**Annealing of oligonucleotides [Ref: 1]:**

1. Add the required volume of H2O to the lyophilized oligonucleotides to obtain a concentration of 100µM. Vortex both tubes for 30 s and incubate them at RT for 5 min to dissolve them.
2. Prepare the annealing mix by adding 45’5µl of H2O, 2’5µl of 1 M NaCl, 1µl of oligo Forward (100µM) and 1µl of the oligo Reverse (100µM) into a PCR tube.
3. Place the PCR tube with the mix in a thermocycler with the following annealing programme: 5 min at 95°C; then, 1 min at 95°C and ramp down 1°C per cycle for 72 cycles and end by keeping the temperature at 10°C.
4. Take 10µl of the annealed F:R oligonucleotides and dilute it with 90µl of H2O to obtain a 0’2µM concentration. The annealed oligonucleotides stocks can be stored at -20°C for future use.

**Ligation of annealing product:**

1µl Plasmid (pSEVA182 digested with *SmaI*)

11µl annealing product (diluted)

1’5µl T4 Ligase

1’5µl Buffer Ligase 10X

[1] Aparicio, T., de Lorenzo, V., & Martínez‐García, E. CRISPR/Cas9‐enhanced ss DNA recombineering for Pseudomonas putida. *Microbial Biotechnology*.