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Community News

Report on the International Workshop on Tomato Genomics University of Hyderabad, Hyderabad, India November 12 - 14, 2006

*by: Rameshwar Sharma and Rajeswari Srinivasan
University of Hyderabad, India*



India is the second largest producer of vegetables in the world. Solanaceous vegetables such as potato, tomato, brinjal, and chilies are a major part of Indian cuisine. Several Solanaceous species are used in the traditional ayurveda medical system of India. To tap the emerging potential of biotechnology to augment agricultural output, India has made major investments in genomics research. With the same token, India joined the International SOL Genome Project and is involved in several areas including sequencing chromosome 5 of tomato, and functional genomics activities related to TILLING, disease resistance, and tomato fruit ripening.

To stimulate interaction within the country and also with the international community, a workshop on tomato genomics was held at Hyderabad, India from November 12 - 14, 2006 under the patronage of the Dept. of Biotechnology, Government of India. Approximately sixty Indian scientists and thirteen foreign scientists met for three days and deliberated on several aspects of tomato structural and functional genomics. The three-day workshop consisted of seven sessions of oral presentations. A general discussion was held at the end of every session to focus on specific issues arising from the session presentations. In the opening talk, "SOL Vision", Dani Zamir presented the SOL concept, and stated that a long-term goal is to generate a bioinformatics framework to link gene sequences with phenotypes.

One of the main ideas of the workshop was to draw a roadmap for a SOL program in India in resonance with the international SOL program. The talks presented helped to outline and also to address several emerging issues. During the presentations on the tomato genome sequencing efforts by the different countries, it was agreed that every country should map 100 seed BACs to speed up progress. The Japanese group presented their work on the development of pools of selective euchromatin rich BACs that were subjected to shot gun sequencing apart from sequencing chromosome 8 in a BAC-by-BAC strategy. Also highlighted was the potential of FISH maps in closing the gaps in genome sequencing.

In addition, there were presentations on technologies being applied in parallel by different members of SOL such as TILLING, for which work is underway in France, Japan (MicroTom), and India (Arka Vikas and M82). It was suggested to coordinate the activities of all three countries by setting up a "TILLING Club". Hiroshi Ezura was designated as the club organizer. There was agreement that similar coordinated clubs should be established for future technologies. Two groups reported improvements in tomato transformation. The Japanese group reported a high-throughput tissue culture system based on a transformation protocol that could be used for generating tag lines and functional genomics. The Indian group reported *in planta* transformation of tomato, which could be used for large-scale applications.

Several participants presented the important role mutants and transgenics will play in regulating tomato development and fruit quality. One of the interesting findings presented was an increase in vitamin-C level in the fruit of *cry-2* mutants. It was also reported that a mutant defective in brassinosteroids called insensitive1 (*bri1*) was used to determine that BRI acts as a receptor for the bioactive brassinolide hormone. In a presentation on fruit ripening, it was shown that delayed ripening was observed after overexpression of SAM decarboxylase and spermidine synthase, and antisense suppression of LeACS2 and Le-ACS4. It was also reported that there is cross talk between ethylene and auxin signals during tomato fruit development. The potential of cDNA and oligo array to investigate transcriptome changes during tomato fruit development was also demonstrated.

A major challenge is to develop varieties with desired pathogen resistance. Jonathan Jones identified several *Rpi* (resistance to *Phytophthora infestans*) genes from wild *Solanum* germplasm, which have the potential to broaden the disease resistance of the *Solanaceae*. Several talks from Indian scientists highlighted the diversity of geminiviruses that infect tomato particularly ToLCV in India and it was felt that a major effort should be launched to study the viral diversity and breeding of lines tolerant to these viruses.

One of the novel features of this workshop was short, oral presentations of posters by young scientists, which was very well received by the senior scientists. They agreed that in future SOL meetings selected posters should be invited for oral presentations to encourage young scientists.

In the final sessions, several core issues of the SOL program were discussed. There was strong agreement that there is a need for sharing germplasm and genomic resources generated under the SOL program, which could be made available to all interested partners and also maintained on a long-term basis for the future. One part of these issues was the importance of how the metabolome influences plant characters and phenotypes. It would be desirable to establish groups to design a strategy for the metabolomics of tomato. It was emphasized that SOL partners should sponsor training workshops for young scientists and also promote exchange visits between the labs of SOL partners in different countries.



SOL-100

Cris Kuhlemeier, Dani Zamir and Sandy Knapp
November 23, 2006

The question

Solanaceae include more than 3,000 species with wide adaptation, form, chemistry, and distribution. Species of the family are of great agricultural, nutritional, horticultural and medicinal importance (e.g., potato, tomato, pepper, petunia and tobacco). This enormous diversity is contrasted by high conservation of gene order and content at the macro and micro levels. Solanaceae genomes can be genetically tied on a common framework linkage map, thus facilitating the identification of genes with homologous phenotypes in the different species. These features make Solanaceae an excellent taxon with which to address a central question in biology:

How can a common set of genes/proteins give rise to such a wide range of morphologically and ecologically distinct species?

The aim

The SOL community will create a common Solanaceae-based genomic framework that includes sequences and phenotypes of 100 genomes encompassing the phylogenetic diversity of the group. Specific objectives are to:

- 1) Tie the emerging tomato, tobacco, potato and Asterid relatives coffee and *Mimulus* (monkey-flower) euchromatic sequences on a common SOL physical map and relate it to other Asterid taxonomic groups, such as *Antirrhinum*, sweet potato and mint.
- 2) Select 100 Solanaceae species and Asterid outgroups (**SOL-100**) for map construction in diversity crosses using Conserved Ortholog Markers (COSII) that represent the evolutionary tree and reflect important human uses.
- 3) Apply emerging novel genome sequencing technologies to **SOL-100** and overlay it on the framework BAC-by-BAC map.
- 4) Genetically map simple and complex phenotypes affecting chemical, morphological and fitness-related traits in the **SOL-100** species.
- 5) Construct bioinformatic vehicles to journey through different levels of the organization of the broad base of biological information in **SOL-100**.

Foster a broadly-based international community of interacting scientists interested in and committed to exploring and conserving natural biodiversity.

Who we are?

The International SOL Genome Project (SOL) is a 'virtual umbrella' aimed at promoting, coordinating and actively seeking additional scientists, countries and funding agencies to participate in an expedition into understanding, utilizing and conserving natural biodiversity (<http://sgn.cornell.edu/solanaceae-project/>). SOL includes scientists from more than thirty countries that are united and excited about the sustainable and equitable use of natural biodiversity in biological research and plant breeding, and in the conservation of these resources for the future. The SOL community is presently sequencing the tomato genome through grants from national funding agencies as well as through international collaborative projects.

THE CLADES OF SOLANACEAE

Indicative list of potential target taxa for sequencing (starred taxa may prove problematic due to rarity or difficulty with cultivation); this initial list of suggestions has been chosen for ease of access to germplasm, cultivation and breadth of phylogenetic coverage.

Clade (after Knapp et al., 2004)	Generic diversity (approx.)	Possible target species
Schwenkia clade	3	<i>Schwenkia americana</i>
Schizanthus	1	<i>Schizanthus pinnatus</i>
Duckeodendron	1	* <i>Duckeodendron cestroides</i>
Goetzea clade	4	<i>Goetzea elegans</i>
Petunia clade	13	<i>Petunia hybrida</i> ; <i>Brunfelsia uniflora</i> ; <i>Nierembergia scoparia</i> ; <i>Calibrachoa parvifolia</i>
Browallia clade	2	<i>Browallia americana</i>
Cestrum clade	3	<i>Cestrum elegans</i> ; <i>Vestia foetida</i>
Salpiglossis clade	2	<i>Salpiglossis sinuata</i>
Nicotiana	1	<i>Nicotiana tabacum</i> (4x – in progress); <i>N. sylvestris</i> (2x)
Anthocercis clade	7	<i>Duboisia hopwoodii</i> ; <i>Anthocercis littorea</i>
Lycium clade	3	<i>Lycium barbarum</i> ; <i>Lycium carolinense</i>
Nolana	1	<i>Nolana humifusa</i> ; <i>Sclerophylax</i> sp.
Jaborosa	1	* <i>Jaborosa integrifolia</i>
Hyoscyamus clade	5	<i>Hyoscyamus niger</i> ; <i>Atropa belladonna</i>
Nicandra clade	2	<i>Nicandra peruviana</i>
Datura clade	2	<i>Datura stramonium</i>
Solandra clade	7	* <i>Juanulloa mexicana</i>
Mandragora	1	* <i>Mandragora officinarum</i>
Solanum clade	4 (Solanum with 13 clades, 1500 spp.)	<i>Discopodium penninervium</i> ; <i>Solanum melongena</i> ; <i>S. nudum</i> ; <i>S. dulcamara</i> ; <i>S. americanum</i> ; <i>S. tuberosum</i> (in progress); <i>S. lycopersicum</i> (in progress)
Iochroma clade	5	<i>Iochroma fuchsioides</i> ; <i>Acnistus arborescens</i> ; <i>Dunalia solanacea</i>
Physalis clade	6	<i>Physalis peruviana</i> ; <i>P. ixocarpa</i> ; <i>Witheringia solanacea</i>
Withania clade	10	<i>Withania somnifera</i> ; <i>Tubocapsicum anomalum</i>
Salpichroa clade	2	<i>Salpichroa organifolia</i>
Capsicum clade	2	<i>Capsicum annum</i>
Potential outgroup taxa from related Asterid families		
Convolvulaceae		<i>Convolvulus tricolor</i> ; <i>Ipomoea batatas</i> (sweet potato)
Plantaginaceae		<i>Antirrhinum majus</i>
Phrymaceae		<i>Mimulus guttatus</i> (in progress)
Lamiaceae		<i>Ocimum basilicum</i> ; <i>Mentha piperita</i> (mint)



- Hans de Jong has offered to carry out FISH experiments for the European partners in the tomato sequencing project. The offer involves the FISH mapping of thirty to forty BACs per country. For further information you can contact Hans (hans.dejong@wur.nl).
- The EU-SOL website, www.eu-sol.net, has a new section on Job Opportunities. If you want to post your advertisements on this website, please email your ads to: eusol@wur.nl.
- Everything you always wanted to know about EU-SOL but were too afraid to ask! On Tuesday, January 16th, René Klein Lankhorst will present an overview of the EU-SOL Project at the *Solanaceae* workshop at the Plant and Animal Genome Conference in San Diego, CA.



**First SOL Spain Meeting
Valencia, Spain
December 20, 2006**
Provided by Antonio Granell

The first SOL Spain meeting took place in Valencia on the 20th of December. Antonio Granell organized the meeting, and it was entitled: "Workshop on Tomato Genomics and The Sequencing of the Tomato Genome. First Meeting of the Spanish Solanaceae Research Community". Over 100 scientists attended including researchers from Spanish public and private research centers, breeding companies, associations of growers, etc. There was a large presence of young scientists at the workshop.

There were four sessions where scientists from different Spanish groups presented their work in twenty-minute time slots. Topics included a progress report on the sequencing and annotation of Chr9, which was presented by Manuel Perez from Sistemas Genómicos and Roderic Guigó from IMIM. There was a general description of the genomics platforms available in Spain developed in the frame of the Spanish ESPSOL project (A. Granell, IBMCP), followed by presentations on implementation of bioinformatics tools to integrate genetic and genomic data by Vicky Martin, UMA, and the analysis of tomato reproductive development which was covered by R. Lozano, UAL. Miguel Botella (UMA) presented a genetic analysis of abiotic stress tolerance in tomato and Eduardo Bejarano from the same university addressed the Virus-Tomato interaction. Jaime Prohens (UPV) presented information on the COMAV *Solanaceae* resources and Rafael Fernandez presented his work on sources of pest resistance derived from *S. pimpinellifolium* TO0937. In addition to presentations by Spanish scientists, the international SOL community was represented by the following people: Dani Zamir (University of Jerusalem, Israel) gave an overview of the International SOL project, Jim Giovanonni (Cornell University, USA) presented his work on tomato fruit ripening, Graham Seymour (University of Nottingham, UK) gave an update on the sequencing of tomato Chr4 in the UK and shared his thoughts on how useful it would have been to know the sequence of the tomato genome at the time they were cloning the *cnr* locus, Ramesh Sharma (University of Hyderabad, India) and Koh Aoki (Kazusa Institute, Japan) gave overviews of the genomic resources available in India and Japan. The SOL Spain workshop also included a presentation by Christian Bachem (Wageningen University) on the sequencing of the potato genome by the PGSC. It was emphasized that it would be mutually beneficial for the potato and tomato sequencing groups to share sequencing and mapping information.

The main conclusions of the meeting were as follows: 1) In order to further advance the tomato genome sequencing project, new seed BACs are needed and the international SOL community has responded with a number of approaches. For example, a to-do list has been circulating amongst the sequencing partners since the India SOL meeting. 2) The tomato and potato sequencing projects should exchange information on sequencing and mapping. 3) This meeting should serve as a starting point for the Spanish SOL community to interact and optimize different genomic platforms that are available. 4) The general impression was that this meeting has positively reinforced the Spanish Community of Researchers in the *Solanaceae* and transmitted the idea of us being an active community with a strong interest in applying genomic tools and knowledge derived from them to the challenges of today's agriculture and also to the understanding of the basic biological processes underlying traits such as fruit quality and disease resistance.

A summary of the meeting has been sent to the representatives of the main financing agencies in Spain.





Tomato Sequencing updates



Chromosomes 1, 10, 11 (US)

Contact: Joyce Van Eck (jv27@cornell.edu)

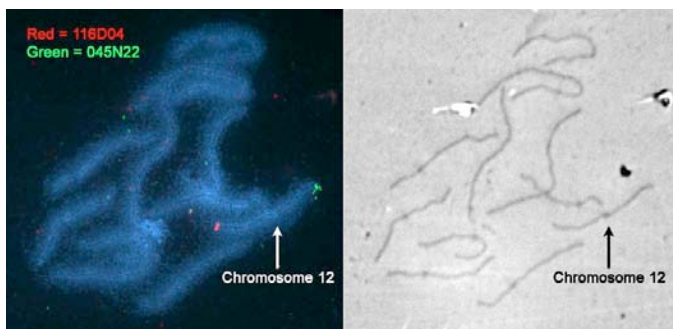
The US team received \$1.8 million from NSF in December to support and expand ongoing activities at SGN, develop a fosmid library and to continue FISH in support of the larger genomics effort.

New tools and resources have been added to SGN (see the "What's New on SGN" section of this newsletter).

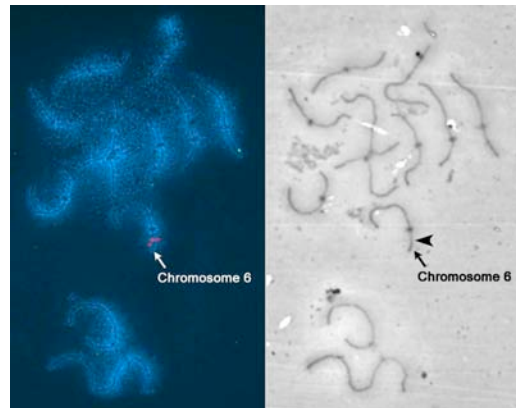
A total of sixty-three BAC clones have now been positioned on tomato chromosomes using FISH by the Stack lab, including seven that have been localized and posted on the SOL Genomics Network website since our last report. Among these clones are two located near the telomeres of the long arms of chrs1 and 12, two located near the euchromatin/heterochromatin borders on the short arm of chr6 and the long arm of chr8, one located in the euchromatin on the long arm of chr7, and two located within the heterochromatic regions of chrs4 (short arm) and 12 (long arm). The recently positioned BACs include:

Chromosome Arm	BAC ID
1Q	088L02
4P	209A01
6P	008F19
7Q	308M01
8Q	225C20
12Q	331D02
12Q	116D04

The figure below illustrates FISH labeling of tomato chr12 with probes near both of the telomeres. BACs Le_HBa045N22 (green) and Le_HBa116D04 (red) are located near the ends of the P and Q arms, respectively.



The following figure shows FISH labeling of chr6 with BAC Le_HBa008F19 (red). The signal for this probe is located close to the euchromatin/heterochromatin border on the short arm of the chromosome. The arrowhead on the phase micrograph indicates the approximate position of this border.



Chromosome 2 (Korea)

Contact: Sanghyeob Lee (sol6793@kribb.re.kr)

Update pending.

Chromosome 3 (China)

Contact: Chuanyou Li (cyli@genetics.ac.cn)

Update pending.

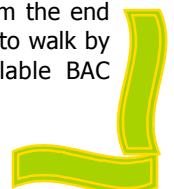
Chromosome 4 (UK)

Contact: Christine Nicholson (ckb@sanger.ac.uk) or Karen McLaren (kb1@sanger.ac.uk)

As of December 14, 2006, 4,977,235 bp of sequence have been generated at the Wellcome Trust Sanger Institute for chr4 after sequencing forty-six BACs from the LE_HBa and SL_MboI libraries. 4,827,176bp of this sequence are unique, i.e., they exclude the overlapping sequence. Eighteen BACs have been fully finished to HTGS phase 3, contributing 1,963,352 bp of sequence. The other accessioned BACs are at HTGS phases 0 to 2 and remain active in our pipeline.

Tilepath selection continues across the FPC contigs containing confirmed chr4 markers and BACs are progressing through the mapping, subcloning and shotgun stages of our sequence pipeline prior to their release into the public databases at EMBL/Genbank/DDBJ where they are accessioned. The development of the chromosome contigs can be viewed via the TPF and AGP assembly files that we are posting monthly at SGN.

A number of markers from the tomato EXPEN-2000 map are un-anchored in the FPC database. Primers are being designed and synthesized from some of these markers to hybridize against the library filters. This is being done with a view to increase coverage. In addition, primer design is underway from the end of sequence contigs using genomic sequence in order to walk by hybridization to extend and bridge gaps. All available BAC libraries will be screened.



Chromosome 5 (India)

Contact: Akhilesh Tyagi (akhilesh@genomeindia.org)

The Indian Initiative on tomato genome sequencing is currently working on forty-four BAC clones from chr5. These BACs are associated with twenty-seven chr5-specific markers (CT101, T1181, T1632, C2-At1g60440, T1252, C2-At1g60200, cLET-8-B23, T0564, cLED-8-G3, T1592, TG432, BS4, C2-At2g01110, C2-At3g55120, TG96, T1360, cLEX-13-G5, T1746, T1777, T1584, TG69, CT130, TG185, TG597 and CT138) covering both chromosomal arms. Six BAC clones have reached phase III level of sequencing, while nineteen and thirteen BAC clones are at phase II and Phase I levels of sequencing, respectively. Six BAC clones are in either the early phase of sequencing or library preparation. The location of all the seed BACs as well as their overlapping BACs on chr5 employed for sequencing was confirmed using chr5-specific Introgression Lines (IL).

Chromosome 6 (The Netherlands)

Contact: Sander Peters (sander.peters@wur.nl)

Since our last report, we finished fifteen BACs to phase 2 and one BAC to phase 3. Except for the phase 2 completed BAC LE_Hba0246E15, chr6 specific marker sequences have been identified for the other fourteen BACs. Currently, one BAC is in the sequence pipeline, and seven candidate seed BAC clones associated with chr 6 specific markers (FER, P27, CD67, T1198, T1500, T1079, and T1063) are in the FISH screening pipeline.

In total, seventy-one chr6 BACs have been sequenced, of which five to phase 3 and sixty-six BACs to phase 2. Of these, sixty-four BACs are from the HindIII BAC library, two from the EcoRI library, and five from the MboI library. From the total set of sequenced BACs, nineteen are extensions, which have been found by STC screening of twenty seed BACs. Of these extensions, twelve are from the HindIII library, two are from the EcoRI, and five are from the MboI library. The total amount of sequence including overlapping sequences now amounts to 8 Mb, of which 5.9 Mb are produced from LE-HBa BACs.

Chromosome 7 (France)

Contact: Farid Regad (regad@ensat.fr)

Update pending.

Chromosome 8 (Japan)

Contact: Erika Asamizu (asamizu@kazusa.or.jp)

We finished forty BACs to phase 3 (total non-overlapping length 4,429,168 bases) of which twenty-five are seed and fifteen are extended clones. Sequence of these clones and of two others at phase 2 have been submitted to GenBank.

We selected 54,831 low-copy BACs by searching the repeat database using the BAC end sequences. The clone list has been sent to SGN and will become available. Among these clones, we pooled 20,000 clones and made shotgun libraries from the mixture of BAC DNA. We are planning to accumulate 2.8 M reads from the selected BAC mixture (SBM) and make shotgun libraries starting in January 2007.

For BAC isolation, we made a 3D DNA-pool of the BAC libraries. This pool enables 4.5-deep screening of the genome. Using the pool, we performed screening of markers that failed in the candidate BAC verification and also framework markers on Tomato-EXPEN 2000 genetic map. As a result, we succeeded in obtaining nine new seed points.

Chromosome 9 (Spain)

Contact: Antonio Granell (agranell@ibmcp.upv.es)

As of today, the sequence of twenty-one BACs has been deposited in the SGN database. Eight are in phase 3 (no gaps), and thirteen are in phase 2. Twelve BACs are in the sequencing pipeline and four new extension BACs have just arrived and are in the process of confirmation.

Two new BACs have been selected. One is from the last candidate of the initial overgo screening made by Cornell, but did not satisfy the stringent criteria of a good seed BAC, the other has been obtained by searching "in silico" BACs containing chr9 markers in their BAC end sequences (BES). These two BACs have been received and confirmed. The first has been mapped to chr9 using Dani Zamir's ILs and the other is in progress.

We are trying to increase the number of seed BACs following different approaches:

1. Searching the BES database with new markers
2. Participating with other countries in mapping BACs containing ORFs in both BES
3. Searching BAC libraries with new chr9 markers

These tasks are to be performed as part of the participation in EUSOL.

The need for additional BAC resources is further revealed by the fact that we cannot extend from six of the positions and in four cases the overlap is larger than 20 kb. In addition to BAC sequencing, a PCR screening of all chr9 markers that had a BAC associated in SGN was been performed. The marker was not found in ten out of the twenty-seven starting BACs initially assigned to euchromatin. The in silico search of chr9 markers against the BES resulted in five candidate BACs, but unfortunately they were too small (less than 20 kb) or the presence of the marker could not be confirmed, and in only one case was confirmed and proceeded to be mapped in Dani's ILs.

Chromosome 12 (Italy)

Contact: Mara Ercolano (ercolano@unina.it)

During these two years of the tomato sequencing project, we set up the following: cytogenetic protocols, a sequencing pipeline, marker analysis strategies, and a number of bioinformatics and molecular resources. According to the chr12 marker mapping information available at SGN, thirty-seven seed BACs were selected to perform verification analysis. The validation of thirteen seed BACs was performed at Naples University on the basis of SNP polymorphisms detected between *S. lycopersicum* and *S. pennellii* using ILs. Primer pairs yielding a single clear PCR product (>500) in all genotypes analyzed were sequenced and analyzed for SNPs and INDELS. Eight additional BACs were validated using an internal sequencing strategy at ENEA and at the University of Padua. However, some difficulties were found such as disagreement of sequence with the original marker, multiple PCR products, contradictory IL mapping data, and incorrect marker/BAC assignment. The location of three BACs was reassigned to other chromosomes as the analysis performed in the Tanksley Lab on the F2 2000 segregating population revealed that BAC Le Hba 152M18 is located on chr11 and BAC Hba 244JO4 is located on chr1 while FISH mapping performed in Stack lab revealed the Bac107D19 is located on chr9. In total, nineteen seed BACs associated to eleven markers mapped onto the short arm and eight mapped to the



long arm of chr12 have been selected as sequencing starting points. To extend the number of seed BACs, Italy will also participate in the SOL India mapping initiative. This may be effective for filling chromosome gaps during the later stages.

Despite the fact that the distribution of chr12 seed BACs is not uniform, small contigs consisting of overlapping BACs started to emerge. Good extension candidate BACs are selected on the basis of the program "**BacEnds Extension v 0.1**" complementary to the SGN Online BLAST Interface. According to the results (unique sequence on both ends; minimal overlapping; appropriate size), more than 100 BACs were picked. These BACs have been mapped using an IL mapping strategy (a total of forty-five specific markers were identified). Extension BACs that show a 100% match of sequence overlaps with connected seeds are being processed. Fourteen sequence islands consisting of two or three overlapping BACs were identified. Eleven seed BACs have been extended in both directions. For seven seed BACs, two or three rounds of extension were performed and overlapping BACs have been merged in sequence islands > 300 kb. The contig built up between markers T1045 and T1211 of approx. 500 kb has been filled. Interestingly, the ratio of physical to genetic distance in this region is 250kb/cM. Currently, fifty-one BACs are in different sequencing phases, and an additional ten extension BACs are under selection. Sequence assembly is in progress. Sequences corresponding to seven BACs were submitted to SGN, and thirty-five additional BACs are in the finishing process.

To provide a preliminary annotation of BAC sequences, a GBrowse at <http://biosrv.cab.unina.it/GBrowse> is available. The platform includes annotation based on similarity searches versus the following libraries: EST/TCs from TomatEST database, EST/TCs from PotatEST database, TIGR LeGI gene index Release 11.0, TIGR StGI gene index Release 10.0, TIGR Solanaceae Repeats v2, SGN Unigene builds: Tomato (200607 build 1) and *Solanum tuberosum* (build 3), SGN tomato EST (version 200607), non-protein coding RNA downloaded from the Rfam database, *Arabidopsis thaliana* RNA downloaded from the NCBI Genomes session).

Moreover, an ftp server is available at (<ftp://cab.unina.it/EST>). We provide a comprehensive set of *Solanaceae* ESTs for related species (*Solanum* spp., *Capsicum* spp., *Nicotiana* spp, *Petunia* spp. and Coffee spp.) (D'Agostino et al., NAR 2007). Unigene builds, generated running the ParPEST pipeline (D'Agostino et al., BMC Bioinformatics 2005), are also accessible at <ftp://cab.unina.it/unigene>. Another bioinformatics task is the building of a *Solanaceae* resistance gene database (<http://srg.tigem.it>). It provides an integrated view of resistance genes in the *Solanaceae* family and represents a useful tool to assist tomato annotation for this gene class. The database contains sequences that have been collected from several sources, for about forty reference genes with information about the sequence of DNA, RNA, protein, related markers, the symptoms of pathogenesis and the taxonomy of plants and of the pathogens. The principal aims of this effort will be illustrated at the next PAG meeting during the *Solanaceae* workshop. Let's see in SAN DIEGO!!!!

Platform Clubs

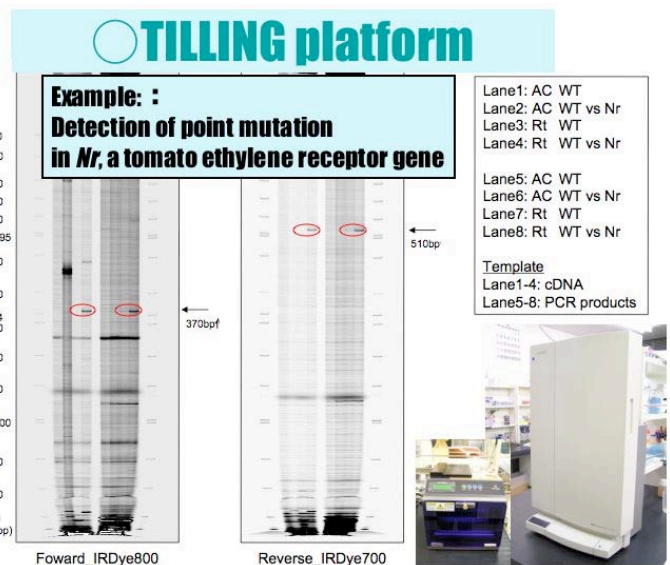
During the SOL India meeting, it was decided that various groups should organize "platform clubs" based on technologies used by members of the SOL community. This article on the TILLING Club is the first in a series of platform club information that will be featured in the SOL Newsletter.

TILLING Club

Contributed by Hiroshi Ezura

TILLING (Targeting Induced Local Lesions IN Genomes) is a method that allows a directed identification of mutations in a specific gene. The method combines mutagenesis by ethyl methanesulfonate (EMS) with a new screening technique that uses the possibility to identify Mismatch-Hybridization. Importantly, this method generates a wide range of mutant alleles efficiently and is applicable to any organism that can be chemically mutagenized. With the accumulation of large-scale sequence data, emphasis in genomics has shifted to elucidating gene function. Therefore, TILLING could be a powerful tool for tomato functional genomics.

I was asked to coordinate a TILLING Club in the SOL community during the SOL India meeting held in Hyderabad, India. Currently, our group is establishing a TILLING platform for a miniature tomato, Micro-Tom, for the Japanese Solanaceae Genomics Consortium (JSOL). We have already generated over 4,000 M2 lines along with M3 seeds, and are preparing DNA pools for screening. In addition, the phenotypic mutant screening, data of the M2 and M3 were deposited in a





pilot mutant database named TOMATOMA (Tomato Mutant Archives) at the National Institute of Genetics. After a TILLING platform is established, we will hold training in the TILLING method for the JSOL community. To my knowledge, several groups (France, Spain, India) in the SOL community are developing TILLING platforms for screening tomato mutants. It is definitely worthwhile to exchange information with each other.

The objectives of the TILLING Club are to share successes, problems encountered, protocols, training opportunities, etc. As coordinator of the SOL Community TILLING Club, I would like to make a network for exchanging such experiences and information. If people are responsible for or interested in the TILLING platform of tomato, please send an e-mail to me (ezura@gene.tsukuba.ac.jp) with the following information: Name, institution, address, and e-mail address. The subject of the e-mail should be 'TILLING CLUB'. I will make a mailing list for the TILLING Club in the SOL community, so that we can exchange information about TILLING in tomato.

Contact Information

Coordinator: Hiroshi Ezura
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Announcements

Publications



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Ezio Portis, I. Nagy, Z. Sasvári, A. Stágel, L. Barchi, and S. Lanteri. 2006. The design of *Capsicum* spp. SSR assays via analysis of *in silico* DNA sequence, and their potential utility for genetic mapping. *Plant Science* available online at www.sciencedirect.com. Will be published in the journal in January 2007.

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New Online Resources

Tomato full-length cDNA database - KaFTom

On December 1, 2006, the Japan Solanaceae Consortium and the Kazusa DNA Research Institute launched a new web site, KaFTom, which provides Tomato full-length cDNA information. KaFTom URL: <http://www.pgb.kazusa.or.jp/kaftom/>

New features of KaFTom include:

- (1) 57,422 5'-end sequences and 326 HTC (high throughput cDNA) sequences from Micro-Tom. Both categories will be updated with the progress of sequencing.
- (2) Similarity search against tomato BAC sequences.
- (3) InterProScan for HTC sequences.
- (4) Similarity search against Arabidopsis and rice genes.

Contact Kentaro Yano (yanoken@kazusa.or.jp) or Koh Aoki (kaoki@kazusa.or.jp) with questions and requests.





SGN mirror launch

We are pleased to announce the release of an SGN mirror site run at the National Coffee Research Center (CENICAFE) in Colombia. The site aims to contribute to the dissemination of genomic data from *Solanaceae* and related species (especially coffee) in Latin America countries and also provide a back-up reservoir of all the information stored at SGN.

CENICAFE bioinformaticians are engaged in the development of molecular tools and databases for the study of the coffee genome and we expect to integrate more coffee sequence data (cDNA and genomic) to SGN in the near future.

The site can be accessed at <http://sgn.cenicafe.org>.

Sequences of RFLP marker regions for chromosome 6

As part of the USAID/MERC and USAID/CRD projects, markers for tomato chromosomes were developed. In many cases, PCR primers were designed from probes, which were sequenced, or directly from the end sequences available at the SGN web site. In some cases primers were developed from sequences available at GenBank. A link will be posted on SGN, but for now, the website is:

<http://www.plantpath.wisc.edu/GeminivirusResistantTomatoes/Markers/TomatoMarkers.htm>

For additional information contact Douglas Maxwell at dpmx@plantpath.wisc.edu.



What's new on SGN?

Phenotypic Database: The recently introduced phenotype information on mapping and mutant populations, associated loci, images, mapping data, and annotations, can now be queried through the web and updated by SGN users. Every individual accession has an associated editor, and images can be uploaded directly online by community members (login required). To become an accession editor, please contact SGN. For an example of an individual detail page, see http://sgn.cornell.edu/phenome/individual.pl?individual_id=4475. The database can be searched using the SGN phenotype search: http://sgn.cornell.edu/search/direct_search.pl?search=phenotypes.

Currently, there are six different populations with more than 5700 associated individuals and more than 8,000 images represented in the phenotype database: the M82 x *Solanum pennellii* introgression lines, TGRC monogenic mutant accessions, tomato and eggplant monogenic mutant populations, and the F2 2000 mapping population. The platform can be used for uploading phenotypic descriptions, images, and genetic information. In the future, it will be possible to calculate phenotype-genotype associations from the information in the database. Any *Solanaceae* population with phenotype and mapping data submitted to us will be added to the database. The data on SGN will be complementary to existing *Solanaceae* phenotype resources and support cross-links to the contributing databases. All information on the SGN phenotype database will remain user-updateable by the original submitter.

If you're interested in submitting your data, please contact SGN for a submitter account and information on data formats at: sgn-feedback@sgn.cornell.edu.

Genes Database: A database of genetic loci has been added to SGN containing descriptions, sequences, mapping data, literature references and annotations for 1,623 tomato loci. Every locus has a designated editor who is an SGN user with special update privileges (contact SGN if you are interested in becoming a locus editor) and SGN users can add information on alleles, annotations and images. The locus database is currently being augmented with information on more than a thousand loci from potato.

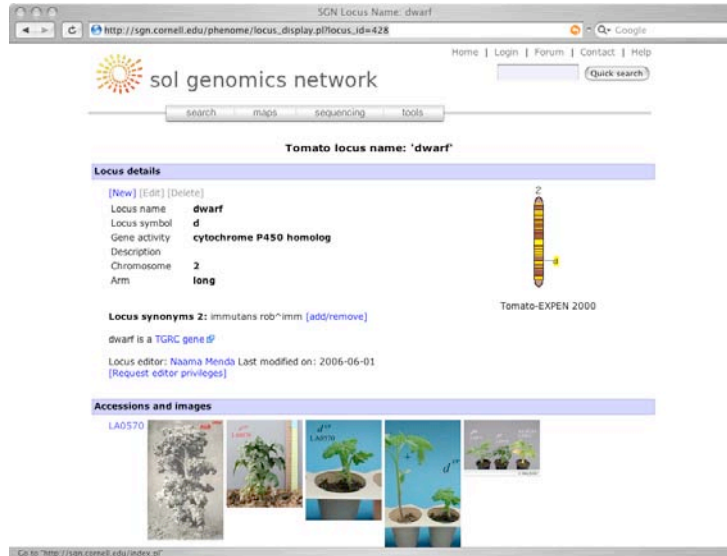




Genes search:

http://sgn.cornell.edu/search/direct_search.pl?search=loci

Sample gene detail page:



Solanaceae Recipes



Ajiaco (Colombian Chicken, Corn, Potato Stew)

Ajiaco Bogotano

Contributed by Marco Cristancho

This is a delicious, thick and rich main dish stew with chicken and potatoes. There are many varieties of this dish (in Colombia there is a saying that there are as many types of Ajiacos as there are Colombians), but their essence is the same. There are some ingredients of the original recipe that are only found in local markets in South America and are difficult to find anywhere else. For instance, three local potato varieties are used for its preparation including "criolla" yellow potatoes (*Solanum phureja*) and they are not easily found fresh out of South America; a local variety of the herb *Galinsoga privaflora* "guasca" that gives a particular taste to the dish is sold in markets in the Andes and is hard to find in other places. However, it is possible to prepare an Ajiaco with the essential ingredients almost everywhere and this is the basic recipe:

INGREDIENTS:

- 1 (3 1/2-4 pound) breast chicken, sliced into medium pieces
 - 1 3/4 teaspoon salt
 - 1 1/2 teaspoon black pepper
 - 2 teaspoons dried oregano
 - 1 1/2 pounds russet potatoes, cut into small pieces
 - 1 cup water
 - 2 pounds yellow potatoes (Yukon gold) peeled and cubed
 - 3 ears of corn, cut into 1 inch pieces
 - Capers
 - Cilantro (coriander)
 - Heavy cream
- Fresh avocados

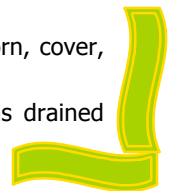
PREPARATION:

Cook chicken with 3/4 teaspoon salt and 1/2 teaspoon pepper in 4 liters of water. Transfer chicken to a plate, debone and slice into medium pieces when cold.

Add sliced chicken, oregano and remaining salt/pepper to pot and sauté about 5 minutes. Peel and cut potatoes and add to pot with chicken and water.

Cover and simmer, stirring occasionally until chicken and potatoes are cooked through, about 25 minutes. Add corn, cover, simmer (stirring) about 5 - 10 minutes.

Serve in bowl with accompaniments (1/2 cup chopped fresh cilantro leaves, 1 cup heavy cream, 3 tablespoons drained capers, 3 avocados, cubed) on the side.





Members of our SGN group participated in a Chili Cook-Off competition sponsored by the Plant Science Department at Cornell University. It was a tough competition, and the SGN team was only two votes short of being the winner. Here is their recipe, which includes several *Solanaceae* family members.

SGN Competition Chili

- 1.5 large cans diced tomatoes (*S. lycopersicum*)
- 1.5 cans tomato paste (*S. lycopersicum*)
- 10 chipotle peppers (*C. annuum*) or to taste
- 1.5 blocks (1.5 lbs?) extra-firm tofu
- 1/2 russet potato (*S. tuberosum*)
- 1 sweet potato (special guest from the Convolvulaceae, *I. batata*)
- a few tbsp olive oil for sauteing
- 1 large green bell pepper (*C. annuum*)
- 2 small red bell peppers (*C. annuum*)
- 2 yellow cooking onions
- 1/2 tsp cayenne powder (*C. frutescens*) or to taste
- 2 stalks celery
- 2 cans corn
- 1 can garbanzo beans
- 2 cans kidney beans
- 2 cans black beans
- a few tbsp flour for thickening

Directions: Add diced tomatoes, tomato paste, chipotles, tofu, and several cups of water (enough to cover) to a large stock pot. Add the potatoes and sweet potatoes.

Meanwhile, chop the onions and bell peppers; saute them in olive oil until the onions are brownish and floppy. Add this mixture to the stock pot once it is cooked.

Add all other ingredients EXCEPT beans and flour; simmer for 30 min or more, tasting as you go. Adjust spiciness to taste.

Refrigerate overnight, if desired. This allows the flavors more time to mingle.

In the morning, reheat the mixture, and add the garbanzos, kidney beans, and black beans. Cook until the mixture, including beans, is hot.

As the very last step, add flour, one heaping teaspoon at a time, to thicken the liquid in the chili. This results in a consistent "gloppy" texture that keeps the veggies and seasonings from separating.

Serve with crusty bread.



Left: Beth Skwarecki; Right: Marty Kreuter

