Whole Genome Amplification Protocol

Pre-Amplification 1:

|  |  |  |
| --- | --- | --- |
| Reagents for Pre-Amplification Master Mix | | |
|  | 1x (µL) | 0 |
| 10x ThermoPol buffer (NEB) | 3.0 | 0.0 |
| dNTP (10 mM) | 1.0 | 0.0 |
| Nuclease free water | 19.5 | 0.0 |
| NG primer (10 µM) | 0.75 | 0.00 |
| NT primer (10 µM) | 0.75 | 0.00 |
| Total | 25.00 | 0.00 |

Note: To update table, change the ‘0’ in the last column of the second row to the number of buffers needed (only enter a numeric value). Then, highlight the entire third column and press ‘F9’.

*Pre-Amplification 1 Cycle:*

1. Samples should already be lysed in 5 µL of lysis buffer (Single Cell Lysis Protocol can be downloaded [here](https://blog.uta.edu/xus/files/2017/11/Cell-Lysis-Protocol-11272017-19ff6d7.docx)).
2. Add 25.0 µL of the pre-amplification master mix solution to each of the samples.
3. Put the samples in the thermal cycler and run a denature cycle:

|  |  |  |
| --- | --- | --- |
|  | **1** | **2** |
| **Temp.** | **94°C** | **94°C** |
| **Time** | **05:00** | **Hold** |

1. **Immediately after the denature cycle, quench the samples in ice.**
2. **Add 0.5** µL of BST DNA polymerase to each sample
3. Put the samples in the thermal cycler and run the Pre-Amplification 1 Cycle **once**:

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| **Temp.** | **10°C** | **10°C** | **15°C** | **20°C** | **30°C** | **40°C** | **50°C** | **65°C** | **95°C** | **95°C** |
| **Time** | **Hold** | **00:45** | **00:45** | **00:45** | **00:45** | **00:45** | **00:45** | **2:00** | **00:20** | **Hold** |

1. **Quench samples on ice immediately.**

Pre-Amplification 2:

Repeat the following three steps **5x times**:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Run Number | 1 | 2 | 3 | 4 | 5 |
| Check when done |  |  |  |  |  |

1. Add 0.5 µL of BST polymerase to the each sample.

Repeat 5x

1. Put the samples in the thermal cycler and run the Pre-Amplification 2 Cycle:

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** |
| **Temp.** | **10°C** | **10°C** | **15°C** | **20°C** | **30°C** | **40°C** | **50°C** | **65°C** | **95°C** | **58°C** | **58°C** |
| **Time** | **Hold** | **00:45** | **00:45** | **00:45** | **00:45** | **00:45** | **00:45** | **2:00** | **00:20** | **00:40** | **Hold** |

1. Quench samples on ice immediately.

Move onto the amplification step when all five runs of pre-amplification 2 are completed.

Amplification (PCR) Step:

|  |  |  |
| --- | --- | --- |
| Reagents for Amplification Master Mix | | |
|  | 1x (µL) | 0 |
| 10x ThermoPol buffer (NEB) | 3.0 | 0.0 |
| dNTP (10 mM) | 1.0 | 0.0 |
| 27mer primer (10 µM) | 1.5 | 0.0 |
| Deep vent (-exo) DNA polymerase | 1.0 | 0.0 |
| Nuclease free water | 23.5 | 0.0 |
| Total | 30.0 | 0.0 |

Note: To update table, change the ‘0’ in the last column of the second row to the number of buffers needed (only enter a numeric value). Then, highlight the entire third column and press ‘F9’.

Amplification Cycle:

1. Add 24 µL of amplification master mix solution to each sample (use all pre-amplification product).
2. Put tubes/wells into the thermal cycler and run the Amplification Cycle:

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | | **22x** | | | |  | |
|  | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| **Temp.** | **94°C** | **94°C** | **59°C** | **65°C** | **72°C** | **72°C** | **4°C** |
| **Time** | **Hold** | **00:20** | **00:20** | **01:00** | **02:00** | **05:00** | **Hold** |

Afterwards, DNA samples may be stored at -20°C.