Primer Design for Restriction Enzyme Cloning (E6901)

Overview

Protocols.io also provides an interactive version of this protocol where you can discover and share optimizations with the research community.

Introduction

Appropriate restriction sites, absent in the target gene, are incorporated in the forward and reverse primers when a target gene is generated by PCR. The choice of the restriction site in the primers determines whether any, or which, extra amino acid residues will be attached to the terminus of the target protein after the cleavage of the intein tag.

Table 2 illustrates some examples of designing forward and reverse primers for pTXB1 and pTYB21. For cloning into pTXB1 one should clone a target gene between the NdeI (forward primer) and the SapI (reverse primer) sites in pTXB1. For the pTYB21 vector the SapI site can be used to clone the 5´ end of the target gene (PstI as the 3´ cloning site for pTYB21 is shown as an example for a reverse primer in the table).

When constructing a N-terminal fusion (pTYB21) a stop codon should be encoded in the reverse primer. The reverse primer for the C-terminal fusion (pTXB1) should not include a stop codon.

We recommend writing out your primers and cloning strategy in order to check SapI (or BspQI) digestion, the reading frames etc. For more information on cloning with SapI, please refer to: IMPACT FAQs

In general, more than 15 bp of target gene sequence is required for PCR (represented by "NNNNNN..."). In Table 2 the restriction site is underlined. The "GGTGGT" sequence at the 5´ end of the primer is a random sequence of 6 bp to ensure efficient DNA cleavage by the restriction enzyme.