

TECHNICAL PROTOCOL

FOR

loxP-amp-loxP

loxP flanked,
Ampicillin Selection Cassette

(A009)

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1. loxP-amp-loxP: PCR template (50 ng/ μ l, 20 μ l)
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Store tube at -20°C

Please read

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Short Description:

“loxP-amp-loxP” cassette is designed to allow ampicillin selection in prokaryotic cells.

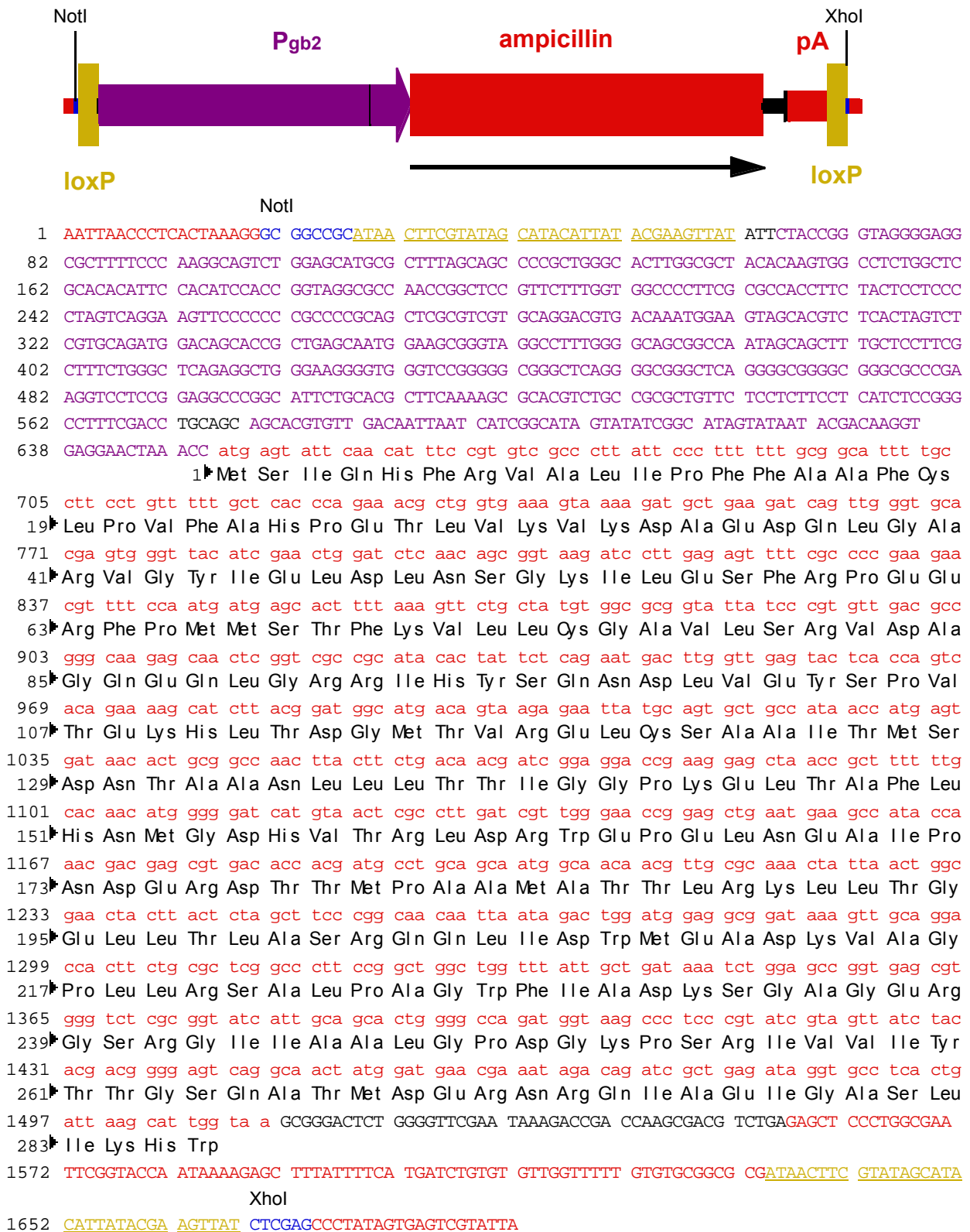
The prokaryotic promoter gb2 driving the gene for ampicillin resistance is a slightly modified version of the Em7 promoter; it mediates higher transcription efficiency than the generally used Tn5 promoter. A synthetic polyadenylation signal terminates the ampicillin expression. The cassette is flanked by loxP sites for later excision by Cre-recombinase. Unique *NotI* and *XhoI* sites flank the cassette for convenient cloning with restriction sites.

Using the provided PCR template one can easily create a loxP-amp-loxP cassette flanked by any other restriction sites to clone the cassette into the vector of choice. The restriction sites can be introduced by adding the corresponding sequence in the PCR primer.

The template can easily be used to engineer the *E. coli* genome by Red[®]/ET[®] Recombination.

The “loxP-amp-loxP cassette” is not linear but plasmid based (3502 bp in size). Due to its R6K origin the plasmid cannot replicate in most *E. coli* strains. The PCR product can therefore be used directly for downstream applications without any further purification.

At least 20 PCR reactions can be performed using 1 µl per reaction as template.



Please take into consideration that the sequence given above does not reflect the complete plasmid but refers to the functional cassette.