BBF RFC 61: Fast multiple gene fragment ligation method based on Type IIs restriction enzyme DraIII

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1. Purpose

With the established BioBrick Assembly standards, ligation of different parts has to be accomplished step by step. It can be time-consuming when dealing with multiple fragment ligation.
BBF RFC 61 is developed aimed at completing the ligation of multiple fragments quickly and efficiently based on Type II restriction enzyme DraIII.

2. Relation to other BBF RFCs

This BBF RFC updates BBF RFC 10.

3. Copyright Notice

4. Description

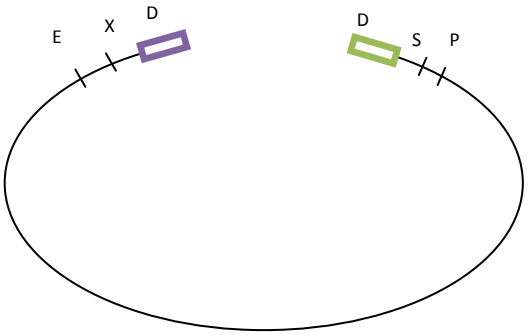
The recognition site of endonuclease DraIII is as follows: 5' CACNNNGTG 3'

Thus, DraIII can generate three base overhangs of any sequence. so the user can design more than one recognition sequence added to multiple fragments and complete the excision in one procedure.

Since the user can specify the overhangs, this method can be used to ligate several different fragments in one step, which is necessary for the construction of multi-fragment part. More importantly, these overhangs can be non-palindromic, which solves the biggest problem faced when trying to do multipart ligations using standard restriction enzymes, the

self ligation of a part, blocing it's incorporation into the construct.

Dra III based ligation method allows high efficiency ligation of up to 4 fragments: 3 inserts and 1 vector. The vector is prepared as this, which is compatible with BBF RFC 10:



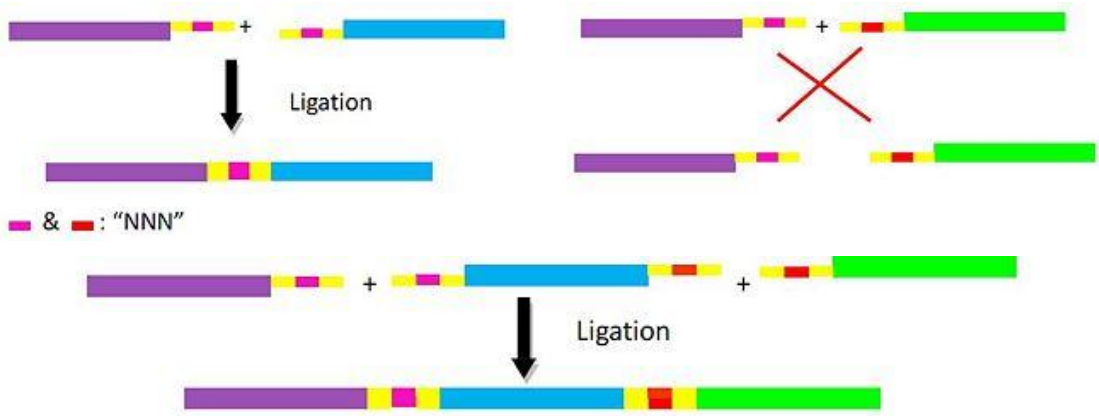
The fragments are designed with different DraIII enzyme cutting site:

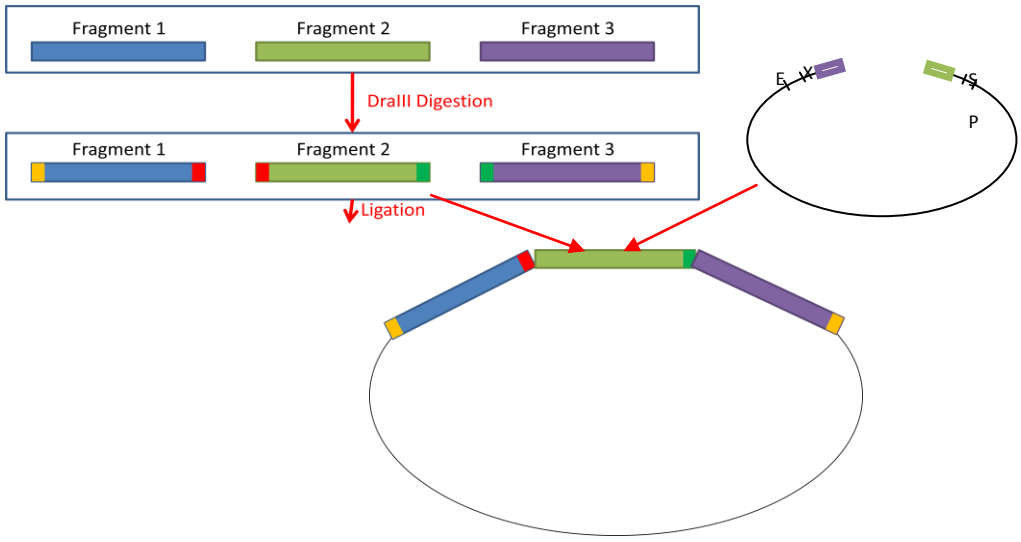
Fragment A : CAC $N_1N_1N_1$ GTG-($n_1n_1n_1$…$n_1n_1n_1$)-CAC$N_2N_2N_2$GTG

Fragment B: CAC $N_2N_2N_2$ GTG-($n_2n_2n_2$…$n_2n_2n_2$)-CAC$N_3N_3N_3$GTG

Fragment C: CAC $N_3N_3N_3$ GTG-($n_3n_3n_3$…$n_3n_3n_3$)-CAC$N_4N_4N_4$GTG

…





5. Protocol

Protocol of Fermentas® FastDigest® DraIII (AdeI) Digestion

1. Prepare the reaction mixture at room temperature in the order indicated:

Component	Volume
Water	17 µl
10X buffer	2 µl
DNA	10 µl (~0.2 µg)
DraIII enzyme	1 µl
Total volume	30 µl

2. Mix gently and spin down.
3. Incubate at 37°C in a heat block or water thermostat for 5 min.
4. Inactivate the enzyme (optional).

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7.References

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[2] **Sambrook J, Maniatis T, Fritsch E F.** *Molecular Cloning: a Laboratory Manual. cold Spring*
Harbor Laboratory Press, Cold Spring Harbor, NY, 3rd ed., 2001.