Primers Used

1st Round Fw Rv

F296 F297

2nd Round

1 PbrR F310 F297

2 MerR F311 F297

3 CueR F312 F297

4 ArsR F313 F297

5 CnrH F405 F297

6 CzcN F404 F297

7 T7 F296 F297

1st Round PCR Setting

|  |  |  |
| --- | --- | --- |
| 95℃  95℃ | 5 min | 1 cycle |
| 95℃ | 20 s | 7 cycles |
| 54℃ | 10 s |
| 72℃ | 20 s |
| 95℃ | 20 s | 35 cycles |
| 72℃ | 30 s |
| 72℃ | 5 min | 1 cycle |
| 4℃ | ∞ | |

2nd Round PCR Setting (use 1st round PCR product as template)

|  |  |  |
| --- | --- | --- |
| 95℃  95℃ | 5 min | 1 cycle |
| 95℃ | 20 s | 35 cycles |
| 72℃ | 30 s |
| 72℃ | 5 min | 1 cycle |
| 4℃ | ∞ | |

|  |  |  |
| --- | --- | --- |
|  | 1st Round  25 uL | 2nd Round  50 uL |
| Q5 Hi-Fi DNA Polymerase | 0.25 uL | 0.5 uL |
| 5X Q5 Reaction Buffer | 5 uL | 10 uL |
| 10 mM dNTSs | 1 uL | 2 uL |
| 10 uM Fw Primer | 1 uL | 2.5 uL |
| 10 uM Rv Primer | 1 uL | 2.5 uL |
| Template DNA | - | 2.5 uL |
| Nuclease-Free Water | 16.75 uL | 30 uL |