Strain

Enclosed are the strains listed below (it is required that the plasmids you requested be shipped separately; however, both shipments were sent on the same day and you should receive them at about the same time):

Please note - all of these recombinogenic bacterial strains should be grown at 32° C because of the temperature-inducible prophage - see protocols on our website for details. Upon arrival you should immediately store the glycerol stocks at -70° C. Cultures can then be initiated directly from the glycerol stocks—there is no need to streak the bacteria on a plate before use.

(Yellow capped vial) SW102: a modified DH10B strain containing the defective lambda prophage;DH10B[*l cl*857(*cro-bio*A)<>*tet*]. In addition this strain contains a fully functional *gal* operon, except for a deletion of *galK*. This feature allows for efficient BAC modification using *galK* positive/negative selection. Derived from the strain DY380. This strain is tetracycline resistant (12.5 ug/ml).

(Red capped vial) SW105: a modified DH10B strain containing the defective lambda prophage and an arabinose-inducible *flpe* gene; DH10B[*l cl*857(*cro-bio*A)<>*ara*C-P_{BAD}*flpe*]. In addition this strain contains a fully functional *gal* operon, except for a deletion of *galK*. This feature allows for efficient BAC modification using *galK* positive/negative selection. Derived from the strain EL250. This strain carries no antibiotic resistance.

(Green capped vial)) SW106: a modified DH10B strain containing the defective lambda prophage and an arabinose-inducible *cre* gene; DH10B[*l cl*857(*cro-bio*A)<>*ara*C- P_{BAD}*cre*]. In addition this strain contains a fully functional *gal* operon, except for a deletion of *galK*. This feature allows for efficient BAC modification using *galK* positive/negative selection. Derived from the strain EL350. This strain carries no antibiotic resistance.

(Blue capped vial) positive contol: SW102 bacteria containing a BAC vector with the galK expression cassette (cloned into the BamHI and HindIII sites of pBeloBAC11).

Please refer to our web site, http://recombineering.ncifcrf.gov, for recombineering protocols.

Plasmid

Enclosed are the plasmids that you requested, all plasmids are at a concentration of ~0.2 ug/ul, **(it is required that the recombinogenic bacterial strains be shipped separately; however, both shipments were sent on the same day and you should receive them at about the same time)**:

pIGCN21: a vector containing the *IRES-eGFPcre-FRT-kan-FRT* cassette
pELO4: a vector containing the *Cam-sac*B cassette
pTamp: a vector containing an Amp cassette for replacing the *lox*P site in pBeloBAC11. Please note that this vector cannot be used to replace either of the two *lox*P sites in the pBACe3.6 backbone.

PL451: *FRT-PGK-EM7-NeobpA-FRT-loxP*
PL452: *loxP-PGK-EM7-NeobpA-loxP*
PL253: Modified *MC1TK*

p*galK*: a vector containing an em7-driven *galK* cassette for use in *galK*-based BAC modification using positive/negative selection

Note: We recommend you store the DNAs at -20° C.

Please refer to our web site, http://recombineering.ncifcrf.gov, for sequences of the enclosed plasmids. If you select "Plasmids" from the box on the left hand side of the opening screen it will take you to a screen with the maps, sequences, and annotated sequences which can be downloaded.

Color Key for Plasmid Tubes

Violet Tube – pIGCN21
Green Tube – pEL04
Yellow Tube – pTamp
White Tube – PL451
Blue Tube – PL452
Orange Tube – PL253
Brown Tube - pgalK

Note)

- Please note that the strains DY380, EL250, and EL350 have been replaced by the new strains SW102, SW105, and SW106, respectively. The new strains are identical to the old ones, except they have been modified to also allow for efficient BAC modification using our new galK positive/negative selection procedure. Please refer to the web site for details: **http://recombineering.ncifcrf.gov/**

- In order to facilitate the implementation of the galK selection system in your lab, we have now started to include an (unpublished) positive control in our package. This control is SW102 bacteria containing a BAC vector with the galK expression cassette (cloned into the BamHI and HindIII sites of pBeloBAC11).

    1. These bacteria can be used to test your Mac+gal+cm plates (the positive control is cm resistant and the colonies will be bright pink, since they are gal^+)
    2. These bacteria can also be used to test your minimal media plates (M63+bio+gal+cm+leu), since they will be able to grow on these plates, whereas SW102 with any other unmodified BAC will not. Make an overnight culture (5 ml LB+cm), wash 1 ml twice in 1xM9 salts, as described in the galK protocol. After the final wash, resuspend in 1 ml 1xM9 salts and make serial dilutions (1:10, 1:100, 1:1000, and 1:10,000), by transfering 100 microliter to a series of 900 microliter 1xM9. Finally plate 100 microliter of the 1:1000 and the 1:10,000 dilutions. If your plates are o.k., colonies will appear after 2-3 days incubation at 32 degrees.
    3. If plated on your negative selection plates (M63+DOG+bio+leu+cm), some colonies will also appear do to deletions of the galK cassette (background)
    4. Of course, this positive control can be used to do a simple BAC recombineering experiment like substituting the galK cassette for a double-stranded oligo.