|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Step** | **T3** | | | | **HS2** | | | |
| **Duration** | **Temperature (˚C)** | **Cycles** | **Duration** | | **Temperature (˚C)** | **Cycles** |
| Initial Denaturation | 2 minutes | 95 |  | 2 minutes | | 95 |  |
| Denaturing | 5 seconds | 95 | 35 | 5 seconds | | 95 | 35 |
| Annealing | 5 seconds | 60 | 5 seconds | | 47 |
| Extension | 25 seconds | 72 | 25 seconds | | 72 |
| Final Denaturation | 5 minutes | 95 | 1 | 5 minutes | | 95 | 1 |
| Melting Curve Initial | 15 seconds | 55 | 15 seconds | | 55 |
| Melting Curve End | 15 seconds | 95 | 15 seconds | | 95 |
| Melt Curve Duration | 20 minutes | | 20 minutes | | |
| Final Hold | infinite | 4 | infinite | | 4 |