SOL NEWSLETTER

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COMMUNITY NEWS

Solanum chrysotrichum Schltdl. In Monte Verde, Costa Rica

SOL-ANDINO: The Genome Project and its Impact

Provided by André Kessler and the SOL-ANDINO launch committee

The *Solanaceae* family is the third most important plant taxon economically, and includes our most valuable horticultural crops (Müller et al., 2005, *Comp. Funct. Genom.* 6:153-158). Due to this utility to humans, national and international programs have significantly advanced *Solanaceae* research in fields such as taxonomy, plant breeding, genomics, metabolomics and proteomics. Recently launched programs such as the International *Solanaceae* Genomics Project (SOL), the European (EU-SOL) and Latin American (LAT-SOL) SOL initiatives and the Planetary Biodiversity Inventory (PBI) Program for the genus *Solanum* will further accelerate genomic discoveries. In contrast to these advances, we encounter a critical shortfall of in-depth *in situ* field research concerning the basic and applied ecology and natural history of *Solanaceae*. Most wild species are only known from a few collections in museums. This is especially problematic when we consider that: "Discoveries of new organisms and new facts about organisms often reset the research cycles of hypothesis testing and theory refinement that underlie good progressive science" (Greene, 2005 *TREE* 20:23-27).

The major goals of each genome project are to understand how organisms function and how a common set of genes give rise to a large diversity of organisms that occupy our planet. But, the answers to these questions about the evolutionary mechanisms can only be found at the interface between historically separated biological research domains (Kafatos & Eisner, 2004, *Science* 303:1257). We are currently experiencing a revolution that aims to realize this consolidation of biological sciences by combining our knowledge on the organism level with that on the community and ecosystem level.

Therefore, the SOL network has launched a new initiative "SOL-ANDINO", which provides an international and integrative research platform for scientists eager to share and enrich resources, visions and capacities relating to biodiversity, ecology, conservation, breeding and sustainable agriculture of *Solanaceae* in the centers of their biodiversity, especially in South America. SOL-ANDINO is a virtual umbrella to link the tremendous SOL genomic efforts to basic and applied biological research of *Solanaceae* in their native habitats. The initiative seeks to facilitate and coordinate networks between researchers and funding agencies to achieve a multidisciplinary expedition into the study of biodiversity and adaptation and by supporting the creation of permanent *in situ* conservation sites in the centers of *Solanaceae* biodiversity. The initiative will integrate expertise, visions, and perspectives of scientists from different biological and agricultural research fields and nationalities into the SOL Network. A detailed list of the objectives is available in a whitepaper on SGN (sgn.cornell.edu).

Building for the future of plant science depends upon the development of an integrated, collaborative and diverse research community. The SOL-ANDINO network will help to provide the framework for the establishment of a shared vision that will enhance both biological and cultural diversity within the SOL community. If successful, this initiative has the capacity to make the SOL genome initiative live beyond its expectations. Partnerships forged across biological disciplines that have traditionally been separated and integrating our knowledge of plants across a broad scientific spectrum have the real potential to take plant science to a new and promising level.

The leaf beetle *Lema trilinea*, a *Solanaceae* specialist, on jimsonweed *Datura wrightii*

Tomato Chloroplast Genome Sequence

Information provided by Sabine Kahlau

The sequencing of the tomato chloroplast genome has been completed. The accession number on the NCBI webpage is NC_007898 and the sequence is available under the following link: http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi??db=nucleotide&val=NC_007898. The corresponding publication is not publicly available at this time, however, the preliminary reference is as follows: Sabine Kahlau, Sue Aspinall, John C. Gray and Ralph Bock, Sequence of the tomato chloroplast DNA and evolutionary comparison of Solanaceous plastid genomes, *Journal of Molecular Evolution* (in press).

TOMATO SEQUENCING UPDATES

Chromosomes 1, 10, 11 (US)

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

To date, fourteen BACs have been sequenced, and five are in the sequencing pipeline.

We have recently localized an additional eight BACs in FISH experiments using tomato SC spreads and will shortly post these data on SGN. This number includes the following BACs:

Chromosome Arm	BAC ID
1Q	309D12
	252G05 (sequenced)
4Q	119A16
	020F17
9Q	099P03
	107D15 (sent for sequencing)
11P	034I10 (sequenced)
	064J13 (sequenced)

The fluorescence micrographs that follow illustrate some of our FISH results. Figure 1 shows the positions of two BACs, 119A16 (green) and 020F17 (red), on the long arm of chromosome 4. In this SC spread, the position of the centromere appears as a bright spot on the axis. The locations of two BACs on chromosome 11, 034I10 (red) and 323E19 (green), are shown in Figure 2.

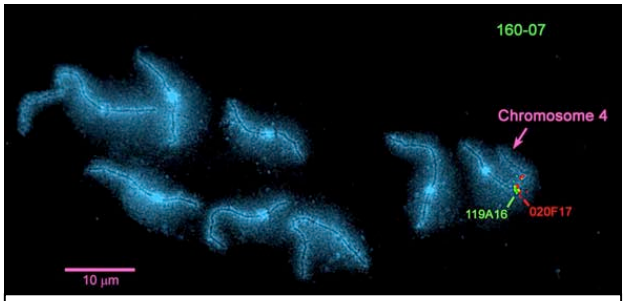


Figure 1: Localization of BACs on chromosome 4.

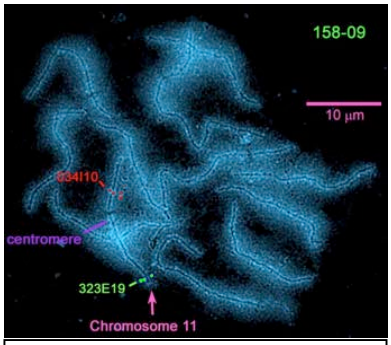


Figure 2: Localization of BACs on chromosome 11.

Chromosome 2 (Korea)

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

To date, we have completed the sequence for fifty-four BACs (fifteen additional BACs from last month). Thirty-five BACs are in the pipeline. Currently, we are focusing on BAC extension using the BAC end sequences and doing Fiber FISH for confirmation of the extended BACs. I am quite interested in the UK's FPC contig because I strongly believe this will accelerate the BAC extension.

Chromosome 3 (China)

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

Update pending.

Chromosome 4 (UK)

Contact: Christine Nicholson (ckb@sanger.ac.uk)

Selection of BACs to sequence the tiling path across chromosome 4 is made using both BES and fingerprint information. To date, a 337 kb contig of three overlapping clones has been sequenced at the Wellcome Trust Sanger Institute to HTGS Phase 3. This contig consists of three LE_HBa BACs: 31H5, 119A16 and 198L24. In the case of neighboring clones in the tiling path, BACs are being finished 2,000 bp into the overlap to minimize redundancy. Three additional LE_HBa BACs have undergone BAC verification by fingerprint and colony PCR confirmation and are currently being transformed into our current sequencing vector in Subcloning prior to Shotgun sequencing. The status of the chromosome 4-attributed BACs are being updated in the BAC Registry.

Two BACs have been localized to chromosome 4 in the Stack Lab and an additional eight candidates have been identified and verified for additional localization.

Our recent focus has been the generation of additional fingerprint data. We have undertaken the fingerprinting of the SL_MboI library, reproducing the original agarose gel fingerprinting technique from AGI that was utilized for the LE_HBa library in order to allow comparison and integration of the two datasets. The number of potential contig merges from the new fingerprint data cannot be predicted at this stage. It is certain that the information will make the SL_MboI clones "visible" in our FPC map and the larger insert size of the SL_MboI clones (135kb compared with 117kb of the LE_HBa library) should provide an efficient substrate for sequencing. At present, the agarose gels are being processed through the Image software. We estimate to incorporate the additional fingerprint data into FPC at the start of May 2006; these data will be made available to the whole community at that time.

Chromosome 5 (India)

Contact: Akhilesh Tyagi (akhilesh@genomeindia.org)

The Indian Initiative on Tomato Genome Sequencing is currently involved in sequencing twenty BAC clones from chromosome 5, anchored to chromosome specific markers (CT101, T1252, C2-At1g60200, cLET-8-B23, T0876, cLED-8-G3, BS4, T1592, T1360, T1777, T1541, T1584, TG69, CT130, TG185, TG597, cLEX-13-G5 and T1746). Sequencing of BACs has progressed to various stages. Whereas, BACs C05HBa0179E24, C05HBa0027B05, C05HBa0058L13, C05HBa0169M21, C05HBa0334K22, C05HBa0166A02, C05HBa0168B11, C05HBa0040C21, C05HBa0131D04, C05HBa0108A18, C05HBa0245E05, C05HBa0251J13 are at phase I level, sequencing of C05HBa0042B19, C05HBa0051A13, C05HBa0006N20, SL_MboI0077G20, C05HBa0006N20, C05HBa0239D11 has progressed to phase II level. Sequencing of C05HBa0191B01, C05HBa0179K09 and C05HBa0261K11 has reached phase III level.

Chromosome 6 (The Netherlands)

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

The Dutch initiative, supported by the Centre of Biosystems Genomics, aims to sequence tomato chromosome 6 by a BAC-by-BAC walking strategy. This strategy is supported using a Sequence Tagged Connector approach as outlined in Peters *et al., Plant Physiol.*, 140:805-814. It involves the use of BAC end sequences from three different BAC libraries made available by the US part of the SOL Initiative, and high resolution non-selective AFLP fingerprinting. Using this approach, we have made progress on identification and extension sequencing. FISH, carried out by the group of Dr. Hans de Jong at the Laboratory of Cytogenetics, Wageningen University Research Centre, has been used to confirm the chromosome 6 locations of seed BACs. At the Plant Research International thus far, thirty-five seed BACs have been sequenced. Currently, thirteen seed BACs are in the sequencing pipeline. BAC sequences are annotated, uploaded and are available at SGN.

With high resolution non-selective AFLP fingerprinting carried out by Keygene, fourteen seed BACs have been screened for possible extension. Using BACs from three different libraries, we have identified five MboI BACs, nine HindIII BACs, and four EcoRI fingerprinted BACs having a minimal overlap and maximal extending insert. From this set we have sequenced three extension BACs and compared their overlapping sequences. The sequence overlaps prove to be without discrepancies and confirms the true nature of the overlapping extension BACs.

Chromosome 7 (France)

Contact: Farid Regad (regad@ensat.fr)

Sixteen seed BACs were selected based on mapping or FISH data. To date, ten of these clones have been sequenced: HBa0002D20, HBa0023C09, HBa0049P16, HBa0095C18, HBa0166A09, HBa0215P04, HBa0230E07, HBa0309B15, HBa0309F18, HBa0325D07 and the remaining clones are currently in the sequencing pipeline: HBa0033O01, HBa0037F23, HBa0059P18, HBa0130B18, HBa0241F16, HBa0308M01.

Seven new clones are in progress of sequencing. Two clones (HBa0002M15, HBa0037G17) were selected based on mapping data and the China FPC update. The five other clones are overlapping BACs selected from the clones mentioned in brackets: HBa0195N01 (HBa0002D20), MboI0031B19 (HBa0325D07), HBa0076M21 (AJ303345, Rossberg *et al*. 2001, *Plant Cell*, 13:979), HBa0172P03 (HBa0062O11, Van der Hoeven *et al*. 2002, *Plant Cell*, 14:1441), MboI0119A22 (HBa0309B15).

Chromosome 8 (Japan)

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

We finished sequencing eighteen BACs making the total length to 2,274,432 bases. One BAC is in Phase 2 and another is in Phase 1. Currently, five additional BACs are in the sequencing pipeline. The first round of BAC extension was performed for fourteen finished BACs. We found that twelve of them could be extended with the maximum overlap of 20 kb. To select additional BACs for sequencing, we are planning to make a 3D DNA pool of LE-HBa, SL-MboI and SL-EcoRI BAC libraries, which enables PCR screening.

Chromosome 9 (Spain)

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

Update pending.

Chromosome 12 (Italy)

Contact: Mara Ercolano (ercolano@unina.it)

To date, fifteen seed BACs have been validated following several verification procedures. Sequencing has been completed to Phase 3 for two seed BACs (Le_HBa0032K07 and Le_HBa0021L02) and to Phase 1 for two other seed BACs (Le_HBa0140M01 and Le_HBa0161H10). An additional three seed BACs (Le_HBa0026C13, Le_HBa0075C18 and Le_HBa0163O04) are currently in the sequencing pipeline. The SGN BAC-end database was screened by BLASTN in order to move out of three sequenced BACs. In all cases, the analyses gave multiple hits from all three BAC libraries and twenty-six candidate overlapping BACs have been ordered. A program complementary to the SGN Online BLAST Interface is being developed to allow a more efficient choice of the overlapping BAC clones. A second sequencing pipeline is being assembled at ENEA and will be ready in the course of the summer.

PRODUCT HIGHLIGHT

TempliPhi Amplification Kits

Contributed by Song-Bin Chang

We thought it would be good to share this information with all of you because it has proven to be very useful for DNA amplification.

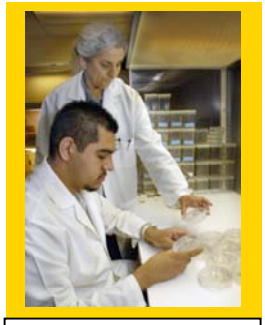
At times, we have had trouble obtaining sufficient amounts of BAC DNA for Fluorescence In-Situ Hybridization (FISH) due to a low yield of BAC DNA after isolation from culture. A few months ago, Dr. Vicky Fernandez from Spain introduced us to TempliPhi Amplification Kits (http://www1.amershambiosciences.com/APTRIX/upp01077.nsf/Content/autodna_templiphi_intro). TempliPhi Kits can multiply nanograms of circular BAC DNA templates to micrograms using bacteriophage Phi29 DNA polymerase and rolling circle amplification. Amplified product from BAC DNA can be used directly for FISHing and amplified DNA from subcloned plasmids can be utilized directly for sequencing. We have done several runs of BAC DNA amplification using the TempliPhi kit, and it always gives sufficient yields of DNA suitable for FISH. It costs less than $10 for a BAC DNA amplification. If a larger amount of template DNA is used, say 40 ng rather than the 10ng recommended, a larger yield is obtained with the same amount of reaction mixture. However, be sure that your DNA is circular!! For more practical details, please contact Dr. Song-Bin Chang (sbchang@lamar.colostate.edu) or the GE Healthcare website listed above.

The Chile Pepper Institute

Contributed by Danise Coon

Diversity in chile pepper germplasm

The Chile Pepper Institute (www.chilepepperinstitute.org) is a research institute in the College of Agriculture and Home Economics at the New Mexico State University (NMSU). It has established itself as the world's premier research based resource center for chile pepper (*Capsicum*), and is the only institute in the world that is specifically dedicated to chile pepper. The goals of the institute include: education through ever-expanding informational resources, disseminating the latest research on new cultivars and disease prevention, publishing "*The Chile Pepper Institute Newsletter,*" providing an international information clearing-house and archive of publications, preserving chile germplasm of both cultivated and wild species, and advancement of chile studies.



Participants of the ASSURED Program

The building where the institute is housed is known as the Chile Pepper Institute's Center for Chile Education. This center has become the premier source of information regarding chile peppers and has a Chile Library with more than 750 books, live chile plant displays, exhibits on current NMSU chile research, chile art, the institute's "Hall of Flame", an interactive informational and educational chile pepper kiosk, and other related educational information. The institute provides an exclusive educational website that houses the greatest wealth of information regarding chile, including digitized downloads of The Capsicum Eggplant Newsletters and the EUCARPIA proceedings.

The institute provides many chile pepper educational programs. The Agricultural Science Summer Undergraduate Research Education and Development (ASSURED) Program was launched in the summer of 2003. It started as a 2-year National Science Foundation grant to help guide students from a farm-labor background into science and a science career path. The program has grown and will be funded for an additional three years. A highlighted program is the annual Teaching and Demonstration Garden, which is a unique environment created to present the wonders of chile peppers in a garden setting. This is an educational facility operated in partnership with the NMSU Chile Pepper Breeding and Genetics Program. It is the world's only garden dedicated exclusively to chile peppers. The garden is in its prime July-October and open to the public for self-guided and guided tours which can be arranged by contacting the institute.

Contact Information

Danise Coon
Program Coordinator - Chile Pepper Institute
New Mexico State University
Phone: (505) 646-3028
e-mail: hotchile@nmsu.org
www.chilepepperinstitute.org

Display of ornamental chile peppers

WHAT'S NEW ON SGN?

A new Gene search feature is available on the SGN search page (http://sgn.cornell.edu/search/direct_search.pl?search=loci). From the "search" menu, choose "genes", and enter search terms for gene symbols, alleles or phenotypes. Gene detail pages provide information about each gene. These entries are currently being curated using controlled vocabularies. On the gene pages, connections related to tomato loci, sequence, and literature information are also available. Your feedback is welcome!

Please note that we will be moving our servers to a new server room during the week of April 3 - 7, 2006. Some of our services, such as FTP, website login, and data uploads will be unavailable during this time. In addition, the website may experience brief service interruptions. Please check the page http://sgn.cornell.edu/help/planned_outages.pl for more information.

ANNOUNCEMENTS

New Publication

Bisbis, B., Delmas, F., Joubès, J., Sicard, A., Hernould, M., Inzé, D., Mouras, A., Chevalier, C. (2005) Cyclin-dependent kinase (CDK) inhibitors regulate the CDK-cyclin complex activities in endoreduplicating cells of developing tomato fruit. *J. Biol. Chem.* 281:7374-7383.

SOLANACEAE RECIPES

In my attempt to find interesting recipes with more than three Solanaceae family members as ingredients, I have reached my goal with this one that requires five different members.

Chicken Stemperata: Stemperata di Pollo
Recipe courtesy Mario Batali, Food Network, www.foodnetwork.com
Cook Time: 1 hour 10 minutes
Yield: 4 servings

1 (3 1/2 pound) chicken, cleaned and cut into 8 serving portions, and breast cut in 2 pieces
Salt and freshly ground black pepper
3 tablespoons plus 1/2 cup extra-virgin olive oil
2 medium russet potatoes, peeled and cut into large cubes
2 red, yellow, or orange peppers, cored, seeded and cut into medium strips
1 stick celery, cut into large pieces
2 small unpeeled eggplants, cut into large cubes
2 medium carrots, peeled and thinly sliced into 1/2-inch thick rounds
2 tablespoons salt packed capers, rinsed and drained
1/2 cup whole pitted Sicilian olives (the green variety)
4 fresh plum tomatoes, cut into large pieces
5 whole chiles
1 1/2 cups dry red wine
1/4 bunch each fresh chopped mint leaves and parsley leaves
Pinch chile flakes

Pat the cleaned chicken dry, and then season the pieces with salt and pepper. In a 12 to 14-inch sauté pan, heat 3 tablespoons olive oil until hot but not smoking. Carefully add the chicken pieces and brown on both sides, about 5 minutes per side. Remove chicken from the pan and set aside.

In the pan with the chicken drippings, add potatoes, peppers, celery, eggplant, carrot, capers, olives, tomatoes and whole chiles. Toss together. Add the wine and chicken, season with salt and pepper and bring to a boil. Cover, lower the heat to a simmer and cook for about 15 minutes until chicken is cooked through. Remove from the heat and stir in chopped mint, parsley and a pinch of chili flakes.

Transfer the cooked stew to a serving dish and allow to cool to room temperature before serving. Drizzle with extra-virgin olive oil and serve.