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Primer Design for Restriction Enzyme Cloning (E6901) V.2

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Guidelines for Primer Design for Restriction Enzyme Cloning (E6901).

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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).

A	B	C
NdeI	5´- GGT GGT CAT ATG NNN NNN... -3´ (forward primer)	pTXB1
SapI (*1)	5´- GGT GGT TGC TCT TCC GCA NNN NNN...-3´ (reverse primer)	pTXB1
SapI (*2)	5´- GGT GGT TGC TCT TCC AAC NNN NNN... -3´ (forward primer)	pTYB21
PstI (*3)	5´- GGT GGT CTG CAG TCA NNN NNN... -3´ (reverse primer)	pTYB21

[Table2 from Construction of the Fusion Plasmid]

**1 SapI digestion creates a 3-nt overhang (GCA) for ligation with the SapI-digested pTXB1 vector (containing a TGC overhang), resulting in an in-frame fusion to the N-terminus of an intein. The SapI site can be used to add one or more extra amino acid residue(s) to the target protein by including an appropriate sequence (e.g. add ACC in the reverse primer corresponding to a GGT codon for a glycine residue). The SapI site is not regenerated after cloning.*

**2 SapI digestion creates a 3-nt overhang (AAC) compatible with the SapI digested pTYB21 (containing a GTT overhang).*
The SapI site is not regenerated after cloning.

**3 A stop codon should be included in the reverse primer when constructing a N-terminal fusion.*
The SapI site is not regenerated after cloning.

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 (see in **Construction of the Fusion Plasmid**). 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.

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MATERIALS

IMPACT KIT **New England Biolabs Catalog #E6901S**

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

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