ghep-isfg

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High-performance LAMP based method for human sex identification using Y chromosome specific genetic markers

WASHINGTON, DC
ISFG 2022
INTERNATIONAL SOCIETY FOR FORENSIC GENETICS

udla Medicina

Germán Burgos Figueroa

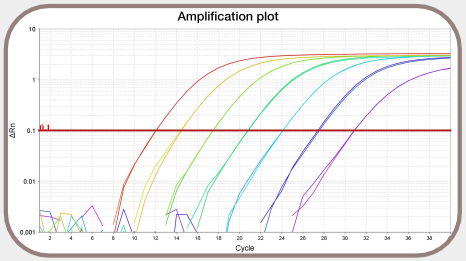
udla | INVESTIGACIÓN Y VINCULACIÓN

01
Introduction

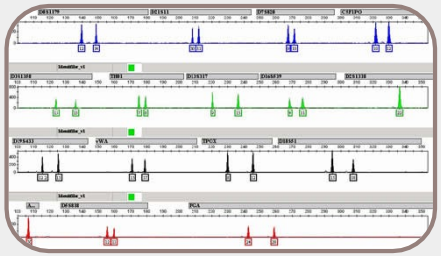
Forensic investigation

Detection of body fluids

Various analyzes



qPCR



Genetic porfile

Human sex determination

Identification of biological samples

1. DNA-based sex determination

2. Forensic caseworks:
   - Mass disaster
   - Missing persons
   - **Sexual assault**

3. Male DNA detection

Male DNA identification

Immunochromatographic techniques

Male antigen detection

Acid phosphatase (AP)

Semenogenin

Prostate Specific Antigen (PSA) o p30

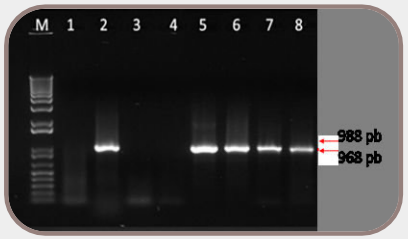
Kits

- PROMEGA → POWERPLEX®
- Applied Biosystems → AmpFlSTR® Identifiler®
- QIAGEN® ThermoFisher Scientific → New Generation

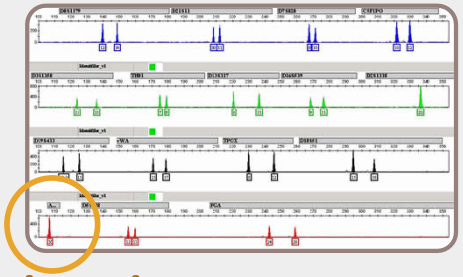
Male DNA identification

Convencional PCR

- Agarose gel electrophoresis



- Capillary electrophoresis



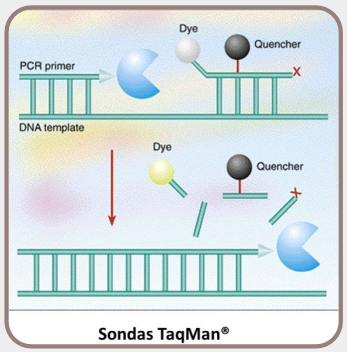
Amelogenin

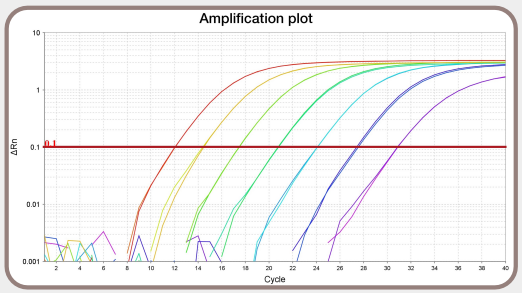
Alternative markers

- Alphoid regions
- TSPY
- DYZ1 (Satellite sequence)

Analysis techniques

Commercial DNA typing kits





Limitations

Sophisticated and expensive equipment

Necessity

Simple and cost-effective molecular tests

Loop-mediated isothermal amplification (LAMP)

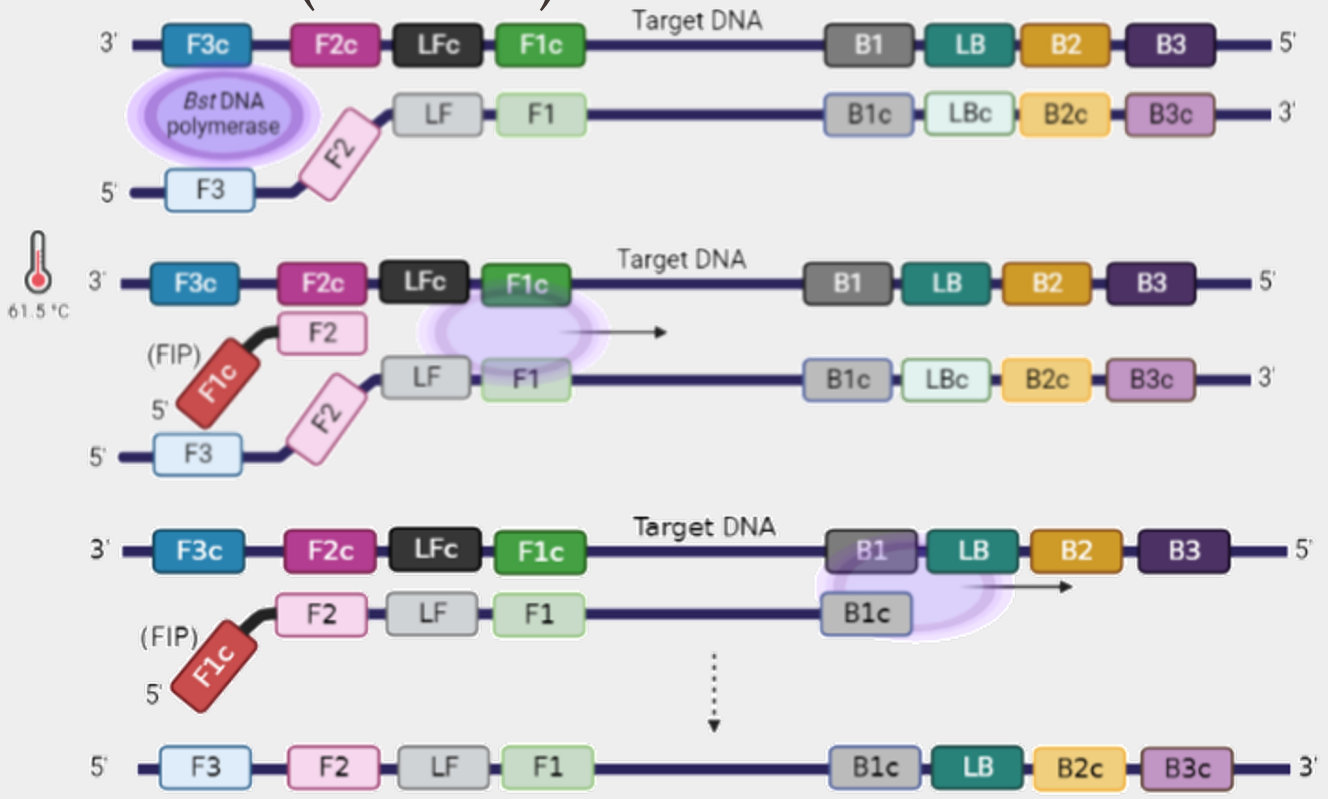
Bst **DNA-pol**

Bacillus stearothermophilus

Auto cycling displacement DNA synthesis

Isothermal conditions (60-65°C)

Molecular diagnostic technique



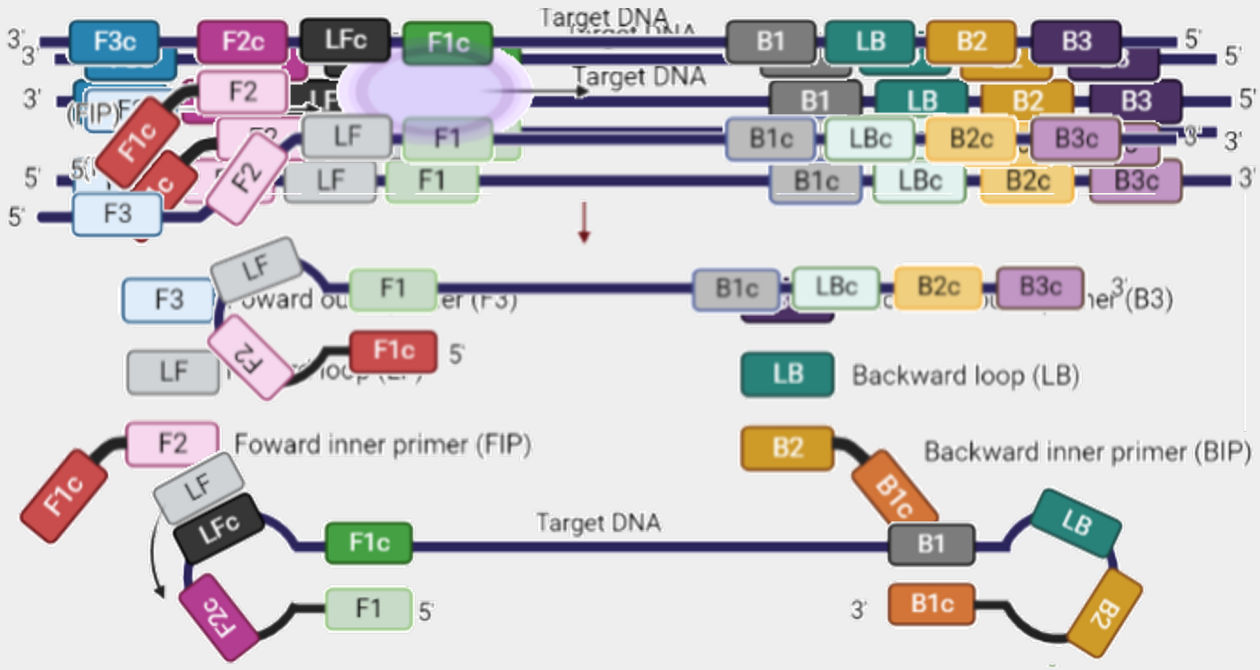
Loop-mediated isothermal amplification (LAMP)

Primers (4-6)

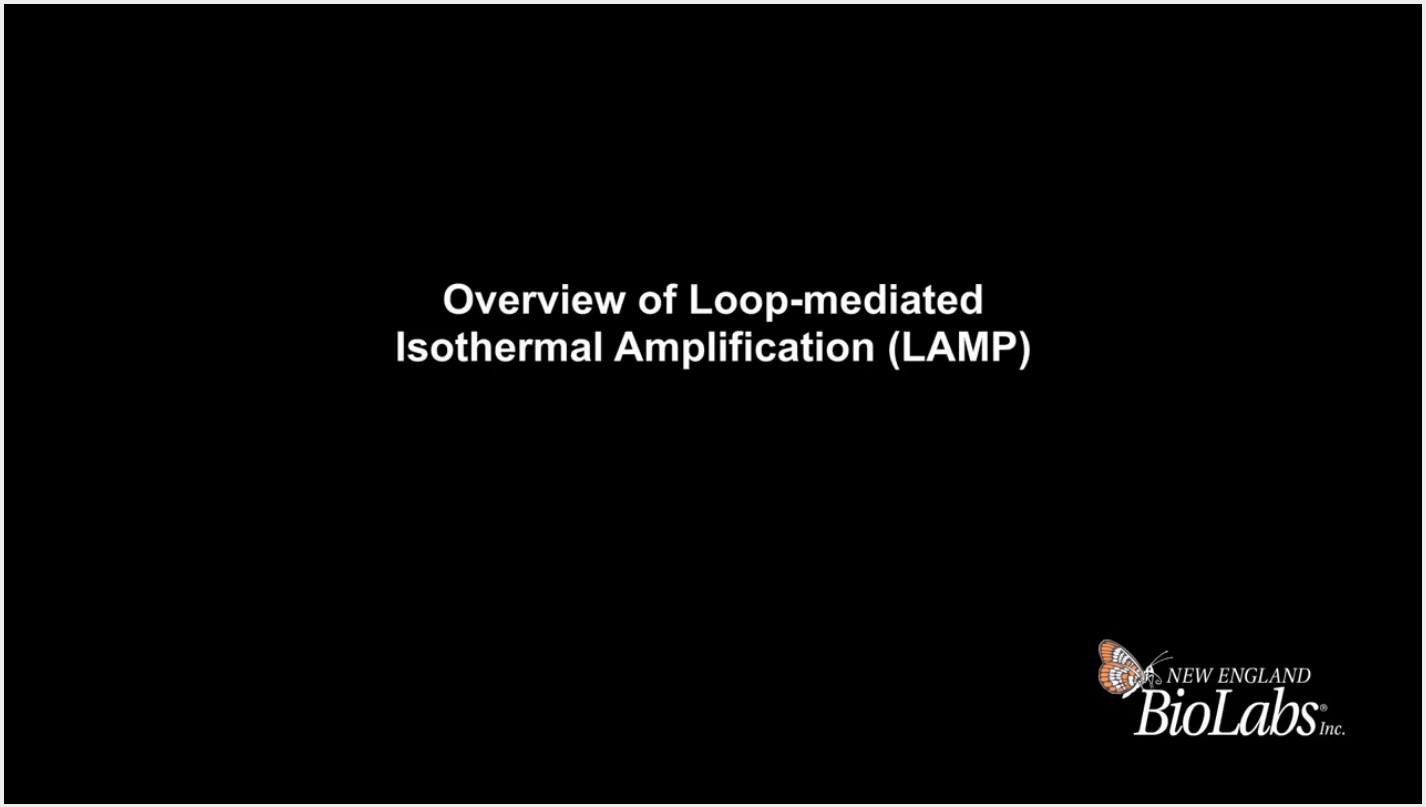
- Outer (F3 –B3)
- Inner (FIP – BIP)
- Loop (FL- BL)

Span over 8 distinct regions in a target gene

Accelerate the speed of the reaction, increase specificity and sensitivity



LAMP Reaction



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Link: https://international.neb.com/tools-and-resources/video-library/loop-mediated-

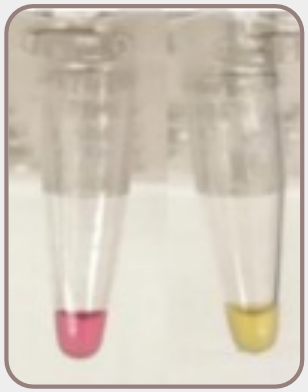
Loop-mediated isothermal amplification (LAMP)

Results

Naked eye

pH sensitive dyes
Phenol red

Mori & Notomi (2009)



Scott et al. (2019)

Color change due to pH variation of the reaction

OBJECTIVES

General

Describe an optimization of a colorimetric LAMP screening protocol to produce highly reliable results of great importance in human DNA identification caseworks.

Specific

Sensibility	5 set of primers
Specificity	6 animal samples

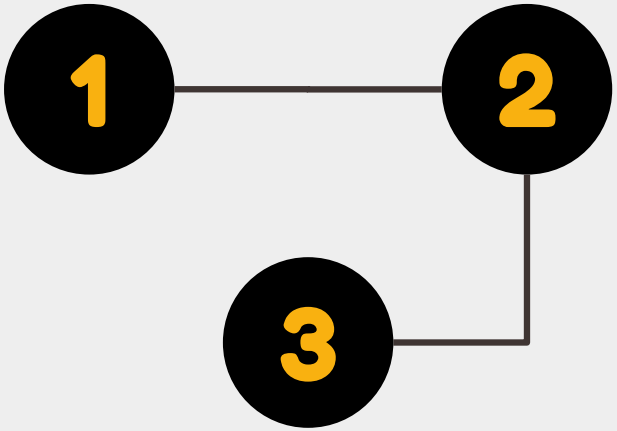
02

Materials and methods

DNA template preparation

DNA extraction

- Blood samples:
  4 men and 4 women
- Salting out procedure
- Spectrophotometry



Sensibility

Male DNA

3,75 ng/uL to 0,0001 ng/µL

+

Female DNA

3,75 ng/uL

1:1

Specificity

Animal samples

- *Felis catus* (domestic cat)
- *Canis lupus familiaris* (domestic dog)
- *Mus musculus* (mouse)
- *Sus scrofa* (pig)
- *Bos taurus* (bull)
- *Equus caballus* (horse)

LAMP set of primers

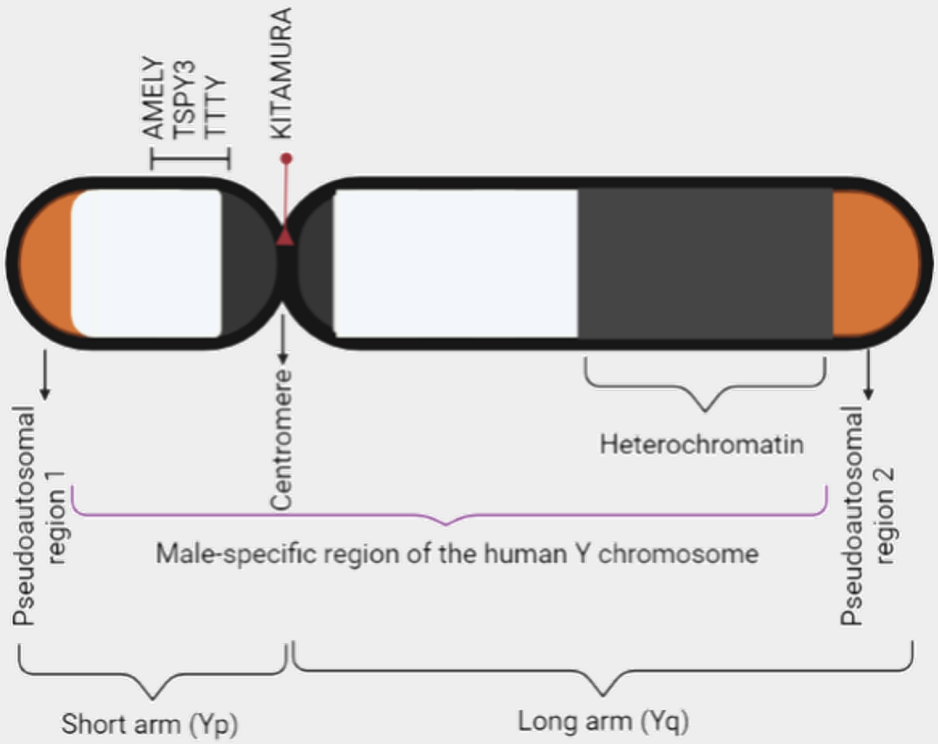


Table 1. Individual LAMP set of primers

AMELY	Amelogenin 6 bp deletion in the first intronic region
TTTY2	Y-linked 2 testis-specific transcript region
TTTY7	Y-linked 7 testis-specific transcript region
TSPY3	Y-encoded testis-specific protein
KITAMURA	Y-chromosome alphoid regions

Primer mix

TSPY3+AMEL
KITAMURA + AMEL
KITAMURA + TSPY3
TSPY3+AMEL+KITAMURA

LAMP colorimetric reaction

Primer mix

1 µL

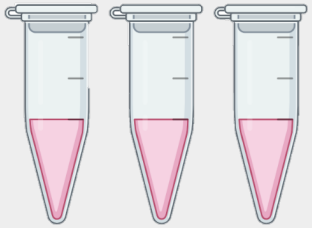
0,2 µM F3/B3
1,6 µM FIP/BIP
0,4 µM loopF/loopR

Master mix

5 µL
WarmStart
Colorimetric LAMP

Sample

1, 5 µL



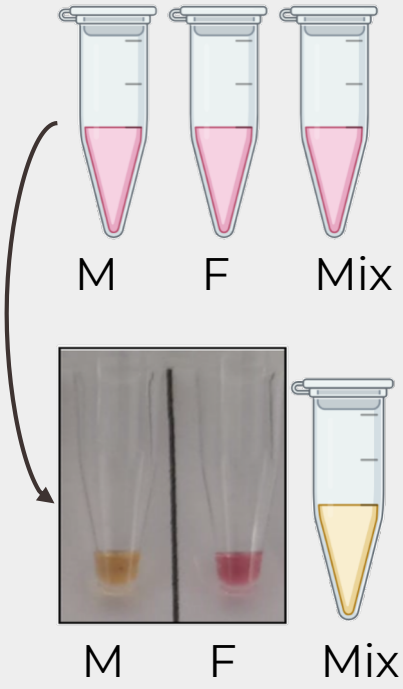
Final volume
10 µL

2, 5 µL H_2O

LAMP colorimetric reaction

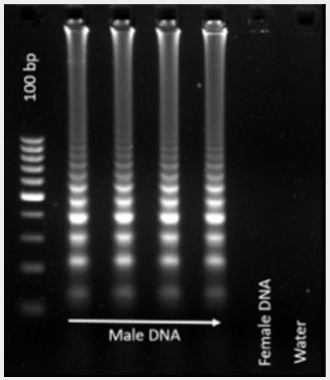
61.5°C
45 minutes

Color change



TBE 2% agarose gel electrophoresis

Confirmation



03

Results and discussion

Sensibility of primers LAMP

Table 2. Sensibility and copy number for each set of primers

Primer Set	Sensibility (ng/uL)	DNA copy number per µL (haploid copies)
TTTY7	0,0586	16,99
TTTY2	0,0293	8,49
TSPY3	0,0073	2,12
Kitamura	0,0073	2,12
AMELY	0,0037	1,07
TSPY3 - AMEL	0,0001	0,03
Kitamura – AMEL	0,0001	0,03
Kitamura- TSPY3	0,0001	0,03
TSPY3 – AMEL - Kitamura	0,0001	0,03

Their copy number and high efficiency in analyzing mixture samples

1 – 6 pg/uL

Commercial male qPCR-DNA quantitation kits

Primer sensibility

Set of primer:

AMELY

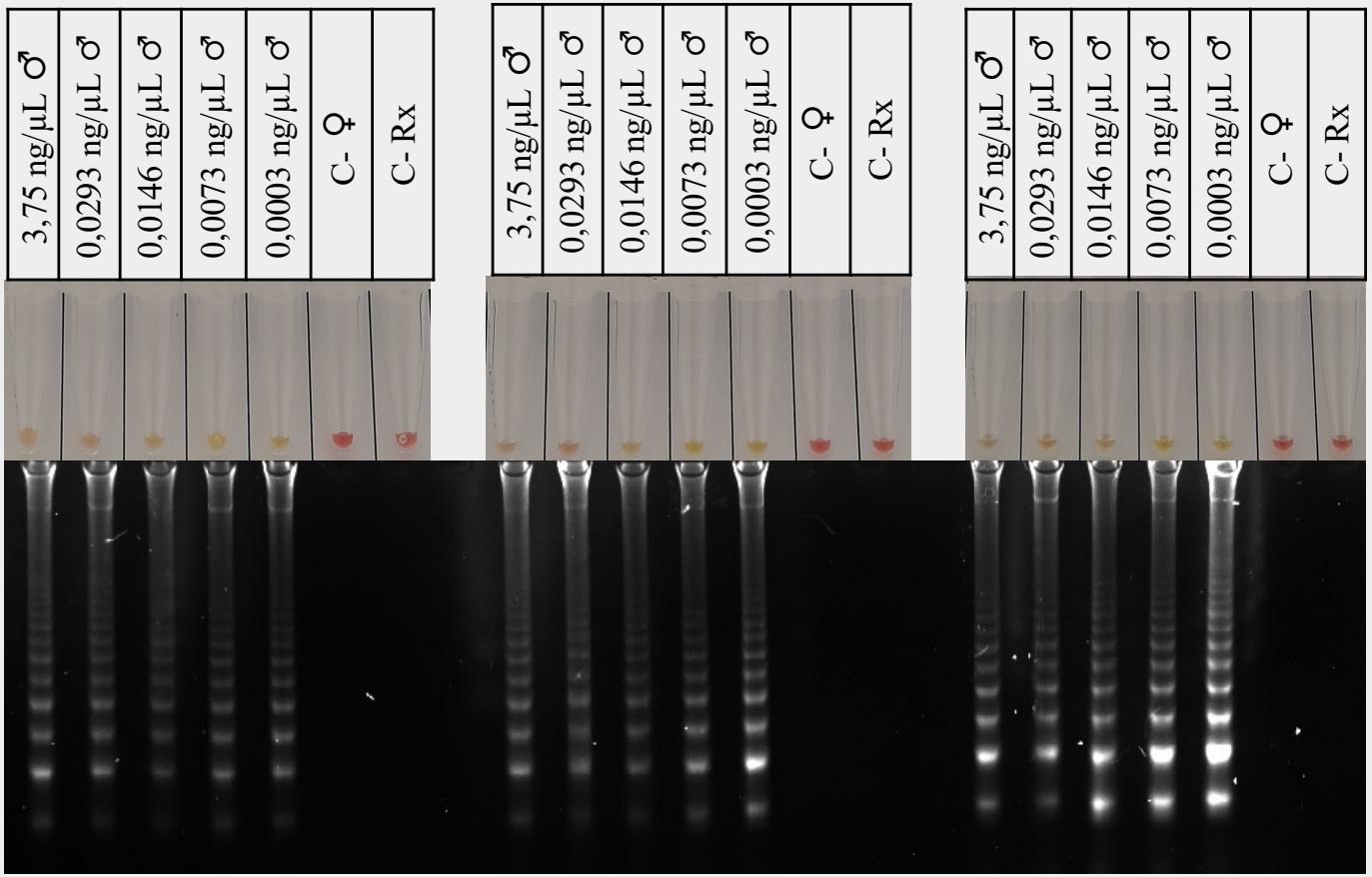


Figure 1. Sensitivity test for LAMP assay: AMELY

Amelogenin gene deficiencies

Table 2 Global scenario of Y deletion and its characteristics involving different populations

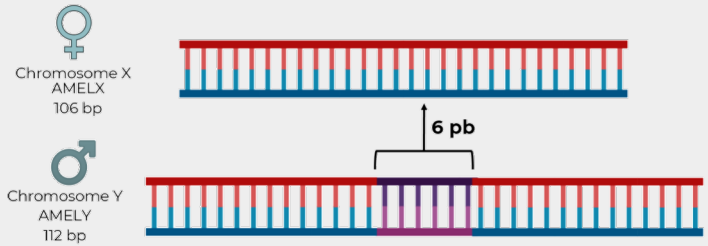
Population	Sample size	AMELY deletion frequency (%)	References
Chinese (North)	79,304	0.0227	[77]
Chinese (South)	8,087	0.037	[93]
Singapore	567	0.705	[127]
Malaysian (mixed)	338	3.6 (Indian) 0.88 (Malay)	[21]
Caucasian	327	0.3058	[106]
Indian	4257	0.23	[60]
Austrain	29,432	0.018	[109]
Indian	270	1.85	[113]
Australian	109,000	0.02	[85]
Nepalese	77	6.49	[18]
Sri Lankan	24	8	[102]
Israeli	96	1.04	[83]
Italian	13,000	0.008	[72]
Chinese	10,526	0.019	[123]
Japanese	500	0.2	[112]

Standard test

Amelogenin gene

Deletions of regions in the ChrY

AMELY allelic deletion



(Dash et al., 2020)

Increased performance

Rare presence of deletions → Coamplification with other Y chromosome markers

Mix of primers

Primer mix

TSPY3+AMEL
KITAMURA + AMEL
KITAMURA + TSPY3
TSPY3+AMEL+KITAMURA

Sensitivity of final mix of primers

Set of primer:

KITAMURA + AMEL + TSPY3

Male DNA
concentration detected

0,0001 ng/uL

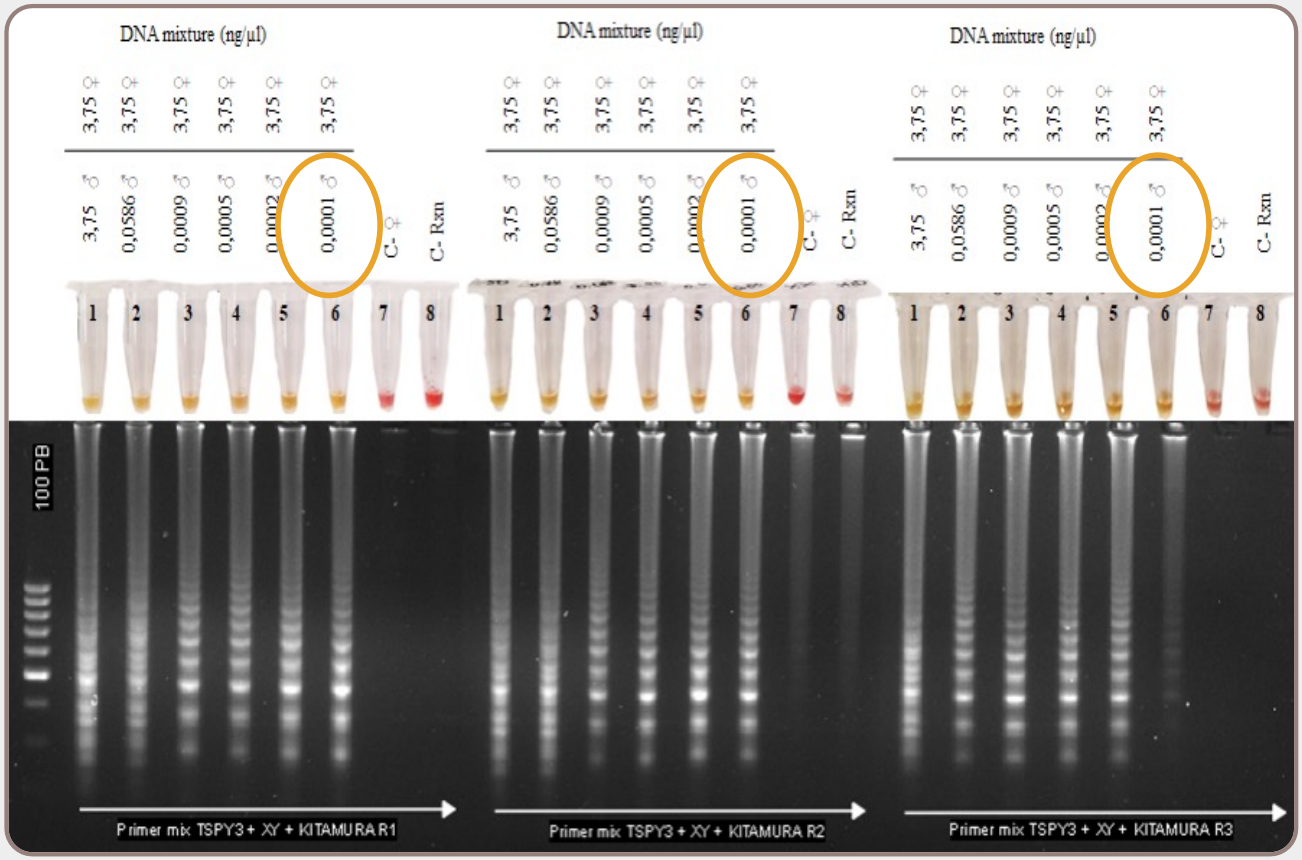


Figure 2. Sensitivity test for LAMP multiplex assay: KITAMURA/AMEL/TSPY3

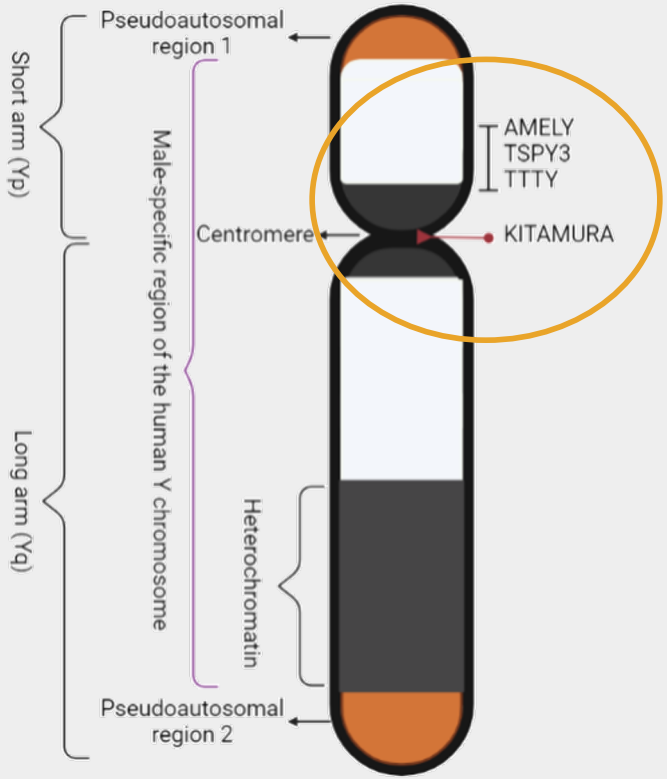
Increased sensitivity

Co-amplification

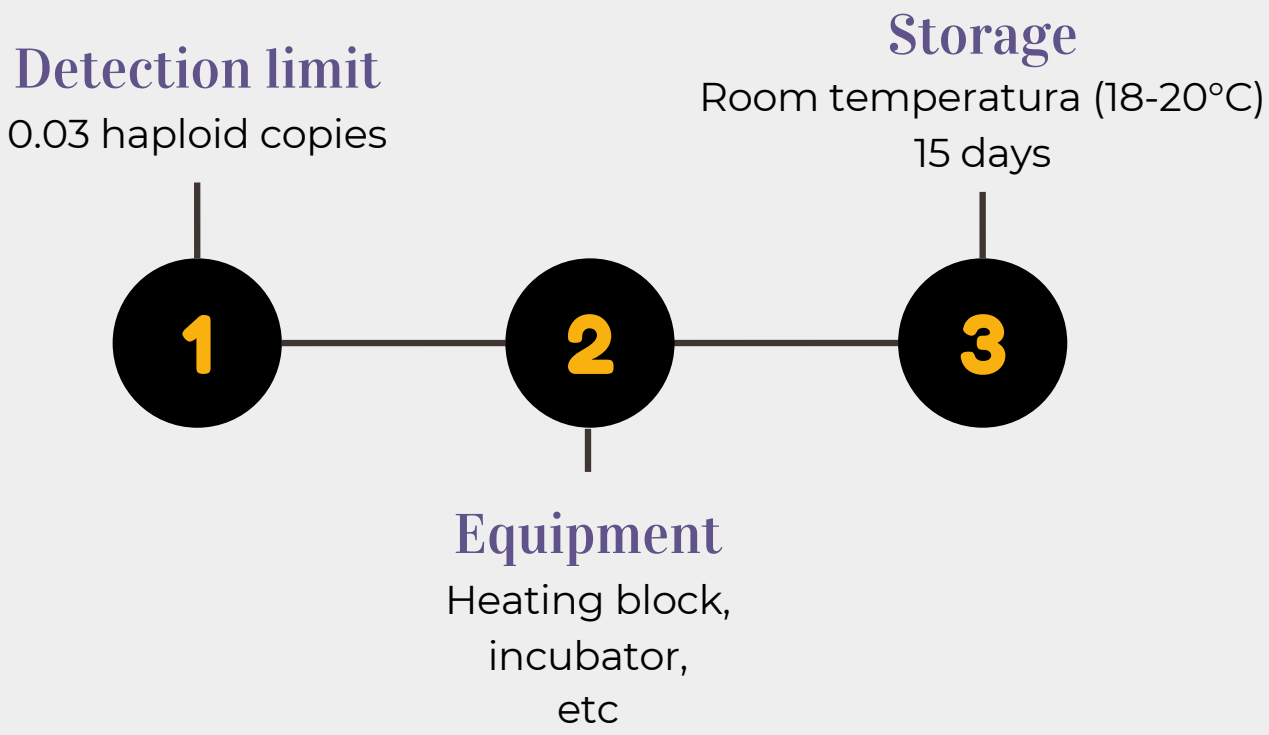
Different target sequences
Multicopy genes

qPCR cuantification kits
Limit: 1-6 pg/µL

10-20 times
LAMP



Versatility



Versatility

Different sample types ——— Search for male components

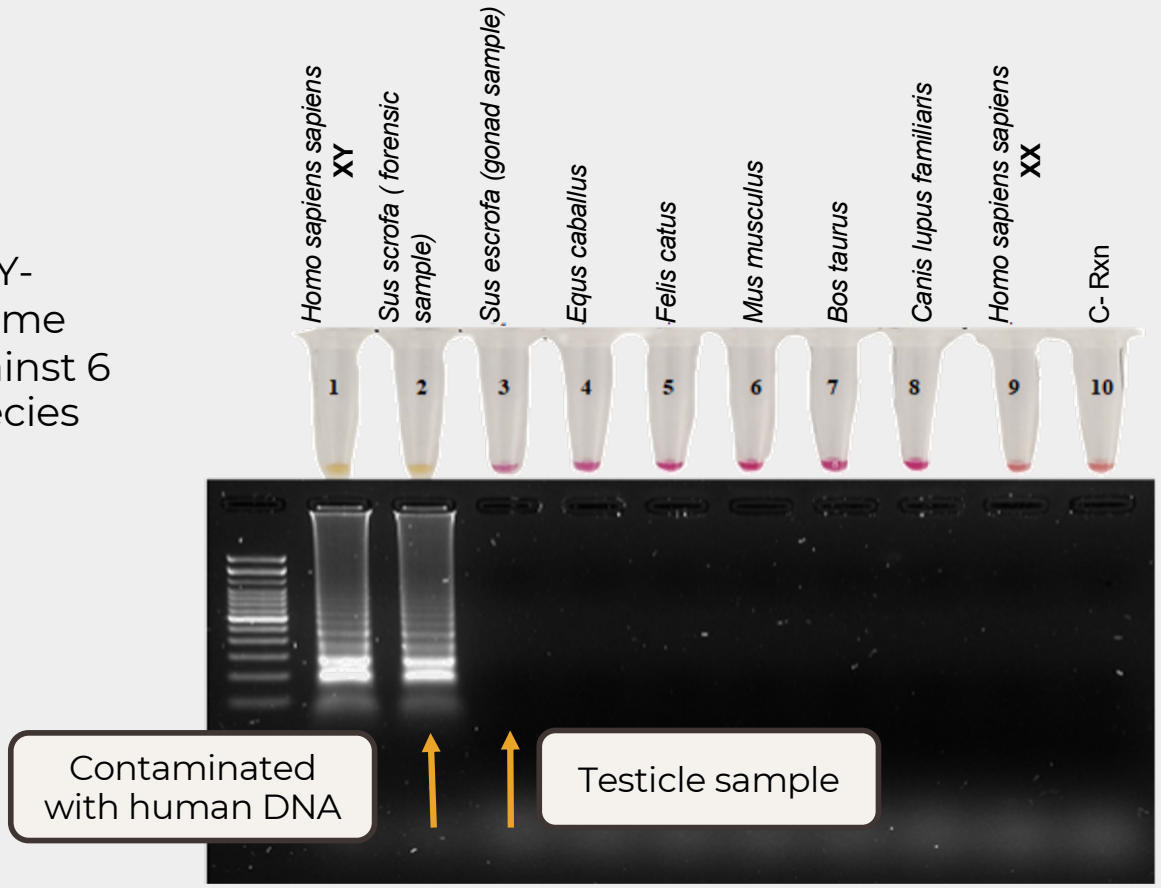
↓

Application in forensic genetics in low complexity laboratories

Non-invasive sex detection in humans with the analysis of free fetal DNA in maternal blood

LAMP specificity

Human Y-chromosome markers against 6 animal species



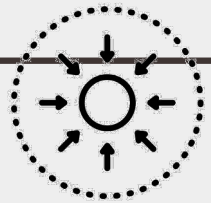
DNA extrated of DNA was extracted from male animals samples

Figure 3. LAMP technique specificity

04

Conclusions

Molecular diagnosis	This report describes a novel design and optimization of a Y-specific markers for sex identification method using versatile loop-mediated isothermal amplification (LAMP).
Sensibility	The sensitivity remarks a higher level of efficacy than real time PCR since our minimal detection was 0,03 haploid copies versus 0.85-1.05 DNA copy number for qPCR kits.
Specificity	The primer sets used in the assay was specific for male human DNA, where no positive results were seen for female human and domestic animal species tested.



05

Recommendations

1 Cross-contamination

Due to the high susceptibility to cross-contamination, it is recommended that rooms are ventilated, different samples are analyzed separately, and the reaction is carried out in separate laboratory spaces to minimize false positives.

2 Future analyzes

The research should be focused on the application of the developed method in a greater number of samples and later, in samples that simulate forensic situations, to determine the applicability in real cases.

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Thank you !
Gracias !
Obrigado !

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Katherin Barrionuevo

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