|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Supplementary table 1. The target value range of each mutant of the quality control** | | | | | |
| Well number | **Amino acid changes** | **Base mutation** | **Positive quality control** | **Negative quality control** | **blank quality control** |
| A | p.G12S | c.34G>A | Ct>33, Tm79.5±2℃ | Ct>35 | Negative |
| B | p.G12R | c.34G>C | Ct>33, Tm80.5±2℃ | Ct>37 | Negative |
| C | p.G12C | c.34G>T | Ct>33, Tm80.0±2℃ | Ct>37 | Negative |
| D | p.G12D | c.35G>A | Ct>33, Tm79.5±2℃ | Ct>35 | Negative |
| E | p.G12A | c.35G>C | Ct>33, Tm80.5±2℃ | Ct>37 | Negative |
| F | p.G12V | c.35G>T | Ct>33, Tm80.0±2℃ | Ct>37 | Negative |
| G | p.G13D | c.38G>A | Ct>33, Tm82.0±2℃ | Ct>35 | Negative |
| H | wild type | -- | Ct 24±2, Tm87.5±2℃ | Ct21±2 | Negative |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Supplementary table 2. The judging rule of the KRAS mutant gene** | | | | | |
| **Well number** | **Amino acid changes** | **Base mutation** | Negative \* | Positive \* | |
| **Ct value** | **Tm value** |
|
| **A** | p.G12S | c.34G>A | Ct>35 or Δ[Mut Ct-Wt Ct]>10 | Ct≤35,and Δ[Mut Ct-Wt Ct]≤10 | Tm79.5±2℃ |
| **B** | p.G12R | c.34G>C | Ct>37 | Ct≤37, | Tm80.5±2℃ |
| **C** | p.G12C | c.34G>T | Ct>37 | Ct≤37 | Tm80.0±2℃ |
| **D** | p.G12D | c.35G>A | Ct>35 or Δ[Mut Ct-Wt Ct] >10 | Ct≤35,and Δ[Mut Ct-Wt Ct]≤10 | Tm79.5±2℃ |
| **E** | p.G12A | c.35G>C | Ct>37 | Ct≤37 | Tm80.5±2℃ |
| **F** | p.G12V | c.35G>T | Ct>37 | Ct≤37 | Tm80.0±2℃ |
| **G** | p.G13D | c.38G>A | Ct>35 or Δ[Mut Ct-Wt Ct] >10 | Ct≤35,and Δ[Mut Ct-Wt Ct]≤10 | Tm82.0±2℃ |
| **H** | wild type | -- | Ct>37 | Ct≤37 | Tm78.5±2℃ |
| 1. Mut Ct, Mutant Ct: refers to the detection result of the mutation reaction well; Wt Ct, wild-type Ct: refers to the detection result of the wild-type reaction well. | | | | | |
| 2. The role of the wild-type detection well: to detect ctDNA in quality control products and samples for monitoring the quality of the samples to be tested. | | | | | |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Supplementary table 3. Detection of the mutKRAS ctDNA in all enrolled patients.** | | | | | | | | |
| **Patients code** | **Gender** | **Age** | **Clinical stage** | **Pathological stage** | **Location** | **CA199(U/mL)** | **mutKRAS ctDNA** | **Outcome(follow up for 2 years)** |
|
|
| PC-1 | Male | 59 | ⅡA | ⅡA | Body and tail | 32.7 | G12R | Died |
| PC-2 | Male | 64 | ⅡB | ⅡB | Body and tail | 1482 | WT | Died |
| PC-3 | Female | 73 | III | ⅡB | Body and tail | 6763 | WT | Died |
| PC-4 | Male | 59 | IB | IB | Head | 144.9 | WT | Survive |
| PC-5 | Male | 64 | ⅡB | III | Head | 614.2 | WT | Died |
| PC-6 | Male | 68 | IB | IB | Body and tail | 178.1 | WT | Survive |
| PC-7 | Female | 47 | ⅡA | IB | Body and tail | 6402 | WT | Died |
| PC-8 | Male | 62 | IB | IB | Head | 276.1 | WT | Survive |
| PC-9 | Male | 65 | ⅡB | III | Head | ＞1000 | G12V | Died |
| PC-10 | Male | 39 | IB | IB | Head | 5.71 | WT | Survive |
| PC-11 | Male | 47 | ⅡB | ⅡB | Head | 3334 | WT | Died |
| PC-12 | Male | 55 | III | III | Head | 14.09 | G12R | Died |
| PC-13 | Female | 79 | III | III | Body and tail | 17.23 | G12V | Died |
| PC-14 | Male | 65 | ⅡB | ⅡB | Head | 63.66 | WT | Died |
| PC-15 | Male | 45 | III | ⅡB | Body and tail | 149.3 | WT | Died |
| PC-16 | Male | 62 | III | ⅡB | Body and tail | 256 | WT | Died |
| PC-17 | Female | 58 | IB | ⅡB | Head | 1508 | WT | Died |
| PC-18 | Female | 58 | ⅡB | IA | Body and tail | 189.2 | G12R | Survive |
| PC-19 | Male | 69 | III | ⅡA | Body and tail | 35.57 | WT | Survive |
| PC-20 | Female | 52 | ⅡB | ⅡB | Head | 495.7 | G12V | Survive |
| PC-21 | Female | 53 | IB | IB | Head | 15.83 | WT | Survive |
| PC-22 | Female | 64 | III | ⅡB | Head | 109.7 | G12R | Died |
| PC-23 | Female | 58 | IB | IB | Head | 17.57 | WT | Survive |
| PC-24 | Male | 61 | IB | IB | Head | 10.35 | WT | Survive |
| PC-25 | Male | 68 | IA | IB | Head | 143.9 | WT | Died |
| PC-26 | Female | 73 | IB | ⅡB | Body and tail | 1346 | WT | Died |
| PC-27 | Female | 64 | IB | ⅡB | Head | 302.2 | WT | Survive |
| PC-28 | Female | 59 | IA | ⅡB | Body and tail | 83.86 | WT | Survive |
| PC-29 | Male | 61 | ⅡB | IA | Head | 29.8 | WT | Died |
| PC-30 | Female | 54 | IB | ⅡB | Head | 79.01 | WT | Survive |
| PC-31 | Male | 45 | ⅡB | III | Head | 210 | WT | Died |
| PC-32 | Male | 52 | ⅡB | ⅡB | Body and tail | 3018 | WT | Died |
| PC-33 | Male | 59 | IB | ⅡB | Head | 83.18 | WT | Survive |
| PC-34 | Male | 57 | IB | IB | Body and tail | 544.1 | WT | Survive |
| PC-35 | Male | 49 | IB | IB | Body and tail | 470.6 | WT | Survive |
| PC-36 | Male | 65 | III | ⅡA | Head | 9.49 | WT | Died |
| PC-37 | Male | 66 | ⅡA | ⅡA | Head | 9.07 | G12D | Survive |
| PC-38 | Male | 54 | ⅡB | IB | Head | 74.21 | WT | Survive |
| PC-39 | Male | 68 | ⅡB | IB | Head | 29.56 | WT | Died |
| PC-40 | Female | 60 | IB | IB | Body and tail | 894.1 | G12D | Survive |
| PC-41 | Male | 52 | IB | ⅡB | Head | 342.3 | G12V | Died |
| PC-42 | Female | 65 | ⅡB | IB | Body and tail | ＞1000 | G12D | Died |
| PC-43 | Female | 55 | ⅡB | IB | Head | 633.5 | G12D | Died |
| PC-44 | Female | 61 | IB | IB | Head | 59.7 | G12C | Died |
| PC-45 | Female | 62 | ⅡB | ⅡA | Body and tail | 49.16 | G12D | Died |
| PC-46 | Male | 69 | IB | III | Head | 63.74 | WT | Died |
| PC-47 | Female | 45 | IB | IA | Head | 24.34 | G12V | Survive |
| PC-48 | Male | 46 | IA | IB | Head | 37.75 | G12D | Died |
| PC-49 | Female | 63 | IB | IB | Head | 155.9 | G12V | Survive |
| PC-50 | Female | 62 | IB | ⅡB | Head | 285.2 | WT | Died |
| PC-51 | Male | 64 | ⅡA | ⅡA | Body and tail | 27.18 | G12R | Died |
| PC-52 | Male | 61 | IB | IB | Body and tail | 67.46 | WT | Died |
| PC-53 | Male | 59 | IB | III | Head | 1195 | WT | Survive |
| PC-54 | Female | 63 | IA | IB | Head | 129.6 | WT | Survive |
| PC-55 | Male | 63 | IA | IB | Head | 189.6 | G12R | Died |
| PC-56 | Female | 53 | IB | IB | Head | 160 | WT | Survive |
| PC-57 | Male | 53 | IA | IB | Body and tail | 139.1 | WT | Survive |
| PC-58 | Female | 65 | IB | IB | Head | 220.8 | WT | Died |
| PC-59 | Female | 68 | IB | IB | Body and tail | 444.7 | WT | Survive |
| PC-60 | Male | 61 | IB | ⅡB | Body and tail | 25.05 | G12C | Died |
| PC-61 | Female | 64 | IB | IB | Head | 152 | WT | Died |
| PC-62 | Male | 63 | IB | ⅡB | Head | 64.8 | G12D | Died |
| PC-63 | Male | 66 | ⅡB | III | Head | 299.2 | G12V | Died |
| PC-64 | Male | 67 | ⅡB | ⅡB | Head | 833.6 | WT | Survive |
| PC-65 | Female | 46 | ⅡB | IB | Head | 338.6 | WT | Survive |
| PC-66 | Male | 61 | IA | IB | Head | 225 | G12R | Died |
| PC-67 | Male | 66 | IB | IB | Head | 1837 | WT | Died |
| PC-68 | Male | 58 | IB | ⅡB | Head | 401.6 | WT | Died |
| PC-69 | Male | 65 | ⅡB | III | Body and tail | 93.77 | G12A | Died |
| PC-70 | Male | 58 | IB | ⅡB | Head | 164.1 | WT | Died |
| PC-71 | Female | 62 | ⅡB | IB | Head | 0.6 | G12R | Died |
| PC-72 | Male | 39 | IB | ⅡB | Head | 568.9 | G12D | Died |
| PC-73 | Male | 64 | IB | IB | Head | 216.6 | WT | Survive |
| PC-74 | Male | 67 | IB | III | Head | 262.7 | G12R | Survive |
| PC-75 | Male | 60 | IA | IB | Head | 22.63 | WT | Survive |
| PC-76 | Male | 68 | IB | ⅡB | Head | 368.4 | WT | Survive |
| PC-77 | Male | 76 | ⅡB | ⅡB | Head | 1414 | WT | Died |
| PC-78 | Female | 56 | ⅡA | ⅡB | Head | 54.93 | G12A | Survive |
| PC-79 | Female | 60 | IB | IB | Body and tail | 134 | WT | Survive |
| PC-80 | Male | 75 | ⅡB | ⅡB | Body and tail | 427.7 | WT | Survive |
| PC-81 | Male | 72 | IB | ⅡB | Head | 268.6 | WT | Survive |
| PC-82 | Male | 39 | IB | IB | Head | 45.14 | WT | Survive |
| PC-83 | Female | 53 | IA | IB | Head | 39.16 | WT | Survive |
| PC-84 | Female | 64 | IB | III | Head | 1263 | WT | Died |
| PC-85 | Male | 59 | IB | ⅡB | Head | 109.7 | WT | Survive |
| PC-86 | Male | 57 | IB | ⅡB | Head | 235.4 | WT | Survive |
| PC-87 | Male | 55 | IB | IB | Head | 87.91 | G12D | Died |
| PC-88 | Female | 71 | IB | IB | Head | 44.53 | WT | Survive |
| PC-89 | Male | 69 | ⅡA | ⅡB | Head | 3847 | G12D | Survive |
| PC-90 | Female | 62 | IB | ⅡB | Head | 200.5 | WT | Survive |
| PC-91 | Male | 68 | IB | ⅡB | Head | 368.4 | WT | Survive |
| PC-92 | Male | 65 | III | IB | Body and tail | 111.9 | WT | Survive |
| PC-93 | Female | 53 | IB | IB | Body and tail | 30.7 | WT | Survive |
| PC-94 | Male | 72 | IB | IB | Head | 25.55 | WT | Died |
| PC-95 | Female | 62 | IB | IB | Body and tail | 2642 | WT | Survive |
| PC-96 | Male | 42 | IB | IB | Head | 436 | G12V | Died |
| PC-97 | Female | 54 | IB | IB | Head | 92.49 | WT | Survive |
| PC-98 | Male | 51 | IB | IB | Head | 174.1 | WT | Survive |
| PC-99 | Female | 60 | IA | IB | Head | 231.3 | WT | Survive |
| PC-100 | Female | 64 | IA | IA | Body and tail | 108.9 | WT | Survive |
| PC-101 | Female | 64 | IB | IB | Head | 431.8 | WT | Survive |
| PC-102 | Female | 52 | IB | IB | Head | 625.5 | WT | Survive |
| PC-103 | Male | 65 | IB | IB | Head | 612.4 | G12A | Died |
| PC-104 | Male | 55 | ⅡB | IB | Head | 9.35 | WT | Survive |
| PC-105 | Female | 64 | IB | IB | Head | 158.6 | WT | Survive |