The protocol for LIC by Exonuclease III

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1. **Design the primers with 15-bp overlap;**
2. **Digest the vector by proper restriction enzyme;**
   For getting high quality vectors, there are some good advices as follows:
   1) **The restriction sites we choose should be 5'-overhangs or blunt ends , but it shouldn't be 3'-protruding ends ;**
   2) **Digestion by double enzymes;**
   3) **Digestion the vector overnight to make sure complete cleavage as possible;**
4. **Quantitate the concentration of the vector through running DNA gel and the vector is prepared for LIC;**
5. **Amplify the insert by PCR with the overlap primers, gel-purified, and quantitation; the insert is also prepared for LIC;（You can also treat the templates with DpnI, if there are scarcely any background bands and the positive PCR bands are dense and special enough.）**
6. **Mix the vector and insert in the reaction system as follows:**

Vector	50-100ng
Insert	50-100ng
10 x ExoⅢ buffer	1ul
Add ddw to	10ul

7. **Place the tube in the ice bath for 5mins**
   (Make sure the mixture in the tube is cooled to the temperature of the ice bath, and the following approach should also operate on the ice);
8. **Add 1ul ExoIII(20units) to the reaction mixture; pipe the mixture for several times ;**
9. **Place the tube on the ice bath or 4℃ for 60mins;**
10. **Add 1ul 0.5M EDTA (pH 8.0) to stop the reaction; pipe the mixture for several times;**
11. **The mixture is then melted at 65℃ for 5mins;**
12. **Place on the ice bath for 5mins;**
13. **Centrifuge to concentrate the mixture;**
14. **Transform the mixture of DNA into DH5α ;**
15. **Incubate the bacteria on the LB plates with proper antibiosis for 16h;**
16. **Pick up single clone for mini-preparation, analyze by restriction enzyme and further analyze by DNA sequencing.**