Strategy for Determining the Activity of a Gene's Promoter

Performed by HC70AL students

Arabidopsis Genomic DNA

↓ **Amplify** the promoter by PCR using High Fidelity DNA Polymerase and a modified FW primer beginning with CACC

Amplified product beginning with *CACC*

Linearized pENTR vector bound by Topoisomerase, also containing GTGG overhang

↓ CACC in PCR product **anneals** to complementary GTGG in pENTR vector

↓ Topoisomerase **seals** the phosphodiester bond and is released from the plasmid

Population of recombinant and non-recombinant plasmid

↓ **Transform** *E. coli* cells using CaCl

↓ **Screen** for colonies containing recombinant plasmid by isolating plasmid DNA and performing a restriction digest and gel electrophoresis

↓ **Sequence** the promoter to make sure there are no mutations

Promoter cloned into pENTR vector

pBGWFS7 T-DNA vector (T-DNA contains toxic gene upstream of *GFP* and *GUS* reporter genes, BastaR gene, and left and right border sequences)

↓ Homologous recombination between the *att* sequences flanking the promoter in pENTR and flanking the toxic gene in pBGWFS7

T-DNA plasmid (T-DNA contains promoter upstream of *GFP* and *GUS* reporter genes, BastaR gene, and left and right border sequences)

↓ **Transform** *E. coli* cells using CaCl

↓ **Screen** for colonies containing recombinant plasmid by isolating plasmid DNA and performing a restriction digest and gel electrophoresis

Promoter cloned into T-DNA plasmid

↓ **Transform** *Agrobacterium tumefaciens* cells containing the Ti Helper plasmid, which encodes genes with T-DNA transfer functions

↓ **Transform** *Arabidopsis* plant with *Agrobacterium* cells containing Ti Helper plasmid and the T-DNA plasmid

↓ **Select** for transformed *Arabidopsis* plants by applying the herbicide Basta

Transformed *Arabidopsis* plants containing the promoter upstream of *GFP* and *GUS* genes