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🌐 Cloning of L0 parts into pUAP-ye for Loop typellS

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We use this protocol and it's working

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1 Design primers as described in the Figure below to add the necessary overhangs for cloning into

pUAP-pe and also the overhangs that correspond to the common syntax.

- 2 DNA parts are PCR amplified from the source DNA part (e.g. plasmid DNA or genomic DNA), using a high fidelity DNA polymerase such as Phusion. Use 10 ng of DNA if the template is plasmid DNA and 100ng if the template is genomic DNA. The cycling conditions are: Denaturation at 98 °C for 30 s. 35 cycles of: denaturation at 98 °C for 10 s, annealing at primer annealing temperature for 30 s, and extension at 72 °C for 15 s/kb. Final extension at 72 °C for 10 min.
- 3 Run the PCR products on a 1.5% (w/v) agarose gel.
- 4 Gel extract the band that corresponds to the size of the amplified DNA part using a kit such as QIAquick Gel Extraction Kit.
- 5 Determine DNA concentration with spectrophotometry (Nanodrop).
- 6 Prepare aliquots of the DNA part at a concentration of 15 nM and of the pUAP-pe acceptor vector at a concentration of 7.5 nM.
- 7 Set up a Type IIS assembly reaction into a 0.2 mL tube according to Table to clone the amplified DNA part into pUAP-pe.

Sterile water	5
pUAP-pe	1
DNA part	1

10x Tang o buffer (Ther mo Fisher)	1
1 mg/m L bovin e seru m album in (NEB)	0.5
T4 DNA ligase (5 U/ μ L) (Ther mo Fisher)	0.25
10m M ATP (SIGM A)	1
Sapl (Lgul) (5 U/ μ L) (Ther mo Fisher)	0.25
Final volum e	10

- 8 Place samples on a thermocycler and use the following program:
Assembly: 26 cycles of 37 °C for 3 min and 16 °C for 4 min.
Termination and enzyme denaturation: 50 °C for 5 min and 80 °C for 10 min.
- 9 Transform 20 μ L of chemically competent E. coli cells (transformation efficiency of 1×10^7 transformants/ μ g plasmid DNA) using 2 μ L of the assembly reaction and then spread on LB agar

plates containing 25 µg/mL chloramphenicol.

- 10** Incubate overnight at 37 °C.
- 11** Select white colonies for sequencing.
- 12** Confirm the presence of the correct insert with Sanger sequencing using the primers UAP_F and UAP_R and any additional DNA part specific primers.
- 13**