Golden Gate Assembly_Perkinsus

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Protist Research to Optimize Tools in Genetics (PROT-G) Perkinsus

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ABSTRACT

The phylum Perkinsozoa is an aquatic parasite lineage that has devastating effects on commercial and natural mollusc populations, and also comprises parasites of algae, fish and amphibians. They are related to dinoflagellates and apicomplexans and thus offer excellent genetic models for both parasitological and evolutionary studies. Genetic transformation was previously achieved for *Perkinsus* spp. but with few tools for transgene expression and limited selection efficacy. We have expanded the power of experimental genetic tools for *Perkinsus* using *P. marinus* as a model. We constructed a modular Golden Gate plasmid assembly system for expression of multiple genes simultaneously as the basis of this effort. This has provided a versatile platform enabling several further developments: efficient selection systems for three drugs, puromycin, bleomycin and blasticidin; eleven new promoters of variable expression strengths; and bi-cistronic transcripts using the viral 2A peptides can couple selection to the maintenance of the expression of a transgene of interest. Collectively, thesenew tools provide great new capacity to genetically modify and study *Perkinsus* as an aquatic parasite and evolutionary model.

ATTACHMENTS

GoldenGate_plasmids.xlsx

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This protocol assumes that

- All modules being assembled have been “domesticated” (are free of internal *BsaI* and *BpiI* recognition sequences)
- All module junctions being assembled have a unique set of compatible overhangs that specifies their order of assembly
- The acceptor plasmid has a different antibiotic resistant gene to that of all of the modules plasmids being assembled into it

It has been found that the isoschizomer *BbsI* (supplied NEB) loses activity very quickly and has a lot of star-activity so it is advised not to use this.

1

Step 1 includes a Step case.

Golden Gate cloning into Level 1

Golden Gate cloning into Levels 0 and 2

step case

Golden Gate cloning into Level 1

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2 To assemble fragments in Level 1 acceptors using *BsaI,* add all of the following to a PCR tube and make the reaction volume up to **20 µL** with sterile distilled water.

- **100 ng** to **200 ng** of acceptor plasmid
- Plasmids containing each module/part to be inserted. Use a 2:1 molar ratio of insert:acceptor. NB: If your sequence is ~1KB or less, it can be added directly to this assembly reaction as a linear PCR amplicon.
- **20 units** BsaI (**1 µL** of BsaI - 5,000 units **New England Biolabs Catalog #R0535L**) CutSmart® Buffer **New England**
- **2 µL** **Biolabs Catalog #B7204S**
- **400 units** T4 DNA Ligase (**1 µL** of T4 DNA Ligase - 20,000 units **New England Biolabs Catalog #M0202S**)
- **2 µL** of **10 millimolar (mM)** ATP

3 Incubate your reaction in a thermocycler for 3 cycles of **00:10:00** **40 °C** , **00:10:00** **16 °C** , followed by a single cycle of **00:10:00** **50 °C** , **00:20:00** **80 °C** , **4 °C** forever. 50m

4 Use **5 µL** of this one-pot digestion-ligation reaction product to transform *E.coli* cells.

5 Positive clones can be selected using LB agar with an antibiotic appropriate to the backbone of the acceptor vector. If your acceptor vector contained the lacZ cassette, include **0.5 millimolar (mM)** IPTG and **0.04 mg/mL** and X-gal. Select 3 -10 (white) colonies for restriction analysis and confirmation by sequencing.

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