# A modified mini-primer set for the mtDNA control region sequence analysis from highly degraded forensic remains

**Na Young Kim, Hwan Young Lee, Myung Jin Park, Woo Ick Yang, Kyoung-Jin Shin**

*Department of Forensic Medicine, Yonsei University College of Medicine*
*Human Identification Research Center, Yonsei University*

YONSEI UNIVERSITY
COLLEGE OF MEDICINE

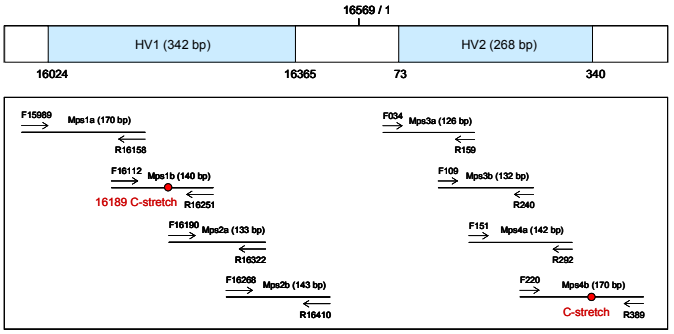
## Mitochondrial DNA

- General properties
  - Maternal inheritance
  - High copy number per cell

  → Powerful tool for forensic identity testing of highly degraded skeletal remains
- Control region : major target for forensic field
  - Hypervariable regions (HV1, HV2 and HV3)
  - Length heteroplasmy and point heteroplasmy

YONSEI UNIVERSITY
COLLEGE OF MEDICINE

# AFDIL mini-primer set




*Gabriel MN et al. (2001) J Forensic Sci. 46:247-253.*

- The different annealing temperature and times for primer pairs make mtDNA analysis labor-intensive.
- A sequence gap on samples with the HV1 length heteroplasmy.
- AFDIL mini-primer produces reduced or inhibited amplification yield by some frequent mutation.

# Primer design of modified mini-primer set

Primer3 (http://www-genome.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi)


- Size : 18 - 27 nucleotide (optimum : 20 nucleotide)
- Temperature : 55℃ - 63℃ (optimum : 60℃)
- GC content : 20% - 80%

**→ Screening PCR**

The final set of primers with high amplification efficiency, relatively short primer length, and high Tm.

# The modified mini-primer sequence

| Control region | Amplicon | Primer sequence (5′ → 3′) | | Amplicon size |
|---|---|---|---|---|
| HV1 | M11 | F15989 | CCC AAA GCT AAG ATT CTA AT | 165bp |
| | | R16153 | CAG GTG GTC AAG TAT TTA TGG | |
| | M12 | F16097 | TAC ATT ACT GCC AGC CAC CA | 137bp |
| | | R16233 | TGA TAG TTG AAG GTT GAT TGC TGT | |
| | M13 | F16159 | CAT AAA AAC CCA ATC CAC AT | 146bp |
| | | R16304 | ACT GTT AAG GGT GGG TAG GT | |
| | M14 | F16247 | ACT CCA AAG CCA CCC CTC A | 164bp |
| | | R16410 | GAG GAT GGT GGT CAA GGG AC | |
| HV2 | M21 | F015 | CAC CCT ATT AAC CAC TCA CG | 173bp |
| | | R187 | CGC CTG TAA TAT TGA ACG TA | |
| | M22 | F120 | CGC AGT ATC TGT CTT TGA TTC C | 166bp |
| | | R285 | GTT ATG ATG TCT GTG TGG AA | |
| | M23 | F220 | TGC TTG TAG GAC ATA ATA AT | 170bp |
| | | R389 | CTG GTT AGG CTG GTG TTA GG | |

YONSEI UNIVERSITY
COLLEGE OF MEDICINE

# PCR amplification efficiency test

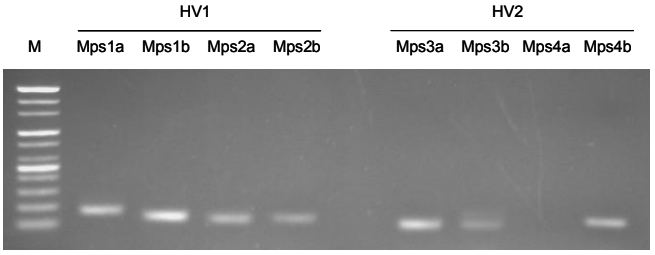
- DNA samples
  - mtDNA haplogroup D4 (16223-16362-73-263-315.1C)
  - HV1 length heteroplasmy
  - Mutations are located at AFDIL's mini-primer annealing site
    → Long bone and molar teeth samples from 55 year-old skeletal remains
    DNA from blood samples
- PCR amplification
  - Total 25.0 μl PCR reaction : 2 μl of DNA, 2.5 μl of STR buffer, 2.5 U AmpliTaq Gold polymerase and primer
- Thermal Cycling

95℃ for 11 min
95℃ for 20 sec
50℃ for 20 sec
72℃ for 30 sec
X 42 cycles (bone)
72℃ for 7 min

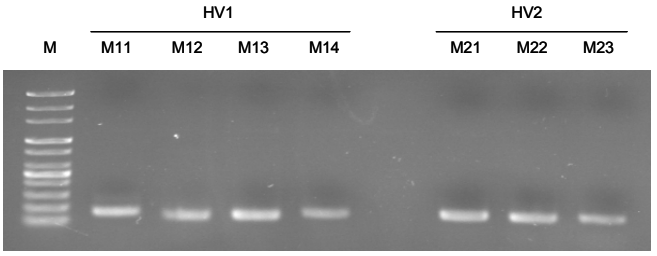
Blood sample : annealing Tm - 56℃, 35 cycles

# Comparison of the PCR amplification efficiency

A. AFDIL mini-primer



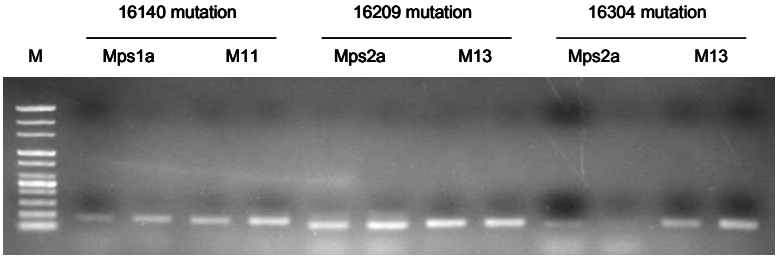

B. modified mini-primer




# PCR amplification efficiency on mutation samples

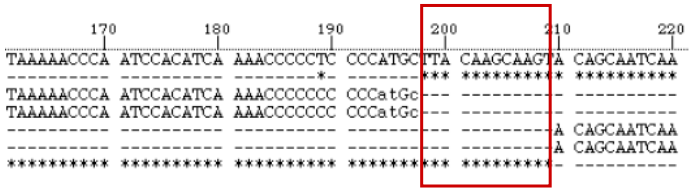
Mutation frequencies

| Population | n.p. 16140 | n.p. 16209 | n.p. 16304 |
|---|---|---|---|
| Caucasians | 0.2% | 1.3% | 8.6% |
| **Asians** | **5.1%** | **3.2%** | **14.7%** |
| African Americans | 0.3% | 6.3% | 0.9% |



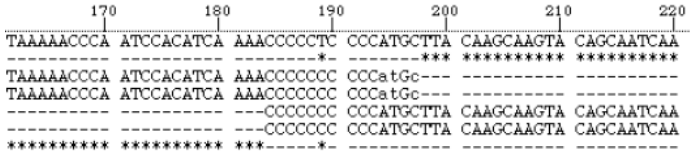
# Comparison of the sequence alignment with the 16189 mutation sample

A. AFDIL mini-primer



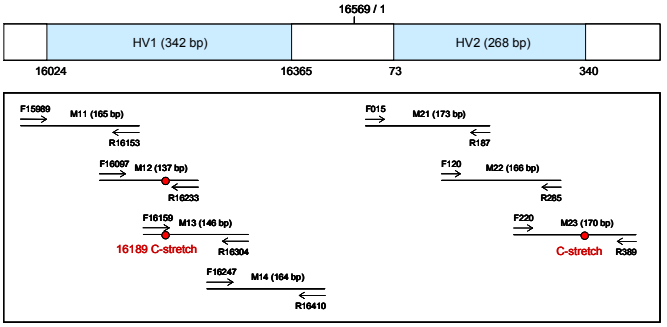

sequencing gap

B. Modified mini-primer






# The modified mini-primer set




- The modified mini-primer set is composed of four and three PCR amplicons.
- The forward primer of M13 was designed to be avoid the possible gap on samples with the HV1 length heteroplasmy.
- Using the FBI mtDNA population database, the first to the third nucleotide from the 3' end of each primer were located at the nucleotide position with a low mutation frequency in the Caucasians, Asians, and Africans.

# Alternative primers

- **In Africans**

  n.p. 16265 mutation frequency : 6.5%

  M14 - F16247 → F16255 (5'-GCC ACC CCT CAC CCA CTA G)

  M13 - R16304 → R16322 (5'-TGG CTT TAT GTA CTA TGT AC)

- **In Caucasians**

  n.p. 239 mutation frequency : 1.9%

  M23 – F220 → F220* (5'-TGC TTG TAG GAC ATA ATA ATA ACA A)



# Conclusion

- We developed the modified mini-primer set which makes up for the weak points of AFDIL mini-primer set.
  - HV1 length heteroplasmy
  - Nucleotide variability
  - PCR amplification condition
- The modified mini-primer set will be a useful tool for mtDNA control region sequence analysis from highly degraded forensic samples.

YONSEI UNIVERSITY
COLLEGE OF MEDICINE

# Acknowledgements

This work was supported by grant number M10640010002-16N4001-00210 from the Korean Ministry of Science and Technology (MOST) and the Korean Science and Engineering Foundation (KOSEF), and grants from MAKRI (Ministry of Naional Defense Agency for Killed In Action Recovery and Identification).

YONSEI UNIVERSITY
COLLEGE OF MEDICINE