**Ultra-fast mode exploration report**

1. **Objective:** Instrument ultra-fast mode exploration and shorten amplification time.
2. **Methods:** Using standard reagents, different amplification procedures are set up to detect repeatability, sensitivity, and linearity.

扩增程序1：37℃ 60s；93℃ 60s；93℃1s，64℃1s（45cycles）；

扩增程序2：37℃ 60s；95℃ 60s；95℃1s，56℃1s（45cycles）；

扩增程序3：37℃ 60s；93℃ 60s；93℃1s，56℃1s（45cycles）；

1. Experimental materials
   1. Reagent: Self-formulated

The fluorescence quantitative PCR amplification kit was purchased from Tiansi (Shanghai) Technology Co., Ltd. (TOTOIVD 5G**®** qPCR Premix, Cat No:QPT-200U); primer probe purchased from homegrown bioengineering (Shanghai) Co., Ltd. (forward primer sequence: AGATTTGGACCTGCGCG; reverse primer sequence: GAGCGGCTGTCTCCACAAGT; probe sequence: TTCTGACCTGAGGCTCTGCGCG; amplified fragment length 70bp).

* 1. Instruments: 1834 1835
  2. Software version: Upper PC: 1.7.1.9, Next PC 1.74

1. Experimental results:
   1. Amplification time statistics

|  |  |  |  |
| --- | --- | --- | --- |
| Instrument number | Amplification procedure 1 | Amplification procedure 2 | Amplification procedure 3 |
| 1834 | 24m49s | 28m18s | 26m52s |
| 1835 | 25m49s | 28m5s | 27m9s |

* 1. Experimental results

4.2.1 Linearity

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| procedure | 1.00E+06 | 1.00E+05 | 1.00E+04 | 1.00E+03 | 1.00E+02 | 1.00E+01 | 1.00E+00 | R value |
| 1834-1 | 20.9 | 23.44 | 27.53 | 32.44 | 36.8 | 37.78 | 38.84 | 0.9783 |
| 1834-2 | 15.95 | 19.27 | 22.66 | 26.47 | 29.75 | 33.53 | 35.29 | 0.9995 |
| 1834-3 | 19.62 | 22.71 | 25.72 | 29.94 | 32.7 | 35.83 | 39.73 | 0.9974 |
| 1835-1 | 20.42 | 23.74 | 27.82 | 31.1 | 35.23 | 38.79 | 40.43 | 0.9992 |
| 1835-2 | 16.45 | 19.18 | 22.53 | 26.24 | 29.81 | 32.89 | 37.53 | 0.9982 |
| 1835-3 | 19.94 | 23.29 | 26.43 | 29.14 | 32.91 | 36.04 | 34.31 | 0.9987 |

4.2.2 Repeatability

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| procedure | passage | 1.00E+03 | 1.00E+03 | 1.00E+03 | 1.00E+03 | average value | standard deviation | CV |
| 1834-1 | FAM | 32.24 | 32.39 | 32.25 | 32.51 | 32.35 | 0.128 | 0.40% |
| VIC | 33.73 | 33.31 | 33.41 | 32.95 | 33.35 | 0.321 | 0.96% |
| ROX | 33.4 | 33.41 | 33.43 | 33.35 | 33.40 | 0.034 | 0.10% |
| CY5 | 33.18 | 33.2 | 33.15 | 32.86 | 33.10 | 0.159 | 0.48% |
| 1834-2 | FAM | 27.27 | 27.07 | 27.47 | 28.15 | 27.49 | 0.47 | 1.71% |
| VIC | 28.95 | 28.74 | 28.99 | 28.98 | 28.92 | 0.12 | 0.41% |
| ROX | 29.36 | 28.95 | 29.51 | 29.4 | 29.31 | 0.25 | 0.84% |
| CY5 | 28.23 | 27.81 | 27.88 | 28.17 | 28.02 | 0.21 | 0.74% |
| 1834-3 | FAM | 31.76 | 31.44 | 30.85 | 30.21 | 31.07 | 0.68 | 2.20% |
| VIC | 32.31 | 31.52 | 30.78 | 30.6 | 31.30 | 0.78 | 2.49% |
| ROX | 32.31 | 31.62 | 31 | 30.67 | 31.40 | 0.72 | 2.30% |
| CY5 | 31.03 | 30.38 | 29.93 | 29.55 | 30.22 | 0.64 | 2.11% |
| 1835-1 | FAM | 32.69 | 32.65 | 31.87 | 31.58 | 32.20 | 0.56 | 1.73% |
| VIC | 32.57 | 32.55 | 32.15 | 31.76 | 32.26 | 0.38 | 1.19% |
| ROX | 32.58 | 32.57 | 32.47 | 32.02 | 32.41 | 0.26 | 0.82% |
| CY5 | 32.06 | 32.18 | 31.43 | 31.4 | 31.77 | 0.41 | 1.29% |
| 1835-2 | FAM | 27.62 | 28.06 | 27.92 | 27.91 | 27.88 | 0.18 | 0.66% |
| VIC | 27.84 | 27.61 | 27.85 | 27.79 | 27.77 | 0.11 | 0.40% |
| ROX | 28.59 | 29.25 | 29.45 | 29.36 | 29.16 | 0.39 | 1.34% |
| CY5 | 27.06 | 27.31 | 27.24 | 27.32 | 27.23 | 0.12 | 0.44% |
| 1835-3 | FAM | 31.68 | 31.02 | 30.55 | 30.64 | 30.97 | 0.51 | 1.66% |
| VIC | 31.69 | 31.24 | 30.73 | 30.57 | 31.06 | 0.51 | 1.64% |
| ROX | 32.12 | 31.35 | 31.34 | 31.22 | 31.51 | 0.41 | 1.31% |
| CY5 | 31.23 | 30.62 | 29.76 | 30.1 | 30.43 | 0.64 | 2.11% |

4.2.3 Sensitivity

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| procedure | passage | 1.00E+00 | 1.00E+00 | 1.00E+00 | 1.00E+00 | Detection rate |
| 1834-1 | FAM | 40.04 | 41.4 | 40.8 | 39.77 | 100% |
| VIC | 40.59 | / | 41.41 | 41.09 | 75% |
| ROX | 39.26 | 40.86 | 40.02 | 39.32 | 100% |
| CY5 | 39.78 | / | 41.03 | 39.89 | 75% |
| 1834-2 | FAM | 37.89 | 38.36 | 36.82 | 36.59 | 100% |
| VIC | 38.26 | 34.72 | 37.85 | 38.46 | 100% |
| ROX | 37.98 | 38.25 | 37.15 | 37.64 | 100% |
| CY5 | 37.6 | 38 | 36.84 | 36.92 | 100% |
| 1834-3 | FAM | 38.37 | 38.3 | 39.87 | 40.42 | 100% |
| VIC | 38.36 | 37.02 | 39.29 | / | 75% |
| ROX | 37.99 | 37.78 | 38.91 | 40.92 | 100% |
| CY5 | 37.41 | 37.24 | 38.62 | 40.6 | 100% |
| 1835-1 | FAM | 39.32 | 40.42 | 40.04 | / | 75% |
| VIC | 38.76 | 39.56 | 39.68 | / | 75% |
| ROX | 38.43 | 39.29 | 39.5 | 40.71 | 100% |
| CY5 | 38.92 | 40.15 | 40.26 | / | 75% |
| 1835-2 | FAM | 37.14 | 37.03 | 36.64 | 37.12 | 100% |
| VIC | 36.92 | 37.22 | 37.08 | 37.73 | 100% |
| ROX | 37.18 | 37.45 | 36.93 | 37.6 | 100% |
| CY5 | 36.65 | 36.71 | 36.02 | 36.9 | 100% |
| 1835-3 | FAM | 39.62 | 39.11 | 38.66 | 39.46 | 100% |
| VIC | 39.32 | 39.04 | 37.08 | 39.32 | 100% |
| ROX | 39.03 | 38.9 | 38.68 | 39.01 | 100% |
| CY5 | 38.94 | 38.7 | 38.46 | 39.03 | 100% |

1. **conclusion**

Similarly predetermined/denatured and annealed time, the smaller the temperature difference between prenaturation and annealing, the shorter the reaction time. Reaction procedure 1 is the shortest time, followed by reaction time 2 and reaction time 3 again. The performance of Reaction Procedure 2 and Reaction Procedure 3 is not much different, and the sensitivity of Reaction Procedure 1 will be slightly affected.