**Dial out PCR for barcodes and miSeq sequencing**

Dial out PCR- With Flow cell barcodes forward and reverse. I used 100ng of vector with 18 cycles where the first 4 is to get the barcode region from the plasmid and the other 14 is to add the illumine miSeq adaptors.

|  |  |
| --- | --- |
|  | **Sample 26-8** |
| **Buffer** | 10ul |
| **dNTPs** | 1ul |
| **Vector diluted 1:10 (total 100ng)** | 1 |
| **Q5 enzyme** | 0.5 |
| **Primer -1 or Primer -2** | 2.5ul |
| **Primer-3** | 2.5ul |
| **Water** | 31.8 |

* note that if you want to run on a nextSeq with phiX use primer 2 instead of primer 1

Dial out PCR protocol:

|  |  |  |
| --- | --- | --- |
| 98°C | 30 Sec |   |
| 98°C | 15 sec | 4 cycles |
| **66** | 15 sec |
| 72°C | 15 sec |
| 98°C | 10 sec | 14cycles |
| 72°C | 15 sec |
| 72°C | 5 sec |
| 72°C | 120 sec |   |
| +4°C |



* Marked in red the tm or the region that aligns to the plasmid

-Spri 1X

 -Qbit- 10.4ng/ul (in 20ul)

**Sequencing library for miseq**

Primers for run :64-Sequencing\_Primer

Preparing Primers (3uM final concentration):

3ul custom primer (100uM starting) into 597ul of HT1

 Custom Read1 primer port: 18

Custom index primer port: 19

\*Note: the miseq will not give you any indication that it is using a custom primer, this is fine. Do not panic, as long it is in the sample sheet, it will use it.

**Primers for plasmid dial out**

|  |  |  |
| --- | --- | --- |
| # | Name |  |
| 1 | PCR\_Dial\_out\_F (for miseq) | AATGATACGGCGACCACCGAGATCTACAC TCTC GGAAAGGACAGTGGGAGTGG |
| 2 | PCR\_Dial\_out\_F (for nextSeq) | AATGATACGGCGACCACCGAGATCTACACTCTC TTCCTGCAGGGTTTGTCTTC  |
| 3 | PCR\_Dial\_out\_R | CAAGCAGAAGACGGCATACGAGAT AAGCAACT GAGCTCAACTTCAAGGAGTGG  |
| 4 | Custom sequencing primer (for miseq) | ATCAGCGGGTTTCCTGCAGGGTTTGTCTT |

* Red font marks sequences that align to the watermelon plasmid