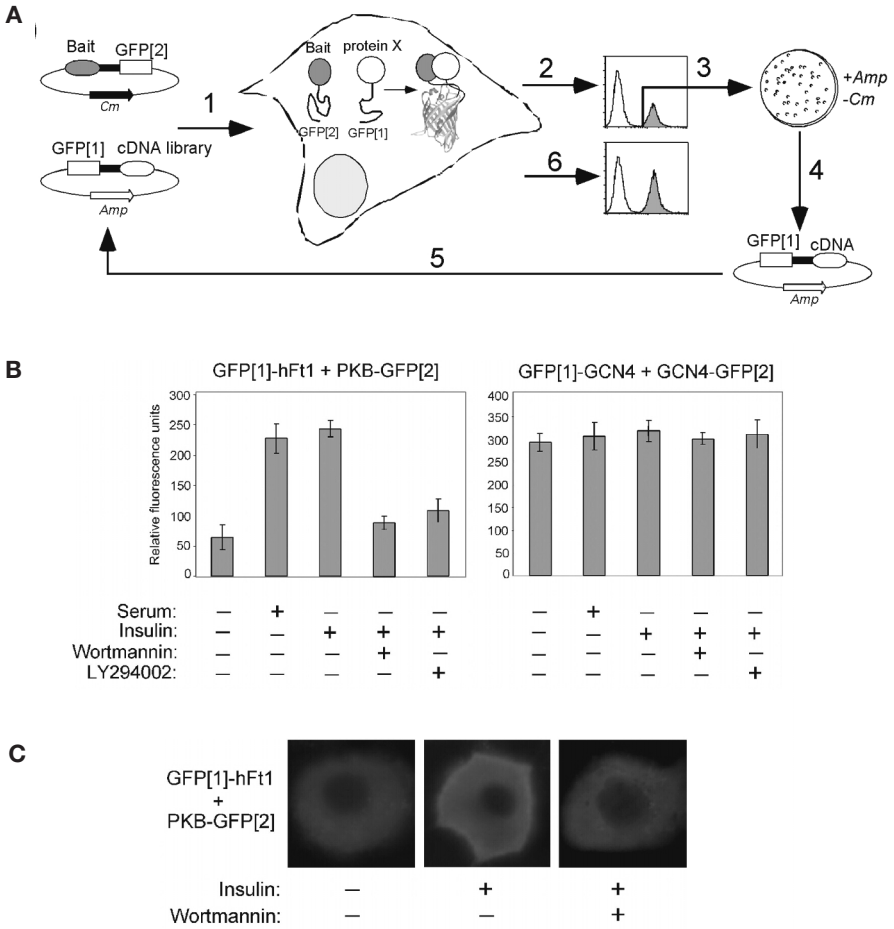

FIGURE 4. The GFP PCA-based library screening strategy. (*A*) A cDNA library is fused to fragment 1 of GFP (GFP[1]-cDNA library) and the full-length bait cDNA to fragment 2 (Bait-GFP[2]), in mammalian expression vectors, harboring *E. coli* selection markers Ampicillin (Amp) and Chloramphenicol (Cm), respectively. In the first step (step 1), COS-1 cells are co-transfected with the plasmids encoding the "bait" and cDNA library "prey" fusions, and a physical interaction between the bait and a prey protein induces the folding and reconstitution of GFP from its fragments, generating fluorescence. Positive clones are collected by FACS (step 2), DNA is extracted from the pools, used to transform *E. coli* which is then grown on plates containing ampicillin to select only for plasmids harboring cDNA (step 3). Clones are picked, plasmids extracted, and interaction of individual proteins with the bait reconfirmed by co-transfecting COS-1 cells with the plasmids encoding bait fusion and individual cDNA fusions (steps 5 and 1) and detection by FACS (step 6). (*B*) Biological validation of a newly identified protein–protein interaction with the GFP PCA. The pharmacological modulation of the interaction between hFt1 (human "fused toes" protein-1) and PKB proteins is shown as an example. HEK293T cells expressing the GFP[1]-hFt1 and PKB-GFP[2] fusions were pretreated with wortmannin or LY294002 and stimulated with serum or insulin. The relative amount of reconstituted GFP, a measure of the interaction between the fused protein partners, was detected by fluorometric analysis in intact cells. The dimerization of GCN4 leucine zipper was used as a control to ensure that cell treatments do not alter protein–protein interactions in a nonspecific way. Fluorescence intensity is given in relative fluorescence units (y axis). (*C*) To determine the cellular location of the PKB/hFt1 protein complex, HEK293T cells were co-transfected with GFP[1]-hFt1 and PKB-GFP[2] fusions and treated with insulin or wortmannin. Fluorescence microscopy was performed on live cells.

Protein–Protein Interactions: A Molecular Cloning *Manual*, 2nd Ed., © 2005 by Cold Spring Harbor Laboratory Press, Chapter 33, Figure 4.