**Inverse PCR DIMPLE/SPINE pipeline:**

Template used: **EV7-71 Clone 9 BB-free** (P7 in WB plasmid midi-maxiprep box).

Preparing the template: Always start from a high-quality plasmid produced from a midi-maxiprep. Dilute plasmid to working concentration (usually to around 10-20 ng/µl) and measure by Qubit high sensitivity to be certain of concentration. For the DIMPLE/SPINE pipeline: Use 100 pg of template per 50 µl PCR reaction. For 20 reactions: 2 µl of 1ng/µl plasmid.

**Note:** 100 pg will be used ensure optimal amplification, but also reduce as much as wild type contamination as possible.

**Note2:** In general cloning methods (not library generation via SPINE/DIMPLE), 1 ng of template is okay to use.

**Setting up the PCR reaction: (work on ice or cooling blocks)**

1. Put in each PCR tube: 2.5 µl of 10 µM forward primer and 2.5 µl 10 µM of reverse primer
2. Create a master mix corresponding to the amount of PCR reactions:

Example:

For 20 reactions:

**Q5 buffer**: 200 µl

**dNTP:** 20 µl

**Fwd primer**: **50 µl (just for calculating water volume)**

**Rev** **primer**: **50 µl (just for water volume)**

**Template DNA**: 2 µl of 1 ng/µl plasmid

**Q5 high fidelity polymerase**: 10 µl

**Water**: 668 µl

1. Mix well the master mix by pipetting up and down
2. Add 45 µl in each PCR tube, mix up and down, change tips. Repeat for each reaction with different primers.
3. Run directly on a PCR machine that has been pre-heated using the following settings:

|  |  |  |
| --- | --- | --- |
| Initial Denaturation | 98°C | 1 min |
| 25–35 Cycles | 98°C 65°C 72°C | 10 seconds 30 seconds 10 min |
| Final Extension | 72°C | 10 min |
| Hold | 4–10°C |  |

1. Run 5ul of PCR on a gel and image using the Azure gel imaging system.
2. If successful, treat PCR reactions with 1ul of DPN1 for 1hr at 37 degrees.
3. Then, run all PCR reactions on a gel and perform gel-purification
4. This is then ready to be used as a backbone for golden-gate cloning.