

### Why pAW8\_Xho?

It has since come to our attention that there has been an oversight while designing the Cre/lox method for gene transfer. In the Cre-expression plasmid pAW8 the first restriction site in the multiple cloning site is SphI (GCATGC). Not only does the site contain an ATG but also forms an ATG at the junction with the loxP site as highlighted:

```

                LoxP           SphI  SacI  Sali  SpeI           LoxM
ATAACTTCGTATAGCATACATTATACGAAGTTATGCATGCGGAGAGCTCGGAGTCGACGAACTAGTATAACTTCGTATATGGTATTATATACGAAGTTAT
I T S Y S I H Y T K L C M R R A R S R R T S I T S Y M V L Y T K L

```

The effect of this is *only* a problem when performing gene transfer where the base strain has the loxP site *directly* upstream of the target gene start codon. The base strain is fine but following cassette exchange the ATGs highlighted above may act as spurious start sites thereby affecting gene transcription.

To get around this problem we have constructed pAW8\_Xho where the SphI site has been replaced by an XhoI site (CTCGAG) and avoids the problems outlined above:

```

                LoxP           Xho  SacI  Sali  SpeI           LoxM
ATAACTTCGTATAGCATACATTATACGAAGTTATCTCGAGGGGAGAGCTCGGAGTCGACGAACTAGTATAACTTCGTATATGGTATTATATACGAAGTTAT
I T S Y S I H Y T K L S R G R A R S R R T S I T S Y M V L Y T K L

```

Please note that XhoI and Sali give compatible overhangs so if you plan to clone your fragment using these two enzymes then you will need to phosphatase the plasmid before ligating the insert.