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|  | **EcoRI Digestion to test insertion**  Mastermix   |  |  |  | | --- | --- | --- | | Reagents | Volume (µL) |  | | EcoRI | 0.5 | 15 | | 10x EcoRI Buffer | 2 | | ddH2O | 12.5 | | DNA | 5 | 5 | | Total | 20 | 20 |   Incubate at 37 for 1hour.  Load samples to 1.5% agorose gel, 100V and run 23min.  Check insert.  Pick the ones that has insert to submit for Sanger sequencing  **Submission forms**  GeneWiz.com  10µL total with 500~1000ng total DNA.  Use 200µL 8-strips.  The submission page will generate the tube number. Mark corresponding tube numbers.  Universal primer SP6 or T7 |