Perspectives Article

CRISPR-based peanut allergen detection: Targeting Ara h1 using DETECTR*

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This project aims to develop a rapid, cost-effective method for detecting peanuts in food. Peanut allergies affect approximately 6.2 million individuals in the United States, posing a serious public health concern due to the risk of accidental exposure. Despite efforts to prevent allergen contamination, more than half of food allergy reactions in restaurants occur even after staff are notified of a customer's allergy. Existing detection methods, such as ELISA (enzyme-linked immunosorbent assay) or PCR (polymerase chain reaction) are sensitive but time-consuming and require specialized equipment. Our method involves using recombinase polymerase amplification (RPA) to amplify the Ara h1 DNA sequence, a major peanut allergen, under isothermal conditions. This would then be followed by CRISPR-Cas12a, which would recognize a chosen target sequence and activate collateral cleavage of a ssDNA reporter linked to the chromoprotein amilCP, producing a visible color change. This reaction will be incorporated into a lateral flow biosensor to create a user-friendly, portable test. By improving accessibility and detection speed, this system has the potential to significantly reduce accidental exposures and improve food safety for individuals with peanut allergies.

Keywords: Peanut allergy detection, CRISPR-Cas12a, DETECTR, food safety, chromoprotein-quencher system

Our goal is to develop a frugal and portable method for detecting peanuts in food. To achieve this, we designed a biosensor using CRISPR-based DETECTR technology, which integrates recombinase polymerase amplification (RPA) and CRISPR-Cas12a for rapid, specific peanut DNA detection. Current methods like ELISA and PCR, while sensitive, are costly, equipment-dependent, and too time-consuming for real-time or field use. These limitations pose risks in settings such as restaurants, schools, or in food manufacturing, where fast decisions are critical for individuals with peanut allergies (Koczula & Gallotta, 2016).

Previous research has demonstrated that combining isothermal amplification techniques, like RPA with CRISPR-Cas12a, is effective for detecting allergens and pathogens (Chen et al., 2018; Li et al., 2018). Unlike PCR, RPA operates at a single temperature and provides faster results without requiring thermocyclers or complex lab setups (Wong et al., 2018). This accessibility makes RPA ideal for low-

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resource or point-of-use applications.

For our biosensor, we selected Ara h1 as the target allergen. Compared to other peanut proteins like Ara h3 and Ara h6, Ara h1 is more heat-stable, allowing it to remain intact even after food preparation or thermal processing. This makes it a reliable marker in diverse food products. While we have not yet identified the exact DNA sequence we will amplify, we plan to select a conserved, stable region of the Ara h1 gene that remains detectable after heating. Establishing such a target is essential for ensuring accurate results in real-world food samples.

For detection, we chose Cas12a due to its ability to recognize specific DNA sequences and trigger collateral cleavage of nearby single-stranded DNA—a property that enables signal amplification (Chen et al., 2018). This cleavage activity is central to our chromoprotein-quencher system, which visually indicates the presence of target DNA.

To convert the molecular signal into a visual readout, we used amilCP, a chromoprotein that produces a strong blue color visible to the naked eye. Chromoproteins like amilCP are preferred over fluorophores in low-resource settings because they do not require UV light or detection equipment. We selected amilCP specifically for its vivid color and rapid maturation, which support quick, clear test results (Ahmed et al., 2022).

Finally, we integrated the reaction into a lateral flow biosensor. Lateral flow assays are widely used in CRISPR-based diagnostics because they are simple, portable, and intuitive to interpret (Koczula & Gallotta, 2016). By combining CRISPR-Cas12a, RPA, amilCP, and lateral flow technology, we aim to develop a cost-effective, user-friendly detection platform that empowers individuals with peanut allergies to make safer food decisions.

To implement this detection system, the following procedure would be followed:

A solution containing the Ara h1 DNA sample, primers specific to Ara h1, recombinase enzyme, single-stranded binding protein, strand-displacing DNA polymerase, reaction buffer, ATP, and other necessary cofactors would be incubated in a sterile microcentrifuge tube at 37°C. This allows primer invasion and amplification of the Ara h1 target sequence. After amplification, Cas12a enzyme, crRNA designed for Ara h1, and the amilCP-quencher system would be added to the same tube. Cas12a would recognize the amplified Ara h1 DNA sequence and activate its trans-cleavage activity, indiscriminately cutting all nearby DNA, including the linker within the amilCP-quencher system. Once the quencher and chromoprotein are separated, the vivid blue color of amilCP becomes visible, and

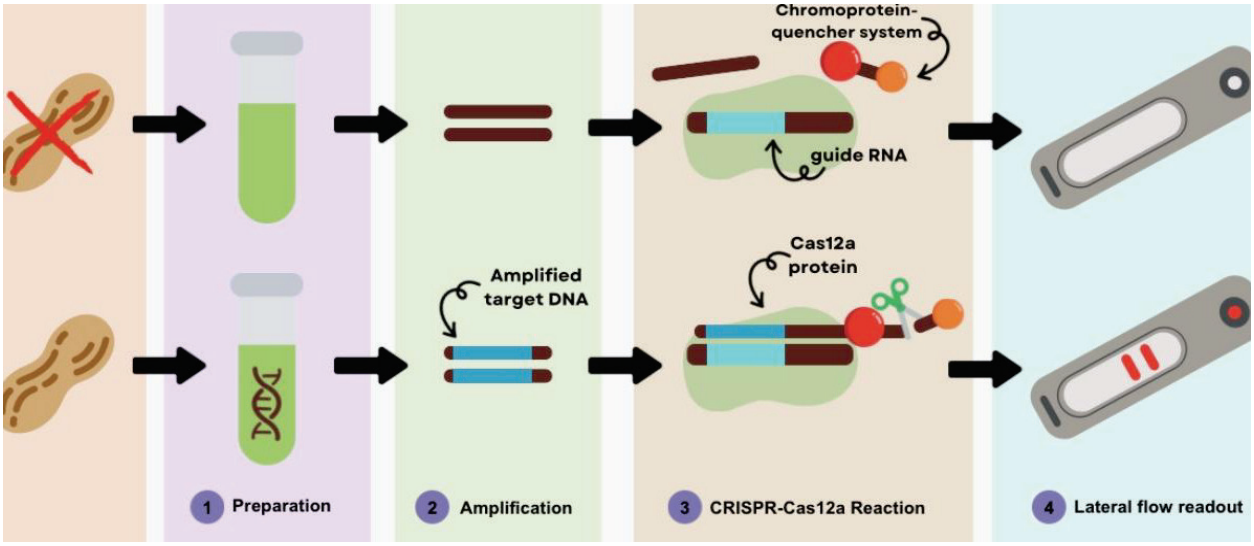

Figure 1. This Figure shows the proposed process of detecting peanut DNA in a scenario where there is peanut DNA, and one where peanut DNA is not present, detailing the steps involved in sample collection, DNA extraction and preparation, amplification, and detection.

the results can be observed through a lateral flow sensor if peanut contamination is present.

Next steps

One crucial factor to consider is how to efficiently crush samples. The finer a sample is, the easier it is to extract proteins for testing. Some possible devices include a manual grinder, a motorized mini-blender, or a rolling press that flattens and crushes food. These are typically used on larger scales, but exploring how they work can be helpful in developing a transportable crushing method. There may also be certain food-safe enzymes available that break down proteins more effectively, releasing peanut allergens.

Another challenge we are addressing is device integration. Using DETECTR and lateral flow sensors in separate devices is not user-friendly, so we want to combine these processes. Some potential solutions include using a microfluidic chip to automate mixing DETECTR's CRISPR reaction with lateral flow detection; a dual-step strip (in which the CRISPR reaction occurs before flowing into a lateral test zone); or a smartphone-based reader to analyze subtle color changes beyond the visual ability of the human eye. These integrations could streamline the workflow for non-laboratory users and improve overall efficiency in fast-paced real-world environments (Koczula & Gallotta, 2016).

We are also considering how to make the device accessible for colorblind individuals. Chromoproteins are used in our project to visually indicate the presence of peanuts, but this may present challenges for colorblind individuals. In such cases, lateral flow sensors could be useful since colorblind individuals would look for the presence of a line rather than a change in color. However, AmiCP's intense blue color alone may be sufficient for colorblind individuals to perceive a change in the device (Ahmed et al., 2022). To ensure that these individuals can confirm peanut detection, we could implement an app that detects changes in light levels, identifies colors, or uses different kits with distinct chromoproteins. To address the needs of blind users, we could also incorporate an auditory feedback system using a smartphone-connected app that provides sound alerts upon a positive detection result.

Another limitation to note is the inability to detect airborne peanut allergens. Our device will not be able to detect airborne peanut proteins. For individuals with severe peanut allergies, simply smelling peanuts may cause life-threatening allergic reactions. Researching a method to detect airborne peanut allergens could expand our kit's usefulness to all allergic individuals. However, airborne peanut proteins are typically present at very low concentrations and are not likely to trigger severe reactions in most cases (Nilsson, 2021).

We are planning several experiments to validate the prototype. After constructing a prototype, it will be crucial to test its sensitivity and specificity. This could be done by using known peanut-contaminated and peanut-free foods on the sensor, comparing the test against existing peanut test kits to measure accuracy, and testing different sample preparation methods to determine which provides the best protein extraction. Studies on DETECTR-based systems have reported sensitivity down to 10 aM (attomolar) and specificity above 95% for target DNA recognition (Chen et al., 2018; Li et al., 2018). These values can serve as performance benchmarks for our biosensor as we optimize detection.

Finally, gathering feedback from real users will be essential. Conducting trials with real users to get feedback on ease of use, speed, and clarity of results could help make the sensor tests more globally accessible. While our system is primarily designed for consumer-facing scenarios such as restaurant use, home use, or food allergy management in schools, it may also be useful for small businesses and food vendors aiming to ensure allergen safety. However, it is not currently designed for high-throughput industrial ingredient screening at facilities like grocery distributors.

Author contributions

C.M. and H.N. led the project. C.M., S.B., and B.Y. wrote the abstract. L.A., H.N., F.V.,

and L.A. wrote the body of the manuscript. L.A. and Y.L. wrote the Next Steps section, which was edited by D.K. C.M. wrote the acknowledgments section. R.T. cited all references. D.K., C.M., and S.P. produced the project video, with C.M. serving as the speaker. H.N. created the graphic. F.V. and S.P. investigated the purpose of the project and gathered data on the prevalence of peanut allergies. B.Y. and L.A. researched various peanut detection methods and different peanut proteins, particularly Ara h 1. S.P. specifically researched Recombinase Polymerase Amplification (RPA), while C.M. researched CRISPR and peanut allergen detection. H.N. and L.A. identified potential guide RNAs, Cas proteins, quenchers, fluorophores, and tags for the biosensor. L.A. and C.M. also conducted research on chromoproteins, CRISPR-Cas12a, and lateral flow biosensors. S.B. focused on current issues and limitations with existing detection methods like ELISA and PCR. Y.L. and S.B. researched detection approaches, different types of biosensors, and their mechanisms. R.T. explored special considerations for individuals with hypersensitive peanut allergies, risks related to peanut-derived products lacking allergenic proteins, methods to override phosphotransferase systems, and the use of fluorescent dyes for peanut protein trace detection.

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