology from laboratory-bound systems to real-world medical diagnostics requires a fundamental shift in how we approach patient interaction and biomarker stability. By engineering a living genosensor capable of autonomous detection within the gut lumen, we address the critical 'translational gap' that currently hinders colorectal cancer screening efforts. This section evaluates the clinical rationale behind choosing a DNA-based detection model and examines how this technology serves as a low-barrier triage tool compared to traditional diagnostic standards.

Addressing the screening fear

The current diagnostic standard, colonoscopy, is highly accurate but suffers from low patient compliance due to its invasive nature and the requirement for bowel preparation. While fecal tests, such as Fecal Immunochemical Testing (FIT) or Cologuard, offer non-invasive alternatives, they often rely on detecting hemoglobin or broad metabolic changes that lead to false positives from diet or inflammation. Our device addresses this by targeting somatic mutations like (*KRAS*, *TP53*, and *APC*) which are definitive signatures of neoplastic growth and thus considered biomarkers for CRC. By providing an at-home, low-cost solution, we aim to identify high-risk individuals who would otherwise forgo screening-funneling them toward necessary clinical interventions. Mitigating operational and financial burdens on primary care infrastructure, while providing more accessible screening pathways that are lower cost and circumvents systemic barriers many uninsured and underinsured demographics when seeking screening.

Rationale for DNA-based sensing

We opted for a genosensor over protein-based biosensors for three main reasons. 1) Stability: Proteins are rapidly denatured and digested by the harsh enzymes of the GI tract. However, DNA is remarkably resilient and can be recovered from fecal matter in sufficient quantities for detection. 2) Specificity: Protein markers for CRC are often elevated in non-cancerous conditions. Particularly in chronic inflammation disorders that are poorly understood. In contrast, the CRISPR-Cas3 system we employ can be programmed to distinguish between a healthy gene and a single point mutation, offering a level of specificity that proteomic sensors have not yet matched. 3) Amplification: While

proteins are static, meaning they can't replicate, the uptake of a single mutated DNA fragment by *A. baylyi* triggers a biological cascade, homologous recombination followed by protein expression, essentially using the bacteria's own machinery to amplify the signal into a visible color change.

Next steps

Several components of this design warrant further investigation during assembly. First it is necessary to determine the minimal amount of mutated DNA required for color change of the pill to occur, *i.e.*, to establish this intervention's sensitivity. Moreover, there is a need to consistently and reproducibly correlate pigment production with cfDNA concentration. Another important component will be the stability of the latter; hence, experiments establishing effectiveness of various methods for preserving the sensing mechanism integrity must occur to ensure stability during distribution and storage. In addition, future iterations could expand the capabilities of a singular capsule to detect multiple genes as an effective detection for several mutations simultaneously with varying reporter types and productions.

Quantification of signal thresholds

The most critical next step is determining the Minimum Detection Limit (MDL). We must establish how many copies of a mutated gene are required to trigger a color change perceptible to the human eye. We propose laboratory trials using synthetic DNA fragments simulating concentrations typically found in the gut lumen. If the *AmilCP* reporter expression is undetectable, we will explore the use of high-copy-number plasmids or stronger synthetic promoters to overexpress the chromoprotein once the mutation is detected. Based on prior studies utilizing analogous systems, the minimum detection limit for mutated DNA is anticipated to be approximately 3–5 pg/μL (Cooper et al., 2023). This concentration is expected to be sufficient to initiate successful recombination events and generate a discernible reporter signal.

Secretion integration

To improve the readability of the result, we plan to test the addition of a secretion peptide, like the *HlyA* secretion system, to the *AmilCP* sequence (Gentshev et al., 2002). By secreting the blue chromoprotein into the surrounding hydrogel matrix rather than keeping it inside the bacterial cytoplasm, we can create a more uniform and intense color change on the pill's surface, reducing user error during interpretation.

Environmental resilience and lyophilization

For the device to be commercially viable, it must survive the 'cold chain' or be shelf-stable at room temperature. Future research will focus on the lyophilization (freeze-drying) of the bacteria-laden hydrogel beads. We will test various cryoprotectants to ensure that the bacteria can be rehydrated and become metabolically active immediately upon ingestion.

Multi-channel logic gates

Finally, we aim to evolve the system from a single-gene detector to a Boolean logic-based sensor. By engineering separate bacterial populations for *APC*, *TP53*, and *KRAS*, we could design a pill that only changes color if two or more mutations are present, further increasing the diagnostic accuracy and reducing the likelihood of false alarms.

Author contributions

IA wrote and researched about the system and parts of the paper and was the key leader in preparing this paper. HB and PS wrote the device level, Discussion, and Next step sections of the paper. IP finished the background portion, LA and AG wrote safety, and all individuals had a role in the ideation of the project.

Acknowledgements

This paper examines the significance of the efforts of a team that focused on a goal and worked hard to achieve it. Ibrahim Abumohsen played a key role in leading the project, with contributions from Luka Ascencio, Havish Bathina, Madeline Boram-Rella, Alyssa Gay, Isaac Pare, and Priyansh Shah.

In addition to our student team, we would like to express our gratitude towards the efforts of Dr. Kaitlin M. Dailey, Mr. Brayden Carty, and Ms. Caitlin Deleo. This research wouldn't have been possible without their constant guidance and mentorship, which has helped us immeasurably. Dr. Dailey constantly contributed by providing us with guidance, information, and editing, which has helped solidify our understanding of the topic and put our ideas on paper. The mentorship of these individuals contributed heavily to our ideation phase and the preparation of our manuscript.

References

About Colorectal Cancer. (n.d.). American Cancer Society National Colorectal Cancer Roundtable. Retrieved April 23, 2026, from <https://nccrt.org/our-impact/about-colorectal-cancer/>

- Cancer Data and Statistics | Cancer*. (2026, January 13). CDC. Retrieved March 29, 2026, from <https://www.cdc.gov/cancer/data/index.html>
- Chan, C. T., Lee, J. W., Cameron, D. E., Bashor, C. J., & Collins, J. J. (2016). 'Deadman' and 'Passcode' microbial kill switches for bacterial containment. *Nature chemical biology*, 12(2), 82–86. <https://doi.org/10.1038/nchembio.1979>
- Chen, T. L., Siu, L. K., Lee, Y. T., Chen, C. P., Huang, L. Y., Wu, R. C., Cho, W. L., & Fung, C. P. (2008). *Acinetobacter baylyi* as a pathogen for opportunistic infection. *Journal of clinical microbiology*, 46(9), 2938–2944. <https://doi.org/10.1128/JCM.00232-08>
- Colorectal cancer statistics, 2023. (n.d.). PubMed. Retrieved April 23, 2026, from <https://pubmed.ncbi.nlm.nih.gov/36856579/>
- Colorectal Cancer Survival Rates | Colorectal Cancer Prognosis. (2026, January 13). American Cancer Society. Retrieved April 23, 2026, from <https://www.cancer.org/cancer/types/colon-rectal-cancer/detection-diagnosis-staging/survival-rates.html>
- Cooper, R. M., Wright, J. A., Ng, J. Q., Goynes, J. M., Suzuki, N., Lee, Y. K., Ichinose, M., Radford, G., Ryan, F. J., Kumar, S., Thomas, E. M., Vrbanc, L., Knight, R., Woods, S. L., Worthley, D. L., & Hasty, J. (2023). Engineered bacteria detect tumor DNA. *Science*, 381(6658), 682–686. <https://doi.org/10.1126/science.adf3974>
- Devers, K. J., Jones, R. M., Kuzel, A. J., & Woolf, S. H. (2010, May). Patient-Reported Barriers to Colorectal Cancer Screening. Retrieved April 15, 2026, from [https://www.ajpmonline.org/article/S0749-3797\(10\)00098-X/abstract](https://www.ajpmonline.org/article/S0749-3797(10)00098-X/abstract)
- Do, T. D., Figueroa-Bossi, N., Black, J. C., Eveno, E., Delaleau, M., Bossi, L., & Boudvillain, M. (2025). Rhod-dependent transcription termination: mechanisms and roles in bacterial fitness and adaptation to environmental changes. *RNA (New York, N.Y.)*, 31(9), 1207–1234. <https://doi.org/10.1261/rna.080486.125>
- Eshed, I., Kushnir, T., Shabshin, N., & Konen, E. (2010). Is magnetic resonance imaging safe for patients with retained metal fragments from combat and terrorist attacks?. *Acta radiologica (Stockholm, Sweden : 1987)*, 51(2), 170–174. <https://doi.org/10.3109/02841850903376298>
- Gentschev, I., Dietrich, G., & Goebel, W. (2002b). The *E. coli* α -hemolysin secretion system and its use in vaccine development. *Trends in Microbiology*, 10(1), 39–45. [https://doi.org/10.1016/s0966-842x\(01\)02259-4](https://doi.org/10.1016/s0966-842x(01)02259-4)
- Giglia, M., & Chu, D. (2016). Familial Colorectal Cancer: Understanding the Alphabet Soup. *Clinics in Colon and Rectal Surgery*, 29(03), 185–195. <https://doi.org/10.1055/s-0036-1584290>
- Küçük, K., Van Gysel, J., Del Marmol, V., & White, J. M. L. (2025). Allergic Contact Dermatitis Induced by Modern Wound Dressings: A Comprehensive Analysis of Risks and Allergenic Components. *International wound journal*, 22(3), e70153. <https://doi.org/10.1111/iwj.70153>
- Künne, T., Kieper, S. N., Bannenberg, J. W., Vogel, A. I. M., Mielliet, W. R., Klein, M., Depken, M., Suarez-Diez, M., & Brouns, S. J. J. (2016). Cas3-Derived Target DNA Degradation Fragments Fuel Primed CRISPR Adaptation. *Molecular Cell*, 63(5), 852–864. <https://doi.org/10.1016/j.molcel.2016.07.011>
- Lutz, R., & Bujard, H. (1997). Independent and tight regulation of transcriptional units in *Escherichia coli* via the LacR/O, the TetR/O and AraC/I1-I2 regulatory elements. *Nucleic acids research*, 25(6), 1203–1210. <https://doi.org/10.1093/nar/25.6.1203>
- Matov, A. (2024). Analysis of Genome-Wide Cell-Free DNA Fragment Length Distributions in Colorectal Cancer. *openRxiv*. <https://doi.org/10.1101/2024.07.17.24310568>
- McKee, A. S., & Fontenot, A. P. (2016). Interplay of innate and adaptive immunity in metal-induced hypersensitivity. *Current opinion in immunology*, 42, 25–30. <https://doi.org/10.1016/j.coi.2016.05.001>
- O'Connell, J. B., Maggard, M. A., & Ko, C. Y. (2004). Colon cancer survival rates with the new American Joint Committee on Cancer sixth edition staging. *Journal of the National Cancer Institute*, 96(19), 1420–1425. <https://doi.org/10.1093/jnci/djh275>
- Ramos, J. L., Martínez-Bueno, M., Molina-Henares, A. J., Terán, W., Watanabe, K., Zhang, X., Gallegos, M. T., Brennan, R., & Tobes, R. (2005). The TetR family of transcriptional repressors. *Microbiology and molecular biology reviews : MMBR*, 69(2), 326–356. <https://doi.org/10.1128/MMBR.69.2.326-356.2005>
- Santala, S., & Santala, V. (2021). *Acinetobacter baylyi* ADP1-naturally competent for synthetic biology. *Essays in biochemistry*, 65(2), 309–318. <https://doi.org/10.1042/EBC20200136>
- Su, Z., Hage, M. E., & Linnebacher, M. (2024, September 15). Mutation patterns in colorectal cancer and their relationship with prognosis. *Science Direct*. Retrieved May 1, 2026, from <https://www.sciencedirect.com/science/article/pii/S2405844024125819>
- Use of Colorectal Cancer Screening Tests | Colorectal Cancer. (2025, June 11). CDC. Retrieved April 23, 2026, from <https://www.cdc.gov/colorectal-cancer/use-screening-tests/index.html>

Vidal-Romero, G., Rocha-Pérez, V., Zambrano-Zaragoza, M. L., Alicia Del Real, Martínez-Acevedo, L., Galindo-Pérez, M. J., & Quintanar-Guerrero, D. (2021). Development and Characterization of pH-Dependent Cel-

lulose Acetate Phthalate Nanofibers by Electrospinning Technique. Nanomaterials, 11(12), 3202–3202. <https://doi.org/10.3390/nano11123202>