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Community News

Syngenta Releases Physical-Genetic Map Alignment in Support of Tomato Genome Sequencing



During the tomato sequencing meeting held at the Plant and Animal Genome (PAG) conference in January, Syngenta announced that they would make resources available to assist the tomato sequencing effort. The Syngenta Applied Genomics group in North Carolina is working with the SOL Genomics Network (SGN; www.sgn.cornell.edu) to release information concerning the location of BACs based on their marker sets from their Ultra High Density map. Approximately 26% of the tomato genome, in a total of 763 anchored BAC contigs, has been assigned to bin positions based on Dani Zamir's introgression lines. The first phase of information can be found on SGN through a link under the "News" section on the homepage.

Syngenta has also offered ad hoc assistance to SGN and genome sequencing scientists, such as manual gap filling, anchoring additional BAC contigs and access to Syngenta SSRs defining specific chromosomal segments.

Keygene Will Provide AFLP-Based Markers to the International Tomato Sequencing Project



Keygene also announced during the tomato sequencing meeting at PAG that they would make available markers to find new seed BACs that are based on AFLP markers. They plan to start with chromosome 6 as a pilot and then make them available to the tomato sequencing consortium.

The tomato sequencing participants were excited about the announcements regarding resources from Syngenta and Keygene because these additional tools will play an important role in moving the project forward. Their generosity in sharing resources they developed is very much appreciated.



Tomato Sequencing Updates



A tomato sequencing project meeting was held during the Plant and Animal Genome Conference in January. An agenda, notes, and presentation files can be found on the SOL Genomics Network at <http://www.sgn.cornell.edu/solanaceae-project/sol-bioinformatics/>.

Chromosomes 1, 10, 11 (US)

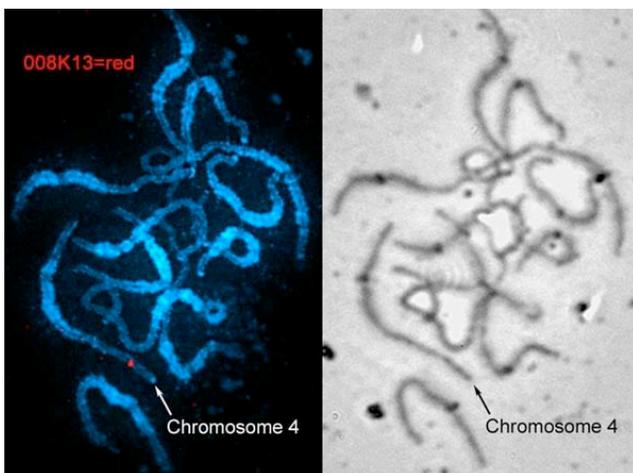
Contact: Joyce Van Eck (jv27@cornell.edu)

To date, nineteen BACs have been sequenced and an additional four BACs are in the sequencing pipeline.

An additional twelve BAC clones have been localized by the Stack lab at Colorado State University and have been posted on SGN (www.sgn.cornell.edu) bringing the total number of fluorescence in situ hybridization (FISH) localized clones to 75. The breakdown is as follows: 17 on chr 1, 4 on chr 2, 3 on chr 3, 11 on chr 4, 6 on chr 5, 5 on chr 6, 4 on chr 7, 4 on chr 8, 9 on chr 9, 2 on chr 10, 6 on chr 11, and 4 on chr 12. The recently positioned BACs include:

<u>Chromosome Arm</u>	<u>BAC ID</u>
1P	LE_HBa0003D15
1Q	LE_HBa0095K03
2Q	LE_HBa0108A18
4Q	LE_HBa0008K13
	SL_MboI0039E17
5P	LE_HBa0147F10
	LE_HBa0189E17
	LE_HBa0309L13
5Q	LE_HBa0138J03
	LE_HBa0169M21
11P	LE_HBa0027B05
11Q	LE_HBa0316E10

The image below illustrates FISH labeling of tomato chr 4 with BAC clone LE_HBa0008K13 (red). The signal is located in the euchromatin of the long arm.



Chromosome 2 (Korea)

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

Update pending.

Chromosome 3 (China)

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

Update pending.

Chromosome 4 (UK)

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

7,231,261 bp of sequence have been generated at the Wellcome Trust Sanger Institute for chr 4 as of February 16th 2007. Of this figure, 6,775,713 bp are unique. The sequence has been produced from fifty-nine BACs originating from the LE_HBa and SL_MboI libraries. We intend to finish all BACs that will contribute to chr 4 to HTGS phase 3 and currently thirty-five BACs that correspond to 4,103,843 bp of sequence have been deposited in the public databases at EMBL/GenBank/DDBJ as phase 3. All other chr 4 BACs with EMBL/GenBank/DDBJ accessions are currently active in our sequencing pipeline at HTGS phases 0 to 2.

With a view to anchoring additional BACs and contigs from the FPC database to chr 4, we have been designing markers for a number of markers from the EXPEN-2000 map that are currently unanchored in the FPC map. A number of PCR hybridizations to filters of the fingerprinted libraries (LE_HBa & SL_MboI) are underway and we will conduct colony PCR verifications to determine if further chromosome markers hybridize to any additional clones and contigs in FPC.

BAC selection continues as suitable sequencing candidates are identified. The progress of chr 4 can be viewed through the development of the TPF and AGP files that we upload monthly to SGN. The TPF indicates the expected relative positions of the BACs along the chromosome and the AGP provides assembly information.





Chromosome 5 (India)

Contact: Akhilesh Tyagi (akhilesh@genomeindia.org)

The Indian Initiative on Tomato Genome Sequencing (IITGS) is currently working on forty-one BAC clones from chr 5. These BACs are associated with twenty-four chr 5-specific markers covering both chromosomal arms. Six BAC clones have reached Phase III level of sequencing, while seventeen and ten BAC clones are at Phase II and Phase I levels of sequencing, respectively. Eight BAC clones are in either early phase of sequencing or library preparation. Seed BACs on six marker positions T0564 (11 cM), C2_At2g01110 (37 cM), T1790 (46 cM), T1360 (73 cM), T1746 (84 cM) and TG185 (119 cM) have been confirmed by FISH analysis. In addition, certain BACs associated with markers shown earlier belonging to chr 5 have now been confirmed on other chromosomes using Introgression Lines and/or FISH data. Some of these clones have been sequenced.

Chromosome 6 (The Netherlands)

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

We have begun finishing of chr 6 BACs. We designed 400 oligos in the first round to close 200 sequence gaps. We have taken gap flanking read pairs and BAC insert sequence overlaps to guide the oligo design and directed PCR amplification of gap sequences. While this phase is still running we closed thirty-two gaps and extended for another twenty gaps. We will also include homology based searches and primer pooling to complete the BAC closure.

BACs H217M17, H222J18, H020O17, H049I02, and M123O13 are currently in the sequence pipeline, and two EcoRI BACs are sequence ready. In addition, we have found thirteen additional extension BACs of which five are from the HindIII BAC library, six from the MboI, and two are from the EcoRI library. After BAC end sequence verification, these BACs will also enter the sequence pipeline.

Chromosome 7 (France)

Contact: Farid Regad (regad@ensat.fr)

Below is a list of twenty-one BACs that have been completed. The corresponding sequence will be available as soon as we receive the NCBI accession numbers.

LE_HBa0001N06; LE_HBa0002M15; LE_HBa0023C09;
LE_HBa0033O01; LE_HBa0037F23; LE_HBa0073N22;
LE_HBa0095C18; LE_HBa0130B18; LE_HBa0163O04;
LE_HBa0184G23; LE_HBa0215P04; LE_HBa0230E07;
LE_HBa0308M01; LE_HBa0309B15; LE_HBa0309F18;
SL_EcoRI0099J13; SL_MboI0017L19; SL_MboI0031B19;
SL_MboI0046H06; SL_MboI0119A22.

Below is a list of fifty-six BACs that are currently in the sequencing pipeline. Among these clones, thirty-eight are at shotgun library stage, thirteen are under sequencing and five are at the gap closure stage.

LE_HBa0002D20; LE_HBa0003E13; LE_HBa0006H17;
LE_HBa0012N15; LE_HBa0018L21; LE_HBa0022P08;
LE_HBa0027M1; LE_HBa0030C22; LE_HBa0030F21;
LE_HBa0033E17; LE_HBa0037G17; LE_HBa0038A02;
LE_HBa0041L08; LE_HBa0046G04; LE_HBa0049C13;
LE_HBa0049P16; LE_HBa0056M08; LE_HBa0059P18;
LE_HBa0060P15; LE_HBa0061J13; LE_HBa0069G15;
LE_HBa0076O09; LE_HBa0079F09; LE_HBa0080A17;
LE_HBa0097F22; LE_HBa0102J11; LE_HBa0103N02;
LE_HBa0104E22; LE_HBa0106F06; LE_HBa0108F03;
LE_HBa0111F22; LE_HBa0114J16; LE_HBa0117J06;
LE_HBa0127J08; LE_HBa0140O20; LE_HBa0162M15;
LE_HBa0166A09; LE_HBa0172P04; LE_HBa0175E07;
LE_HBa0184E04; LE_HBa0222K11; LE_HBa0224G23;
LE_HBa0226J04; LE_HBa0236D20; LE_HBa0241F16;
LE_HBa0242B17; LE_HBa0284C08; LE_HBa0287B22;
LE_HBa0325D07; SL_EcoRI0019G22;
SL_EcoRI0020F06; SL_EcoRI0067O11;
SL_EcoRI0097H15; SL_EcoRI0111B06;
SL_MboI0024K03; SL_MboI0093E04.

In order to generate more seed BACs, ten COS markers (C2_At1g04970; C2_At2g04842; C2_At2g06925; C2_At2g16060; C2_At3g14910; C2_At5g56940; T0671; T0837; T1366; T1651) will be tested for BAC overgo hybridizations at Cornell.

Chromosome 8 (Japan)

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

We finished fifty-two clones to Phase 3 (total non-redundant length 5,525,452 bases) of which twenty-eight are seed BACs and twenty-four are extended clones. This month, we have initiated shotgun sequencing of a selected low-copy BAC mixture at the speed of 40,000 reads per week. We have also started development of new microsatellite markers on the EXPEN-2000 map, and so far identified over 200 markers showing polymorphism between the parents among which 173 are co-dominant markers. We expect the final number of new microsatellite markers to be 1000.





Chromosome 9 (Spain)

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

Currently, the sequences of twenty-five BACs have been completed and sixteen more are in progress at different finishing stages. Some of the BACs are AT-rich and repetitive sequence rich which makes finishing more difficult. Six additional extension BACs have been ordered.

The *in silico* search has identified eight additional seed BACs with BAC end sequences containing chr 9 markers. These markers have been mapped to chr 9 using Dani's ILs and allelic polymorphisms were identified by sequencing.

Some candidate BACs for chr 9 markers whose sequences did not show sequence polymorphisms (even in 1400 bp) in the ILs will be confirmed by FISH. Two candidates for FISH are a BAC located at 2 cM to rule out that it is already in the telomeric region and one on the short arm that contains repetitive and Ty3 transposon sequences that together with an approximate location makes it a good candidate for the euchromatin/heterochromatin border. Additional BACs contained markers that mapped at 4 cM and showed some overlapping.

A list of ten chr 9 markers has been sent to Cornell to identify new seed BACs by overgo screening. We will use the information from Syngenta to identify additional BACs mapped to chr 9.

Chromosome 12 (Italy)

Contact: Mara Ercolano (ercolano@unina.it)

Since our last report, two new seed BACs have been validated and three new BACs have been selected to extend minimal tiling paths. Currently, forty-eight BACs are in different sequencing phases and seven were submitted to the SGN repository. Forty-two BACs are in the finishing process and an additional six BACs are in the sequencing pipeline. Of these, thirty-five are extension BACs, which have been selected from fifteen starting points. Tile path selection continues across the established sequence islands. Twelve seed BACs have been extended in both directions and three in one direction. Moreover, we are trying to increase the number of seed BACs in order to fill gaps between anchor markers.



Solanaceae Poetry

The following haiku was written by Paul Covey. He is a graduate student in Pat Bedinger's group at the Colorado State University. Paul won the Colorado State University Department of Biology Haiku Contest in 2006 with this haiku. Congratulations, Paul!

I say Solanum
You say Lycopersicum
Just say tomato





Announcements

Publications

Autar K. Mattoo, Anatoly P. Sobolev, Anil Neelam, Ravinder K. Goyal, Avtar K. Handa, and Anna L. Segre. 2006. Nuclear magnetic resonance spectroscopy-based metabolite profiling of transgenic tomato fruit engineered to accumulate spermidine and spermine reveals enhanced anabolic and nitrogen-carbon interactions. *Plant Physiol* 142:1759-1770.

Alka Srivastava, Sang Ho Chung, Tahira Fatima, Tatsiana Datsenka, Avtar K. Handa, and Autar K. Mattoo. 2007. Polyamines as anabolic growth regulators revealed by transcriptome analysis and metabolite profiles of tomato fruits engineered to accumulate spermidine and spermine. *Plant Biotechnology* (will be published in the March issue).

Nuray Z. Unlu, Torsten Bohn, David Francis, Steven K. Clinton, and Steven J. Schwartz. 2007. Carotenoid absorption in humans consuming tomato sauces obtained from tangerine or high- β -carotene varieties of tomatoes. *J Agric Food Chem* 55:1597-1603.

Conferences



The 4th Solanaceae Genome Workshop

Ramada Plaza, Jeju Island, Korea

September 9 – 13, 2007

<http://www.solanaceae2007.org/>



Abstracts Submission Deadline - June 30, 2007

Registration Deadline - July 31, 2007



Potato Association of America

Shilo Inn Conference Center Idaho Falls, Idaho, USA

August 12 - 16, 2007

<http://www.conferences.uidaho.edu/PAA/>





Solanaceae Recipes

The following recipe was submitted by Theresa Fulton. If you have any *Solanaceae* recipes you would like to share, send them to Joyce Van Eck at jv27@cornell.edu.

Spicy Peanut Stew with Ginger and Tomato from the New York Times, January 24, 2007

- | | |
|--|--|
| 1 medium-size eggplant, peeled and cut into 1/2 inch cubes | 1 small (14.5 oz.) can diced tomatoes, preferably roasted |
| 1 tsp salt, more to taste | 4 C vegetable stock or water |
| 1 tsp ground cumin | 1/2 C natural sweetened peanut butter (creamy or crunchy) |
| 1 tsp ground coriander | 1 medium-size zucchini, 6-8 oz, cut in quarters lengthwise, then sliced 1/2 inch thick |
| 1/4 tsp turmeric | 2 Tbsp freshly squeezed lemon juice (1-2 lemons) |
| 1/8 tsp cayenne | 1/3 C coarsely chopped cilantro leaves, plus whole leaves for garnish |
| 1/4 C peanut oil | Cooked rice, for serving |
| 2 shallots, thinly sliced | Chopped roasted salted peanuts, for garnish (optional) |
| 2 inches fresh ginger, peeled and minced | |
| 1 to 2 jalapeno chilies, seeded and minced | |
| 1 onion, chopped | |
| 1/3 C tomato paste | |

1. In a colander, toss eggplant with 1 tsp salt; set aside for 30 min. Rinse, drain well, and set aside. In a small bowl, combine coriander, turmeric and cayenne; set aside.
2. In a large pot, heat 3 Tbsp oil over medium high heat. Add shallots and fry, stirring often, until soft, crisp and caramelized, about 10 min. Using a slotted spoon, transfer shallots to a large bowl, leaving oil in pot. Raise heat to high and add eggplant. Cook, stirring often, until lightly browned and just tender, about 10 min. Transfer to bowl with shallots.
3. Add remaining 1 Tbsp oil to pot and heat over medium-high heat. Add ginger and chilies and cook, stirring for 30 seconds. Add spices and cook, stirring, 30 seconds more. Add onion and cook, stirring to scrape up any browned bits, until softened and translucent, about 5 min. Add tomato paste and cook, stirring, 1 min.
4. Add diced tomatoes, stock or water, eggplant, shallots and a sprinkling of salt. Bring to a boil and cook 5 min. Place peanut butter in a medium bowl, add one or two ladlefuls of hot soup, and stir until emulsified, then pour mixture back into soup.
5. Reduce heat to simmer, add zucchini, cover and cook 10-15 min, until vegetables are tender. Turn off heat and stir in lemon juice and chopped cilantro. Let cool slightly and taste; add salt if necessary. Serve in bowls with rice, garnished with cilantro leaves and chopped peanuts, if desired.

Yield: 8 servings

