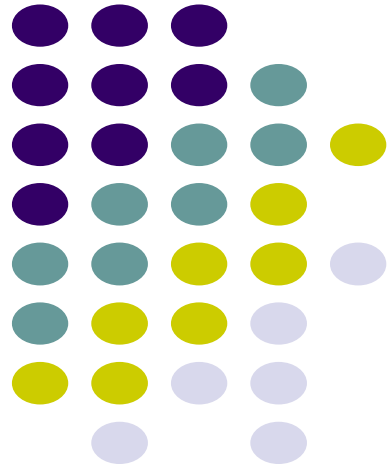
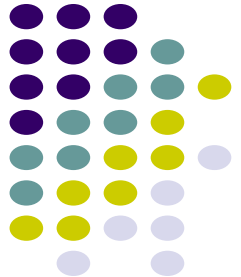
Advanced DNA-Based Point-of-Care Diagnostic Methods

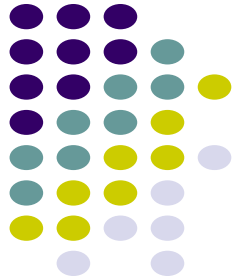


DNA-Based Point-of-Care Diagnostic Methods



- Point-of-care (POC) diagnostic assays which do not require sophisticated equipment and can be rapidly and cheaply performed in the field are in high demand.
- PCR based methods have multiple advantages over other technologies but require an electricity supply to perform the temperature changes required for DNA amplification; seriously limiting its adequacy for POC applications. As an alternative, isothermal DNA amplification methods are ideally suited to overcome this limitation
- For instance, isothermal amplification combined with lateral flow strips and portable fluorometers has been successfully used for POC detection of pathogen DNA

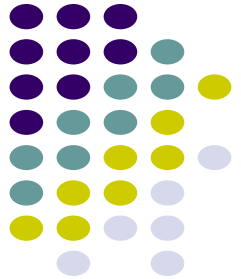
POINT-OF-CARE DNA EXTRACTION METHODS



- In plant tissues, the DNA extraction method requires the ability to efficiently remove a number of chemicals that can inhibit the DNA amplification reaction
- A lateral flow device (LFD) DNA extraction method has been reported as rapid and efficient for POC testing and has been successfully used in plant pathogen detection
- This method involves sample disruption in extraction buffer using metal ball bearings before transferring the lysate onto the release pad of a LFD nitrocellulose membrane.
- A small piece of membrane is then excised and added into the DNA amplification reaction such as PCR or other isothermal amplification methods. The isolated DNA is very stable on the membrane at room temperature which allows the extraction to be performed in the field

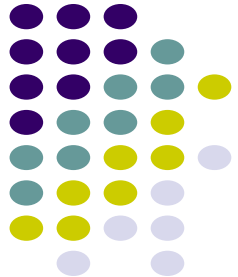
- Another method is use of simple cellulose-based dipstick that allows plant sample processing in as little as 30 s. Plant tissues are macerated by shaking in a tube containing extraction buffer and 1–2 ball bearings for 8–10 s. A cellulose dipstick is inserted in the tube containing the sample before washing it three times in a second tube containing wash buffer and finally into the tube containing the amplification mix.
- The technology works efficiently in multiple cultivated species including rice, wheat tomato and sorghum as well as notoriously difficult tissues such as leaves from mature trees (mandarin, lime, and lemon). It can be used to detect pathogen DNA as well as RNA from infected tissues and works with multiple amplification methods such as PCR, LAMP, and RPA.

APPLICATION OF NUCLEIC ACID ISOTHERMAL AMPLIFICATION TECHNIQUES IN PLANT DISEASE DETECTION



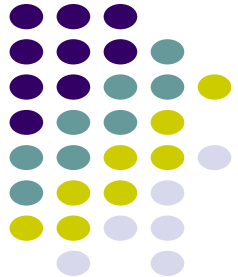
- Although PCR is highly sensitive and robust, it is constrained by a number of technical limitations. For instance, specificity is highly dependent on the primers used and its inherent sensitivity makes it prone to false positives due to sample cross-contamination.
- Besides, PCR also requires electrically powered equipment to perform the thermal cycling which limits its use for point-of-care diagnostics. A number of alternative isothermal techniques are now available that can obviate the need for a thermal cycler.

Loop-mediated isothermal amplification (LAMP)

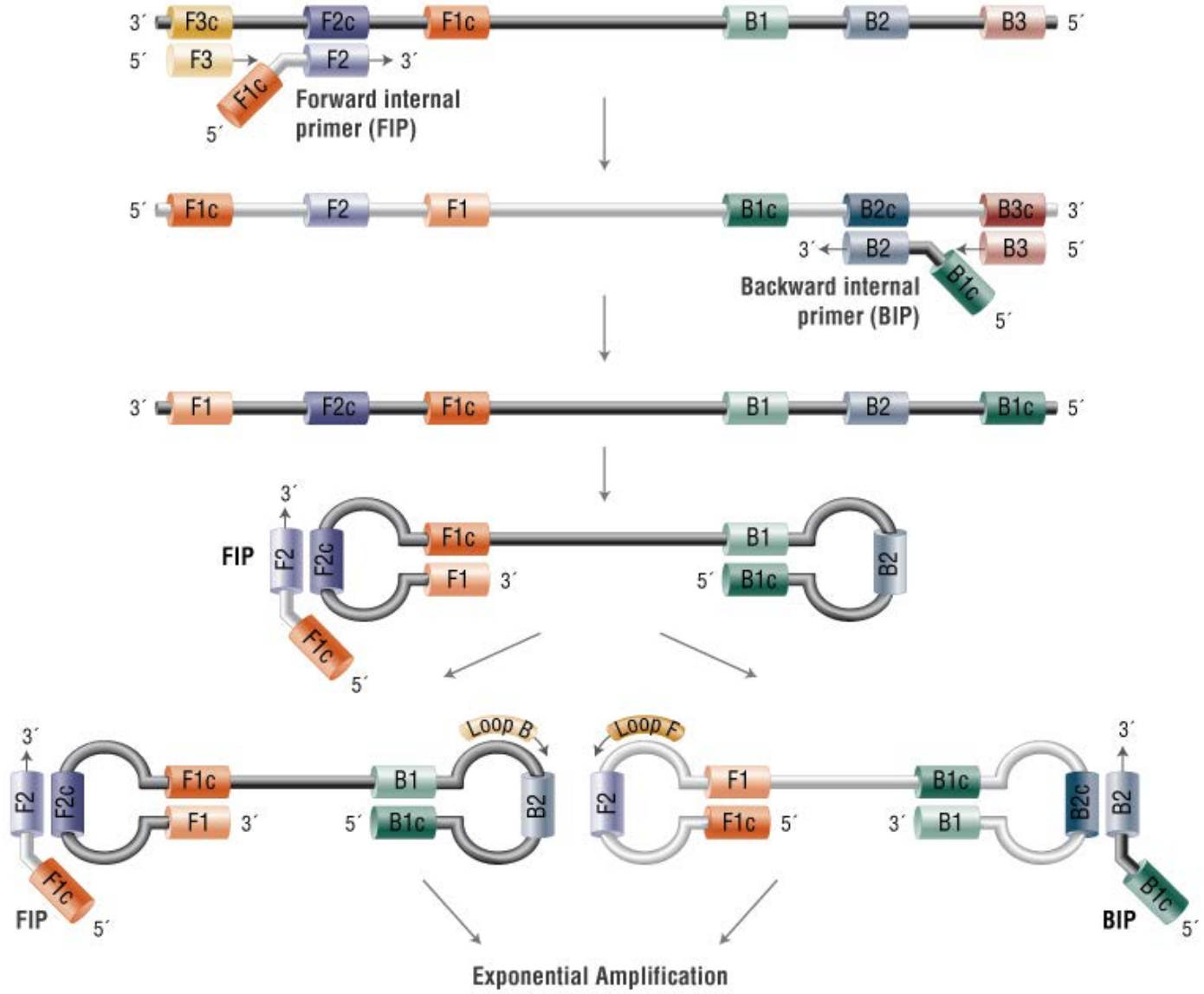


- It has been widely used due to its high efficiency, specificity, simplicity and quickness.
- LAMP requires two long outer primers and two short inner primers that recognize six specific sequences in the target DNA.
- The first inner primer containing sense and antisense sequences in the DNA will hybridize the target sequence and initiate DNA synthesis.
- Next, the outer primer carries out the strand-displacement DNA synthesis and produces a single stranded DNA which works as a template for the second inner and outer primers producing a DNA molecule with a loop structure.
- The unremitting cycling reaction accumulates products with repeated sequences of target DNA of different sizes.
- The reaction tube is incubated at 63-65°C in a regular laboratory water bath or heat block that helps in maintaining a constant temperature.
- The amplified product can be detected by naked eye as a white precipitate or a yellow-green color solution after addition of SYBR green to the reaction tube.

Three major advantages of LAMP



- Firstly, it can be carried out at a constant temperature with a short reaction time. This rapid isothermal process makes it ideal for point-of-care detection of plant pathogens in the field.
- Secondly, it has very high amplification efficiency and sensitivity as it generates large amounts of PCR product with low amounts of input DNA.
- Finally, this method is relatively cost effective as it requires simple equipment to perform the assay. Furthermore, there have been reports stating that LAMP generates amplicons with several inverted repeats which could be potentially used to increase the sensitivity in hybridization assays, such as LAMP-ELISA hybridization and LAMP incorporated with colorimetric gold nanoparticle hybridization probes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Exponential Amplification

- The integration of LAMP with the electrochemical sensor offered a robust platform for pathogen detection as it was highly sensitive, detecting as low as 10 copies of pathogen genomic DNA
- LAMP-biosensor technology has a strong potential for in field testing, detection and identification of plant diseases