



TECHNICAL PROTOCOL
FOR
loxP-PGK-gb2-hygro-loxP
**loxP flanked,
Pro- and Eukaryotic
Hygromycin Selection Cassette**
(A011)

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1 Eppendorf tubes + manual

1. loxP-PGK-gb2-hygro-loxP: PCR template (50 ng/μl, 20μl)
2. This manual

Store tube at -20°C

Please read

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Short Description:

“loxP-PGK-gb2-hygro-loxP” template is designed to allow hygromycin selection in prokaryotic and eukaryotic cells.

The loxP-PGK-gb2-hygro-loxP template encodes the hygromycin resistance gene which combines a prokaryotic promoter (gb2) for expression in *E.coli* with a eukaryotic promoter (PGK) for expression in mammalian cells.

The prokaryotic promoter gb2 is a slightly modified version of the Em7 promoter; it mediates higher transcription efficiency than the normally used Tn5 promoter. The promoter of the mouse Phosphoglucokinase gene (PGK) is used as eukaryotic promoter. A synthetic polyadenylation signal terminates the hygromycin expression. The cassette is flanked by loxP sites for later excision by Cre-recombinase.

Using the provided PCR template one can easily create a loxP-PGK-gb2-hygro-loxP cassette flanked by any 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 generate targeting constructs mediated by a single Red/ET Recombination step.

The “loxP-PGK-gb2-hygro-loxP template” is not linear but plasmid based (3643bp in size). Due to its R6K origin it can not replicate in most of the *E. coli* strains. The PCR product can therefore be used directly for downstream applications without any further purification.

We recommend growing the recombined cells between 4 and 24h under non-selective conditions before streaking the culture on plates conditioned with 100 µg/ml hygromycin plus the antibiotics for the selective marker of the target vector (e.g. 15 µg/ml chloramphenicol for BAC clones).

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

Map: loxP-PGK-gb2-hygro-loxP cassette

