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Domestication of L0 parts for Loop type IIS (BsaI and SapI)

Forked from Domestication of L0 parts for Loop type IIS (BsaI and SapI)

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Protocol status: Working
We use this protocol and it's working

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Abstract

Domestication and cloning of L0 parts into pUAP4 for **Loop type IIS assembly**

Materials

MATERIALS

Phusion high-fidelity PCR kit **Thermo Scientific** **Catalog #**F553S

MinElute Gel Extraction Kit **Catalog #**28606

Domestication Guide.

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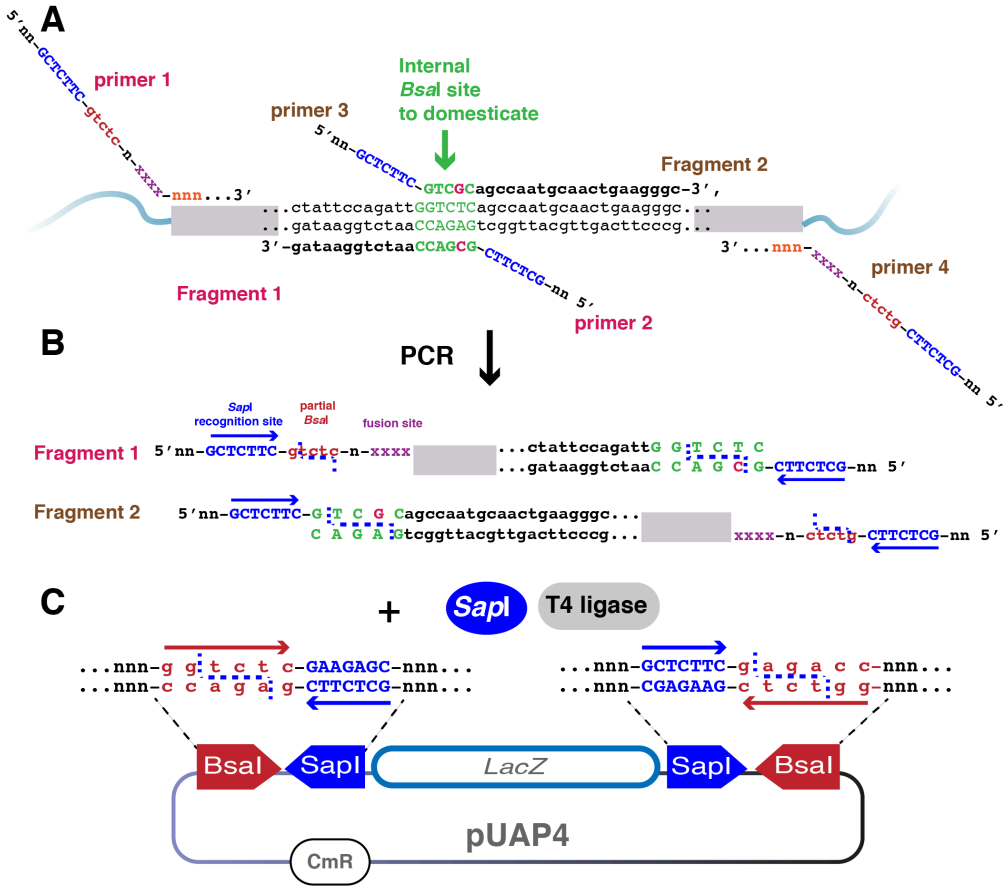

Domestication of internal BsaI and SapI sites is necessary when a DNA sequence of interest contains a BsaI or SapI recognition site. For domestication, overlapping PCR can be used. Briefly, two PCR fragments are amplified upstream (fragment 1, with primers 1 and 2) and downstream (fragment 2, with primers 3 and 4) of the sequence to domesticate, which will be part of the reverse primer (primer 2) of fragment 1 and of the forward primer (primer 3) of fragment 2. Both primer 2 and 3 will be specially designed with a single nucleotide mismatch to alter the sequence to domesticate (taking care to not alter amino acid composition if the region is in the coding sequence). Primers will also contain SapI recognition sites to allow the two fragments to be ligated together into pUAP4 using SapI type IIS assembly. Primer 1 and primer 4 are designed according to the guidelines for **cloning of L0 parts into pUAP4**. Blue arrows: SapI recognition site. Blue dashed lines: SapI cleavage site. Red arrows: BsaI recognition site. CmR: chloramphenicol bacterial resistance cassette. LacZ: lacZα cassette for blue-white screening of colonies.

PCR and amplified fragments purification

2 Amplify the parts using your preferred polymerase (e.g. Phusion, Thermo Scientigic™) following the manufacturer instructions.

3 Run your PCR product on agarose gel.

4 Extract the amplified fragments using a PCR purification kit (e.g. Qiagen MinElute™).

Fragments Assembly

5 Set up the assembly of the fragments as in **cloning of L0 parts into pUAP4**

[pUAP4]= plasmid length/200
[fragment]= fragment length/100

The formulas above will give you the required concentration in ng/µl.