**Source:** Elizabeth Specht, Stephen Mayfield Lab, University of California San Diego

**Plasmid name:** pHR18

**Vector:** pBluescript

**Host strain:** DH5a *E. coli*

**Origin:** Created by seamless cloning of several different fragments; fully sequence-verified.

**Insert:** This construct contains two intact cassettes and one truncated cassette. The first intact cassette is the ARS2 coding sequence, driven by the chimeric HSP70-RBCS2 promoter and followed by its endogenous 3’ UTR; the CDS and 3’ UTR were amplified from cDNA harvested from a strain under sulfur starvation conditions. The second intact cassette is the ARG7 CDS, surrounded by its endogenous promoter/5’ UTR and 3’ UTR, which were amplified from genomic DNA of wild type CC1010 strain. The final, truncated cassette contains the 3’ half of the CDS of hygromycin resistance gene followed by the RBCS2 3’ UTR; upstream of this lies a 1.6kb intron that was amplified from CC1010 genomic DNA.

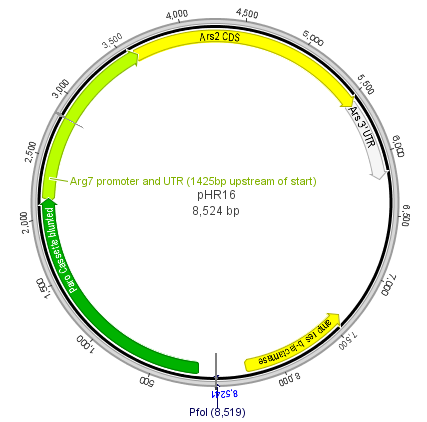
Total insert size is 9,093 bp.

**Selection:** Ampicillin resistant in *E. coli*; restores arginine prototrophy in arg7 *Chlamydomonas* mutants.

**Comment:** This plasmid represents one half of the pair of plasmids designed to detect homologous recombination in *C. reinhardtii.* Upon recombination with its partner, pHR23, it will produce an intact, functional hygromycin resistance cassette. pHR18 is the plasmid that has been transformed into CC-1820 to create the strain B12.

**Reference:** Currently under review for publication*.*

**Plasmid Map:**



**Sequence (see also .gb file with all annotations in the sequence):**

GACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATAGGGCGGCCGCCAGCTGGAATTCATCCCACACACCTGCCCGTCTGCCTGACAGGAAGTGAACGCATGTCGAGGGAGGCCTCACCAATCGTCACACGAGCCCTCGTCAGAAACACGTCTCCGCCACGCTCTCCCTCTCACGGCCGACCCCGCAGCCCTTTTGCCCTTTCCTAGGCCACCGACAGGACCCAGGCGCTCTCAGCATGCCTCAACAACCCGTACTCGTGCCAGCGGTGCCCTTGTGCTGGTGATCGCTTGGAAGCGCATGCGAAGACGAAGGGGCGGAGCAGGCGGCCTGGCTGTTCGAAGGGCTCGCCGCCAGTTCGGGTGCCTTTCTCCACGCGCGCCTCCACACCTACCGATGCGTGAAGGCAGGCAAATGCTCATGTTTGCCCGAACTCGGAGTCCTTAAAAAGCCGCTTCTTGTCGTCGTTCCGAGACATGTTAGCAGATCGCAGTGCCACCTTTCCTGACGCGCTCGGCCCCATATTCGGACGCAATTGTCATTTGTAGCACAATTGGAGCAAATCTGGCGAGGCAGTAGGCTTTTAAGTTGCAAGGCGAGAGAGCAAAGTGGGACGCGGCGTGATTATTGGTATTTACGCGACGGCCCGGCGCGTTAGCGGCCCTTCCCCCAGGCCAGGGACGATTATGTATCAATATTGTTGCGTTCGGGCACTCGTGCGAGGGCTCCTGCGGGCTGGGGAGGGGGATCTGGGAATTGGAGGTACGACCGAGATGGCTTGCTCGGGGGGAGGTTTCCTCGCCGAGCAAGCCAGGGTTAGGTGTTGCGCTCTTGACTCGTTGTGCATTCTAGGACCCCACTGCTACTCACAACAAGCCAAAATGGACGATGCGTTGCGTGCACTGCGGGGTCGGTATCCCGGTTGTGAGTGGGTTGTTGTGGAGGATGGGGCCTCGGGGGCTGGTGTTTATCGGCTTCGGGGTGGTGGGCGGGAGTTGTTTGTCAAGGTGGCAGCTCTGGGGGCCGGGGTGGGCTTGTTGGGTGAGGCTGAGCGGCTGGTGTGGTTGGCGGAGGTGGGGATTCCCGTACCTCGTGTTGTGGAGGGTGGTGGGGACGAGAGGGTCGCCTGGTTGGTCACCGAAGCGGTTCCGGGGCGTCCGGCCAGTGCGCGGTGGCCGCGGGAGCAGCGGCTGGACGTGGCGGTGGCGCTCGCGGGGCTCGCTCGTTCGCTGCACGCGCTGGACTGGGAGCGGTGTCCGTTCGATCGCAGTCTCGCGGTGACGGTGCCGCAGGCGGCCCGTGCTGTCGCTGAAGGGAGCGTCGACTTGGAGGATCTGGACGAGGAGCGGAAGGGGTGGTCGGGGGAGCGGCTTCTCGCCGAGCTGGAGCGGACTCGGCCTGCGGACGAGGATCTGGCGGTTTGCCACGGTCACCTGTGCCCGGACAACGTGCTGCTCGACCCTCGTACCTGCGAGGTGACCGGGCTGATCGACGTGGGGCGGGTCGGCCGTGCGGACCGGCACTCCGATCTCGCGCTGGTGCTGCGCGAGCTGGCCCACGAGGAGGACCCGTGGTTCGGGCCGGAGTGTTCCGCGGCGTTCCTGCGGGAGTACGGGCGCGGGTGGGATGGGGCGGTATCGGAGGAAAAGCTGGCGTTTTACCGGCTGTTGGACGAGTTCTTCTGAGCGTTCTGGCAGCAGCTGGACCGCCTGTACCATGGAGAAGAGCTTTACTTGCCGGGATGGCCGATTTCGCTGATTGATACGGGATCGGAGCTCGGAGGCTTTCGCGCTAGGGGCTAGGCGAAGGGCAGTGGTGACCAGGGTCGGTGTGGGGTCGGCCCACGGTCAATTAGCCACAGGAGGATCAGGGGGAGGTAGGCACGTCGACTTGGTTTGCGACCCCGCAGTTTTGGCGGACGTGCTGTTGTAGATGTTAGCGTGTGCGTGAGCCAGTGGCCAACGTGCCACACCCATTGAGAAGACCAACCAACTTACTGGCAATATCTGCCAATGCCATACTGCATGTAATGGCCAGGCCATGTGAGAGTTTGCCGTGCCTGGAATTCGCTGAGGGTTTAATGTCGACGAGGAGGAGGTGCAAGGGGGATACCAGCGCGTGTTTCTCAGGGCCTGTGTGGGACACCGAAACGTGGTAAAAGAGACCCGCCCGCGAACTGTGTATGTGGAGTAGCGTGGCGTGTGCGGCCGGACCGACAAGGCAGCTTGTGGACTGCCCCACGTTGCAGAGTCAGCTGACAACGACACGTGCGCCTTCCTGTCATTGCCCGTGCGCACGCACGTCCTCCGCACTCCCAACAAATTGACAGCGACACGTGCGCCTTCCTATAAGCCTATGCCCGCACACGCTCCCGCGCCCTCAGGTGTCGGGCCAGACCACAGACCGGTTGGTCCACGAGTGCGAGGAGGATGAGGCGGGCGGCTGCGGCGGCGCCGGCGGGGCGGCGGGCGGCGAGGAGGACGGCCTGGGACTGGGCATCACAGGTGGGTGGCAGGCTGGCAGGGACTCACGCATGGGCCTTGTACGTGACTGCGGTTCTGCATGGCTAGTGGCTCACGCGCTGCGCACGTTCACGTACGGCTTGTGGGCATGCAGTGCCTTGACGTGAGGCTGCGCTGCCTTGCTGCTGCCGCCTTGCCCCGCTCCCTGCACACACTGCAGCCGGCTTCGGGCGCTACTTCACCGCGGGCTACGAGTGCGAGAACGCGCAGCAGCTCAACAGGCTGCTGGGGTACAAGGCGCTGTGAGAGCGCGCCGCAGGGGGAGTGTGTTCATATTGTGGTTGTTTGGGCCGTGGGCGCGGGCTGCATGTGCGTATTGCACGCGTACAGCATTGGTGACTGGTCAGGTGTAAGCGGCCGGCAGTGCGCCGCGAGGCGCTGCAGCGAGTTGTGGGGCATGCGTCATGCGCAGACGGCCCCTGGACGACAAGGCGTTGAGTTGGCGTTTGGAGGTGTGGGACGACGTGGGGTTTGTGCCGTCAAAGCACAGAACAGAAGGCGTGACCGTTTTACGAGCTCGTATGATGTAGCATGGATTGAATAATGACATGTGATTTTTGTTACAAGCGACGAATGCGTGGGGTTTTGGATGGCAGGGGTTTCAGTCGCCCGATTGCGCATGCACACGTGACCAAATTTATGCTCAACGACGTGACCATTGCTTTATACATACTTGTGTATCGGTTGGCACTTATAACAATTGGCTCGTCAAATTGACGCGAGGCTGCACTTCGATCCTGAAAGCCCCAGTTCAACAAGTCGGATAGCCAAATGGCCCCGCTCGCTCTCCAGCATCAAGGGGCCTCTAAGTGCCTCGCGGCAACCCAGCGCAAGTGTGCTCGCGTTGCGGTGAGCTGGACTCGTGCACTTGTCGACGCCGTCGGCACCGCAATCGAAAGACGCGTGCGTCGAGCAATTGTGGAAGCCGCTGACGAATTGTCCGCATGTGACATTGCAGGCTCGCGTCCCCGCTCGTCTCAGCGTCATGGGTGCCCTCGCGGTGTTCGCCGTCGCTTGCCTCGCGGCAGTGGCGTCGGTTGCGCATGCGGCCGACACCAAAAAGCCCAACTTTGTGGTGATCTTCACCGATGACCAGGACGCCATTCAGAACAGCACCCACCCGCACTACATGCCCAGCCTGCACAAGTACATCCGCTACCCGGGAGTGGAGCTGTCTCAGTACTTCGTCACCACCCCCGTGTGCTGCCCCTCGCGGACAAACCTGTGGCGCGGCCAGTTCGCCCACAACACCAACTTCACCAGCGTGCTGCCTCCCTACGGTGGCTGGGCCAAGTGGAAGGGCCTGGGCATCGACCAGTCCTACCTGCCGCTGTGGCTCAAGGACCAAGGCTATAACACCTACTACGTGGGCAAGTTCCTTGTGGACTACTCGGTCAGCAACTACCAGCAGGTGCCCGCGGGCTGGGACGACATCGATGCCCTGGTCACCCCCTACACCTTTGACTACAACACCCCCGGCTTCAGCCGCAACGGCGCGACCCCCAACATCTACCCCGGCGAGTACAGCACTGACGTCATTCGCGACAAGGGCATTGCTCAGATCAAGTCGGCCGTGGCTGCCGGAAAGCCCTTCTACGCGCAGATCTCGCCCATCGCGCCGCACACCTCCACCCAGATTTCCACCGACCCCGTCACCGGAGTGACGAGGTCCTTCTTCTACCCGCCCATCCCCGCCCCCCGCCACTGGCAGCTGTTCTCCGACGCCAACCTGCCCGGCGGCACGCCCAACAAGAACCTGTACGAGGTGGACGTGAGCGACAAGCCCGCCTGGGTCCGCGCCCTGCCGCTGGCCCAGCAGAACAACCGCACCTACCTGGAGGAGATCTACCGCCTGCGCCTGAGGTCGCTGGCGGCCGTGGACGAGCTGATTGAGCAAGTCGTCAAGACCCTGGATGAGGCGGGTGTGCTTGACAACACCTACATCATCTACAGCGCTGACAACGGCTACCACGTGGGTGCCCACCGCTTCGGCGCGGGCAAGACCACGGGCTATGAGGAGGACCTGCGTGTGCCCTTCCTCATCCGCGGCCCAGGCATCAAGGCCAGCCAGTCCGACAAGCCGCAGAACAGCAAGGTTGGCCTGCACGTGGACTTTGCGCCCACCATTCTCAGCCTGGCCGGCGCCTCGCACCTGCTCGGGGACAAGGGGCTGGACGGCACCCCGCTGGGCCTGTACGCCAACGACGACGGCACTCTTCGCTCCGACTACCCTCGTCCGGAGCAGCACCGCCAGCAGTTCCAGGGCGAGTTCTGGGGCGGCTGGAGTGATGAGCTGCTGCAGAACCTCAGGTCCCAGCCCAACAACACTTGGAAGGTGGTGCGCACGTATGACGAGAGCAGCAAGCAGGGATGGAAGCTCATCGCGCAGTGCACCAACGAGCGCGAGCTGTACGACCTGCGCAAGGACCCCGGTGAGCTGTACAACATCTACGACAAGGCCAAGCCCGCCGTGCGCAGCCGCCTGGAGGGGCTGCTGGCGGTGCTGGCCGTGTGCAAGGGGGAGAGCTGCTCCAACCCGTGGAAGATCCTGCACCCCGACGGCACCGTCAAGAACTTCACCCAGGCACTCAACTCCAAGTACGACCGCATCTACAACGCAATCCGCCCCTTCACCTACAAGACGTGCCTGCAGTACCTGGATTGGGACAACGAGGACAGTCAGTTTAAGACGCAGATCCGCGGCGCCAACCCCGCAGCCGGCGTGGGCCACCACCGCCTGCTCACCGCCGCCAGCGAGCGCGCCATCGCCACCCGCCGCCGCGCCCAGGCCGCCGTCAGTGCCGAGCTGGCGGAGCGGCCGGCTGTGTTCCAGGCAAAGGTCGAGGAGAAGTCGGTGCCGGTGCCCCAGGACATCCTGAAGGCCGACGTGGAGAAGTGGTTCGCCTTCAACAATGCCGAGTACTACCTGGCTTAGATGGTCGATATTATATAAAAGCCAATGCAAGCGCGCATGGACATAGCGCATCGACCAAGCGCCACCATGGCTTGGGTTTCTTTGATACGGTTGGGCTAAGTTTGATATGTGGGTTTTGGACGTGGCCGCTTGGTCAGTAAGCGGTCCACGTGGTAATGCCGTGTGCGTGATCCCCCCTGGAGTGGTGTTGGGGTAGGTTAATGACAAGGTAAAGCAGTGGGTACATGCACGCACAATTGCGTCGGACAGAAGAGTACCGGGACGTGATCCATGAAGAAATGGTATAAGGCGCCTCATGCATCCGTAGATGGCGCTCACGTGCGCTTAATTGCATGCGCGCCGTCACTTGTTTGTTGATTGCGGAATTAAGTGGTTAGGCCACTTGGTTGCGAAGAGTGTTGTGCGCCGCCCTCGGTAGTTCGGTGCGCCGCTGGAAACTTGCGTTGGTGTTCTGAGCTGCGGAGCTCTGGTTGGTCACTTGGTCTGCTGTTGTGCCTGTATGTTAAGAGGTGCTGGGTAAAGAAGTGGGCTTGCGTGGATGTTGACTGGCTGGCAGATAGGACTGTGCAGCGGCCTTGCTGCCGCGTGGTAAAGACTGAGAAAGGTATGTACCCGGCGTGGTGCCATGGAGCCATGGAACGAAGCATTAAGACCTCAGCCTGCAGAGTACTGCGGCCGCGAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGA