Triplex affinity capture of a single copy clone from a yeast genomic library

Takashi Ito, Cassandra L. Smith* and Charles R. Cantor

Department of Molecular and Cell Biology, University of California and Division of Chemical Biodynamics, Lawrence Berkeley Laboratory, Berkeley, CA 94720, USA

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Library screening methods based on nucleotide sequences usually employ *in situ* filter hybridization to colonies and plaques (1). These methods are useful and reliable, but involve labor-intensive steps like filter preparation and manipulation and the re-isolation of positive clones from the original bacterial plates. Triplex affinity capture (TAC) is an alternative screening method based on direct DNA isolation (2). TAC was used to isolate microsatellite clones from a human genomic library as potentially useful DNA markers for genetic linkage mapping (2). In TAC, plasmid DNA prepared *en masse* from a library is incubated with a biotinylated homopyrimidine oligonucleotide probe under mildly acidic conditions to form an intermolecular triplex between the target DNA and the probe. Subsequently, streptavidin-coated magnetic beads are used to capture this complex and separate it from bulk DNA. Double-stranded target DNA is recovered by incubating the beads under mildly alkaline conditions that selectively destabilize the triple helix but not the Watson-Crick double helix. Since TAC is performed in several small tubes, large numbers of clones or several libraries may be screened simultaneously. TAC yields intact, double-stranded DNA that can readily be used for enzyme digestion, electrophoresis or transformation.

Here we demonstrate the utility of TAC to isolate a single copy clone from a yeast genomic library. Genomic DNA from a *Saccharomyces cerevisiae* strain containing a triplex-forming site integrated at the *LEU2* locus (3), was digested with *Bgl*III and cloned into the *Bam*HI site of pTZ19RG vector, a variant of pTZ19R containing rare-cutter sites (T.I., unpublished). Plasmid DNA (~2 µg) from the library was isolated, digested with *Not*I and incubated with 10 pmol of 5'-biotinylated CT₂CT₄CT₂C-T₃CT₅CT₂ in 2 M NaCl, 0.1 M sodium phosphate buffer (pH 6.0) for 2 hr at 50°C. Then, ~0.2 mg of streptavidin-coated magnetic beads (DynaL Streptavidin M-280) were added, collected by a magnet after 1 hour of incubation and washed extensively with the same buffer. Captured DNAs were released by incubation in 1 M Tris-HCl (pH 9), 0.5 mM EDTA for 20 min, recovered by ethanol precipitation, recircularized and used for transformation of competent DH5α cells. Plasmid DNA isolated from the cultures was analyzed by gel electrophoresis. One purification cycle at pH 6.0 yielded an enriched library with a complex electrophoretic pattern, although some bands were significantly enriched compared to the original library. A second TAC cycle at more stringent condition (pH 6.2) provided a simple population composed of only four DNA species, named A-D

according to their sizes. Analysis of twelve randomly chosen clones showed that they contained two A, six B, two C and two D. Restriction mapping and PCR using primers specific to the *LEU2* locus of this strain showed that clone B was the desired target. Other clones (A, C and D) may be derived from pseudo-target sites in this strain, as suggested by previous affinity cleaving experiments (3). An additional cycle of TAC where all steps were performed at 50°C distinguished the target clone from the pseudo-clones, although the yield was low. Conditions for a single step purification of B from the total library were not identified.

TAC is clearly useful for the isolation of triplex-forming single copy sequences from yeast, and presumably for the effective enrichment of equivalent single copy sequences from more complex genomes using appropriate probes (for a discussion of probe length see ref. 4). The use of G and a novel artificial base analog (5) in the third strand has broadened the triplex recognition capability to allow one to find a target for TAC in natural, non-tagged sequences with much ease. Furthermore, the use of RecA protein dependent triplex formation (see 6-8) has the potential to extend this purification technique to any DNA sequence.

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* To whom correspondence should be addressed