STR Typing of Degraded DNA using a Repair Enzyme and Whole Genome Amplification

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Characters of degraded DNA

- Forensic samples are exposed to unstable environments
- DNA could be damaged
  - It may be degraded into fragments smaller than amplicon size
  - It may contain only small amounts of genomic DNA
  - Therefore, it may result in failure of PCR amplification at some of STR loci and produce incomplete DNA profile

⇒ MiniSTR: PCR amplicon size can be reduced

Repair enzyme, Whole genome amplification

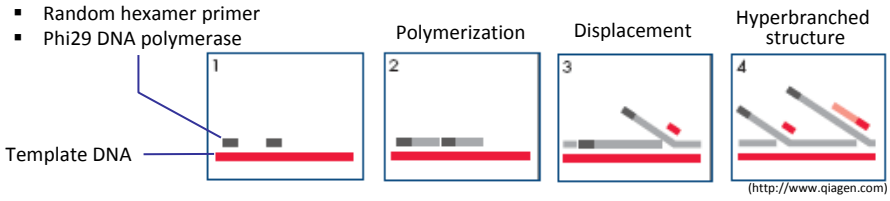
Repairing Enzyme

- Within living cells, the integrity of DNA molecules is continually maintained by enzymatic repair processes.
- By using repairing enzyme, DNA repair of living cell can be carried out in vitro.
- Commercial reagent : PreCR Repair Mix
  - (Uracil-DNA Glycosylase, formamidopyrimidine-DNA glycosylase, Endonuclease IV, Endonuclease VIII, T4 Endonuclease V, Bst DNA Polymerase, Taq DNA ligase)
- In previous studies, only UV damaged DNA and DNA from skeletal remains were evaluated.

Repair enzyme should be evaluated in various types of damaged DNA and forensic samples

Whole Genome Amplification (WGA)

- WGA is a technique to specifically increase the DNA quantities originating from samples with limited DNA contents.
  - Preimplantation genetic diagnosis (PGD)
- Isothermal WGA method
  - MDA (Multiple Displacement Amplification)



After repairing of degraded DNA, MDA based WGA method can be applied to repaired DNA.

Aims of This Study

- Validation of the availability and efficacy of repairing enzyme in various types of degraded DNA
- To present the possibility of application of WGA to forensic field
- To increase the success rate of DNA typing in damaged sample

Materials and Methods: Sample preparation

- **Artificially degraded DNA**
  - 9948 standard DNA, K562 high molecular DNA
  - UV radiated DNA: UV radiation during 2 minutes by CL-1000 Ultraviolet cross-linker
  - Oxidized DNA: Hydroxyl radical by fenton reaction using Fe^{2+}, H_2O_2
  - Acid/heat damaged DNA: NaCl, Sodium-Na, 70˚C, 10 hours incubation
  - DNase I treated DNA: 0.006 unit DNase I treatment for 10~15 min
- **Naturally degraded DNA**
  - Dried blood spot, dried saliva spot from **5 applicants: IRB approval,** 10uL, sunshine exposure and air dry during 2 weeks
    - QIAamp DNA Investigator Kit
  - Old skeletal remains: over 60 years
    - DNA extracted by Lee *et al*. (*Forensic Sci Int Genet.* 2010; In press)
- All of the samples were prepared in quintuplicate.

Materials and Methods: Repair of degraded DNA

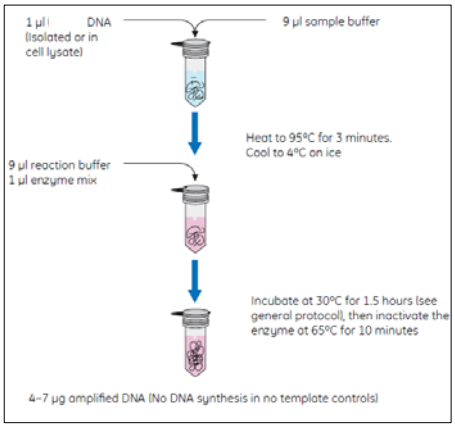
- Amplification of autosomal STR: AmpFℓSTR Identifiler Kit

Reagents	Repair		Control	
dH_20	6.0 ㎕	1.5 ㎕	9.0 ㎕	2.8 ㎕
Identifiler Reaction Mix	9.5 ㎕	3.8 ㎕	9.5 ㎕	3.8 ㎕
10× NAD^+	2.5 ㎕	1.0 ㎕	-	-
AmpliTaq Gold DNA polymerase (5 unit/㎕)	0.5 ㎕	0.4 ㎕	0.5 ㎕	0.4 ㎕
PreCR Repair Mix	0.5 ㎕	0.3 ㎕	-	-
Demaged DNA	1.0 ㎕	1.0 ㎕	1.0 ㎕	1.0 ㎕
Incubation at 37˚C, 20 min for repairing			-	-
Identifiler Primer Set	5.0 ㎕	2.0 ㎕	5.0 ㎕	2.0 ㎕
Total reaction volume	25.0 ㎕	10.0 ㎕	25.0 ㎕	10.0 ㎕
Thermal cycling for PCR amplification 95˚C for 11 min; 94˚C for 1 min: 59˚C for 1 min: and 72˚C for 1 min × 28 cycles; a final extension at 60˚C for 60 min.				

In case of DNA obtained from skeletal remains, 3.5 U of Gold Taq enzyme and 2.0 uL of template DNA were used with 5 more amplification cycle.

Materials and Methods: WGA

- **Sensitivity test:** Serial dilution samples (10 ng, 1 ng, 500 pg, 250 pg, 125 pg, 62.5 pg, 31.3 pg)
- **Performing of WGA:** GenomiPhi V2 Amplification Kit (MDA based WGA)
- **Quantification:** TBS-380 Mini-Fluorometer
- **Amplification of autosomal STR:** AmpFℓSTR Identifiler Kit



(http://www.gelifesciences.com)

Materials and Methods: Combination of Repair and WGA

- Whole volume of repaired DNA should be used for WGA
- Combination of the repairing step and WGA

Process	Reagents	Volume
DNA repairing	dH_20	5.7 ㎕
	10×ThermoPol Reaction Buffer	1.0 ㎕
	1mM dNTP	1.0 ㎕
	10× NAD^+	1.0 ㎕
	PreCR Repair Mix	0.3 ㎕
	Demaged DNA (1 ng/㎕)	1.0 ㎕
	Total reaction volume	10.0 ㎕
	Incubation at 37˚C, 20 min for repairing	
Reducing volume	Vacuum dry during 15 min	
WGA	95˚C, 3 min denaturation, ice Add Reaction Buffer 9㎕, Enzyme 1㎕ Elongation: 30˚C, 1.5 hour incubation Enzyme inactivation: 65˚C, 10 min	

- **Using naturally degraded DNA,** applicability of combining step **was evaluated**.
- **Quantification:** TBS-380 Mini-Fluorometer
- **Amplification of autosomal STR:** AmpFℓSTR Identifiler Kit

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Results : Repair of Degraded DNA

UV radiated DNA

: Pyrimidine dimer

Oxidized DNA

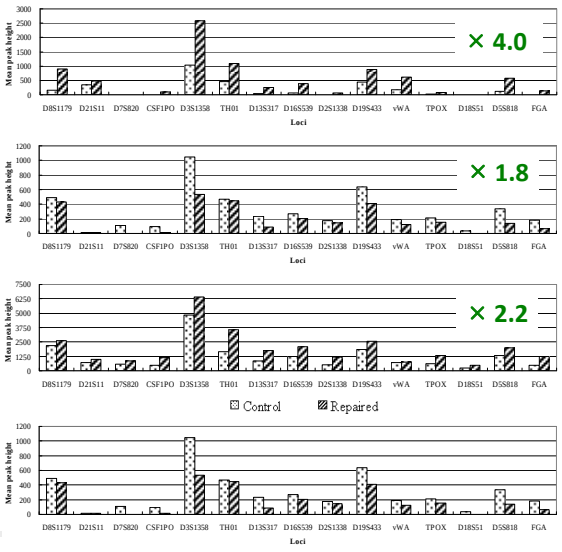
: Guanine → 8-oxo-Guanine, nick, fragmentation

Acid/heat DNA

: Apurine/apyrimidine site, deamination, nick, fragmentation

DNase I treated DNA

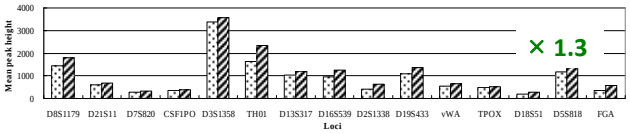
: Nick, fragmentation



Results : Repair of Degraded DNA

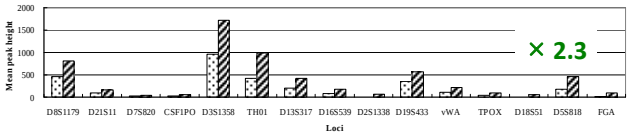
Dried blood spot

; UV damage, desiccation, oxidation



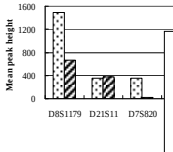
Dried saliva spot

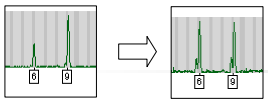
; UV damage, desiccation, oxidation

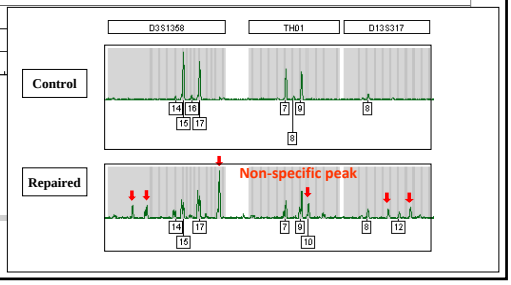


Skeletal remains

Peak imbalance was decreased (50%)



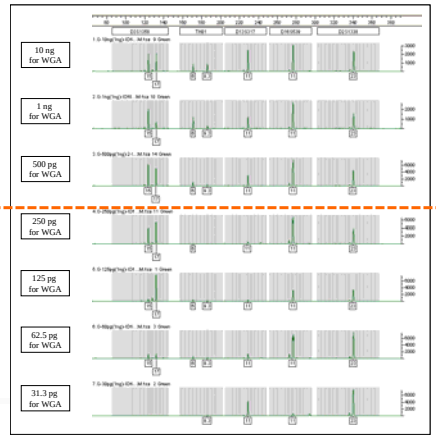




Results : Sensitivity Test of WGA

- Mean concentration of amplified DNA by WGA in sensitivity test

Input DNA for WGA	10 ng	1 ng	500 pg	250 pg	125 pg	62.5 pg	31.3 pg
Concentrations (ng/uL)	191.9	179.8	148.5	112.7	34.7	46.4	18.8



Peak imbalance was shown
- between loci
- between alleles

Allele drop out

Results : Combination of Repair and WGA

- Establishment of combination of repair and WGA
- Evaluation of combining method using naturally degraded DNA

Source of DNA	Dried blood spots	Dried saliva spots	Skeletal remains
DNA concentration of WGA (ng/uL)	–	–	16.1

- Combining method was difficult to apply for degraded DNA.
  - MDA based WGA did not work in degraded DNA.
  - It may be due to the possibility that repair effect was not big enough to overcome the limitation of MDA.

Conclusion

- UV damaged DNA, oxidized DNA, acid/heat damaged DNA can be repaired by repair enzyme. Therefore, repair enzyme would be useful for analyzing forensic samples such as damaged DNA.
- Whole genome amplification could be used for low quantity intact DNA. However, when using very low quantity of DNA for WGA, allele drop out can be observed.
- In the future, studies on WGA for degraded DNA will be necessary, since MDA based WGA is difficult to apply for degraded DNA.