Filling-in protocol

This protocol is based on the one described in "A method for filling in the cohesive ends of double-stranded DNA using Pfu DNA polymerase", Yang, S. et al., 2005.

1) Digestion:
   In a microcentrifuge tube mix the following:
   - 12,5 µL water;
   - 10 µL DNA (NB: the DNA quantity in the final tube should be 4,37µg);
   - 2,5 µL 10X digestion buffer;
   - 1 µL SpeI enzyme;
   - Incubate 10 min at 37°C;
   - Incubate 20 min at 80°C in order to inactivate the enzyme;

2) Purification:
   - Add 100 µL EtOH 100% and incubate 30 min at -20°C;
   - Centrifuge at 11000g for 30 min at 4°C;
   - Discard the supernatant;
   - Add 200 µL EtOH 70%;
   - Centrifuge at 11000g for 30 min at 4°C;
   - Discard the supernatant;
   - Dry the pellet by vacuum centrifugation for 10 min;
   - Resuspend the pellet in 42 µL TE buffer;

3) Filling-in:
   - Add 2 µL dNTP at 2,5 mM (so that the final concentration is 0,1 mM)
   - Add 5 µL 10X Pfu buffer supplemented with MgSO₄;
   - Add 1,6 µL Pfu polymerase;
   - Incubate at 72°C for 15 min;

4) Purification:
   - Add EtOH 100% and incubate 30 min at -20°C;
   - Centrifuge at 11000g for 30 min at 4°C;
- Discard the supernatant;
- Add 200 µL EtOH 70% ;
- Centrifuge at 11000g for 30 min at 4°C;
- Discard the supernatant;
- Dry the pellet by vacuum centrifugation for 10 min;
- Resuspend the pellet in 9 µL TE buffer;

5) **Ligation:**
- Add 1 µL of T4 DNA ligase buffer ;
- Add 1 µL of T4 DNA ligase;
- Incubate at room temperature over night;
- Incubate at 70°C for 5 min to inactivate the ligase;