**KAPA HiFi HotStart PCR**

*Walker Orr, 03/27/2023*

**Application**

Amplifying SPINE backbones and other long (~10 kbp) amplicons with high fidelity.

**Required Equipment**

PCR Block

**Required Reagents**

Kit reagents for NEB Q5 High-Fidelity DNA Polymerase (M0491):

**Sample Workflow**

Total time: Variable

Active time: 15 minutes

**General Comments**

1. Prepare PCR master mix. The reaction MUST be set up on ice!! Thaw, vortex, and spin buffers; thaw and flick or pipette primers. Master mix will contain all reaction components common to all PCR reactions. See “Reaction Volumes,” below.
2. Transfer the appropriate volumes of PCR master mix, template, and primers to individual PCR tubes or wells. Mix well (multichannel pipette is helpful) and spin down.
3. Perform the PCR; see “Reaction Conditions,” below.

**Reaction Volumes**

|  |  |  |  |
| --- | --- | --- | --- |
| **Component** | **[Final]** | **25 µL reaction** |  |
| Nuclease-free water |  | To 25 µL | To 50 µL |
| 5x Q5 Reaction Buffer | 1x | 5.0 µL | 10.0 µL |
| 10 mM dNTPs | 200 µM | 0.5 µL | 1.0 µL |
| 10 µM Forward primer | .5 µM | 1.25 µL | 2.5 µL |
| 10 µM Reverse primer | .5 µM | 1.25 µL | 2.5 µL |
| Template DNA | <1,000 ng | As required | As required |
| Q5 High-Fidelity DNA Polymerase | 0.02 U/µL | 0.25 µL | 0.5 µL |
| 5x Q5 High GC Enhancer (if using) | 1x | 5 µL | 10 µL |

Considerations:

1. Q5 High-Fidelity DNA Polymerase may be diluted in 1x Q5 Reaction Buffer just prior to use in order to reduce pipetting errors.

**Reaction Conditions**

|  |  |  |  |
| --- | --- | --- | --- |
| **Step** | **Cycles** | **Temperature** | **Duration** |
| Initial denaturation | 1 | 98°C | 1 minute |
| Denaturation | 15-**35** | 98°C | 5-**10** seconds |
| Annealing | 50-72°C (**65°C**) | 10-**30** seconds |
| Extension | 72°C | 20-30 seconds/kb  **10 minutes** |
| Final extension | 1 | 72°C | 1 min/kb (**10 minutes**) |

Considerations:

1. For a standard (~10 kbp) EV-A71 inverse PCR reaction, use the settings in **bold**, above.