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A COMBINED STRATEGY FOR BAC EXTENSION: APPLICATIONS TO THE SEQUENCING OF TOMATO CHROMOSOME 12

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In the frame of the International Tomato Sequencing Project, the first seed BACs of chromosome 12 have been sequenced and submitted to the Solanaceae (SOL) Genomics Network. A preliminary annotation was derived from the Italian bioinformatic platform for the Tomato Genome (Chiusano, SIGA 2006). In order to choose the right clones for extending these seed BACs, a combined computational and experimental strategy was undertaken.

Considering EST-genome alignments, potential gene tags were located on these sequenced seed BACs. Indeed, in some cases partial matching at the ends of the BACs with tentative *consensus* sequences from tomato and potato cluster of ESTs (D'Agostino, SIGA 2006) were considered to support BAC selection for further sequencing.

In particular, at the 3' end of the seed BAC Le_HBa0032K07 4 tomato ESTs belonging to a 3' end of a tentative consensus made of 24 sequences supported the design of primer pairs exploiting the consensus sequence landing out of the BAC. The primers were used to check the presence of the gene, putatively encoding a serine-threonine kinase, into the tomato genome, by amplifying both genomic DNA from *Solanum lycopersicum* and *S. pennellii* and cDNA from leaf tissue. The sequencing of the cDNA fragment and its alignment with the *consensus* sequence confirmed the reliability of the computationally defined tentative *consensus* and the expression of the gene.

The same primer pairs were then used for PCR experiments to detect four putative extending BACs, previously selected from the SGN BAC ends database. The amplification of expected sized fragments, and their sequencing results allowed one BAC to be selected as the best one to extend the contig, thus evidencing the success of the proposed strategy.

The same approach is being used to extend other seed BACs.