IRB Tomato News

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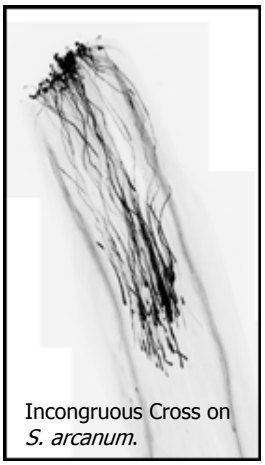
Of Special Interest

Updated IRBT website; release of iTRAQ Proteomics and pFCM100.

IRBT represented at FSPRIII in Tuscon, AZ.



Incongruous Cross on *S. arcanum*.

Serving interest in Interspecific Reproductive Barriers of Tomato (IRBT) and the plant reproduction community.

Proteomics and FISH Cassette for Mutagenesis

IRBT is proud to release the pre-publication data of a style and pollen iTRAQ proteomics meta-endeavourer, as well as a publicly accessible broad spectrum mutagenesis tool called pFCM100 (see pg 2).

Proteomics

A major goal of IRBT is to identify the molecular components involved during interspecies reproduction. iTRAQ analysis utilizes specific signature labeling of up to four separate peptide samples and allowed us to take a powerful comparative look at peptides of **unpollinated styles** and **pollen** from four different tomato accessions LA0407, LA1777 (both *Solanum habrochaites*), LA0716 (*S. pennellii*), and M82 (*S. lycopersicum*).

These accessions are unique in their reproductive traits and have long been used as sources of biodiversity for domestication. The domestic tomato, M82, lacks SRNase based gametophytic self incompatibility and is readily fertile with the other three wild tomato accessions. However, its pollen is rejected by *S. hadrochaties* and *S. pennellii*. LA0407 is unique because it is incongruous with tomato in a different way than that of LA1777. Well characterized isogenic lines from both parental accessions of LA1777 and LA0716 have been widely used for QTL mapping and trait domestication.

iTRAQ also allows us to identify peptides that are hydrophobic, low abundant, or post-translationally modified. Because four samples can be analyzed together we can use the levels of relative quantification in each sample to identify putative candidate proteins involved during prezygotic reproductive barriers.

Multiple peptides were expressed in all the samples. Uniquely identified peptides represent close to 37% of the total peptide spectra (769 from pollen and 493 from Mature Unpollinated Styles). SGN-unigene numbers and GO terms can be used search the *xls*. data and all spectral data is listed as raw *.wiff* files on the website irbtomato.org. Please also read the abstract presented at the 2008 ASPB meeting in Merida, Mexico by Gloria Lopez Casado for more information.

A parallel 454 sequencing effort is under way with pollen and mature unpollinated styles of M82 and LA0716. Once complete, this will increase the number of tissue specific genes in our gene bank and strengthen our iTRAQ results.

Fishing for a mutant

FISH Cassette for Mutagenesis (pFCM100)

Tackling interspecific barriers requires looking at this complex topic from multiple angles. Here we use a forward genetics approach to identify mutants with changes in there reproductive traits.

We have completed the assembly of a versatile insertional mutagenesis tool (pFCM100) and are in the process of making available M82 lines that carry the transgene on each arm of the 12 tomato chromosomes. This transgene can be tracked in transgenic plants, using FISH (**F**luorescent ***In Situ*** **H**ybridization) technology. The three main objectives of this tool are to;

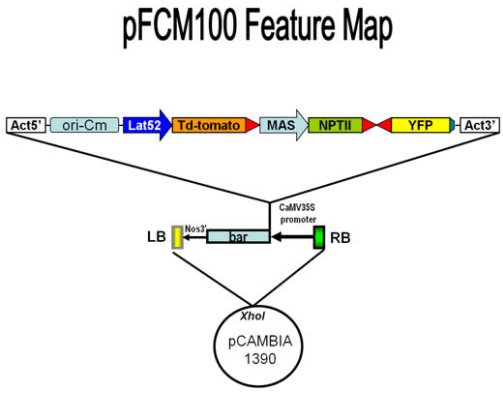
1) Identify genes involved in tomato reproductive biology (in particular, inter-specific reproductive barriers) by following the segregation distortion of a dominant maker, Kan^{r}.

2) Use a pollen-expressed fluorescent protein to follow transgene segregation & visualize pollen tube growth in styles.

3) Make the tomato genome more accessible to the tomato community by providing a highly efficient global insertional mutagenesis approach.

We brought together several useful features from different sources such as pJASM13 (Gidoni et al., 2003). We foresee the utility of pFCM100 in other plant species, as well. Interested labs may contact *IRBT* on the web or email Subbaiah Chalivendra @ (schalive@lamar.colostate.edu).

The power of insertional mutagenesis is enhanced in pFCM100 by combining T-DNA and transposon tools with a gene trap (a promoter-less **YFP** gene). The FISH cassette has kanamycin resistance (**NPTII**) driven by the **MAS** promoter as the plant transformation marker. After the excision of the Ds element, Basta resistance (**bar** gene) comes under the regulation of **35S** promoter to select for transposition events. Ac lines are available in multiple tomato cultivars, such as VF36, VFNT cherry, Money maker and microtom. The pollen-genotyping cassette consists of an enhanced fluorescent protein, **Td-Tomato**, under the control of a pollen-specific promoter **LAT52**, which allows tracking transgene segregation. We have also incorporated a plasmid rescue cassette (**ori-cm**) to clone flanking plant genomic DNA in *E. coli*, using convenient restriction enzyme sites and selecting transformants on chloramphenicol.



IRBT makes it to the FSPRII meeting in AZ

Recently members representing three IRBT laboratories attended the 2008 Frontiers In Sexual Plant Reproduction III (FSPRIII) meeting held in Tuscon, Arizona.

Dr Patricia Bendinger from Colorado State University presented a talk titled “Unilateral Incongruity in Tomato: Role of Selfincompatibility Factors”. A second talk titled “Endocytosis in S-RNase-Based Self-Incompatibility” was presented by Dr. Bruce McClure from the University of Missouri. Post Doc. Wentao Li from the University of Riverside, California, presented a research poster titled “Fine Mapping of a Pollen Unilateral Incompatibility Locus in Tomato”. In it he describes a chromosome 6 gene, *ui6.1*, that acts as a gametophytic factor in a two gene system involving the chromosome 1 QTL. Preliminary results show *ui6.1* maps to a 0,10 cM region on the short arm of chromosome 6. Physical mapping of this gene using BAC and cosmid libraries are in progress.

To read the full abstracts go to the Publications link at **www.irbtomato.org**