|  |  |  |
| --- | --- | --- |
| **Step** | **Key Details** | Y |
| Inverse PCR | * Start with concentrated template, dilute, and use 100 pg / rxn * 50 ul reactions: Q5 |  |
| Diagnostic gel |  |  |
| Dpn1 digestion | -1 ul Dpn1 to 45 ul PCR |  |
| Gel cleanup | -Fresh TAE  -Elute 9 ul 50C water |  |
| Oligopool PCR | -20 cycles  -25 ul reactions: KAPA HiFi  -1 ng template per reaction |  |
| TapeStation | -D1000 High Sensitivity  -Dilute 1 ul PCR product in 9 ul water |  |
| PCR Cleanup | -5:1 ratio of binding buffer  -Elute in room temp water 9 ul |  |
| Golden Gate | -2 ul BsmBI |  |
| Electroporation | -Clean up the golden gate reaction first, 2:1 ratio binding buffer  -Plate 1 ul and 10 ul dilutions |  |
| Midiprep |  |  |

Sublibrary Numbers:

Dates: