



We also have been involved in the improvement steps of this assembly by filling gaps with BAC sequences and with alternative assemblies. We also built Superscaffolds using clone-end information (BAC and fosmid ends).

We also participate to the generation of the official annotation for the tomato genome provided by the International Tomato Annotation Group (ITAG), a multinational consortium, funded in part by the [EU-SOL project](#).

## Funding

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[See the INRA press release](#).

## The initial approach description

The [International Tomato Genome Sequencing Project](#) aimed in a first approach to sequence the gene-rich euchromatic portions of the twelve tomato chromosomes. This International program involves 12 different countries among which France is devoted to the sequencing of chromosome 7. With an estimated length of 27 Mbases of gene-dense euchromatin, the coverage of chromosome 7 is expected to require the sequencing of more than 250 BACs. The strategy is to identify and sequence a minimal tiling path of BAC clones through this approximately 27 Mb euchromatin.

### ***Seed BACs selection using FISH and ILs mapping***

The initial pool of seed BACs provided by Cornell (USA) was enlarged with new putative seed BACs whose location on chromosome 7 was verified by two methods before proceeding to the sequencing step (performed by Cogenics, Grenoble, France) :

- \* Screening of the multi-species [introgression lines](#) (ILs) for BAC-end sequence polymorphism.
- \* BAC-FISH technology on mitotic chromosomes (INRA Rennes) or on pachyten meiotic chromosomes (Wageningen).

### ***DNA pools and Macroarray filters generation***

In order to increase our capacity to select positive clones for the sequencing pipe, the following new tools were generated:

- \* Macroarray filters were generated by the French Plant Genomic Resource Centre ([CNRGV](#), INRA-Toulouse) for all the 3 BAC libraries available : *HindIII*, *Mbol* and *EcoRI* and half of the fosmid library (Giovannoni, Cornell - USA).
- \* Three-dimensional DNA pools (3D-pools) were produced in collaboration with the CNRGV. The *HindIII* 3D-pools, made of half of the *HindIII* library, corresponds to 7.8 x coverage of the tomato genome while the *Mbol* 3-D pools are made with the entire *Mbol* library, corresponding to 7.5 x genome equivalent. This lay-out allows the screening of *HindIII* and *Mbol* libraries respectively with 448 and 384 PCR reactions. The 3D-pools have been tested and validated by PCR screening using specific primers for chromosome 7 markers (seed BAC selection) or designed from the BAC-End Sequences (overlapping BAC selection). The results confirmed the high sensitivity and specificity of the method. These tools are now available for all the partners of the Multinational Tomato Genome Sequencing Project and can be ordered at the [CNRGV](#).