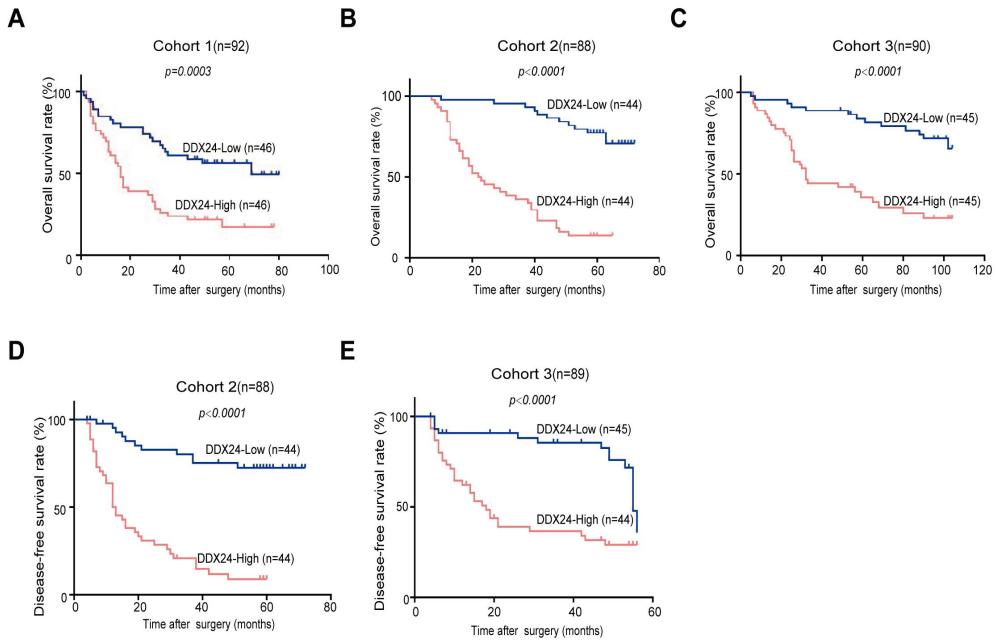


RNA helicase DDX24 stabilizes LAMB1 to promote hepatocellular carcinoma progression

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27 **Supplementary Figures 1.** A-C Kaplan-Meier analysis of overall survival (OS)
28 performed according to the DDX24 protein levels in cohorts 1 (A), 2 (B), and 3 (C),
29 respectively. D-E Kaplan-Meier analysis of disease-free survival (DFS) performed
30 according to the DDX24 protein levels in cohorts 2 and 3, respectively.

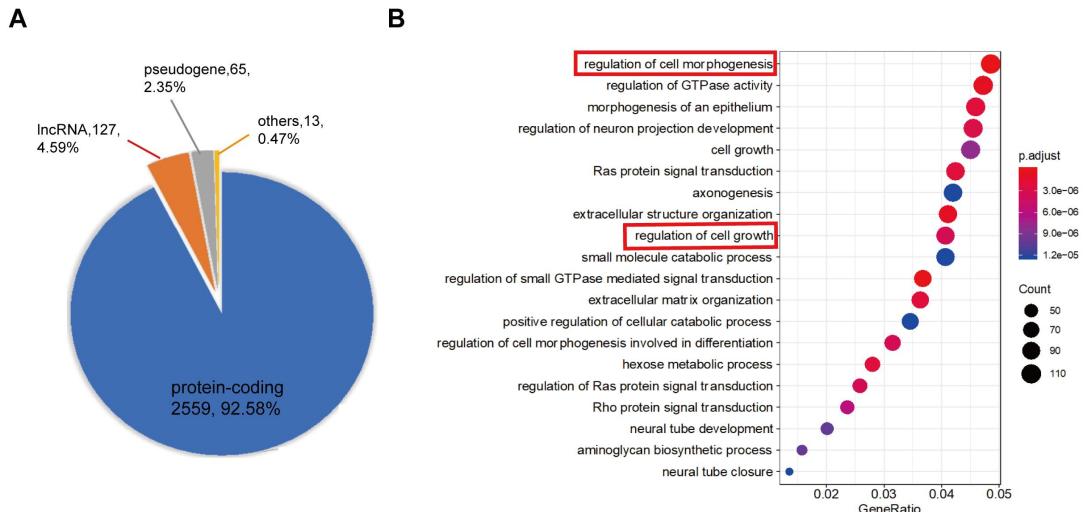
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37 **Supplementary Figure 2. Analysis of RIP-seq using DDX24 antibody.** A Pie chart
 38 displayed the RNA distribution from RIP-seq using DDX24 antibody. B The top 20 of
 39 enriched pathway from RIP-seq.

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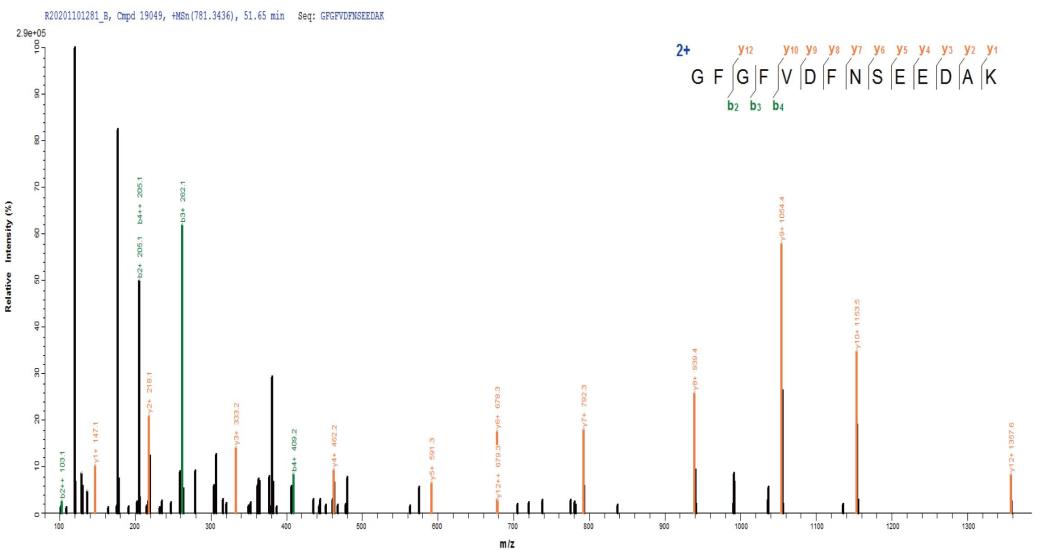
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47 **Supplementary Figure 3. The putative DDX24 targeted protein sequence.**

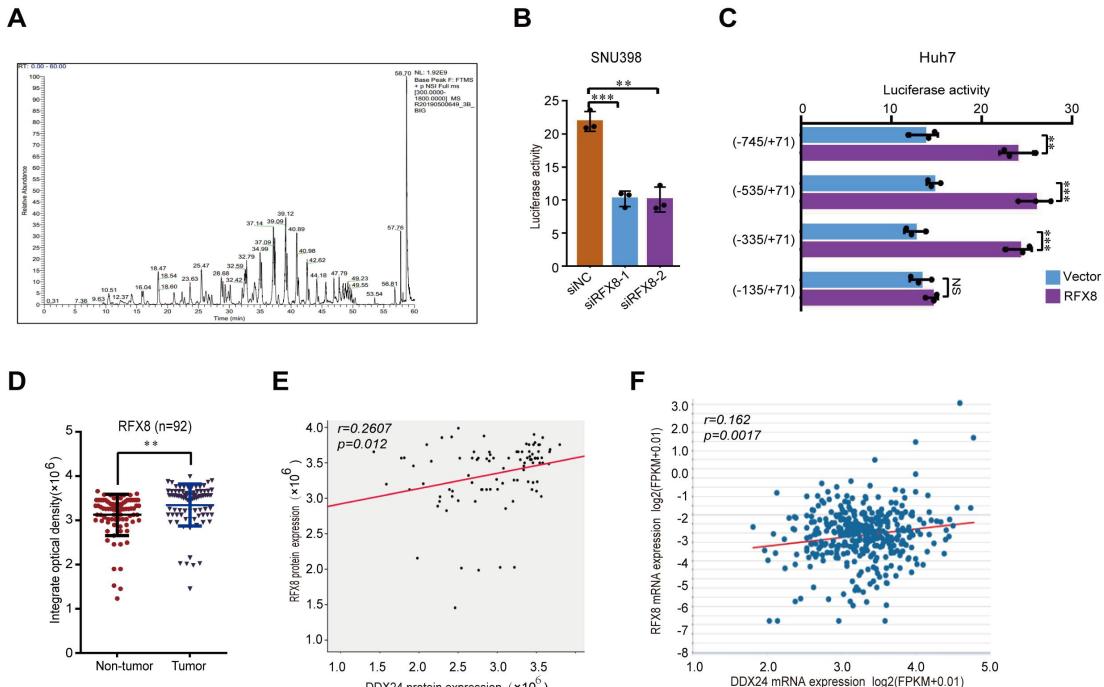
48 MALDI-TOF/TOF mass spectrometry that was used to identify the putative DDX24
49 targeted protein sequence was GFGFVDFNSEEDAK. The assigned b and y ion peaks
50 on the spectrum are marked with their corresponding m/z values.

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56 **Supplementary Figure 4. MALDI-TOF/TOF mass spectrometry identifying the**

57 **putative DDX24 promoter targeted protein sequence.** **A** The peak figure of the

58 putative DDX24 promoter-binding protein from MALDI-TOF/TOF mass

59 spectrometry. **B** Relative DDX24 promoter activity in SNU398 cells with RFX8

60 knockdown was determined by dual-luciferase assay. **C** Dual-luciferase reporter

61 assays to determine RFX8 binding region. **D** Expression analysis of RFX8 from the

62 92 HCC tissues and non-tumor tissues (cohort 1). **E** Correlation between RFX8 and

63 DDX24 expression in 92 HCC tissue samples (cohort 1). **F** Correlation between

64 RFX8 and DDX24 expression in 374 HCC patient tissues from the TCGA database.

65 The results are shown as means \pm SD, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS = not

66 significant.

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Supplementary Table 1. Primer sequences used in the study

| Primer name | Primer sequences |
|---|---|
| Primers for real-time PCR: | |
| DDX24 | Purchased from GeneCopoeia, Hs-QRP-23370 |
| RFX8 | Purchased from GeneCopoeia, Hs-QRP-23954 |
| Flag- sense | 5'- AAGTCCGTTCACTCCTAAAGG -3' |
| Flag- antisense | 5'- TCATCGTCATCCTTAGTCGA -3' |
| LAMB1-sense | 5'- ATT CCTGGAGGAAATCCTTG -3' |
| LAMB1-antisense | 5'- TCATTGCTTAAGCCCCAGTG -3' |
| ITPR1-sense | 5'- ACAGCACCAACAGACGCAGT -3' |
| ITPR1-antisense | 5'- ATTACGGTCCCCAGCAATT -3' |
| CRIM1-sense | 5'- GCAATCCCTTGAGTTCCA -3' |
| CRIM1-antisense | 5'- CTTCAGGACAACGTGGAGAGA -3' |
| FBN1-sense | 5'-GGCTACCTCCAGCACTACCA -3' |
| FBN1-antisense | 5'- ACATGCACTTGTAGCTCCC -3' |
| FBN2-sense | 5'- AATCGGAAGCTTCAAATGCC -3' |
| FBN2-antisense | 5'- CAGGAGAAATCCTGCACTCG -3' |
| TRIO-sense | 5'- CTGGAAGACCGGATTCAAGA -3' |
| TRIO-antisense | 5'- AGCTCCTCCAGGCCACGTC -3' |
| DYNC1H1-sense | 5'- GTATGCAGAGCCGCTCACTG -3' |
| DYNC1H1-antisense | 5'- CACCTAGTCATTCACGGGG-3' |
| NC(SEC62)-sense | 5'- ACCAATATGATGGGTACCCG -3' |
| NC(SEC62)-antisense | 5'- GCCTTGCCCCACTTGAATC -3' |
| Primers for RT-PCR: | |
| Fragment-1 of DDX24 promoter region-sense | 5'- AAGTGAGAGATGTGCGACTC -3' |
| Fragment-1 of DDX24 promoter region-antisense | 5'- ACGTGTGATGAGTGTTCCTTGT -3' |
| Fragment-2 of DDX24 promoter region-sense | 5'- AGGAACACTCATCACACGTC -3' |
| Fragment-2 of DDX24 promoter region-antisense | 5'- GCTCCCGCACAGTAACGTA -3' |
| Fragment-3 of DDX24 promoter region-sense | 5'- AACTTACGTTACTGTGCGGG -3' |
| Fragment-3 of DDX24 promoter region-antisense | 5' - TCCCGCCCCATGGCAAA -3' |
| Fragment-4 of DDX24 promoter region-sense | 5'- GGTAGCTGAGTTGCCATAGG -3' |
| Fragment-4 of DDX24 promoter region-antisense | 5'- CGAGTGAAGAACCTCAGAAC -3' |

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Supplementary Table 2. The characteristics of antibodies

| Protein | Application | Origin | City/State/Country | Dilution |
|----------------|--------------------|--------------------------------------|---------------------------|-----------------------------|
| DDX24 | WB, IF | A300697A, Bethyl Laboratories | Montgomery/TX/USA | 1:1000 & 1:200 |
| DDX24 | IHC, IP | HPA002554, Sigma-Aldrich | St Louis/MO/USA | 1:50 |
| RFX8 | WB, IHC, IF | TA330886, OriGene Technologies | Rockville/MD/USA | 1:1000 & 1:50 & 1:200 |
| LAMB1 | WB, IHC | GTX100787, Genetex | San Antonio/TX/USA | 1:1000 & 1:100 |
| p-SRC | WB | 44-660G, ThermoFisher | Rockford/IL/USA | 1:1000 |
| SRC | WB | #2109S, CST | Danvers/MA/USA | 1:1000 |
| Histone H3 | WB | #4499S, CST | Danvers/MA/USA | 1:1000 |
| β-actin | WB | #4970S, CST | Danvers/MA/USA | 1:1000 |
| GAPDH | WB | #5174S, CST | Danvers/MA/USA | 1:1000 |
| NCL(Nucleolin) | WB | #14574, CST | Danvers/MA/USA | 1:1000 |
| NCL(Nucleolin) | IP | #14574, CST | Danvers/MA/USA | 1:50 |
| FLAG | IP | #14793, CST | Danvers/MA/USA | 1:50 |
| FLAG | WB | #14793, CST | Danvers/MA/USA | 1:1000 |

73 WB, Western blotting; IF, Immunofluorescence staining; IP, immunoprecipitation; IHC,

74 Immunohistochemical (IHC) staining; CST, Signaling Technology

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77 **Supplementary Table 3. Clinical characteristics of 11 HCC patients**

| Case | Gender | Age (years) | T stage | N stage | M stage | AJCC stage |
|------|--------|-------------|---------|---------|---------|------------|
| 1 | Male | 49 | T2 | N0 | M1 | IVB |
| 2 | Male | 68 | T3 | N0 | M0 | IIIA |
| 3 | Male | 65 | T1b | N0 | M0 | IB |
| 4 | Male | 60 | T3 | N0 | M0 | IIIA |
| 5 | Male | 49 | T1a | N0 | M1 | IVB |
| 6 | Female | 43 | T1a | N0 | M0 | IA |
| 7 | Male | 60 | T1b | N0 | M0 | IB |
| 8 | Male | 67 | T1a | N0 | M0 | IA |
| 9 | Male | 77 | T1b | N0 | M0 | IB |
| 10 | Male | 64 | T1b | N0 | M0 | I |
| 11 | Male | 55 | T2 | N0 | M0 | IVB |

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Supplementary Table 4. Clinical characteristics of 270 HCC patients.

| Patient characteristics | Cohort 1 | Cohort 2 | Cohort 3 |
|---|-----------|----------|-----------|
| | Tissues | Tissues | Tissues |
| No. of patients | 92 | 88 | 90 |
| Gender (male/female) | 83/9 | 72/16 | 80/10 |
| Age, years (mean, range) | 53, 25-73 | 53,27-84 | 52, 31-78 |
| Edmondson Grade (I+II/III+IV) | 60/32 | 58/32 | 44/46 |
| AJCC Grade (1+2/3+4) | 51/41 | 88/0 | 88/2 |
| Liver Cirrhosis (present/absent) | 36/56 | 76/12 | 81/9 |
| Tumor Size, cm ($\leq 3 / > 3$) | 22/70 | 25/63 | 36/54 |
| T stage (T1+T2/T3+T4) | 51/41 | 88/0 | 87/3 |
| N stage (N1+N2/N3+N4) | 91/1 | - | - |
| M stage (M0/M1) | 90/2 | - | - |
| Portal Vein Tumor Thrombus (present/absent) | 84/8 | - | - |
| ALT, U/L ($\leq 45 / > 45$) | - | 48/40 | 59/31 |
| HBcAb (positive/negative) | - | 77/11 | 81/9 |
| AFP ug/L ($\leq 25 / > 25$) | - | 27/61 | 38/52 |
| HBsAg (positive/negative) | - | 75/13 | 71/19 |
| HCV (present/absent) | - | 1/87 | 2/88 |
| TBIL, umol/L ($\leq 20 / > 20$) | - | 74/14 | 75/15 |
| Cirrhosis Nodules ($\leq 3 / > 3$) | - | 49/39 | 56/34 |
| Tumor Capsule (present/absent) | - | 45/43 | 42/48 |
| Tumor Recurrence (present/absent) | - | 52/36 | 49/41 |
| Tumor Number ($\leq 1 / > 1$) | - | 73/15 | 79/11 |

102 **Supplementary Table 5. Correlation between DDX24 expression and HCC**

103 **clinicopathologic features in 270 patients.**

| | DDX24 expression levels | | <i>P</i> |
|-----------------|-------------------------|----------------|---------------------|
| | high expression | low expression | |
| Gender | | | |
| Male | 123 | 112 | 0.0463 ^a |
| Female | 12 | 23 | |
| Age (years) | | | |
| ≤55 | 86 | 76 | 0.2141 |
| > 55 | 49 | 59 | |
| Edmondson Grade | | | |
| I+II | 77 | 85 | 0.3203 |
| III+IV | 58 | 50 | |
| AJCC Grade | | | |
| 1+2 | 108 | 119 | 0.0673 |
| 3+4 | 27 | 16 | |
| Tumor Size(cm) | | | |
| ≤3 | 33 | 50 | 0.0249 ^a |
| > 3 | 102 | 85 | |
| T | | | |
| T1/T2 | 107 | 119 | 0.048 ^a |
| T3/T4 | 28 | 16 | |

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105 ^a*p* values less than .05 were considered statistically significant.
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123 **Supplementary Table 6. Correlation between DDX24 expression and HCC**124 **clinicopathologic features in 92 patients: Cohort 1.**

| | DDX24 expression levels | | <i>p</i> |
|----------------------------|-------------------------|----------------|---------------------|
| | high expression | low expression | |
| Liver Cirrhosis | | | 0.3928 |
| with | 16 | 20 | |
| without | 30 | 26 | |
| Portal Vein Tumor Thrombus | | | 0.0264 ^a |
| No | 39 | 45 | |
| Yes | 7 | 1 | |

125 ^a*p* values less than .05 were considered statistically significant.

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Supplementary Table 7. Correlation between DDX24 expression and HCC

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clinicopathologic features in 88 patients: Cohort2.

| | DDX24 expression levels | | <i>p</i> |
|-------------------|-------------------------|----------------|----------------------|
| | high expression | low expression | |
| Liver Cirrhosis | | | 0.2140 |
| with | 40 | 36 | |
| without | 4 | 8 | |
| ALT | | | 0.3918 |
| ≤45 | 26 | 22 | |
| > 45 | 18 | 22 | |
| HBcAb | | | 0.3336 |
| positive | 40 | 37 | |
| negative | 4 | 7 | |
| AFP | | | 0.8172 |
| ≤25 | 13 | 14 | |
| > 25 | 31 | 30 | |
| HBsAg | | | 0.3674 |
| positive | 36 | 39 | |
| negative | 8 | 5 | |
| HCV | | | 0.3145 |
| with | 1 | 0 | |
| without | 43 | 44 | |
| TBIL | | | 0.0803 |
| ≤20 | 40 | 34 | |
| > 20 | 4 | 10 | |
| Cirrhosis Nodules | | | 0.8301 |
| ≤3 | 25 | 24 | |
| > 3 | 19 | 20 | |
| Tumor Capsule | | | 0.1355 |
| with | 26 | 19 | |
| without | 18 | 25 | |
| Tumor Recurrence | | | <0.0001 ^a |
| with | 35 | 17 | |
| without | 9 | 27 | |
| Tumor Number | | | 0.1564 |
| ≤1 | 39 | 34 | |
| > 1 | 5 | 10 | |

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^a*p* values less than .05 were considered statistically significant.

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157 **Supplementary Table 8. Correlation between DDX24 expression and HCC**158 **clinicopathologic features in 90 patients: Cohort 3.**

| | DDX24 expression levels | | <i>P</i> |
|-------------------|-------------------------|----------------|----------------------|
| | high expression | low expression | |
| Liver Cirrhosis | | | |
| with | 39 | 42 | 0.2918 |
| without | 6 | 3 | |
| ALT | | | |
| ≤45 | 31 | 28 | 0.5057 |
| > 45 | 14 | 17 | |
| HBcAb | | | |
| positive | 42 | 39 | 0.2918 |
| negative | 3 | 6 | |
| AFP | | | |
| ≤25 | 21 | 17 | 0.3933 |
| > 25 | 24 | 28 | |
| HBsAg | | | |
| positive | 38 | 33 | 0.1965 |
| negative | 7 | 12 | |
| HCV | | | |
| with | 1 | 1 | >0.9999 |
| without | 44 | 44 | |
| TBIL | | | |
| ≤20 | 38 | 37 | 0.7773 |
| > 20 | 7 | 8 | |
| Cirrhosis Nodules | | | |
| ≤3 | 23 | 33 | 0.0297 ^a |
| > 3 | 22 | 12 | |
| Tumor Capsule | | | |
| with | 18 | 24 | 0.2049 |
| without | 27 | 21 | |
| Tumor Recurrence | | | |
| with | 34 | 15 | <0.0001 ^a |
| without | 11 | 30 | |
| Tumor Number | | | |
| ≤1 | 38 | 41 | 0.3343 |
| > 1 | 7 | 4 | |

159 ^ap values less than .05 were considered statistically significant.

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162 **Supplementary Table 9. The half-time of LAMB1 mRNA in each group**

| HCC cell lines | Half-time (hours) | p value |
|-----------------|-------------------|----------|
| Hep3B NC | > 10 | |
| Hep3B shDDX24-1 | 2.613±0.1291 | < 0.0001 |
| Hep3B shDDX24-2 | 3.948±0.3587 | < 0.0001 |

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168 **Supplementary Table 10. The five-year survival rate and average survival time**169 **(months).**

| Protein expression level | Five-year survival rate (Ave, OS by months) |
|--------------------------|---|
| DDX24 low | 56.52% (60.64) |
| DDX24 high | 19.57% (32.64) |
| LAMB1 low | 67.39% (47.85) |
| LAMB1 high | 6.52% (18.65) |
| DDX24 low, LAMB1 low | 76.67% (52.63) |
| DDX24 high, LAMB1 high | 3.33% (16.77) |

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175 **Supplementary Table 11. The b and y values of MALDI-TOF/TOF mass
176 spectrometry**

| Ion Mass | D | I | L | R | N | V | R |
|----------|----------|----------|----------|----------|----------|----------|----------|
| b+ | 116.0342 | 229.1183 | 342.2023 | 498.3034 | 612.3464 | 711.4148 | |
| b++ | 58.207 | 115.0628 | 171.6048 | 249.6554 | 306.6768 | 356.2110 | |
| y++ | | 770.4995 | 657.4155 | 544.3314 | 388.2303 | 274.1874 | 175.1190 |
| y++ | | 385.7534 | 329.2114 | 272.6693 | 194.6188 | 137.5973 | 88.0631 |

177 **Short Tandem Repeat (STR) Profile Report in Hepatoma Cell Lines**

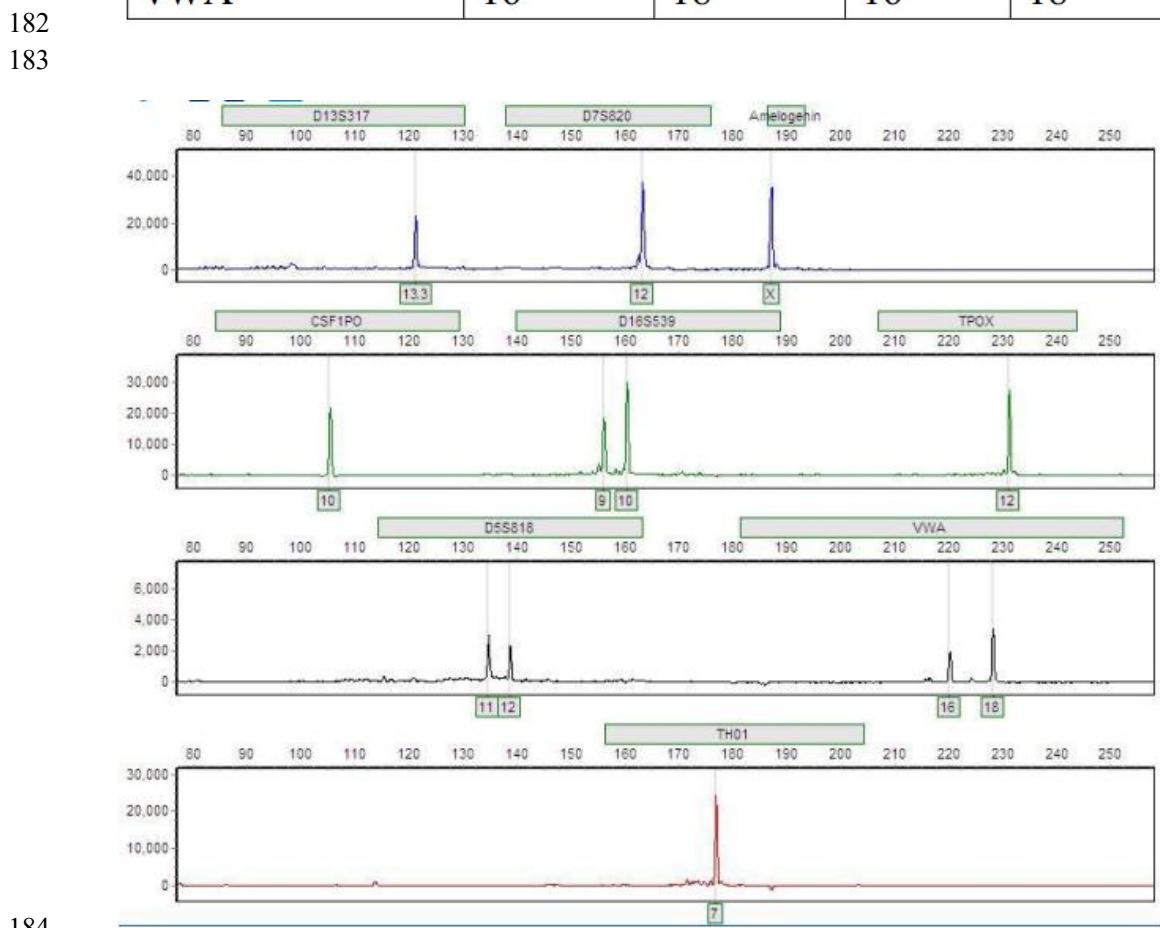
178 STR report for L02: Percent match between the sample and the database profile:

179 100%.

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181 STR Profile:

| Genetic Site | Customer sample | | ExPASy | |
|--------------|-----------------|------|--------|------|
| | L-02 | L-02 | X | X |
| Amelogenin | X | X | X | X |
| CSF1PO | 10 | 10 | 10 | 10 |
| D13S317 | 13.3 | 13.3 | 13.3 | 13.3 |
| D16S539 | 9 | 10 | 9 | 10 |
| D5S818 | 11 | 12 | 11 | 12 |
| D7S820 | 12 | 12 | 12 | 12 |
| TH01 | 7 | 7 | 7 | 7 |
| TPOX | 12 | 12 | 12 | 12 |
| vWA | 16 | 18 | 16 | 18 |



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185 STR report for SNU449: Percent match between the sample and the database

186 profile: 94.4%.

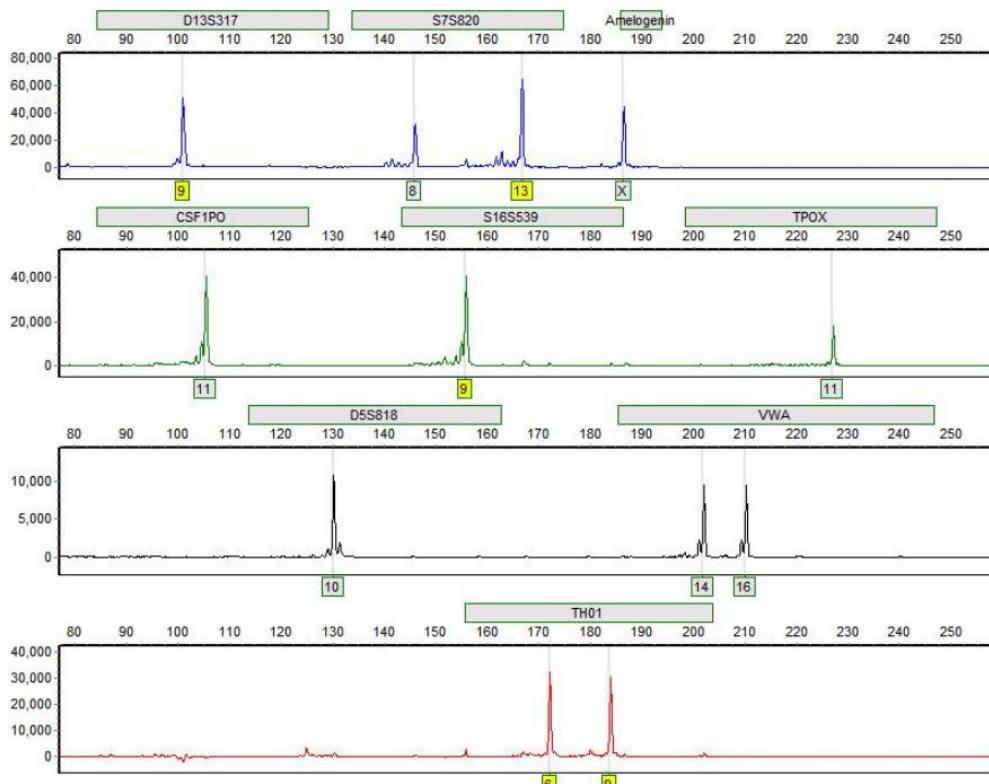
187

188 STR Profile:

| Genetic Site | Customer sample | | ATCC | |
|--------------|-----------------|----|---------|----|
| | SNU-449 | | SNU-449 | |
| Amelogenin | X | X | X | Y |
| CSF1PO | 11 | 11 | 11 | 11 |
| D13S317 | 9 | 9 | 9 | 9 |
| D16S539 | 9 | 9 | 9 | 9 |
| D5S818 | 10 | 10 | 10 | 10 |
| D7S820 | 8 | 13 | 8 | 13 |
| TH01 | 6 | 9 | 6 | 9 |
| TPOX | 11 | 11 | 11 | 11 |
| vWA | 14 | 16 | 14 | 16 |

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193 STR report for Huh7: Percent match between the sample and the database

194 profile: 94.4%.

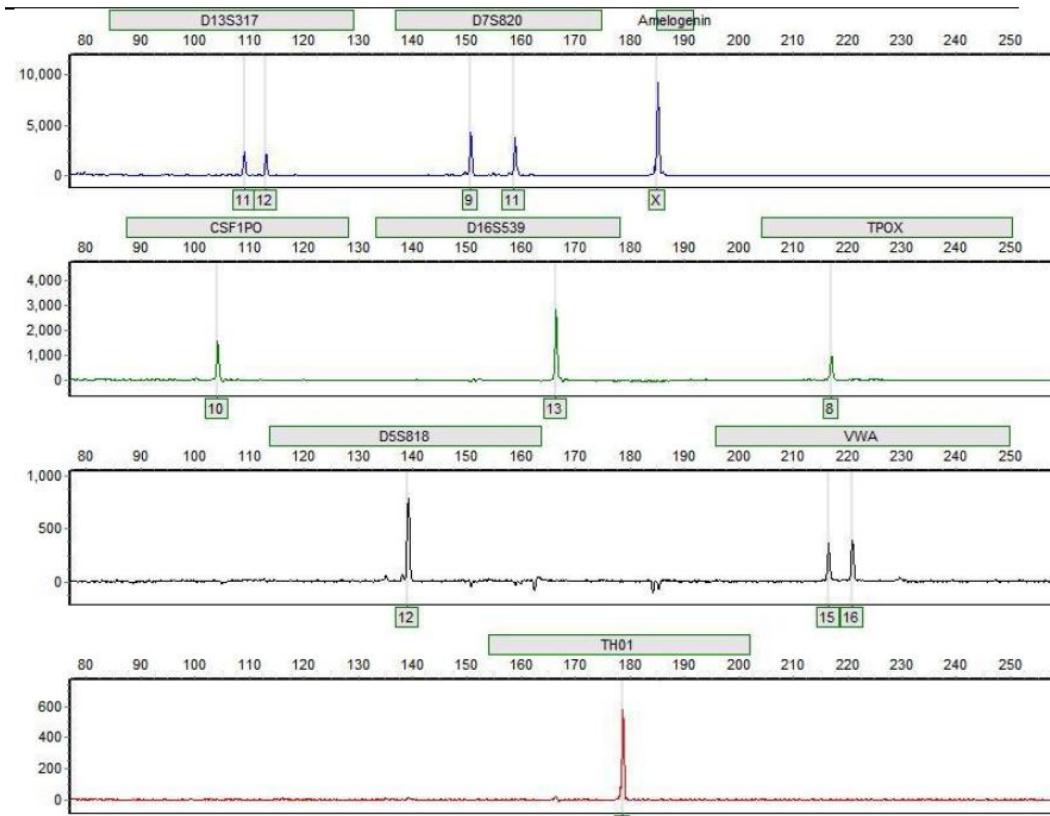
195

196 STR Profile:

| Genetic Site | Customer sample | | JCRB | |
|--------------|-----------------|----|-------|----|
| | HUH-7 | | HUH-7 | |
| Amelogenin | X | X | X | X |
| CSF1PO | 11 | 11 | 11 | 11 |
| D13S317 | 10 | 11 | 10 | 11 |
| D16S539 | 10 | 10 | 10 | 10 |
| D5S818 | 11 | 12 | 12 | 12 |
| D7S820 | 11 | 11 | 11 | 11 |
| TH01 | 7 | 7 | 7 | 7 |
| TPOX | 8 | 11 | 8 | 11 |
| vWA | 16 | 18 | 16 | 18 |

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201 STR report for PLC/PRF/5: Percent match between the sample and the database

202 profile: 100%.

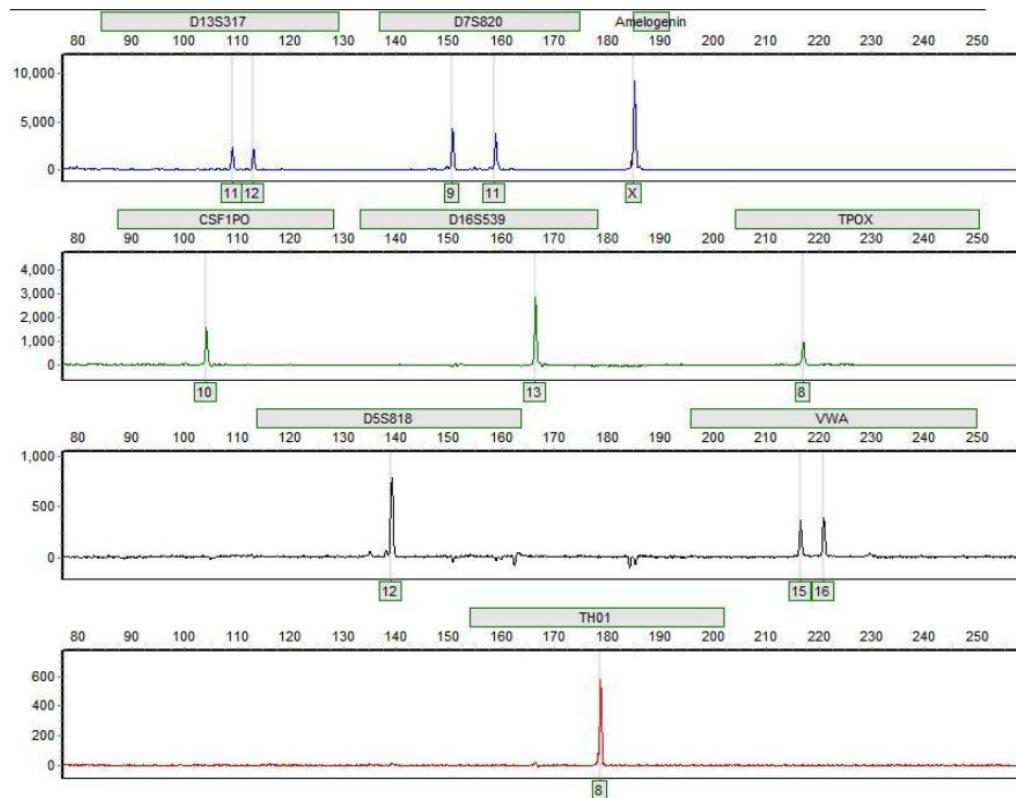
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204 STR Profile:

| Genetic Site | Customer sample | | ATCC | |
|--------------|-----------------|----|-----------|----|
| | PLC/PRF/5 | | PLC/PRF/5 | |
| Amelogenin | X | X | X | X |
| CSF1PO | 10 | 10 | 10 | 10 |
| D13S317 | 11 | 12 | 11 | 12 |
| D16S539 | 13 | 13 | 13 | 13 |
| D5S818 | 12 | 12 | 12 | 12 |
| D7S820 | 9 | 11 | 9 | 11 |
| TH01 | 8 | 8 | 8 | 8 |
| TPOX | 8 | 8 | 8 | 8 |
| vWA | 15 | 16 | 15 | 16 |

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211 STR report for SNU398: Percent match between the sample and the database

212 profile: 100%.

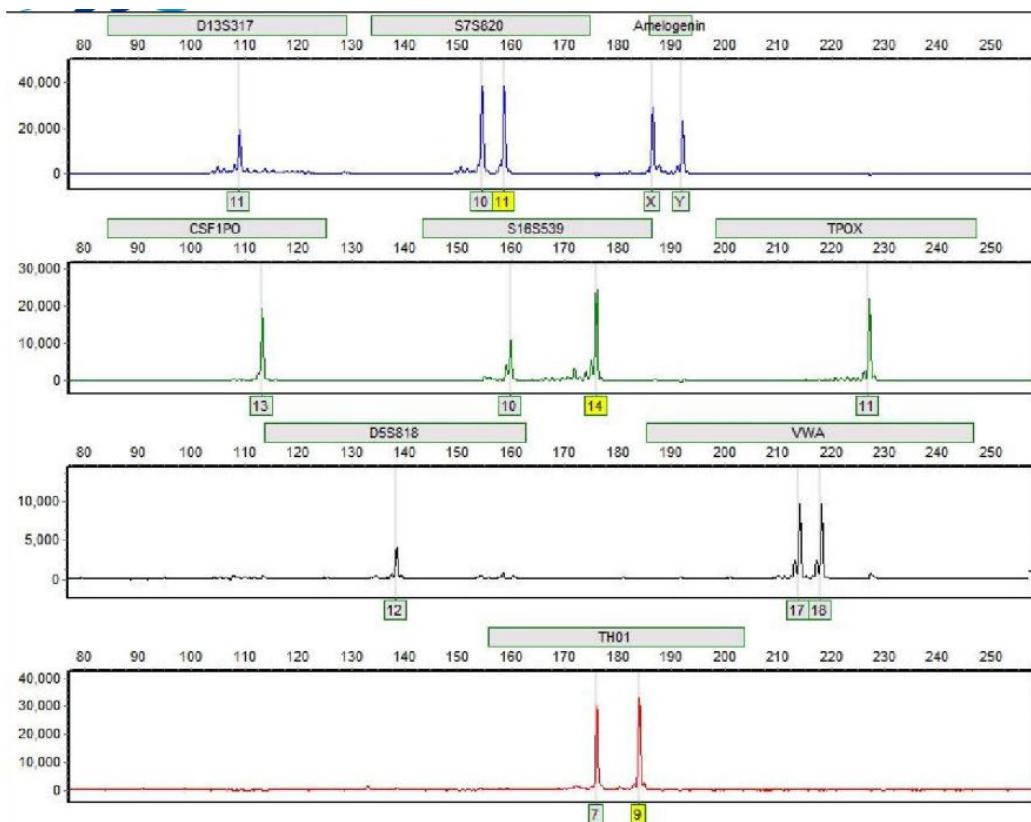
213

214 STR Profile:

| Genetic Site | Customer sample | | ATCC | |
|--------------|-----------------|----|---------|----|
| | SNU-398 | | SNU-398 | |
| Amelogenin | X | Y | X | Y |
| CSF1PO | 13 | 13 | 13 | 13 |
| D13S317 | 11 | 11 | 11 | 11 |
| D16S539 | 10 | 14 | 10 | 14 |
| D5S818 | 12 | 12 | 12 | 12 |
| D7S820 | 10 | 11 | 10 | 11 |
| TH01 | 7 | 9 | 7 | 9 |
| TPOX | 11 | 11 | 11 | 11 |
| vWA | 17 | 18 | 17 | 18 |

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STR report for Hep3B: Percent match between the sample and the database

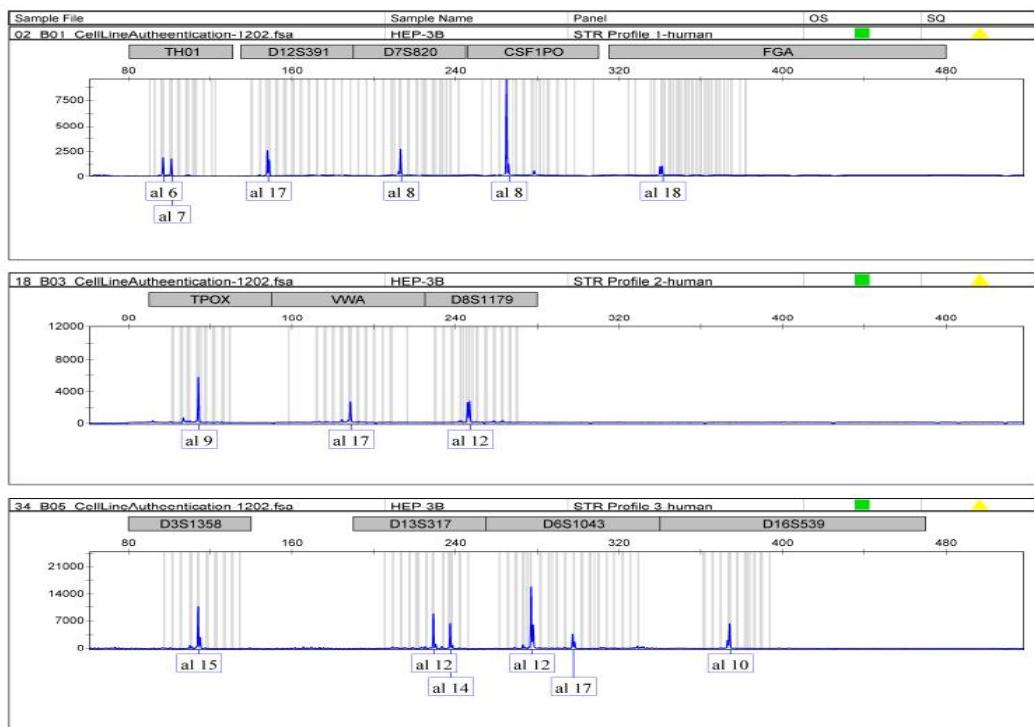
222 profile: 98%.

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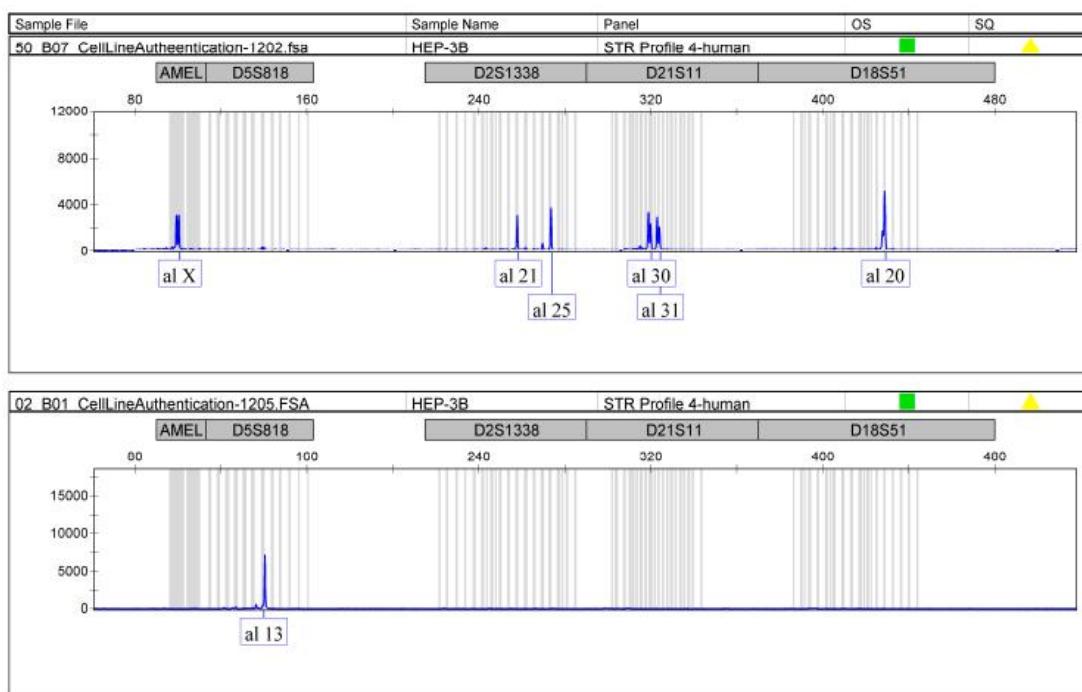
STR Profile:

| Marker | Hep3B | | | | ATCC | | |
|---------|---------|---------|---------|---------|---------|---------|---------|
| | Allele1 | Allele2 | Allele3 | Allele4 | Allele1 | Allele2 | Allele3 |
| D5S818 | 13 | 13 | | | 13 | 13 | |
| D13S317 | 12 | 14 | | | 12 | 14 | |
| D7S820 | 8 | 8 | | | 8 | 10 | |
| D16S539 | 10 | 10 | | | 10 | 10 | |
| VWA | 17 | 17 | | | 17 | 17 | |
| TH01 | 6 | 7 | | | 6 | 7 | |
| AMEL | X | X | | | X | X | |
| TPOX | 9 | 9 | | | 9 | 9 | |
| CSF1PO | 8 | 8 | | | 8 | 8 | |
| D12S391 | 17 | 17 | | | | | |
| FGA | 18 | 18 | | | | | |
| D2S1338 | 21 | 25 | | | | | |
| D21S11 | 30 | 31 | | | | | |
| D18S51 | 20 | 20 | | | | | |
| D8S1179 | 12 | 12 | | | | | |
| D3S1358 | 15 | 15 | | | | | |
| D6S1043 | 12 | 17 | | | | | |
| PENTAE | 5 | 16 | | | | | |
| D19S433 | 12.2 | 14 | | | | | |
| PENTAD | 12 | 14 | | | | | |

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