

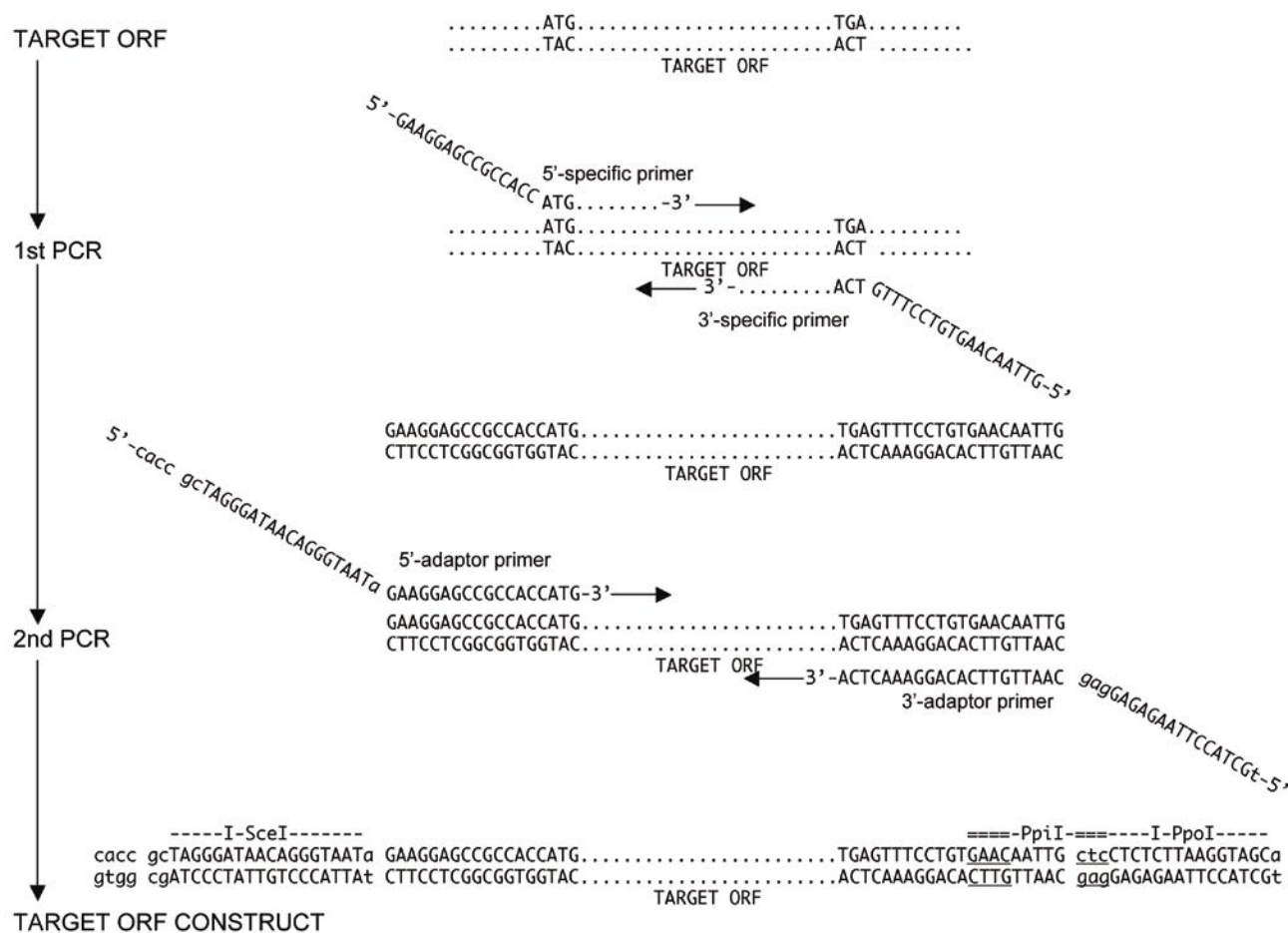
SUPPLEMENTARY MATERIAL FOR:

Constructing ORFeome resources with removable termination codons

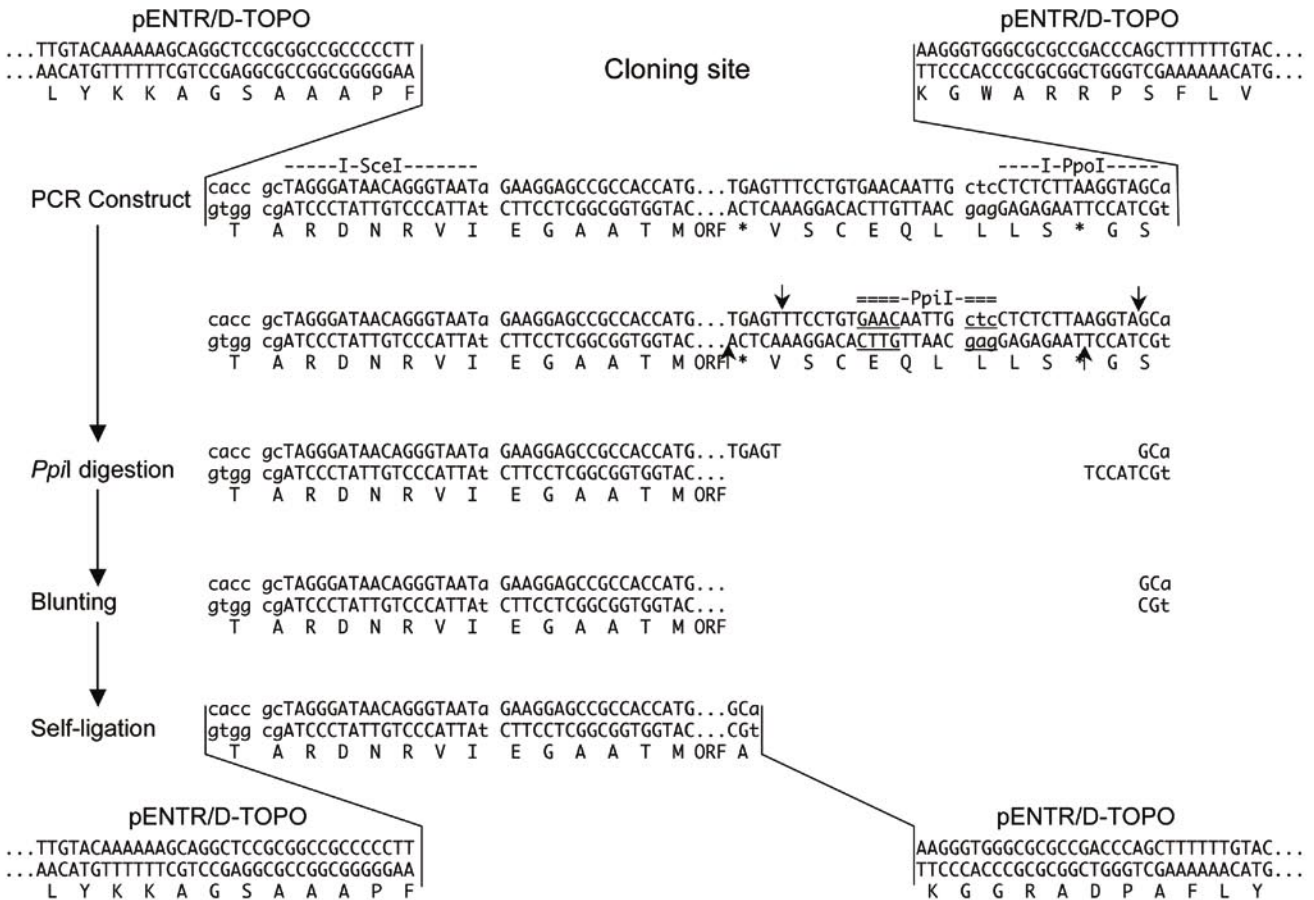
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Supplementary Figure S1. The construction of target open reading frame (ORF) fragment. The target ORF is amplified by the pair of primers that are specific to the target ORF sequence. Then the PCR product is used as the template for a second PCR with a pair of adaptor primers carrying homing endonuclease and type-IIS restriction endonuclease sites. The final product is suitable for cloning into the pENTR-TOPO vector.



Supplementary Figure S2. The stop codon elimination from the target open reading frame (ORF). The plasmid carrying the target ORF is digested with the type-IIIS restriction endonuclease, *PpiI*. The termini of the digest are blunted with T4 DNA polymerase in the presence of dNTPs, then self-ligated with T4 DNA ligase at a dilute DNA concentration in order to favor intramolecular ligation. The resulting plasmid will lack its original stop codon and will be in-frame with the C-terminal *attL2* site of the Gateway vector.



Supplementary Figure S3. Theoretical construction of target open reading frame (ORF) fragment with attB sites at both ends. The target ORF is amplified by a pair of primers that are specific to the target ORF sequence. Then the PCR product is used as the template for a second PCR, with the pair of adaptor primers carrying *I-PpoI* and *PpoI* sites. Finally, the product is used as template for a third PCR with another set of adapter primers designed to attach *attB* sites at the both ends. The final product would be suitable for the pDONR vectors carrying *attP* sites. This construction scheme has not been experimentally validated.