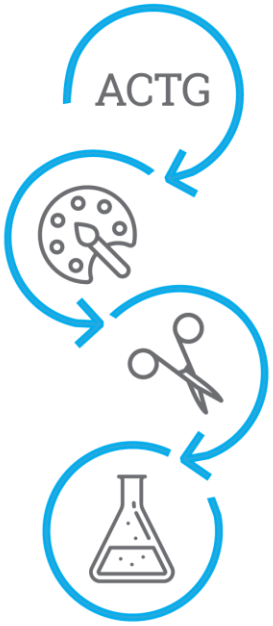


DNAdots

Simple explanations
of modern genetic techniques

minipcr

Loop-mediated Isothermal Amplification (LAMP)

What it is:

DNA amplification without thermal cycling

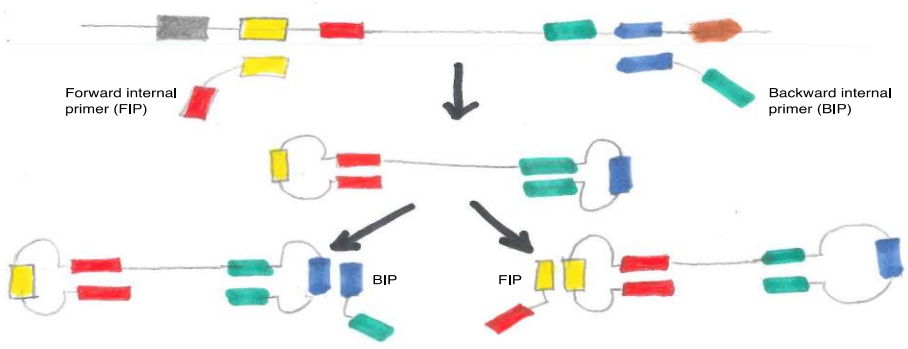
For over 30 years, the gold standard for detecting DNA and other nucleic acids has been PCR (the Polymerase Chain Reaction). In PCR, an enzyme called DNA polymerase makes exponential copies of a target DNA molecule. Much of modern biology and medicine depends on DNA amplification by PCR, for example to identify species, diagnose infections, or to detect contamination of a food or water source. The catch is, DNA amplification by PCR requires cycles of heating and cooling, usually 30 *thermal cycles,* to generate enough DNA for detection.

Like PCR, LAMP (loop-mediated isothermal amplification) produces many copies of a specific region of the genome from a tiny amount of starting material. However, LAMP does not require thermal cycling; amplification occurs at a constant temperature (usually between 60 and 65 degrees Celsius). LAMP can be used for creating quick and portable genetic tests that can be carried out using simple equipment, such as a heater or water bath.

How it works:

Using multiple sets of primers to create stem-loops

LAMP works at a single temperature (*isothermal*) using DNA polymerases that can read through double-stranded DNA without the need for heat to open up the double helix ("strand displacement" activity). To start the copy reaction, LAMP uses specially designed *primers* (short pieces of DNA complementary to the target) that form stem-like "loop" structures. These loops allow the polymerase to initiate DNA copy reactions at multiple locations, creating large amounts of DNA in a short period of time. As opposed to PCR which typically uses just one pair of primers, LAMP makes use of 2 or 3 sets of primers. The step-by-step process is hard to explain in text format – a helpful visualization of how LAMP works can be found here.



Unlike PCR which makes billions of identical copies of DNA, the final products of LAMP are stem-loop DNAs of various lengths and cauliflower-like structures with multiple loops, adding up to a remarkable amount of DNA!

How it is used:

When may LAMP be preferable to PCR?

PCR is certainly routine and powerful, but it's not perfect for every place. Because LAMP doesn't depend on temperature changes, it can be enabled in low-resource settings that don't have access to a thermal cycler. Another advantage is that detecting LAMP-amplified DNA can be simple with color-based or turbidity-based modifications to LAMP. In colorimetric LAMP, the changing pH of the amplification reaction is used to drive a color change, making DNA detection as easy as telling pink from yellow. In turbidity-based LAMP, the reaction byproduct magnesium pyrophosphate precipitates making the solution cloudy. Users can tell how successful the LAMP amplification was based on color change (colorimetric LAMP) or how cloudy the liquid became (turbidity). This eliminates the need for gel electrophoresis or other downstream detection techniques to verify the results.

While there are advantages, there can also be hurdles to using LAMP. The main challenge is assay design; because of the more complex primer configuration, it can be difficult to design successful LAMP primer sets. For any desired target site of amplification, 2 or 3 sets of primers must be designed in a complicated relationship. Therefore it usually takes more time and effort to develop new experiments to detect DNA targets using LAMP.

The future:

Genetic assays in space and other remote settings

LAMP is already being used for easy detection of DNA targets in new places, from farms to doctors' offices. It has the potential to bring fast and simple genetic tests to detect disease in field settings. Teams of researchers are using LAMP to diagnose infectious diseases in humans (e.g. malaria) and plants (e.g. fungal infections) directly in the field with minimal instrumentation. Very recently, thanks to Genes in Space, LAMP was successfully used aboard the International Space Station (ISS). Up on the ISS, a colorimetric LAMP experiment designed by high school student Julian Rubinfien enabled astronauts to directly detect DNA targets simply by reading the color of the tubes, using a miniPCR machine to incubate the samples!

Learn more:

- "Loop-mediated isothermal amplification." *Wikipedia.* https://en.wikipedia.org/wiki/Loop-mediated_isothermal_amplification
- "Loop-mediated Isothermal Amplification (LAMP)." *New England Biolabs.* https://www.neb.com/protocols/2014/06/17/loop-mediated-isothermal-amplification-lamp
- Tanner, N.A.. et. al. "Visual detection of isothermal nucleic acid amplification using pH-sensitive dyes." *Biotechniques.* 2015. https://www.ncbi.nlm.nih.gov/pubmed/25652028