LAMP FOR FOOD

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Loop mediated isothermal amplification (LAMP)

DNA amplification based on loops

Using Bst polymerase (*Bacillus stearothermophilus*)

4-6 primer, 60-65 °C, strand displacement
Loop mediated isothermal amplification (LAMP)

- discovery in early 2000s
- widely applied for detection and analysis of nucleic acid
- Mainly pathogenic microorganisms
- viruses, bacteria, fungi and worms

- a vast number of protocols have been published
Applications of LAMP

**Virus detection:** SARS and West Nile Virus. Common in clinical diagnosis of more prominent virus classes like Herpesviridae, Hepadnaviridae, and Retroviridae.

**Bacteria:** *E. coli*, *Salmonella* spp., or *Mycobacterium* spp.

**Protozoa:** foodborne and waterborne parasitic pathogens (*Cryptosporidium*, *Giardia*, *Toxoplasma*).
Applications of LAMP

Further development of LAMP assays dedicated to the diagnosis of

- cancer
- genetic disorders
- sex determination
LAMP has been performed on various food samples:

- unpasteurized milk
- raw eggs
- meat

which are prone to bacterial contamination causing gastroenteric disease or which may serve as a way of ingesting pathogens
LAMP Reagents and Heating Devices

### Primers
- FIP; BIP
- F3;B3
- LF; LB

### LAMP Buffer
- dCTP
- dTTP
- dATP
- dGTP

### Bst DNA polymerase

### DNA

### 25 µl

### Distilled water

### Amplification
60°C ~ 65°C
Maximum 60 min

### Heating Devices
LAMP (Loop-mediated isothermal amplification of DNA)

- Rapid, simple, highly sensitive
- Use 4 or 6 primers and Bst DNA polymerase
- Use a heat block or a water-bath under isothermal conditions
- Visual detection
LAMP

Samples

DNA/RNA extraction

(Reference time)

DNA/RNA extraction from samples

About 30 min

Amplification

1. Prepare master mix.

About 60 min

2. Add DNA/RNA samples

3. LAMP amplification

Detection

4. Detection
   - visual detection (by fluorescence)
   - real-time turbidity detection

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Equipment for amplification

- Water bath
- Heat block
- LAMP device

Detection of the amplification

- Agarose Gel Electrophoresis
- Turbidity
- Fluorescency
The composition of the reaction mixture

**Primers**
- FIP primer (F1c, F2)
- F3 primer
- BIP primer (B1c, B2)
- B3 primer

*Bst* DNA polymerase

dNTPs

reaction buffer

**Products**

The final products are stem loop DNAs with several inverted repeats of the target and cauliflower like structures with multiple loops.
Primers generally used for LAMP

4 specially designed primers, 2 inner and 2 outer, can recognize 6 distinct sequences on the target gene.

BIP: B1c, a TTTT linker and B2
FIP: F1c, a TTTTT linker and F2c

The outer primers are smaller and called B3 and F3.
nPCR + LAMP electrophoresis

nPCR

LAMP
General strategy and incorporation of LAMP for food investigations

Primer design based on specific genes of the target

Evaluation of specificity and sensitivity of LAMP

Application of common methods and compare with LAMP in samples collected from various food material

Spiked material

Natural material

Rapid tests for food control in import / export
Routine screening by LAMP

Prevention of distribution of food borne diseases and epidemics via food
Important cycles of transmission

Questionmarks indicate uncertainty regarding the frequency of interaction between cycles.
Foodborne and waterborne pathogens
CRYPTOSPORIDIUM

1. Thick-walled oocyst (sporulated) exits host
2. Contamination of water and food with oocysts.
3. Thick-walled oocyst ingested by host
Cryptosporidium generated LAMP products

Sensitivity tests after oocysts’ LDM followed by DNA extraction and LAMP

1. $10^6$ oocysts
2. $10^5$ oocysts
3. $10^4$ oocysts
4. $10^3$ oocysts
5. $10^2$ oocysts
6. $10^1$ oocysts
7. $10^0$ oocysts
8. $10^{-1}$ oocysts
9. Negative control

Fig. 4.

Amplification curves of 12 fecal samples using SAM-1 LAMP assay
TOXOPLASMA

Foodborne and waterborne outbreaks of toxoplasmosis
Toxoplasma Transmission to Humans

HUMANS

FOOD
WATER
SOIL

Tissue cysts

Oocysts
# Toxoplasma LAMP generated products

<table>
<thead>
<tr>
<th>Line</th>
<th>LAMP - PCR</th>
<th>LAMP</th>
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<tr>
<td></td>
<td>B1 gene</td>
<td>TgOWP gene</td>
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**LAMP specificity**

|          |          |          |          |          |
|----------|----------|----------|----------|
| 1        | 2        | 3        | 4        |
|          |          | 5        | 6        |
|          |          | 7        | 8        |
|          |          | N        | M        |

- Babesia gibsoni
- Trypanosoma brucei
- Neospora
- Cryptosporidium parvum
- Giardia lamblia
- Toxoplasma AHC1 (oocysts)
- Toxoplasma PLK (tachyzoites)
- Toxoplasma RH (tachyzoites)
- Negative control (DDW)
- Marker
**Toxoplasma LAMP generated products**

**LAMP specificity**

**A**

*Toxoplasma gondii* B1 gene (AF179871)

**B**

*Toxoplasma gondii* putative oocyst wall protein COWP (AY465428)

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**Markers**

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Toxoplasma generated LAMP products

LAMP sensitivity

LAMP PCR reaction with Primers F3-B3
What is in your food?

LAMP will be an alternative method to include food safety and food control.
Recent examples found for LAMP application

Parasitology Research, June 2013, Volume 112, pp 2171-2175

Evaluation of the initial and chronic phases of toxocariasis after consumption of liver treated by freezing or cooling
Recent examples found for LAMP application


LAMP: Methods for plant species identification in food

SPICES: caraway, celery, cumin, mustard
Perspectives and questions
Thank you for your attention!