

## Whole genome amplification effect for low template DNA in hybrid capture-based next generation sequencing of identity SNPs

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## Backgrounds

- **Forensic genotyping system for low template DNA**
  - CE-based STR multiplex system (PowerPlex, GlobalFiler, ...)
  - NGS-based targeted SNP system
    1. PCR-based target enrichment  
(Precision ID, ForenSeq, QIAseq, ...)
    2. Hybrid capture-based target enrichment  
followed by Low-input DNA Library preparation

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## Backgrounds



Article

### Applications of Probe Capture Enrichment Next Generation Sequencing for Whole Mitochondrial Genome and 426 Nuclear SNPs for Forensically Challenging Samples

Shelly Y. Shih <sup>1</sup>, Nikhil Bose <sup>1,2</sup>, Anna Beatriz R. Gonçalves <sup>1,3</sup>, Henry A. Erlich <sup>1</sup> and Cassandra D. Calloway <sup>1,2,\*</sup>



Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: [www.elsevier.com/locate/fsigen](http://www.elsevier.com/locate/fsigen)

Research paper

Target capture enrichment of nuclear SNP markers for massively parallel sequencing of degraded and mixed samples

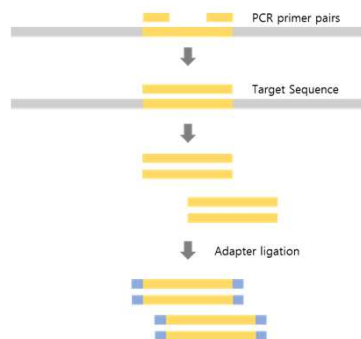
Nikhil Bose<sup>a,b</sup>, Katie Carlberg<sup>a,c</sup>, George Sensabaugh<sup>b,d</sup>, Henry Erlich<sup>a</sup>, Cassandra Calloway<sup>a,b,\*</sup>

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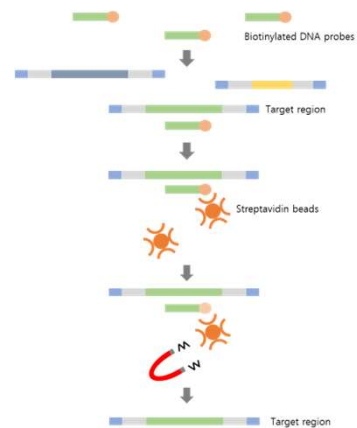
## Introduction (continued)

- **PCR-based target enrichment**

- Specific primer binding site
- PCR primer interference



### Hybrid capture-based target enrichment



**Flexible expansion of the SNP panel**

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## Introduction (continued)

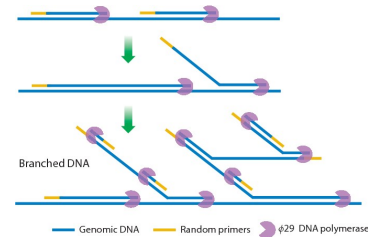
- **Low-input DNA Library preparation kits**
  - Require about 100pg of input DNA
  - Low library complexity



**Whole genome amplification**

-> Genome recovery  
from single cell

### Multiple Displacement Amplification

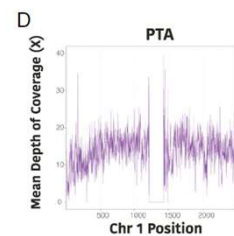
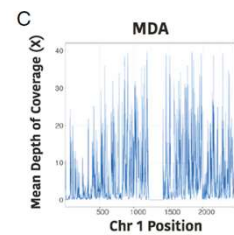
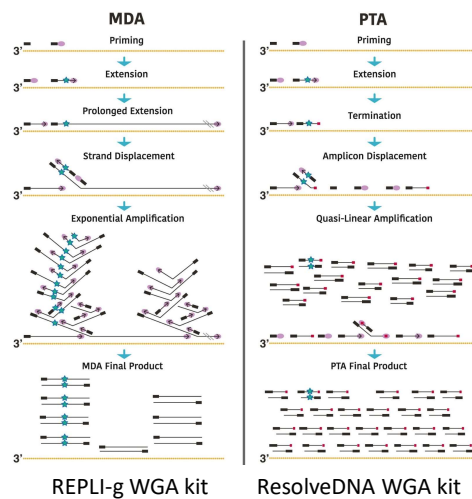


Annual review of genomics and human genetics, 2015

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## Introduction (continued)

### Multiple Displacement Amplification (MDA) vs Primary Template-directed Amplification (PTA)



Gonzalez-Pena Et al. PNAS (2021)

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## Aims of Study

**Can whole genome amplification increase the efficiency of hybrid capture-based NGS for low template DNA?**



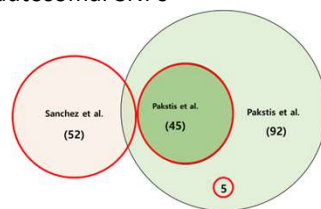
- Development of **hybrid capture NGS** system of Identity SNPs for **trace amounts of DNA**
- Comparison of **hybrid capture efficiency** between **genomic DNA** and **WGA DNA**
- Investigation of **lower limit for the amounts of input DNA** that can be analyzed

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## Materials and Methods

### Target SNPs

- **136 Identity SNPs**
- 102 autosomal SNPs



- 34 Y-SNPs  
from Precision ID Identity panel

- **Hybrid capture**

- KAPA HyperChoice custom probe (Roche)
- 120bp (including target SNPs)

### Samples

- **5 male samples** from 4 populations
- **Input DNA**

**Genomic DNA**

**WGA DNA**

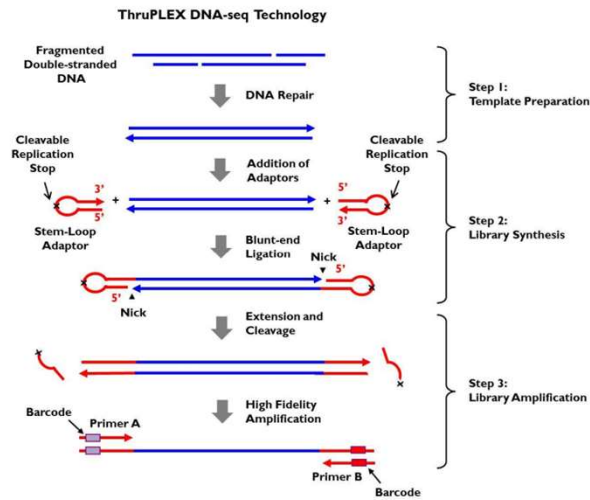
Ref. **10ng**

1ng	.....	1ng
500pg	.....	500pg
200pg	.....	200pg
100pg	.....	100pg
50pg	.....	50pg
25pg	.....	25pg
		12.5pg
		6.25pg

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## Materials and Methods (continued)

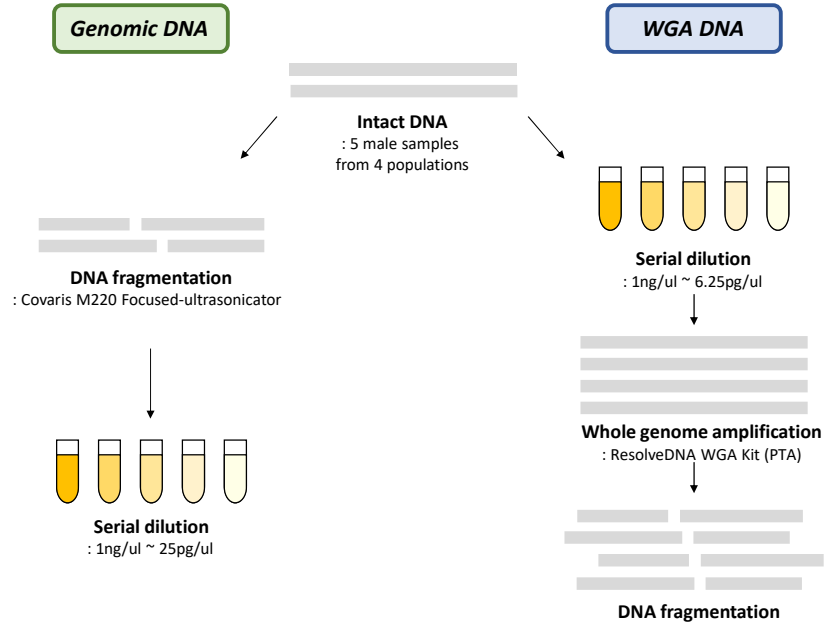
### ThruPLEX® DNA-seq Kit (50pg ~ 50ng)



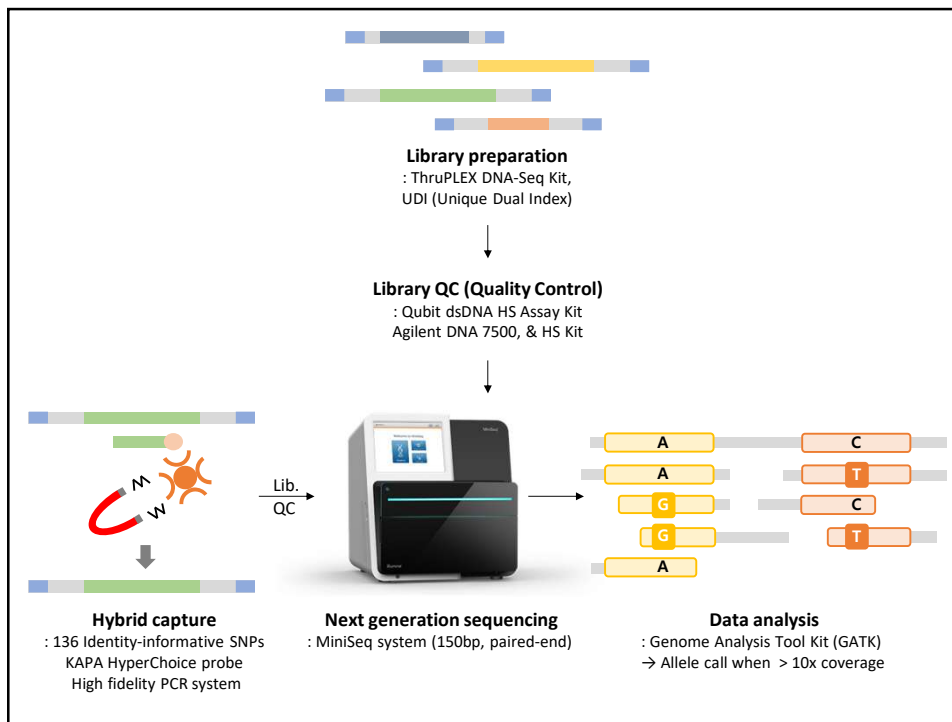
<https://www.takarabio.com/>

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## Workflow



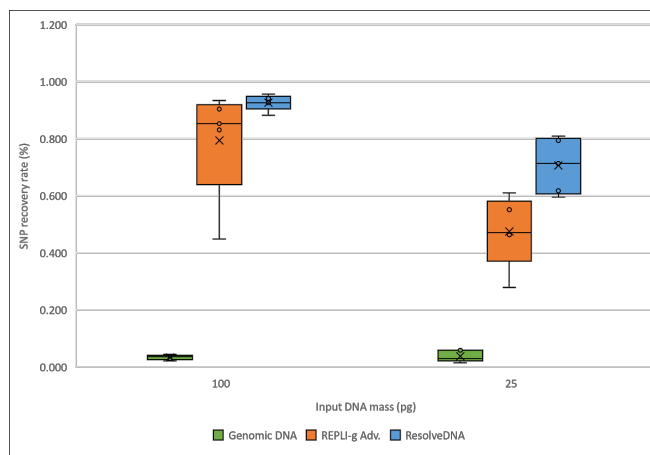
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## Result & Discussion (continued)

### Comparison of MDA-based WGA and PTA-based WGA



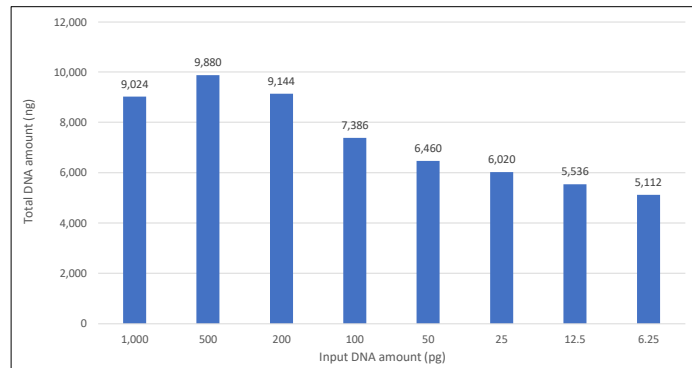
*Not published data*

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## Result & Discussion (continued)

### 1. Performance of *PTA-based Whole genome amplification*

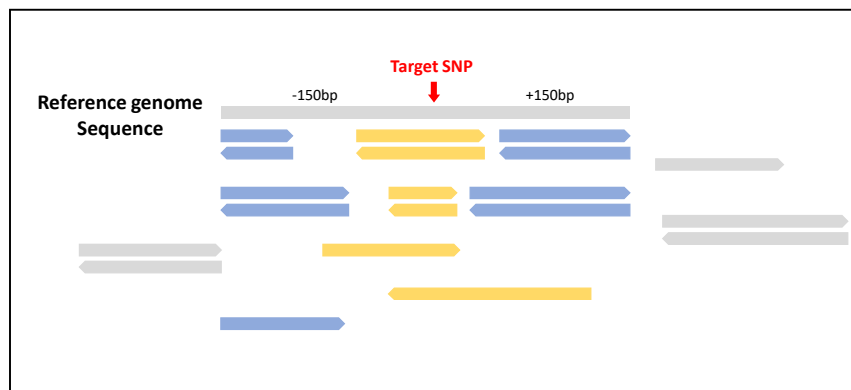
- *ResovleDNA WGA kit* was performed well even with **6.25pg of input DNA**.



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## Result & Discussion (continued)

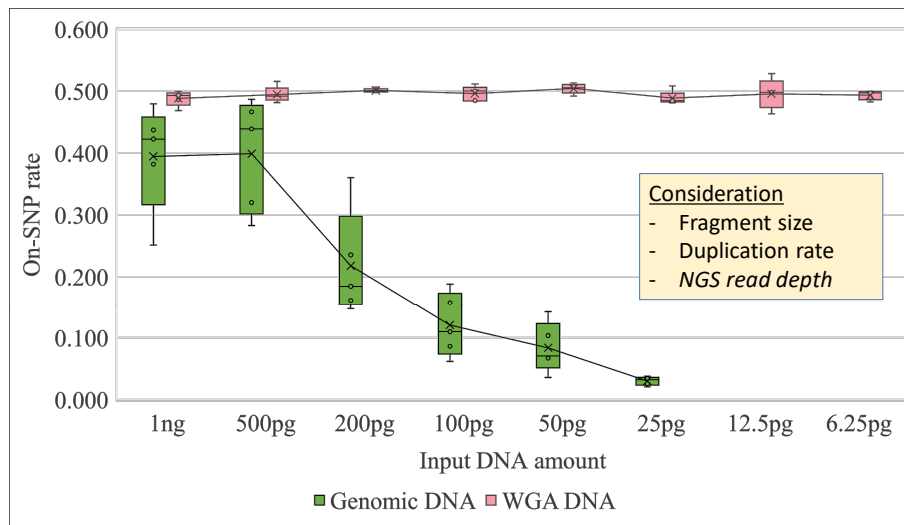
### *On-target rate, On-SNP rate and PCR duplicates*



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## Result & Discussion (continued)

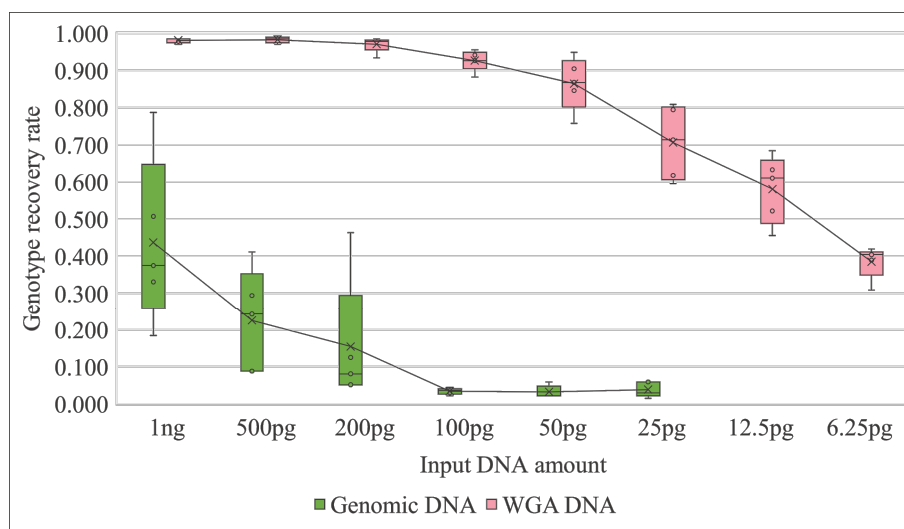
### 2. On-SNP rate of hybrid capture-based NGS system



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## Result & Discussion (continued)

### 3. The genotype recovery rate

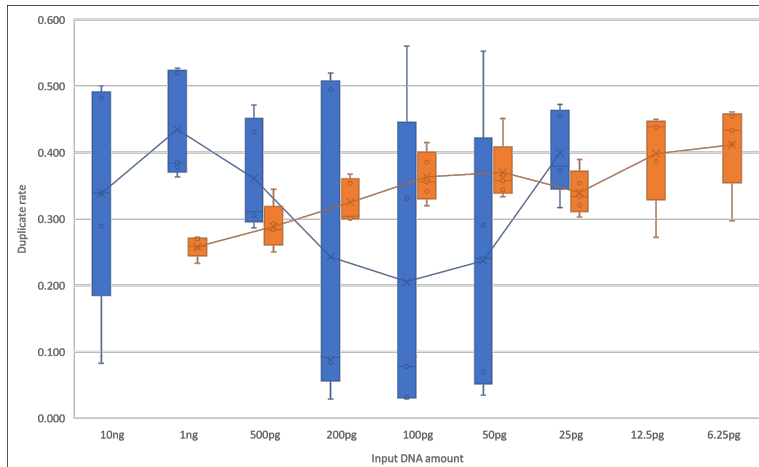


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## Result & Discussion (continued)

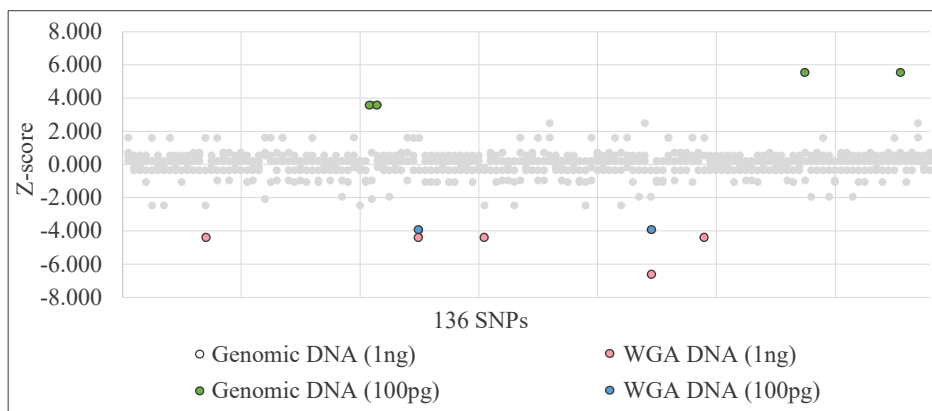
### 4. Duplicate rate



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## Result & Discussion (continued)

### 5. Genotype recovery rate of 136 SNPs according to Z-scores



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## Conclusion



We developed **hybrid capture NGS** system of Identity SNPs for **trace amounts of DNA**

- Around **40%** of genotype recovery was showed with **1ng of genomic DNA**.
- **More than 80 %** of genotype recovery can be obtained with **50pg of input DNA by WGA**.



*The **utility of WGA** was demonstrated in hybrid capture-based NGS for low template DNA*

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## Ongoing Study

- Increase number of SNPs to > 1,200

A compilation of tri-allelic SNPs from 1000 Genomes and use of the most polymorphic loci for a large-scale human identification panel

C. Phillips<sup>a,\*</sup>, J. Amigo<sup>b</sup>, A.O. Tillmar<sup>c,d</sup>, M.A. Peck<sup>e</sup>, M. de la Puente<sup>a</sup>, J. Ruiz-Ramírez<sup>a</sup>, F. Bittner<sup>e</sup>, Š. Idrizbegović<sup>e</sup>, Y. Wang<sup>f</sup>, T.J. Parsons<sup>g</sup>, M.V. Lareu<sup>h</sup>



- Use more efficient library preparation kit with smaller sized fragment to get higher On-SNP rate
- Replicated analysis to increase accuracy of recovered genotypes of SNPs

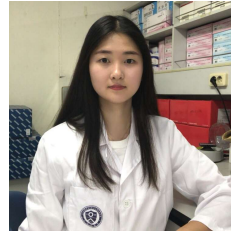
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## Acknowledgements

### Members of Yonsei DNA profiling group



Su Min Ju  
MSc candidate



Ye-Lim Kwon  
PhD candidate

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