Recommended PCR conditions:

Additive (µl in a total volume of 15 μl mixture): 2.5-5.0; Loop 2 program: 68°C/30”; 66°C/30”; 64°C/30”; 62°C/30”; Loop 2 cycle number: N=?

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| --- | --- | --- | --- |
| Max GC% in any 100 bp at the target region | Min GC% in any 100 bp at the target region | Additive | Loop 2 program |
| GC% ≥ 95 | GC% ≤ 10 | 6.5-9.0 | 72°C/30”; 68°C/30”; 66°C/30”; 64°C/30”; 62°C/30”; 58°C/30”; |
| 10 < GC% ≤ 30 | 6.5-9.0 | 72°C/30”; 68°C/30”; 66°C/30”; 64°C/30”; 62°C/30”; 59°C/30”; |
| GC% ˃ 30 | 6.5-9.0 | 72°C/30”; 68°C/30”; 66°C/30”; 64°C/30”; 62°C/30”; 60°C/30”; |
| 95 ˃ GC% ≥ 90 | GC% ≤ 10 | 6.0-8.5 | 71°C/30”; 68°C/30”; 66°C/30”; 64°C/30”; 62°C/30”; 59°C/30”; |
| 10 < GC% ≤ 30 | 6.0-8.5 | 71°C/30”; 68°C/30”; 66°C/30”; 64°C/30”; 62°C/30”; 60°C/30”; |
| GC% ˃ 30 | 6.0-8.5 | 71°C/30”; 68°C/30”; 66°C/30”; 64°C/30”; 62°C/30”; 61°C/30”; |
| 90 ˃ GC% ≥ 85 | GC% ≤ 10 | 5.5-8.0 | 70°C/30”; 68°C/30”; 66°C/30”; 64°C/30”; 62°C/30”; 60°C/30”; |
| 10 < GC% ≤ 30 | 5.5-8.0 | 70°C/30”; 68°C/30”; 66°C/30”; 64°C/30”; 62°C/30”; 61°C/30”; |
| GC% ˃ 30 | 5.5-8.0 | 70°C/30”; 68°C/30”; 66°C/30”; 64°C/30”; 62°C/30”; |
| 85 ˃ GC% ≥ 80 | anyway | 3.5-6.0 | 69°C/30”; 68°C/30”; 66°C/30”; 64°C/30”; 62°C/30”; |
| 80 ˃ GC% ≥ 75 | anyway | 3.0-5.5 | 68°C/30”; 66°C/30”; 64°C/30”; 62°C/30”; |
| GC% < 75 | anyway | 2.5-5.0 | 68°C/30”; 66°C/30”; 64°C/30”; 62°C/30”; |

**N= 1+ ⌈Amplicon length/3.0 kb-0.3⌉**