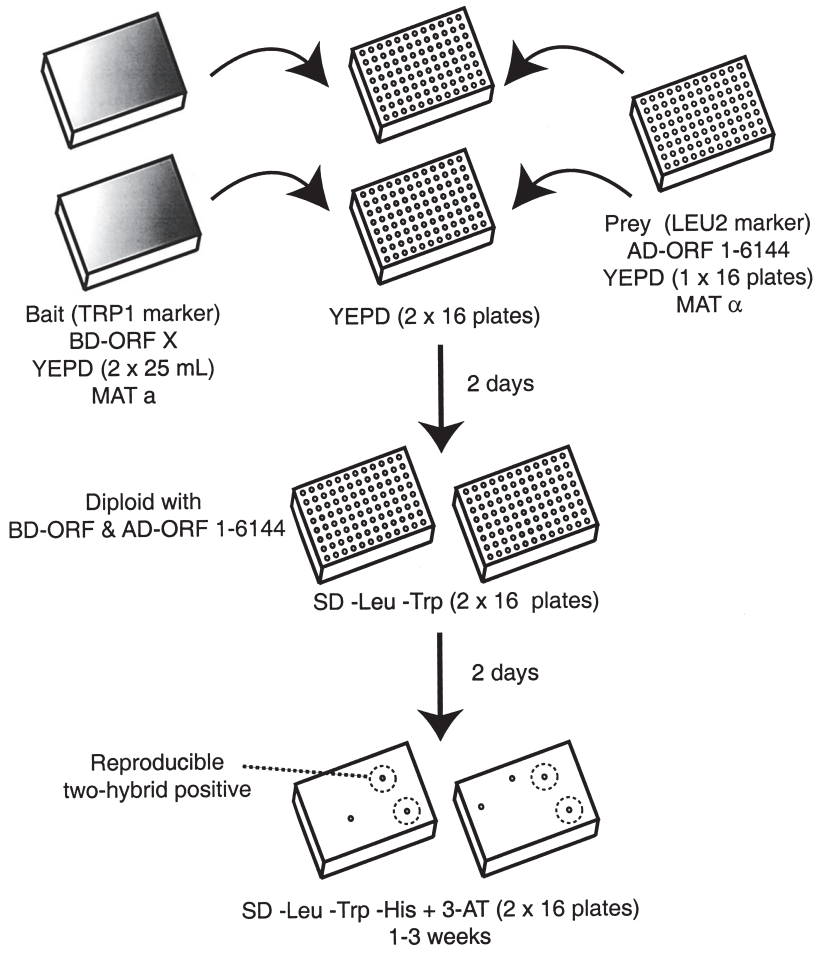

FIGURE 5. Flowchart depicting the array-based screening procedure outlined in Protocol 3. Each replicating step is achieved by using a high-density replicating tool. The figure depicts 96-spot format plates, although we use 384-spot plates to represent the yeast genome. The screens are performed in duplicate starting with two transformants of the identical bait strain cultured in yeast extract-peptone-dextrose (YEPD) liquid media. The bait strain harbors a plasmid with the *TRP1* marker, and the prey strain harbors a plasmid with the *LEU2* marker. The haploid bait and prey strains are overlaid together on solid YEPD media to allow them to mate. The resulting diploids are then selected on SD medium lacking leucine and tryptophan (SD –Leu –Trp) (diploids will have both plasmids and will therefore survive in the absence of those nutrients). Selection of two-hybrid positives is performed on SD lacking leucine, tryptophan, and histidine (SD –Leu –Trp –His) supplemented with 3-aminotriazole (3-AT). The positives that appear on both plates of the duplicate screen are considered reproducible positives, whereas the single positives usually do not reproduce upon retesting.

Protein–Protein Interactions: A Molecular Cloning *Manual*, 2nd Ed., © 2005 by Cold Spring Harbor Laboratory Press, Chapter 37, Figure 5.