



In this Issue

In this issue you will find brief status reports from each group participating in the tomato genome sequencing effort, items related to SGN and bioinformatics, and a new section called Community News. The purpose of this new section

is to provide groups doing research on the various Solanaceous crops with the opportunity to post information. Information can be sent by e-mail to Joyce Van Eck at jv27@cornell.edu.



Tomato Sequencing Updates



Chromosomes 1, 10, 11 (US)

The first 75,000 BACs were sent for end sequencing. The sequencing company has currently submitted 43,000 chromatograms from 21,500 clones. In addition, 2,000 random sheared genomic clones have also been generated and are in the process of being sequenced. This will provide a snapshot of the nature of euchromatic versus heterochromatic regions of the genome, including information on the nature of repeat sequences, through comparison with BAC ends.

The past several months have been spent training people who will be involved in FISH and purchasing equipment that includes a new Leica UV/phase microscope to use for FISH. Song-Bin Chang will join Steve Stack's lab at Colorado State University as a post-doctoral fellow in January. Song-Bin has done extensive work on tomato FISH in Hans de Jong's laboratory in Wageningen.

Chromosome 2 (Korea)

We received 520 BACs anchored to 60 markers on chromosome 2. We are currently confirming that the BACs are located on chromosome 2 using overgo probe hybridization and PCR. We have already finished confirmation of 38 BACs. In addition, we also did FISH using 5 BACs, which are currently being sequenced.

There have been some difficulties encountered during the overgo probe hybridization and FISH. For the overgo probe hybridization, some BACs didn't show any band or showed different band patterns among the members of BACs. As for FISH using the 5 BACs, one BAC hybridized to all chromosomes, and could possibly have repetitive sequences. We are working with the Tanksley group to determine how to proceed on these situations.

Chromosome 3 (China)

Using seed BACs from chromosome 3 provided by the Tanksley Laboratory, we have initiated a FISH mapping effort in order to: 1) Confirm the physical chromosome location of each of the genetically anchored seed BACs, and 2) Position the most distal and most proximal mapped BACs on chromosome 3. This work will help to estimate the amount of euchromatic DNA that needs to be sequenced in each interval and to estimate the distance to the telomere and centromeric heterochromatin. It turned out that most of the seed BACs were clustered on the distal long arm, with only one BAC positioned on the short arm among the 40 seed BACs tested. As a prelude for large-scale BAC sequencing, 20 of the confirmed BACs were selected to be sequenced.

Chromosome 4 (UK)

Official notification of funding was recently received. Work will begin in late 2004 or early 2005.

Chromosome 5 (India)

In addition to ongoing programs on tomato, new proposals on sequencing chromosome 5 and functional genomics of tomato were submitted to the funding agency in India. These were considered by the appropriate committee and the final decision is expected soon.



Chromosome 6 (The Netherlands)

The CBSG tomato sequencing programme aims at the sequencing of chromosome 6 of the tomato cultivar Heinz 1706. After an initial slow start, the entire sequencing effort is now well on track: BACs and BAC contigs are mapped on a high-density AFLP map of Keygene and subsequently retrieved from the Heinz 1706 BAC library. Next, the physical positions of these BACs are confirmed by FISH. This confirmation by FISH appears to be an indispensable step in the sequencing programme as it was found that the deduced order of BACs on the genetic map in many cases deviates from the BAC order on the physical map. This phenomenon is particularly observed close to heterochromatic regions on the chromosome. Once confirmed by FISH, the BACs are sequenced to a 6X – 8X coverage and the obtained sequences are subsequently assembled into contigs. Finally, contigs are annotated using a fully automated BLAST pipeline.

In the current reporting period, a total of 48 BACs has been mapped on chromosome 6 using AFLP markers and also some available RFLP markers. After FISH analyses, a total of 25 BACs was found suitable for sequencing. The remaining BACs were either derived from heterochromatic regions on chromosome 6, or were derived from different chromosomes. Of the 25 selected BACs, 8 BACs have been completed and a further 12 BACs are currently in the sequencing pipeline.

Of the completed BACs, only BAC 250I21 has been fully closed and annotated. All other BACs were sequenced up to an approximately 6X coverage. When assembling these BACs into contigs, it showed that most of the BACs contain approx. 23% - 30% low/medium repetitive DNA which tend to pile-up during the assembly process.

This causes an apparent reduction in obtained BAC length of approx. 25% - 30%. The assembly of BAC clones at a 6X coverage resulted in an average 11 gaps per 100 kb of DNA. This number is in range with the statistically expected number of obtained contigs at the given coverage. However, a large spread in number of gaps per 100 kb of DNA was observed ranging from 17 gaps/100 kb for BAC 112G05 to 2 gaps/100 kb for BAC 308F14.

Chromosome 7 (France)

The sequencing project of chromosome 7 is currently under development. A final decision on funding is expected by the end of this year.

Chromosome 8 (Japan)

Update pending

Chromosome 9 (Spain)

A proposal was submitted earlier this month to Genoma España. Decisions on funding will be made by the end of this year.

Chromosome 12 (Italy)

Sequencing of chromosome 12 has been financed by the Italian Ministry of Agriculture with an initial grant of 600.000 Euros. Partners involved are the University of Naples, the Italian Agency for New technologies, Energy and the Environment (ENEA, with own funds) and The University of Padua. The BACs have been received from SGN. The actual sequencing is expected to start before the end of 2004 and it will involve the full euchromatic region of chromosome 12 and the chloroplast genome.

Mitochondrial genome (Argentina)

An update will be provided in a future SOL newsletter.

Community News

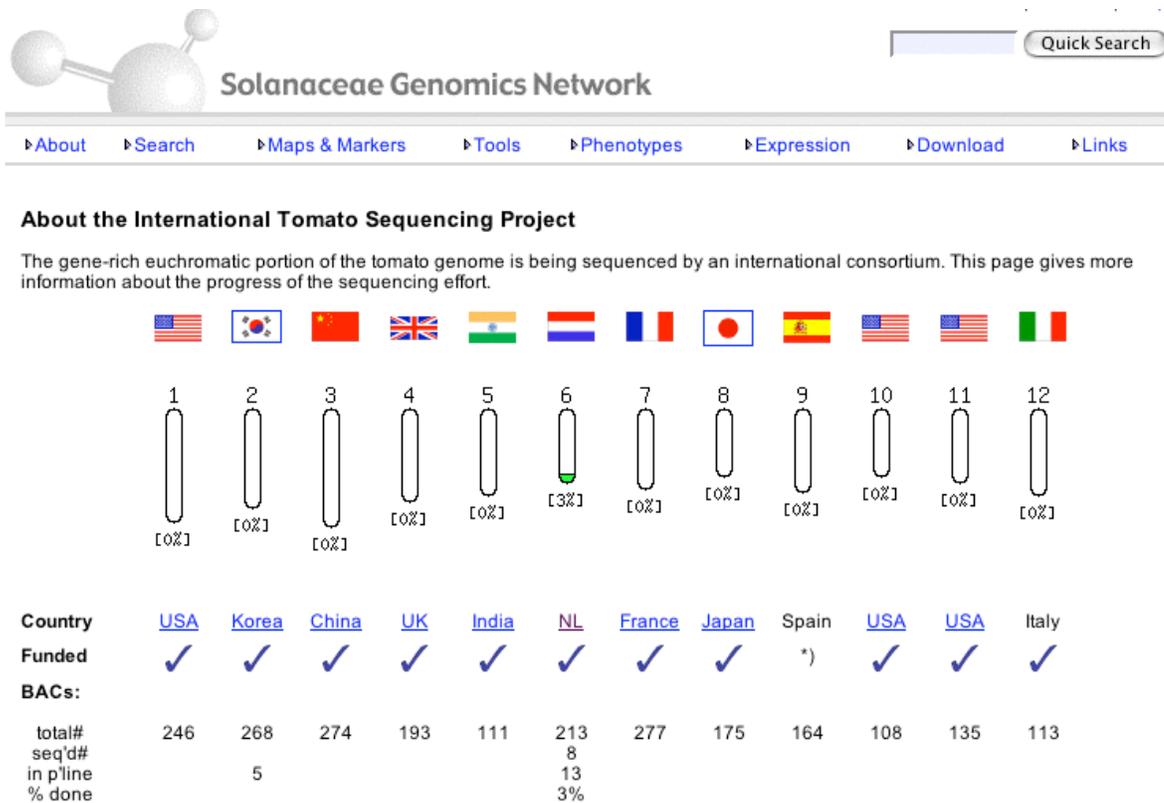


Petunia News from Tom Gerats: In the happy surroundings of SOL, we, as a Petunia community have organized ourselves a bit further. As a result we have initiated a website that intends to comprehensively cover the developments within and between groups that foster Petunia as their pet research object: <http://www.petuniaplatform.net>

What's New on SGN?

-The BAC end sequences will be posted soon (before the end of the year) on the SGN FTP site.

-There is a new tomato sequencing summary page on SGN, at http://sgn.cornell.edu/help/about/tomato_sequencing.html. A screenshot of the page is included here:



Bioinformatics

A reminder about the upcoming SOL bioinformatics meeting being held during the Plant and Animal Genome Conference

International Plant and Animal Genome XIII Conference (PAG-XIII)
 January 15-19, 2005
 Town & Country Hotel in San Diego*

*Lukas Mueller has organized a SOL bioinformatics meeting on Saturday, January 15, 2005 from 1:00PM-6:00PM in the Dover room. The meeting is not limited to bioinformaticians. It is open to everyone who is involved in sequencing. Please let Lukas know if you will attend and what topics you would like to see discussed. Lukas's e-mail address is lam87@cornell.edu.