***PCR Cycling Parameters***

1. **PCR Cycling Parameters for EF1**

Tm of Elongation Factor-1 (EF1) = 60.1°C

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Step** | **Temperature** | **Time** |
| **STAGE 1** | Initial Denaturation | 94 °C | 00:03:00 |
| **STAGE 2****(30 cycles)** | Denaturation | 94 °C | 00:00:40 |
| Annealing | 55 °C | 00:00:40 |
| Extension | 72 °C | 00:01:00 |
| **STAGE 3** | Final Extension | 72 °C | 00:10:00 |
| Hold | 1. °C
 | ∞ |

1. **PCR Cycling Parameters for BT2**

Tm of β-Tubulin = 70.3°C

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Step** | **Temperature** | **Time** |
| **STAGE 1** | Initial Denaturation | 94 °C | 00:03:00 |
| **STAGE 2****(30 cycles)** | Denaturation | 94 °C | 00:00:40 |
| Annealing | 65 °C | 00:00:40 |
| Extension | 72 °C | 00:01:00 |
| **STAGE 3** | Final Extension | 72 °C | 00:10:00 |
| Hold | 4 °C | ∞ |