

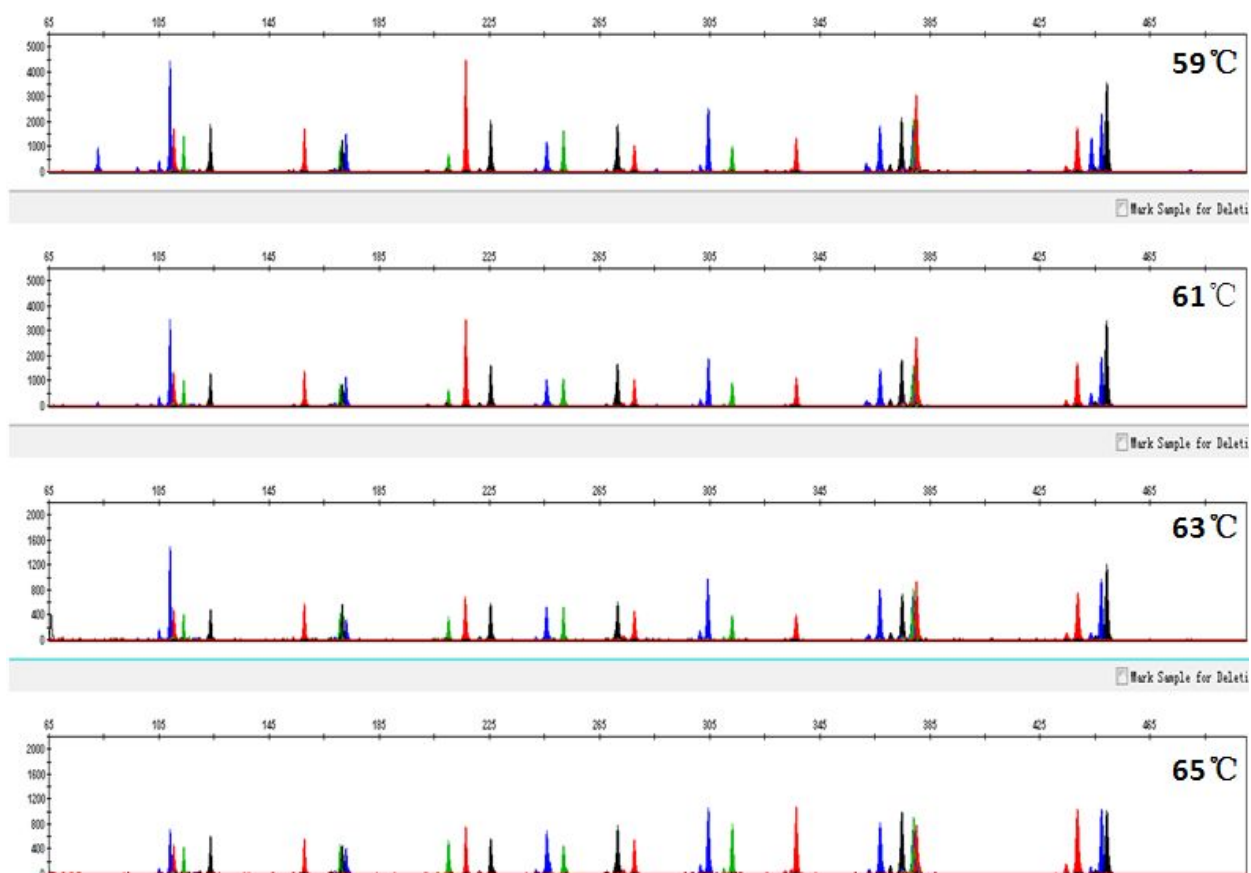
# Developmental Validation of a novel 5 dye Y-STR System comprising the 27 YfilerPlus loci

Rufeng Bai<sup>1,2,\*</sup>, Yaju Liu<sup>3,\*</sup>, Zheng Li<sup>4</sup>, Haiying Jin<sup>4</sup>, Qinghua Tian<sup>1</sup>, Meisen Shi<sup>1,2</sup>, Shuhua Ma<sup>5</sup>

<sup>1</sup>Key Laboratory of Evidence Science (China University of Political Science and Law), Ministry of Education, Beijing 100088, P.R.China; <sup>2</sup> Collaborative Innovation Center of Judicial Civilization, Beijing 100088, P.R.China; <sup>3</sup>Institute of Criminal Sciences and Technology, Municipal Public Security Bureau of Xuchang, Xuchang 461000, P.R.China; <sup>4</sup> HEALTH GeneTech, Ningbo 315000, P.R.China; <sup>5</sup>Department of Radiology, First Affiliated Hospital, Medical College of Shantou University, Shantou 515041, P.R.China

\*These authors contributed equally to this work

Correspondence and requests for materials should be addressed to Meisen Shi and Shuhua Ma (email: [shimeisen2000@163.com](mailto:shimeisen2000@163.com), [mashuhua6699@163.com](mailto:mashuhua6699@163.com))



**FigureS1. Representative electropherograms for reactions using annealing temperatures from 59°C to 65°C**

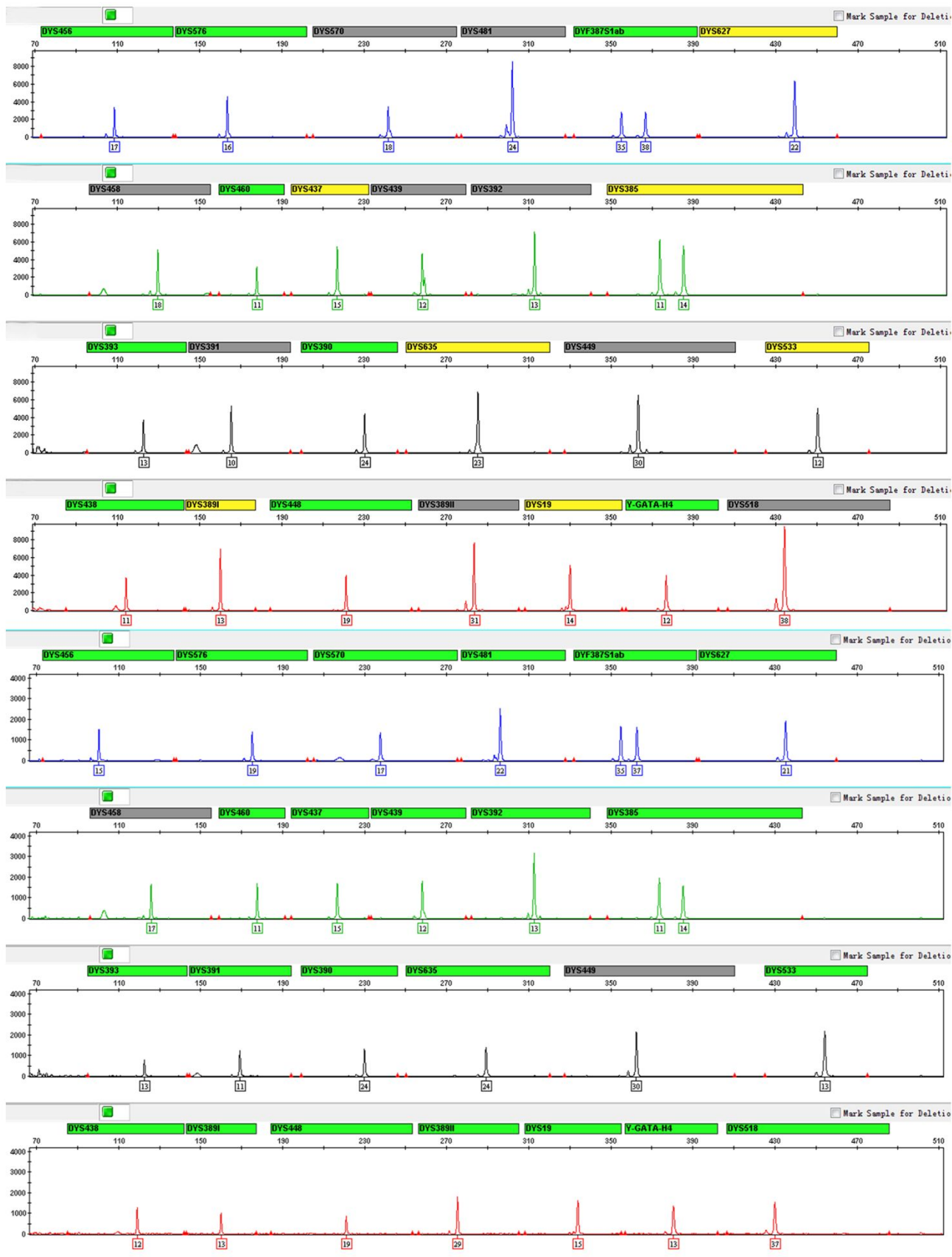
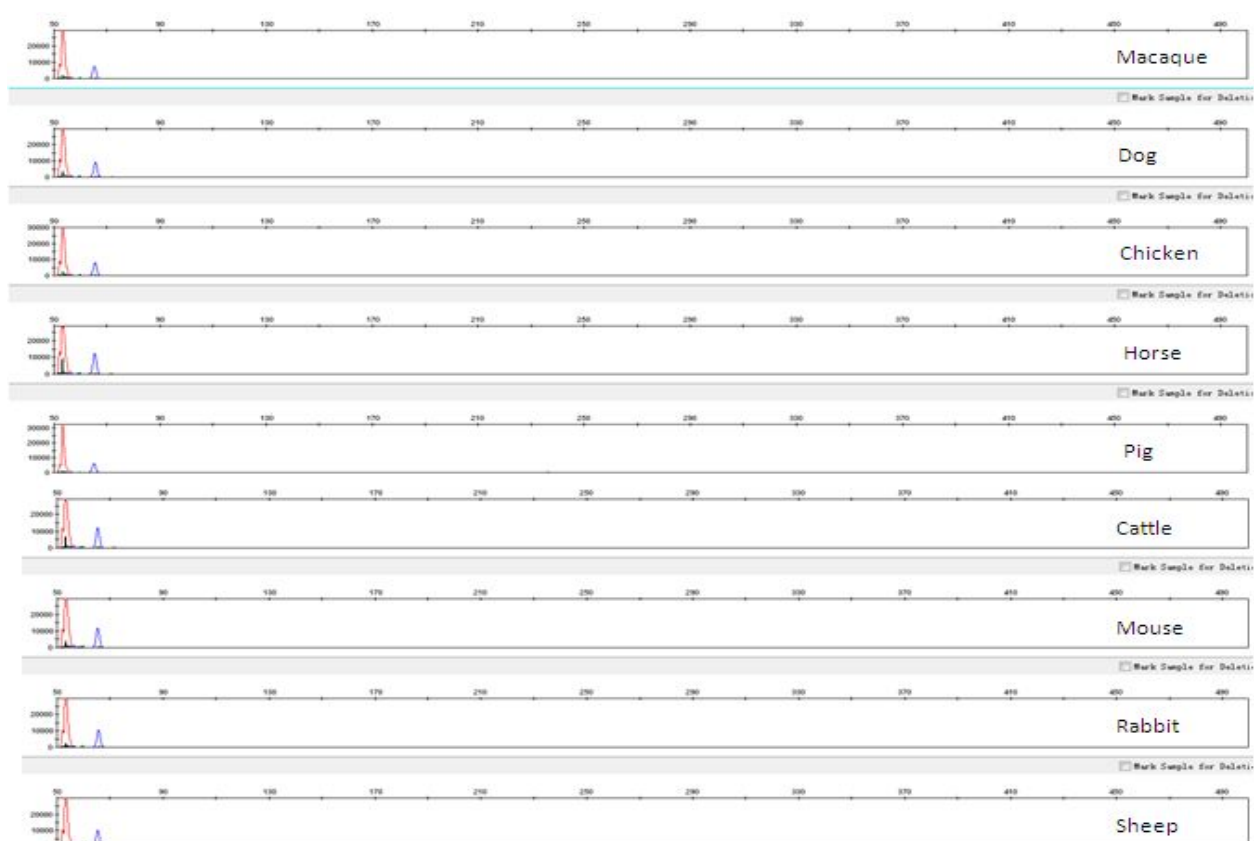
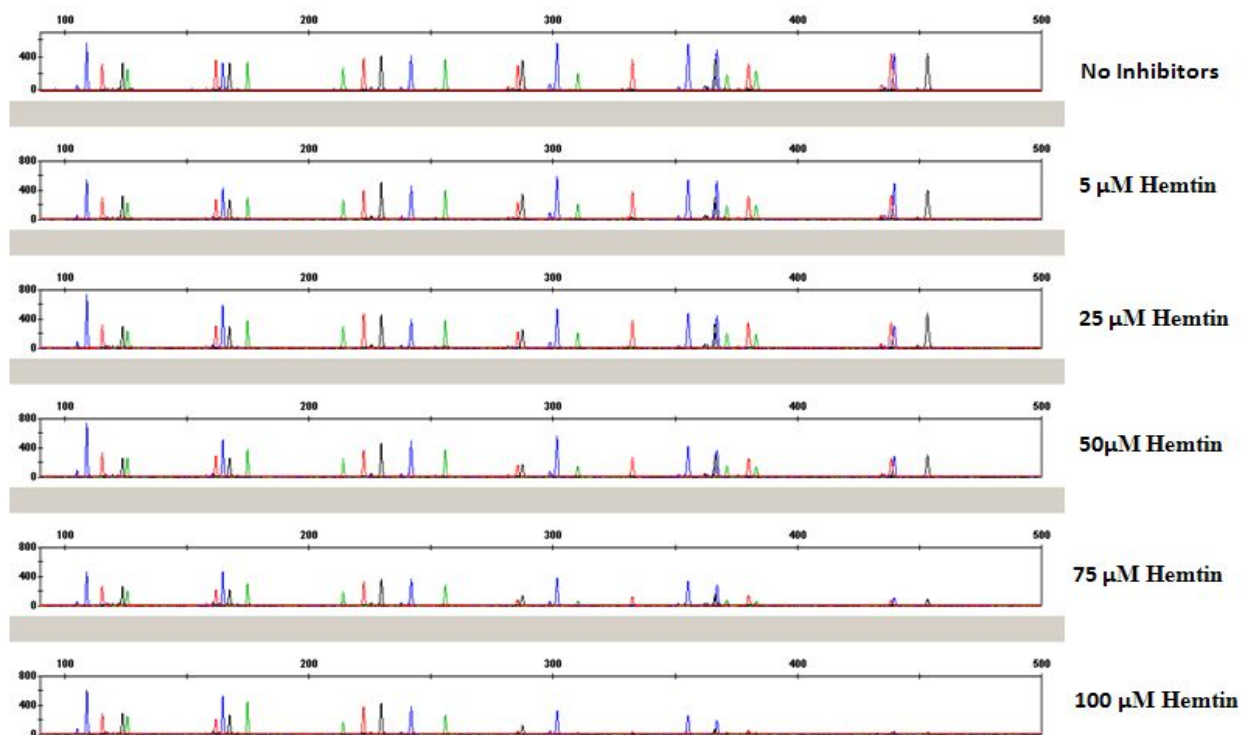


Figure S2. Electropherogram of the Control DNA 9948 (upper) and 007 (lower) amplified by STRtyper-27 system



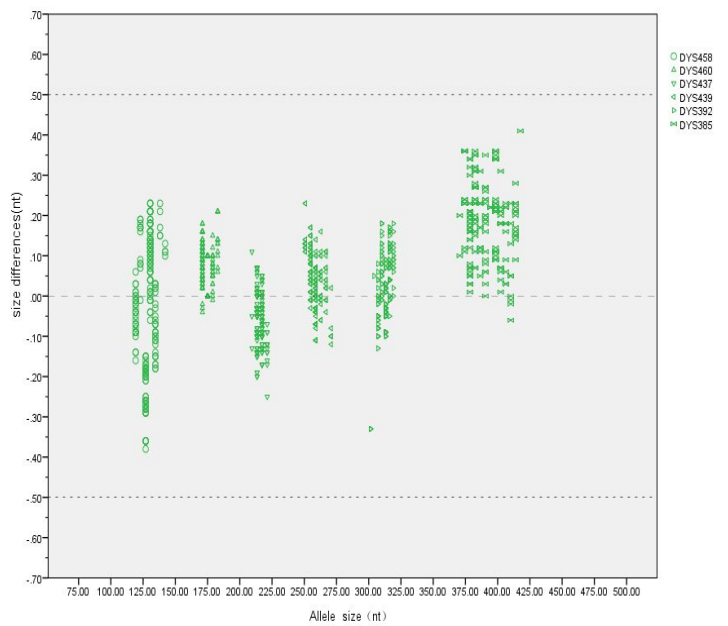
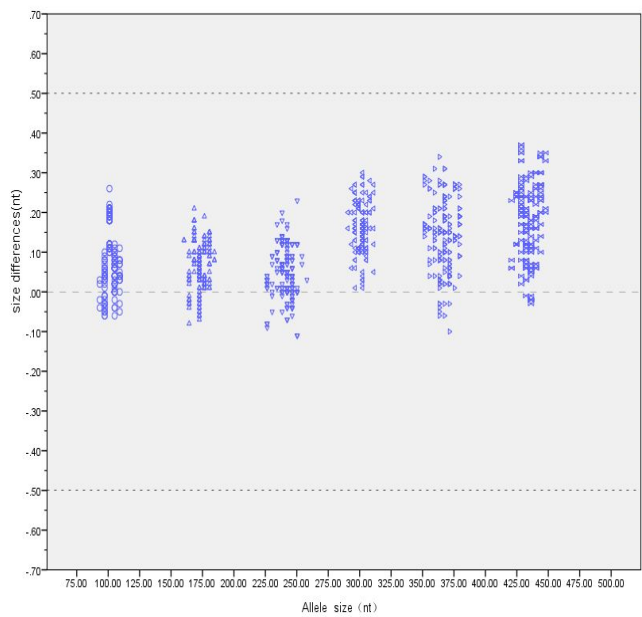
**Figure S3. Representative results of the species specificity assessment**

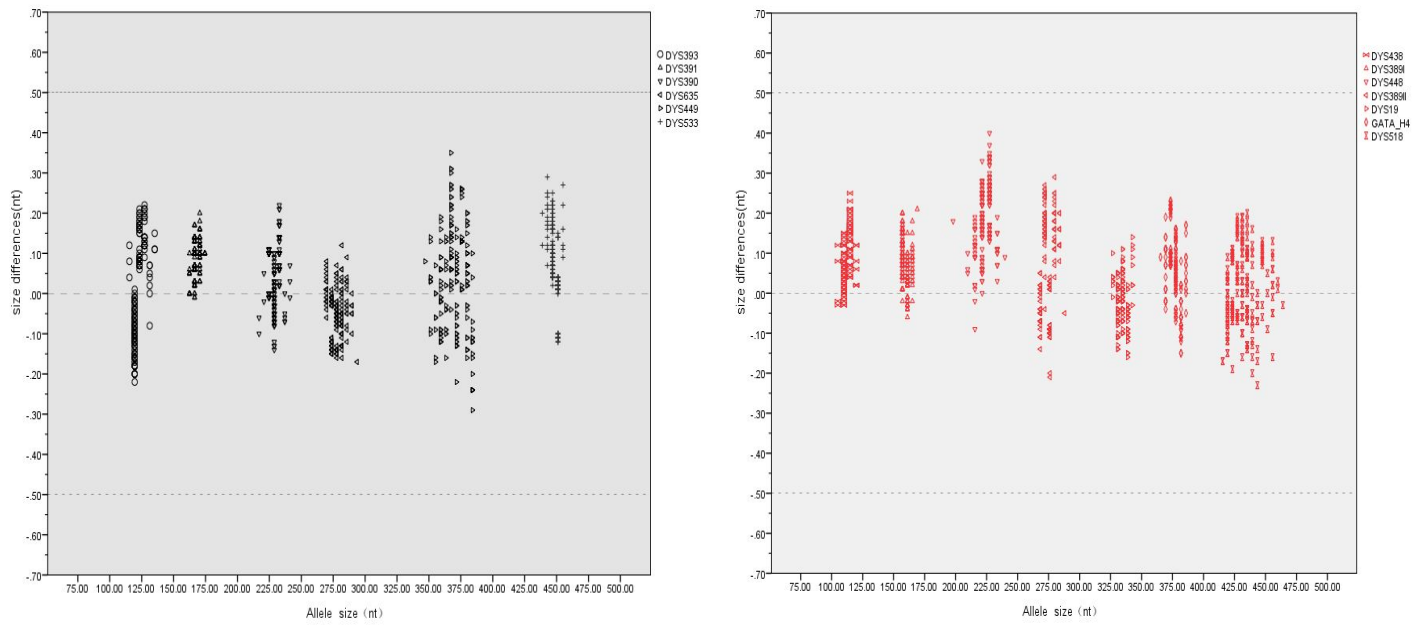


**Figure S4. Representative electropherogram of 1ng 9948 amplified in the various concentrations of the hematin in the 25μL system.**

| Locus     | Stutter range(%) | Stutter average(%) | Standard Deviation(%) |
|-----------|------------------|--------------------|-----------------------|
| DYS456    | 2.03-14.41       | 10.32%             | 1.88%                 |
| DYS576    | 8.20-13.57       | 10.61%             | 1.05%                 |
| DYS570    | 6.74-13.48       | 9.08%              | 0.97%                 |
| DYS481    | 1.91-23.15       | 18.19%             | 1.91%                 |
| DYF387S1  | 4.45-18.28       | 10.97%             | 2.32%                 |
| DYS627    | 6.24-11.01       | 8.57%              | 1.24%                 |
| DYS458    | 1.80-13.00       | 10.06%             | 1.34%                 |
| DYS460    | 3.75-9.62        | 5.70%              | 1.31%                 |
| DYS437    | 3.98-9.15        | 6.40%              | 1.28%                 |
| DYS439    | 4.76-10.90       | 6.81%              | 1.18%                 |
| DYS392    | 3.27-17.06       | 9.79%              | 2.38%                 |
| DYS385    | 5.08-14.16       | 9.14%              | 2.51%                 |
| DYS393    | 1.21-11.71       | 7.99%              | 1.58%                 |
| DYS391    | 5.22-12.97       | 7.51%              | 1.04%                 |
| DYS390    | 6.45-12.91       | 8.89%              | 1.00%                 |
| DYS635    | 4.49-12.30       | 7.83%              | 1.34%                 |
| DYS449    | 11.41-20.79      | 15.70%             | 1.81%                 |
| DYS533    | 4.17-7.73        | 6.06%              | 0.85%                 |
| DYS438    | 1.46-10.47       | 4.57%              | 2.27%                 |
| DYS389I   | 5.35-9.71        | 6.90%              | 0.98%                 |
| DYS448    | 2.80-5.43        | 3.85%              | 0.76%                 |
| DYS389II  | 10.05-16.18      | 12.96%             | 1.20%                 |
| DYS19     | 4.74-8.18        | 6.31%              | 0.95%                 |
| Y-GATA-H4 | 5.34-10.28       | 7.42%              | 1.04%                 |
| DYS518    | 11.12-21.42      | 14.42%             | 2.00%                 |

**Table S1 Observed stutter values for 27 Y-STR loci**

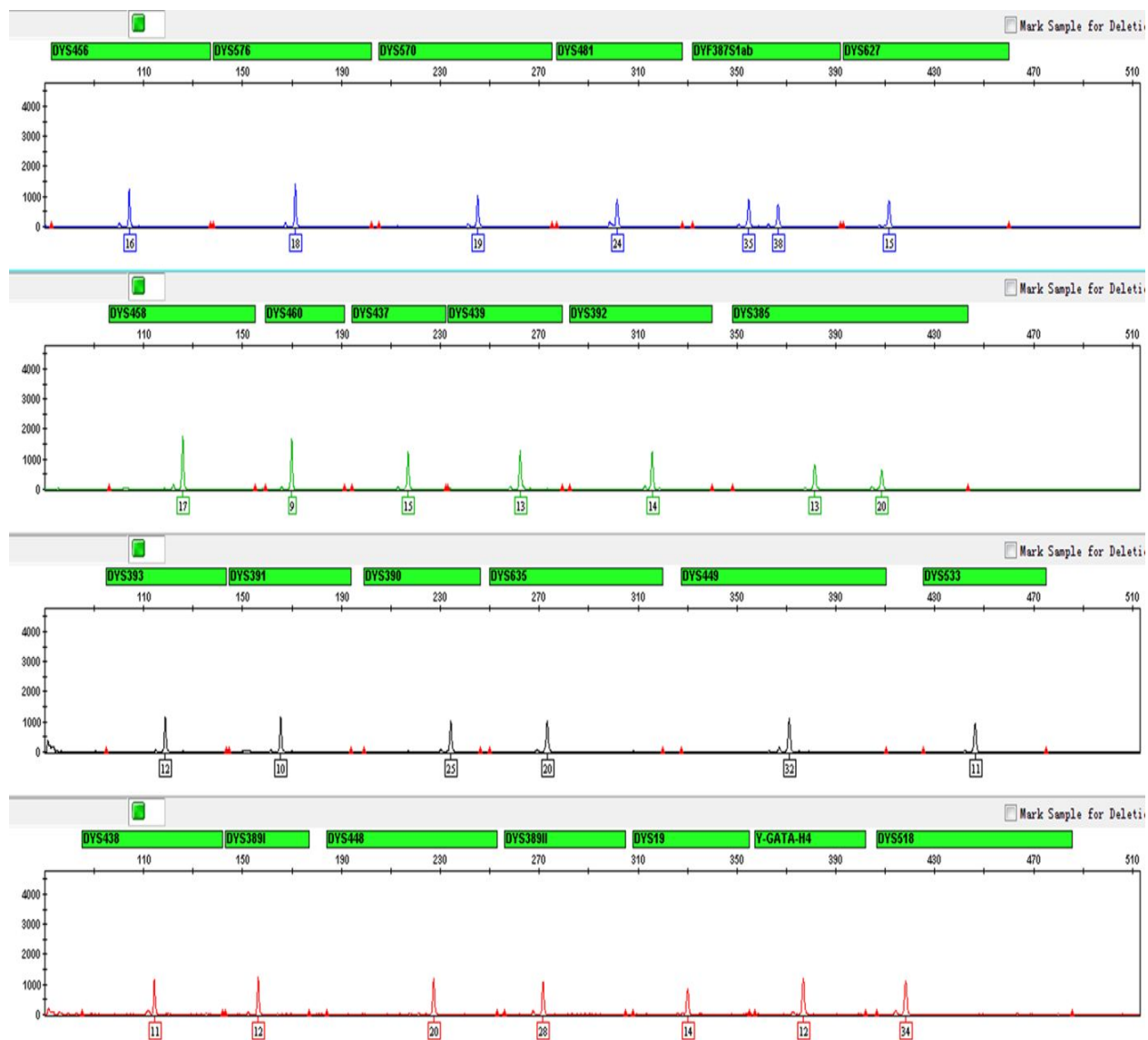




**Figure S5. The size deviation of the extracted DNA samples**

| 25 $\mu$ L system | Control DNA 9948      |                 | Control DNA 007      |                |
|-------------------|-----------------------|-----------------|----------------------|----------------|
| 9948:007          | 9948 alleles detected | % complete 9948 | 007 alleles detected | % complete 007 |
| 19:1              | 27                    | 100%            | 14                   | 51%            |
| 18:2              | 27                    | 100%            | 24                   | 88%            |
| 16:4              | 27                    | 100%            | 27                   | 100%           |
| 14:6              | 27                    | 100%            | 27                   | 100%           |
| 12:8              | 27                    | 100%            | 27                   | 100%           |
| 10:10             | 27                    | 100%            | 27                   | 100%           |
| 8:12              | 27                    | 100%            | 27                   | 100%           |
| 6:14              | 27                    | 100%            | 27                   | 100%           |
| 4:16              | 27                    | 100%            | 27                   | 100%           |
| 2:18              | 21                    | 77%             | 27                   | 100%           |
| 1:19              | 10                    | 37%             | 27                   | 100%           |

**Table S2. Detected alleles of the different proportions from the mixture in the 25 $\mu$ L system**



**Figure S6. STRtyper-27 electropherograms obtained from one degraded DNA bone sample(0.1ng)**