Colorimetric LAMP: Visual Detection for Simple Diagnostics

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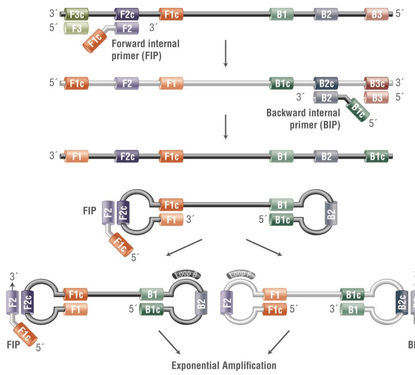
INTRODUCTION

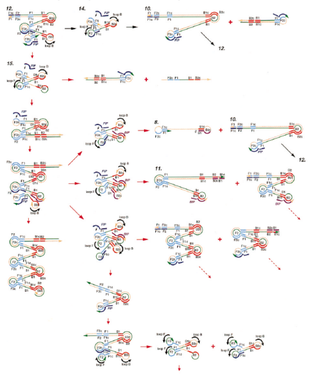
Much of modern biology and medicine depends on the ability to accurately determine the identity of a target, whether virus, tumor, mystery infection, or even an uncertain type of food. Fortunately, all living things and viruses carry unique identifiers in their DNA or RNA genomes. Once a sequence identifier is determined, we still need to be able to find it, using a nucleic acid amplification method for precisely identifying a particular sequence. This amplification can be analyzed to see what is made and how much of a particular DNA was present in the sample to begin with. And only the specific target of interest will be amplified, enabling accurate detection of the sequences that we are looking for. With demands and applications for nucleic acid identification growing all the time, new methods have been developed to allow detection more easily, rapidly, and in more settings. A popular example is loop-mediated isothermal amplification (LAMP) which works at a single temperature (isothermal) using DNA polymerases with a special ability to go through double-stranded DNA without heating (“strand displacement” activity. NEB developed a novel colorimetric format that reacts to DNA synthesis by changing color from pink to yellow as a direct visual response to DNA polymerase adding bases to the growing DNA products. This simple readout of amplification, paired with the speed and robustness of LAMP make for a useful diagnostic tool, with LAMP being used for easy detection of targets everywhere from farms to doctors’ offices, and recently, the International Space Station! If it has DNA or RNA, we can find it, and LAMP will let it be done easily, rapidly and right where you need the answer. While there are plenty of those places on Earth, we’ll soon have them in space too, and with methods like LAMP our astronauts, their food supplies, and their homes can be kept safe.

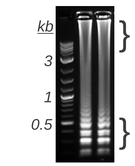
Loop-Mediated Isothermal Amplification: LAMP



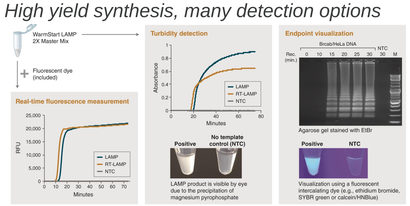
- 6 core regions of target sequence identified
- Internal (FIP, BIP) primers target F2/B2 regions with reverse complement tails
- Displacement from outside (F3, B3) primers releases product with self-complementary ends
- “Loops” form, molecules allow for duplication and initiation at multiple sites
- Each molecule produces longer subsequent products, reaction grows exponentially
- Huge products (>20,000 bp) from small (~150 bp) LAMP structure

Example product expansion

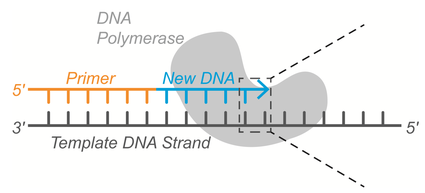




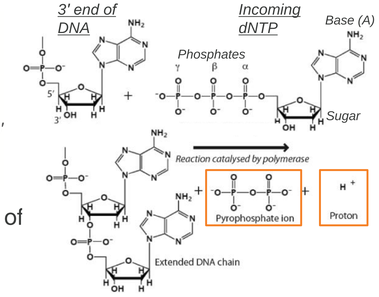
High yield synthesis, many detection options



DNA POLYMERASE REACTION AND PRODUCTS



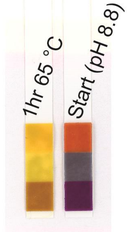
- DNA polymerase adds dNTP to 3′ end of DNA strand
- Some methods detect PPi production, require additional metal (Mn) or visualizing precipitation of Mg•PPi (hard)
- Detection of H^+ used in electronic DNA sequencing (Ion Torrent™), but requires sophisticated equipment



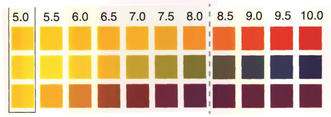
- DNA_n + dNTP → DNA_{n+1} + Pyrophosphate + H^+
- Each incorporation produces 1 H^+

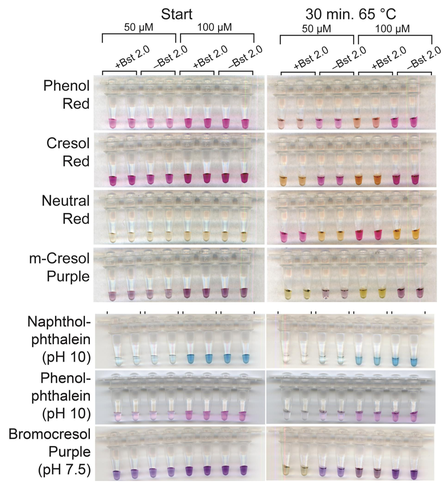
LAMP REACTIONS PRODUCE pH CHANGE

- LAMP makes a lot of DNA, can we see H^+ products?
- Perform reactions without buffer, adjust pH to ~ pH 8.8
- Incubate with LAMP primers, target, *Bst* 2.0 DNA polymerase
- After reaction pH dropped > 2 pH units!



pH Key:





- Use pH indicator? Add into reaction, see pH change by eye
- Variety of indicators, best transition in pH 7-8 range
- Higher/lower range OK, but reactions take longer (polymerase activity preference)
- Choose based on color, solubility, safety, etc.

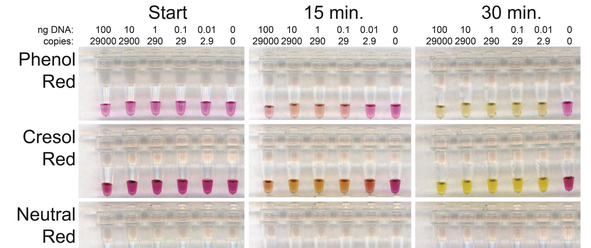
COLORIMETRIC LAMP

SPECIFICITY

- Important to tell positive from negative reactions
- No-template control (NTC) reactions must keep initial color
- Time, dye, assay show specificity variation, use to select best product candidates

SENSITIVITY

- Want to detect lowest possible # copies
- Maintain fast reactions, discriminate from NTC
- *Can detect <5 copies by eye in <30 min*

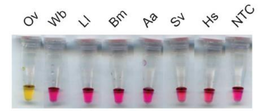


Colorimetric LAMP Master Mix

- Optimized with phenol red
- DNA and RNA detection
- Enables simple, field and point-of-need assays

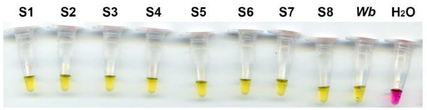
Negative *Positive*

Example Colorimetric Tests



- *Onchocerca volvulus*: causes onchocerciasis, “river blindness”
- Detect parasite directly from black flies, use for vector surveillance; screen patients for therapy

- *Wuchereria bancrofti*: cause of lymphatic filariasis, “elephantiasis”
- Detected using blood samples of infected patients



Other examples: Zika (mosquitos, urine); Lyme (ticks)

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