

SCREENING OF A BAC LIBRARY OF *CITRUS SINENSIS* BY MEANS OF OVERGO PROBES FOR THE IDENTIFICATION OF CLONES CONTAINING GENE SEQUENCES WITH RELEVANT ROLES IN FRUIT QUALITY

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Compared with the most commonly used gene-specific hybridization probes (subcloned DNA fragments, PCR amplicon products or DNA oligonucleotides), the overgo hybridization method (Ross et al., 1999) has several advantages (better hybridization kinetics, higher specificity labeling, low background of hybridization, low rate of false positives, high throughput, and ease of handling). The foundation of these advantages is the short sequence that is needed for an overgo probe, which increases the likelihood of finding suitable single copy regions from EST sequences or unigenes. Overgo probes are designed to 36/40-bp regions of cDNA that have been prescreened to mask out all known repeat elements. This assumes the availability of a comprehensive repeat element database that allows repeat masking of the target sequence. HarvEST:Citrus (<http://harvest.ucr.edu/>), an EST database-viewing software developed at the University of California – Riverside, displays 89 libraries and 229,570 ESTs from Citrus and Poncirus. We used the C38 assembly (36,980 unigenes) and the OligoSpawn software (<http://138.23.191.145/>) to design the overgo probes employed to screen a *Citrus sinensis* 'Vaniglia' BAC library. OligoSpawn software was used to select 22,444 "unique" oligos (36-bp oligonucleotides each of which appears in one unigene but does not occur, exactly or approximately, in any other) that were filtered against a repeat database (<http://int-citrusgenomics.org/usa/ucr/Files.php>). For 8,786 unigenes, no unique oligos are found, either because the C38 assembly occasionally places alternate alleles in different unigenes, or because very similar paralogs occur in the citrus genome. In these cases OligoSpawn software selected 36-bp oligonucleotides which occur in more than one putative unigene, resulting in a total of 31,230 unigenes covered by overgo probes. For BAC library screening we used 89 overgo probes associated with unigenes that putatively code for structural enzymes of the selected pathways (flavonol, anthocyanin, carotenoid, chlorophyll, cellulose, starch, ascorbic acid, aromatic amino acid and lignin biosynthesis; sucrose catabolism; glycolysis; oxidative/nonoxidative pentose phosphate pathway; fatty acid biosynthesis and oxidation; Krebs cycle). In this paper, we describe the methods and we report on experimental results for the overgo probes designed.

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