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| --- | --- | --- | --- | --- |
|  | **Amplicon PCR** | |  | **Index PCR** |
| Reagent | Concentration | Volume/rxn |  | Volume/rxn |
| DNA | Full | 1.0 |  | 1.0 |
| H2O | - | 10.3 |  | 23.0 |
| Q5 Mastermix | 2x | 12.5 |  | 25.0 |
| Forward Primer | 10μM | 0.6 |  | 0.5 |
| Reverse Primer | 10μM | 0.6 |  | 0.5 |
|  | PCR Volume | 25.0 |  | 50.0 |
|  | Temperature (°C) | Time (seconds) | | Time (seconds) |
| Initial Denature | 98 | 120 |  | 120 |
| Denature | 98 | 10 |  | 15 |
| Annealing | 51/54 | 10 |  | 15 |
| Extension | 72 | 15 |  | 25 |
| Final Extension | 72 | 120 |  | 120 |
|  | PCR Cycles | 22 |  | 22 |
|  |  |  |  |  |