September 2009
Issue Number: 24
Editor: Joyce Van Eck

The Sol Newsletter

In this issue.....

Community News

Potato Genome Sequencing Consortium

Contributed by Jeanne Jacobs, Robin Buell, Christian Bachem and Sanwen Huang (members of the PGSC steering committee), on behalf of the PGSC

Potato is a key member of the Solanaceae. It is the world's third most important crop and the most important vegetable crop. The potato genome has twelve chromosomes and is estimated to be 840 Mb. The global Potato Genome Sequencing Consortium is generating the high quality genome sequence of potato.

The Potato Genome Sequencing Consortium (PGSC) was an initiative of the Plant Breeding Department of Wageningen University & Research in the Netherlands. The PGSC is a global consortium of research groups from fourteen countries (Fig. 1).

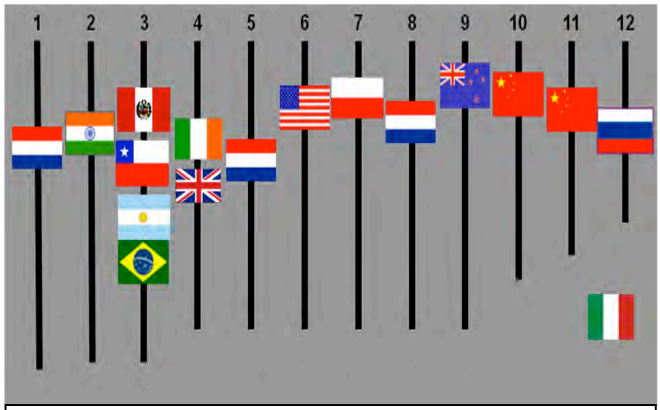


Figure1: Chromosomes as allocated early in the project. Chromosome 1, 5, 8 the Netherlands; 2 India; 3 Peru, Chile, Argentina, Brazil; 4 Republic of Ireland, United Kingdom; 6 USA; 7 Poland; 9 New Zealand; 10, 11 China; 12 Russian Federation. Italy joined in recent months.

At the onset, the approach was to use BAC-by-BAC sequencing of the (heterozygous) diploid line RH89-039-16 (RH) via conventional Sanger sequencing. The diploid RH is one of the parental lines of the mapping population SHxRH, used to develop a high density genetic map (van Os et al. Genetics 173:1075-1087, 2006). A physical map of RH was under development. The various partners in the PGSC were allocated chromosome(s). However, in the past two years, Next Generation Sequencing technologies developed significantly, leading to a change of approach within the PGSC and in 2008, sequencing of the doubled monoploid DM1-3 516R44 (DM) of *Solanum phureja* was initiated as a complementary project to sequencing of *S. tuberosum* RH. In June 2009, PGSC members came together at Teagasc in Carlow (Republic of Ireland) to plan the final phases of the project (Fig. 2).

Currently, we are busy with finalizing the sequence data for both RH and DM with the goal to generate a high quality genome sequence of potato. Coverage is greater than 70X using a combination of sequence data generated by Sanger sequencing (complete BAC clones, BAC end sequences from various libraries, fosmid end sequences), Illumina (single read and mate pair reads of genomic DNA, transcriptome libraries from 32 different tissues), and Roche 454 (single read, paired end reads, long jump libraries, and transcriptome). This is supplemented by an improved physical map of RH using Whole Genome Profiling™ and the development of an anchored genetic reference map based on DM being undertaken by a number of PGSC members.

Figure 2: Participants of the PGSC workshop in Carlow, June 2009.

Hybrid assembly will take place at Beijing Genomics Institute and Wageningen University & Research, combining Next Generation Sequencing data with Sanger sequencing data for RH as well as for DM. This will generate three virtual molecules for each haplotype. A first draft assembly of DM based on Illumina short reads has been generated. The total assembled genome is 720 Mb, with a N50 contig size of 22 kb and a N50 scaffold of 255 kb.

The first draft assembly is available at www.potatogenome.net (subject to acceptance of the data access agreement). Updates will be made over the next six months as additional data is generated including annotation of the genes, identification of the transcriptome, and analysis of genes critical to potato projects.

Updates on progress will be presented at the SOL conference in New Delhi, India, in November and at the Plant & Animal Genome XVIII congress in San Diego, CA, USA in January.

Contact details for PGSC members can be found at www.potatogenome.net.

The 23rd International Conference on Coffee Science ASIC 2010

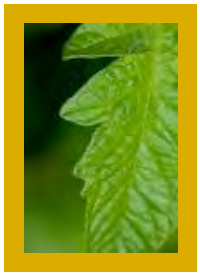
Provided by A. Charrier

The most important conference on coffee research worldwide will take place in Indonesia from October 3rd to 7th, 2010 at the Grand Ball Room of the Bali Grand Hyatt Hotel, Nusa Dua, Bali, Indonesia.

ASIC 2010 will focus on the latest international findings in scientific and technological researches in all areas of the coffee industry. Particularly, the current themes "Coffee Genomics" and "Coffee Biotechnology" will be on the conference agenda, enabling participants to find out about and discuss the important progress in these areas.

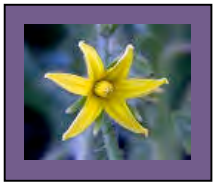
Participants interested in presenting an oral or poster communication will be required to submit a summary by March 31st, 2010 at the latest to:

Dr. Rémy Liardon (Scientific Secretary, ASIC)
Ch. de Grand-Vennes 5B
CH-1010 Lausanne (Switzerland)
Phone: +41 21 653 0745
E-mail: coffee-science@asic-cafe.org
www.asic-cafe.org



Eating Tomato Leaves…..Really?

Dina St. Clair, a tomato breeder at the University of California at Davis, told me about an article she read in the New York Times about cooking with tomato leaves. I thought I would share it with you. I never even considered cooking anything with tomato leaves in it, but looks like some people have used them based on this article. If any of you have ever used tomato leaves in your cooking, send me an e-mail (jv27@cornell.edu), and I'll share your comments in the next newsletter. Here's the url for the article: http://www.nytimes.com/2009/07/29/dining/29curi.html?_r=1&ref=dining.

Tomato Sequencing Updates

ANNOUNCEMENT – FISH Localizations

The laboratory of Dr. Stephen Stack at Colorado State University is once again offering to use FISH to localize BACs for labs in other countries that are participating in the tomato genome sequencing project. The Stack lab will attempt to localize ten BACs per country at no charge. These BACs would be in addition to any that may have been previously localized for these countries. Those who wish to submit BACs for localization should prepare a minimum of 5 mg of DNA, either dried down free from buffers, EDTA, etc., or dissolved in sterile distilled water. The identity, concentration, and solvent for the DNA must be clearly indicated. For further information please contact Dr. Stack at Stephen.Stack@colostate.edu.

Chromosomes 1, 10 (US)

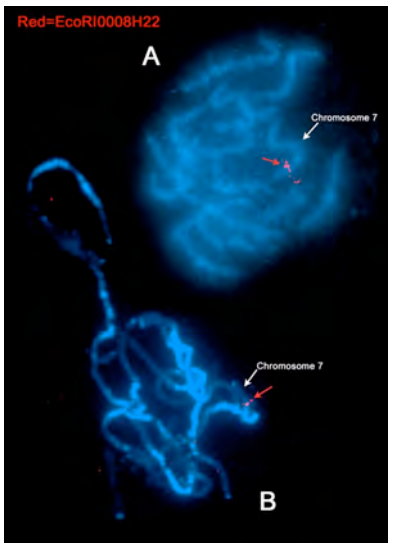
Contact: Joyce Van Eck (jv27@cornell.edu)

In the fall of 2008, the International Tomato Genome Sequencing Consortium agreed to initiate whole genome shotgun sequencing of tomato to both facilitate the high quality euchromatin effort and to capture the bulk of heterochromatin sequence that would otherwise be missed. We will contribute 10x sequence generated by 454 GS FLX with Titanium chemistry. We have prepared 3 - 4 Kb paired-end libraries that are entering the sequencing queue with nearly 1 Gb from this resource generated to date with apx. 3 Gb from 8 -10 kb paired-end libraries for a total of approximately 4.5 Gb (or apx. 5 genome equivalents). In addition, 20 Kb paired-end libraries are being prepared for us by 454 Roche. We have also initiated creation of a new physical map through collaboration with the Arizona Genomics Institute to facilitate genome sequence completion. The majority of BACs are already fingerprinted and a draft assembly is anticipated before the end of 2009.

A total of 188 BAC clones have been localized on tomato pachytene synaptonemal complex spreads using fluorescence in situ hybridization (FISH). They are distributed among the chromosomes as follows: 1 – 36; 2 – 16; 3 – 14; 4 – 16; 5 – 11; 6 – 9; 7 – 18; 8 – 5; 9 – 18; 10 – 27; 11 – 13; 12 – 5. This number includes twenty BACs (listed below) that have been localized since the last newsletter. Data for these BACs have been posted on the SOL Genomics Network website.

Chromosome Arm	BAC ID
1P	LE_HBa0177F14
1P	LE_HBa0032H01
1Q	LE_HBa0092H13
1Q	LE_HBa0131F15
1Q	LE_HBa0126F09
3Q	LE_HBa0007J09
5P	LE_HBa0303C11
7P	SL_EcoRI0008H22
7Q	LE_HBa0226J04
7Q	LE_HBa0179K09
7Q	LE_HBa0227C07
7Q	LE_HBa0079F09
8Q	LE_HBa0034L18
10P	LE_HBa0060A06
10P	LE_HBa0111D09
10Q	LE_HBa0057G01
10Q	LE_HBa0005D06
10Q	LE_HBa0188N09
10Q	LE_HBa0021O12
10Q	LE_HBa0049K02

The figure on the right illustrates FISH labeling of tomato chr7 with BAC clone SL_EcoRI0008H22 (red) on both a pachytene synaptonemal complex spread (A) and 3:1 EtOH:Acetic acid fixed pachytene chromosome spread (B). The signal is located at the euchromatin/heterochromatin border on the short arm.



Chromosome 2 (Korea)

Contact: Sunghwan Jo (shjo@kribb.re.kr)

To date, 195 BAC clones have been sequenced corresponding to 15.5 Mbp. The assembly consists of forty-two supercontigs. The size of the longest contig is 1,346,425 bp and N50 of contig size is 742,118 bp long. All contigs are anchored along the linkage group 2. To extend chr2 contigs, we have sequenced additional candidates with 454 FLX Ti and 3kb paired end. As a result, 9 Mb of non-redundant sequence was obtained from a pool of ninety-five fosmids and eighty-three BACs, which may be localized on chr2. Fifty-four supercontig ends have been extended with the sequence from twenty-one ends and among them we could find the next extension BACs and/or fosmids. We are planning to determine the sequence with the same method using 454 FLX Ti and 3kb paired end.

Chromosome 3 (China)

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

Our effort is currently focused on sequencing ninety-five BACs, which were confirmed to be on chr3 by introgression line (IL) mapping. To date, seventy-three BAC clones were completely finished and the rest of the BACs were completed as HTGS phase 2 with the Sanger sequencing platform. At the same time, we initiated an effort to sequence 100 BACs using the next-generation sequencing platform the Illumina Genome Analyzer after making a label to each BAC. So far, about seventy BACs were confirmed on chr3 by IL mapping. Among them, thirty BACs were sequenced with nearly 1000-fold coverage. Each BAC was constructed with two paired end subclone libraries of 500 bp and 6 kb. The primary assembly results have been achieved.

Chromosome 4 (UK)

Contact: Gerard Bishop and R. Lopez-Cobollo (g.bishop@imperial.ac.uk, r.lopez-cobollo@imperial.ac.uk*)*

We are currently identifying BACs to extend existing contigs and also screening for BACs with chr4 markers that are absent from the current chr4 BAC sequences. This is being carried out by both the analysis of the BAC/fosmid end sequences and by screening the 3D BAC superpools.

Currently, we have eleven BACs and one fosmid being sequenced by Cogenics that were confirmed to be on chr4 by IL mapping. BACs: SLMboI0090M22, LE_HBa0091, LE_HBa0054E15, SL_MboI0048I03, SL_EcoRI0091C05, SL_MboI0069J23, SL_MboI0105G02, LE_HBa0051E04, LE_HBa0102P20, LE_HBa0028J23, LE_HBa0025G05 and a fosmid :: SL_FOS0162E05. Phase II sequence will be available soon.

Twenty-seven of the 141 markers for which the corresponding BACs have not been identified have been analyzed. Eight new BACs were identified from this screening. We have identified another six BACs from BAC extension (SL_EcoRI0041M09, LE_HBa0012C21, SL_MboI0028K07, LE_HBa0114K12, SL_EcoRI0049J22, LE_HBa0052C01) and two fosmids (SL_FOS0279J18 SL_FOS0010B17) and these are ready for sequencing. We are verifying chr4 location of another four BACs prior to sending them for sequencing.

Bioinformatic analysis using our Gbrowse viewed Golden Path (AGP based) is helping us to extend contigs and fill gaps (via fosmid and BAC end sequence overlaps). We are also currently in the process of both reordering and changing the relative orientation of contigs on chr4.

We are also generating a SOLID3 ~7-8kb mate pair run (X2 slides) that will soon be available for the whole genome next generation shotgun approach to sequence the tomato genome.

Chromosome 5 (India)

Contact: Akhilesh Tyagi (akhilesh@genomeindia.org)

At the Indian Initiative on Tomato Genome Sequencing, we have confirmed positions of ninety-five BACs on chr5. Till now fifty-one BACs have been sequenced to phase III level, nineteen BACs are at phase II level and sixteen BACs are at phase I level of sequencing. The remaining nine BACs are in the early phase of sequencing or library preparation. A search is on to find new extension BACs by performing overgo hybridization on the filters available for the three tomato libraries, PCR screening on the 3-D DNA pools of *Hin*dIII and *Mbo*I BAC libraries, the fosmid end sequences and SBM (selected BAC mixture) shotgun data.

Chromosome 6 (The Netherlands)

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

Update pending.

Chromosome 7 (France)

Contact: Murielle Philippot (murielle.philippot@ensat.fr)

To date, 186 BACs and seven fosmids have been selected and validated on chr7. Among these there are ninety-two "seed BACs" and 101 overlapping BACs and fosmids. 149 BACs have been sequenced to phase 2 or 3 and thirty-eight are in phase 0 or 1. We submitted to Genbank and SGN a total of 161 BAC sequences anchored to chr7 and three BACs allocated to chr0. Overall, 17.9 Mb of sequences were generated of which 15.4 Mb are non redundant (61% of the total estimated euchromatin of chr7). The BACs are organized in forty contigs on chr7. The contigs contain from two to nineteen members.

We are also engaged now in the Whole Genome Sequencing project, which aims to produce a complete draft sequence of the tomato genome using Next Generation Sequencing technologies. The assembly of the whole genome will be mainly based on the sequences produced by 454. To date, we produced eleven 454 Titanium runs and will release nine more runs within the upcoming weeks. It is important to mention that in order to better cope with the assembly tasks to which we are contributing, we reinforced our bioinformatics team and are now taking advantage of the expertise and infrastructure of the Toulouse Bioinformatic platform (Bioinfo-Genotoul) and especially the 256 Go RAM computing facility.

Chromosome 8 (Japan)

Contact: Shusei Sato (ssato@kazusa.or.jp)

As of August 20, 2009, 196 BAC clones (112% of initial target) have been completed as Phase 3 that produced a non-redundant length of 19,010,595 bp and an additional seven BAC clones are in the sequencing pipeline.

We have accumulated 4.24 million files of Selected BAC Mixture (SBM) shotgun data, which reached to 2.3 Gb of total length. These shotgun sequences have been assembled into 100,784 contigs covering approximately 540 Mbp regions of the genome.

Chromosome 9 (Spain)

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

We have produced a 5 kb SOLiD library and 600 million mate paired reads (35 bp in length) obtained from it using SOLiD sequencing. In collaboration with the Barcelona Supercomputing Center and the other SOLiD sequence producers (Giorgio Valle in Padova, Gerard Bishop in the UK, and Roeland van Ham in the Netherlands), we are trying de novo assembly of SOLiD data. In addition, this data will be used in combination with other sequencing data in the generation of the first draft of the tomato genome.

Chromosome 11 (China)
Contact: Zhonghua Zhang (zhangzh.ivf@caas.net.cn) or Sanwen Huang (huangsanwen@caas.net.cn)
Update pending.

Chromosome 12 (Italy)
Contact: Mara Ercolano (ercolanao@unina.it)
Currently, eighty-two chr12 BACs have been fully sequenced using either the Sanger method or 454-based approach and submitted to GenBank/SGN. Of these, twenty-seven are in HTGS3, thirty in HTGS, and twenty-five in HTGS1 phase. All the BACs underwent genetic mapping through IL to confirm their position on chr12. In addition, six Titaniun-454 runs have been performed producing around a 2X sequence coverage of the tomato genome. Two slides of a 10kb pair-end library have been run on the SOLiD system v.3, producing around 630 million reads for both F and R primers. The SOLiD paired-end reads will be quite useful for building up the scaffold of the tomato genome, using as input data for the contigs generated by means of the assembly of the 454 runs.

Announcements

Publications

Bhattacharjee S, Zamora A, Azhar MT, Sacco MA, Lambert LH, Moffett P (2009) Virus resistance induced by NB-LRR proteins involves Argonaute4-dependent translational control. Plant J 58:940-951.

Carli P, Arima S, Fogliano V, Tardella L, Frusciante L, Ercolano MR (2009) Use of network analysis to capture key traits affecting tomato organoleptic quality. J Exp Bot 60:3379-3386.

Cillo F, Mascia T, Pasciuto MM, Gallitelli D (2009) Differential effects of mild and severe *Cucumber mosaic virus* strains in the perturbation of microRNA-regulated gene expression in tomato map to the 3′ sequence of RNA 2. Mol Plant Microbe In 22:1239-1249.

Fatima T, Rivera-Dominguez M, Troncoso-Rojas R, Tiznado-Hernandez ME, Handa AK, Mattoo AK (2008) Tomato. In: Compendium of Transgenic Crop Plants: Transgenic Vegetable Crops Vol. 6 (Kole C, Hall TC, eds.), Blackwell Publishing, Oxford, UK pp. 1-46.

Mattoo AK, Yachha SK, Fatima T (2008) Genetic manipulation of vegetable crops to alleviate diet-related diseases. In: Improving the Health-Promoting Properties of Fruit and Vegetable Products (Tomas-Barberan FA, Gil MI, eds.), Woodhead Publ. Ltd., Cambridge, pp. 327-345.

Nambessan S, Handa AK, Mattoo AK (2008) Polyamines and regulation of ripening and senescence. In: Postharvest Biology and Technology of Fruits, Vegetables and Flowers (Paliyath G, Murr DP, Handa AK, Lurie S, eds.), Willey-Blackwell Publ., Ames, IA, pp. 319-340.

Ribeiro APO, Picoli EAT, Lani ERG, Vendrame WA, Otoni WC (2009) The influence of flask sealing on *in vitro* morphogenesis of eggplant (*Solanum melongena* L.). In Vitro Cell Dev Biol-Plant 45:421-428.

Schilmiller AL, Schauvinhold I, Larson M, Xu R, Charbonneau AL, Schmidt A, Wilkerson C, Last RL, Pichersky E (2009) Monoterpenes in the glandular trichomes of tomato are synthesized from a neryl diphosphate precursor rather than geranyl diphosphate. PNAS 106:10865-10870.

Yokotani N, Nakano R, Imanishi S, Nagata M, Inaba A, Kubo Y (2009) Ripening-associated ethylene biosynthesis in tomato fruit is autocatalytically and developmentally regulated. J Exp Bot 60:3433–3442.

Conferences

SOL 2009, The 6th Solanaceae Genome Workshop
November 8 - 13, 2009
New Delhi, India
www.sol2009.org

Plant and Animal Genome Conference XVIII
January 9 - 13, 2010
San Diego, CA
www.intl-pag.org

Potato Association of America
August 15 - 19, 2010
Corvallis, Oregon
http://potatoassociation.org

Capsicum and Eggplant Breeding 2010, Working Group Meeting
August 30 - September 1, 2010
Valencia, Spain
e-mail: jprohens@btc.upv.es
www.comav.upv.es/capsicumeggplant

Solanaceae Recipes

Curried Eggplant with Tomatoes

http://www.aubergines.org/recipes.php

2 pounds eggplant, preferably the slender oriental variety, sliced 1/4-inch thick
3 tablespoons oil
2 teaspoons ground coriander
1 teaspoon ground cumin
1 teaspoon salt, or to taste
1/2 teaspoon cayenne pepper
1/4 teaspoon ground turmeric
1/2 teaspoon fennel seeds
1/4 teaspoon black mustard seeds
2 cups (about 1-1/2 pounds) chopped ripe tomatoes
2 teaspoons minced ginger
2 teaspoons minced garlic

1. Preheat a grill. Brush eggplant slices with half the oil and grill, turning once, until lightly browned.
2. Combine coriander, cumin, salt, cayenne pepper, and turmeric in a small dish. Set aside.
3. Heat remaining oil in a large skillet. Add fennel seeds and mustard seeds, and when they begin to pop (a few seconds), add tomatoes, ginger, garlic, and the spice mixture.
4. Cook over medium-high heat, stirring constantly, until the mixture thickens and turns a slight orange color.
5. Add grilled eggplant and mix to combine with other ingredients. Cover and cook over medium-low heat about 10 minutes. Serve hot or at room temperature.

New World Lasagna

Provided by Leslie Wanner

Ingredients in this recipe that have their origins in the Americas are indicated in **bold type**; those that come from the Solanaceae are in **bold red type**.

For a 9" X 12" baking dish (serves 6). This recipe is very easily scaled up or down to fit different sizes of baking dishes.

The Mole Sauce

Combine and liquefy in a blender or food processor one 24 oz. jar of a good prepared medium or hot salsa (**tomatoes, chili peppers**) and ½ C. unsweetened **cocoa** powder.

The Filling

2 Tbs. olive oil (or **corn** oil)
2 cloves garlic, crushed
1 fresh jalapeno **chili pepper** (or other **chili pepper** type), finely chopped
1-2 Tbs. cumin powder
4 large or 6 medium-sized Yukon Gold or other yellow waxy **potato**, cut into small cubes
1 large (1 lb. 13 oz.) can **black beans,** rinsed (or 2 C. home-cooked); **pinto** or other types of **beans** may be substituted for **black beans**
1 large handful of fresh cilantro leaves, finely chopped

Sauté the **potatoes**, garlic, **chili pepper**, cumin in the olive oil until the **potatoes** are browned on most sides. Add the rinsed and drained **black beans**, and salt to taste. Heat 2 minutes more until **beans** are heated through. Stir in the finely chopped cilantro.

Putting it all together

1. Pour a thin layer of **mole sauce** in baking dish.
2. Make a layer of fresh **corn tortillas**; cover with half of the filling.
3. Sprinkle with a couple handfuls of grated sharp cheddar cheese, and dribble **mole sauce** over it all----more sauce to produce a juicier lasagna, or less for a drier lasagna. Repeat with another layer.
4. End with a third layer of **tortillas**, covered with **mole sauce** and grated cheddar.
5. Cover and bake at 350°F until the lasagna is heated through and sauce and cheese are bubbly, about ½ hour. Uncover and bake 5-10 minutes.