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Plasmid construction

In 1 collection

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ABSTRACT

Via restriction enzyme digest and ligation

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1 Design PCR primers such that they contain a BglII restriction site upstream of the gene, and SalI downstream of the gene. Design cut sites such that gene will still be in frame post ligation.

2 Acquire purified pmCherryC1 stock, and cleave using BglII and SalI using the standard NEB protocol. Simultaneously cleave PCR product

3 Purify both vector and insert using 1% agarose gel, and compare to uncut controls to make sure that restriction digest went to completion. Gel extract vector and insert.

4 Ligate with T4 ligase at room temperature overnight following standard NEB protocol.

5 Transform into XL10 Gold cells, plate on ampicillin selective medium, grow overnight at 37 C

6 Pick colonies, grow 5 mL overnight culture, and purify plasmid using a commercially available plasmid purification kit.