AtetX

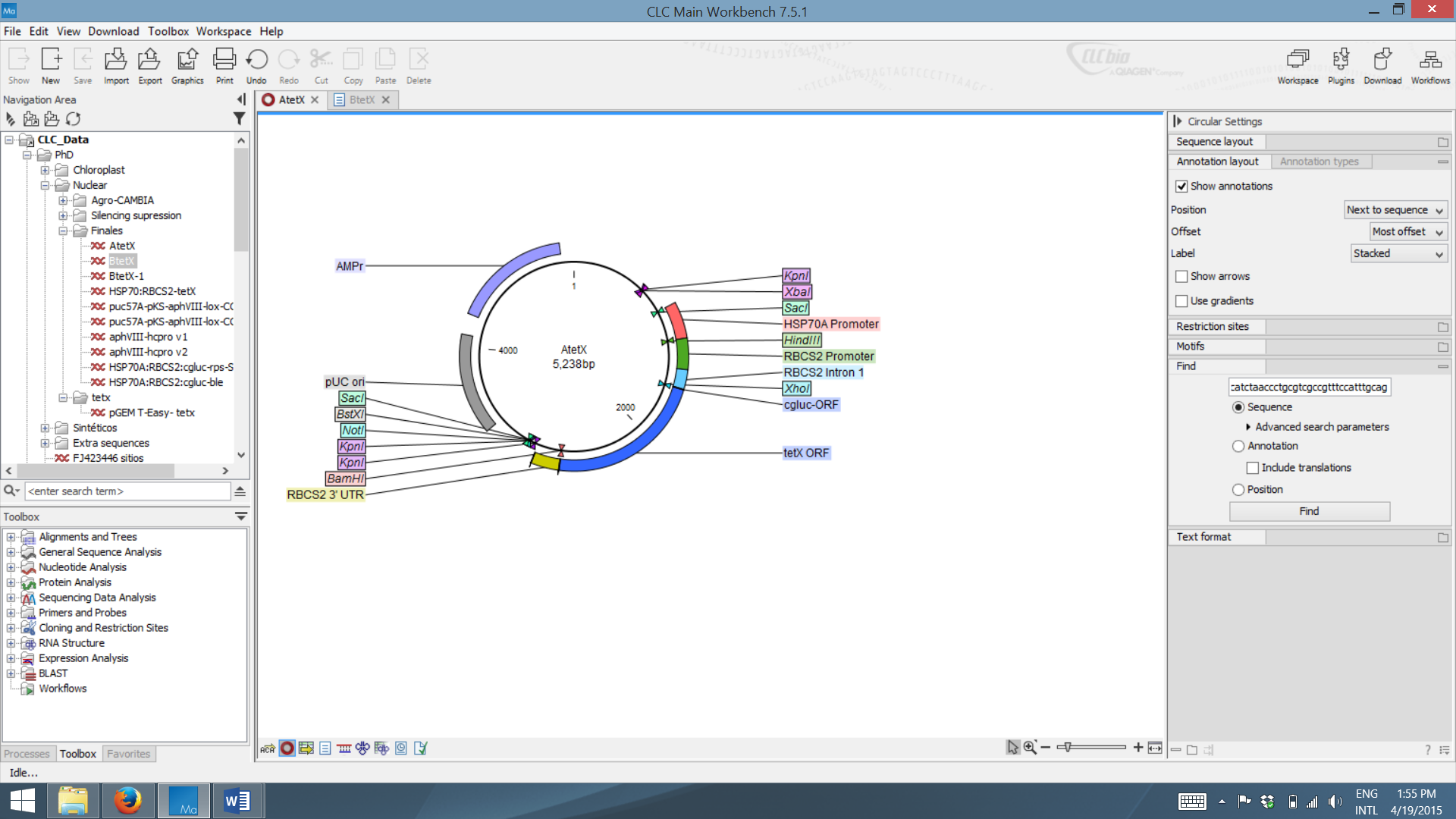
Deposited by Sergio García and Guy Cardineau.

Vector backbone: pHsp70A\_RbcS2-cgLuc (Chlamydomonas resource center). Host strain: DH5α. Resistance: ampicillin

The construct was generated by Polymerase Chain Reaction, amplifying the open reading frame of tetX from the BtetX plasmid (Deposited at the Chlamydomonas resource center) with a proof-reading high fidelity (1 error/100,000 bp = 0.001%) enzyme and oligos that carried *Xho*I and *Bam*HI sites in their 5’ends. The amplicon was digested with *Bam*HI and *Xho*I, and cloned into the corresponding sites of plasmid pHsp70A/RbcS2-cgLuc, replacing the luciferase ORF with that of *tet*X.

Comment:The AtetX plasmidcan be used to select nuclear transformants of *Chlamydomonas reinhardtii*. It has been proven to work in the cell wall deficient strain CC-849, however the wild-type cell-walled strain CC-125 is as sensitive to tetracycline as strain CC-849. The transformants should be selected in in TAP plates containing 15 µg/mL of tetracycline and maintained below a light intensity of 24 µmoles m-2 s-1. Transformed colonies will be visible after 8 days and can resist up to 100 µg/mL of tetracycline.

Plasmid Map



LOCUS AtetX 5238 bp DNA circular

DEFINITION

FEATURES Location/Qualifiers

enhancer 900..1172

/label="HSP70A Promoter"

promoter 1173..1406

/label="RBCS2 Promoter"

intron 1407..1551

/label="RBCS2 Intron 1"

CDS 1553..>1556

/cds\_type=ORF

/label=cgluc-ORF

CDS <1557..>2729

/cds\_type=ORF

/label="tetX ORF"

3'UTR join(2730..2733,2734..2940,complement(2941..2944))

/label="RBCS2 3' UTR"

oriT 3339..4095

/label="pUC ori"

CDS 4253..5130

/label=AMPr

ORIGIN

CACCTGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGCTTACAATTTCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTGTAATACGACTCACTATAGGGCGAATTGGGTACCTCGCGAATGCATCTAGAATCCCCAGCATGCCTGCTATTGTCTTCCCAATCCTCCCCCTTGCTGTCCTGCCCCACCCCACCCCCCAGAATAGAATGACACCTACTCAGACAATGCGATGCAATTTCCTCATTTTATTAGGAAAGGACAGTGGGAGTGGCACCTTCCAGGGTCAAGGAAGGCACGGGGGAGGGGCAAACAACAGATGGCTGGCAACTAGAAGGCACAGTCGAGGCTGATAGCGAGCTCGCTGAGGCTTGACATGATTGGTGCGTATGTTTGTATGAAGCTACAGGACTGATTTGGCGGGCTATGAGGGCGGGGGAAGCTCTGGAAGGGCCGCGATGGGGCGCGCGGCGTCCAGAAGGCGCCATACGGCCCGCTGGCGGCACCCATCCGGTATAAAAGCCCGCGACCCCGAACGGTGACCTCCACTTTCAGCGACAAACGAGCACTTATACATACGCGACTATTCTGCCGCTATACATAACCACTCAGCTAGCTTAAGATCCCATCAAGCTTGCATGCCGGGCGCGCCAGAAGGAGCGCAGCCAAACCAGGATGATGTTTGATGGGGTATTTGAGCACTTGCAACCCTTATCCGGAAGCCCCCTGGCCCACAAAGGCTAGGCGCCAATGCAAGCAGTTCGCATGCAGCCCCTGGAGCGGTGCCCTCCTGATAAACCGGCCAGGGGGCCTATGTTCTTTACTTTTTTACAAGAGAAGTCACTCAACATCTTAAAATGGCCAGGTGAGTCGACGAGCAAGCCCGGCGGATCAGGCAGCGTGCTTGCAGATTTGACTTGCAACGCCCGCATTGTGTCGACGAAGGCTTTTGGCTCCTCTGTCGCTGTCTCAAGCAGCATCTAACCCTGCGTCGCCGTTTCCATTTGCAGGATGCTCGAgATGACCATGCGCATCGACACCGACAAGCAGATGAACCTGCTGTCCGACAAGAACGTGGCGATCATCGGCGGCGGCCCCGTGGGCCTGACCATGGCCAAGCTGCTCCAGCAGAACGGCATCGACGTGTCGGTGTACGAGCGCGACAACGACCGCGAGGCGCGCATCTTCGGCGGCACCCTGGACCTGCACAAGGGCTCCGGCCAGGAGGCCATGAAGAAGGCGGGCCTGCTCCAGACGTACTACGACCTGGCCCTGCCCATGGGCGTGAACATCGCGGACAAGAAGGGCAACATCCTGAGCACCAAGAACGTGAAGCCCGAGAACCGCTTCGACAACCCGGAGATCAACCGCAACGACCTGCGCGCCATCCTGCTGAACAGCCTGGAGAACGACACCGTGATCTGGGACCGCAAGCTGGTGATGCTGGAGCCCGGCAAGAAGAAGTGGACCCTGACGTTCGAGAACAAGCCGTCGGAGACCGCCGACCTGGTGATCCTGGCGAACGGCGGCATGTCCAAGGTGCGCAAGTTCGTGACCGACACGGAGGTGGAGGAGACCGGCACGTTCAACATCCAGGCCGACATCCACCAGCCCGAGATCAACTGCCCGGGCTTCTTCCAGCTGTGCAACGGCAACCGCCTGATGGCGTCCCACCAGGGCAACCTGCTGTTCGCCAACCCCAACAACAACGGCGCGCTGCACTTCGGCATCAGCTTCAAGACCCCGGACGAGTGGAAGAACCAGACGCAGGTGGACTTCCAGAACCGCAACTCCGTGGTGGACTTCCTGCTGAAGGAGTTCAGCGACTGGGACGAGCGCTACAAGGAGCTGATCCACACCACGCTGAGCTTCGTGGGCCTGGCCACCCGCATCTTCCCCCTGGAGAAGCCGTGGAAGTCGAAGCGCCCCCTGCCGATCACGATGATCGGCGACGCCGCGCACCTGATGCCCCCGTTCGCGGGCCAGGGCGTGAACAGCGGCCTGGTGGACGCCCTGATCCTGTCGGACAACCTGGCGGACGGCAAGTTCAACAGCATCGAGGAGGCCGTGAAGAACTACGAGCAGCAGATGTTCATGTACGGCAAGGAGGCGCAGGAGGAGTCGACGCAGAACGAGATCGAGATGTTCAAGCCCGACTTCACCTTCCAGCAGCTGCTGAACGTGTGAGGATCCCCGCTCCGTGTAAATGGAGGCGCTCGTTGATCTGAGCCTTGCCCCCTGACGAACGGCGGTGGATGGAAGATACTGCTCTCAAGTGCTGAAGCGGTAGCTTAGCTCCCCGTTTCGTGCTGATCAGTCTTTTTCAACACGTAAAAAGCGGAGGAGTTTTGCAATTTTGTTGGTTGTAACGATCCTCCGTTGATTTTGGCCTCTTTCTCCATGGGCGGGCTGGGCGTATTTGAAGCGGGTACCCCATAACTTCGTATAATGTATGCTATACGAAGTTATGGTACCGCGGCCGCCACCGCGGTGGAGCTCCAGCTTTTGTTCCCTTTAGTGAGGGTTAATTTCGAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGC