|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Time: 2h  Prepare 30m  Ligation 1h | 4. Cloning of BS treated DNA fragments into sequencing plasmid  The enzyme we used for second round amplification is Fermentas HotShot Taq. Which adds A to 3’ end. Check whether you need to do A-tailing before TA cloning.  Ligation  pGEM-T Easy Vector System  1. Brief spin the pGEM-T Easy Vector and Control Insert DNA tubes to collect contents to the bottom of the tube.  2. Set up ligation master mix:   |  |  |  |  | | --- | --- | --- | --- | | Reagents | Sample | Positive control | Negative control | | 2x Rapid Ligation buffer | 5 |  |  | | pGEM®-T Easy Vector (50ng) | 1 |  |  | | PCR product | 3 | 2 | 3 | | T4 DNA Ligase  (3 Weiss units/μl) | 1 |  |  | | H2O | - | 1 | - | | Total | 10 | 10 | 10 |   3. Mix the reactions by pipetting. Incubate for 1 hour at room temperature (~25°C). Or incubate overnight at 4°C for maximum number of transformants. |