Minutes v2

Peanut Genome Consortium (PGC) at Atlanta, GA; 7 December 2015

PARTICIPANTS

Steve Brown	Corley Holbrook	David Bertioli	Dan Ward
Mark Burow	Scott Jackson	Brian Scheffler	Jeremey Schmutz
Kelly Chamberlin	Peggy Ozias-Akins	Barry Tillman	Darlene Cowart
Charles Chen	Bob Parker	Lutz Froenicke	Bob White
Jim Elder	Howard Valentine	Soraya Bertioli	
Baozhu Guo	Richard F. Wilson	Jeff Elhers	

Representation in absentia via proxy to (name of voting member) or via teleconference:

Richard Michelmore (Froenicke)	Steven Cannon (Wilson)	George Birdsong, (teleconference)
Rajeev Varshney (Wilson)	Tom Stalker (Wilson)	Andrew Farmer (teleconference)
Victor Nwosu (Wilson)	Graeme Wright (O'Conner),	Jeff Ehlers (teleconference)
Ray Schnell (Nwosu)	O'Conner (teleconference)	
Howard Shapiro (Nwosu)	David Hoisington (Wilson)	
	T. Radhakrishnon (Wilson)	

Jackson convened the meeting. A quorum was established. Agenda and Minutes-v2 (4 November 2015) from Brisbane, AU were approved. Wilson reported that Tom Stalker is recovering from surgery on November 10.

PGC Membership

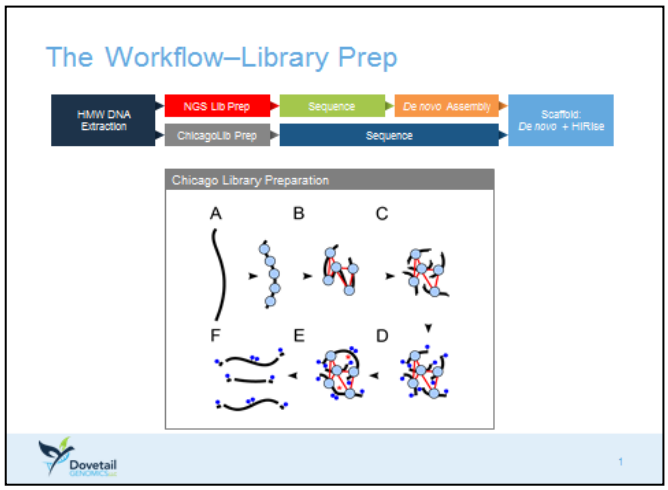
Guillermo Seijo (Instituto de Botanica del Nordeste, Cordoba, Argentina) was welcomed to PGC membership. Wilson presented a slate of potential candidates for the 2016 election of PGC officers (chairpersons). Valentine raised a point-of-order that candidates could not be self-nominated; however, the objection was dismissed because Section 2.02(b4) of the PGC Policies & Procedures does not require independent nominations, and prior PGC elections have allowed write-in candidates who may be self-nominated. Scheffler moved that candidates should contest for three positions (chairperson, co-chairperson-at large, co-chairperson-plant breeding). The motion passed. Wilson shall draft/distribute an electronic ballot and request validated votes be returned to him prior to January 8, 2016.

New members: Kelly Chamberlin (USDA, ARS, Stillwater OK) and Barry Tillman (University of Florida, Marianna, FL) were welcomed to membership without objection from two-thirds of voting members. Nominations for 2016 Chair positions: Jackson, Ozias-Akins, Michelmore (by acclamation). Holbrook (by Valentine), Tillman (by Kelly Chamberlin), Stalker (by Steve Brown), Burow (by Jackson),

The PGC Executive Committee now consists of twenty-nine (29) members plus 10 Ex Officio members. Future PGC business will require 20 members for a simple majority, and 26 affirmative votes for two-thirds majority. Wilson will revise the PGC Policies & Procedures to reflect these changes.

Update on Diploid & Tetraploid Genome Assemblies

Progress on DoveTail project: Froenicke reported that enhanced DoveTail technology has increased assembly contiguity of several genomes (alligator-265x, potato-8x, lettuce-3x). The improved workflow for library preparation involves: A: Pure DNA (black strand) is reconstituted into chromatin with addition of histones (blue circles). B: Reconstituted chromatin is crosslinked in solution forming chromatin aggregates/globular molecules. Crosslinks (red lines) formed between nucleosomes stabilize the chromatin and form scaffolds. C: Chromatin aggregates are cut with restriction endonucleases. DNA fragments from original molecule remain associated due to crosslinked nucleosome scaffolds. D: Cut ends are blunt ended and marked with biotin (small blue circles). E: Blunt ends are randomly ligated, forming short,



medium, and long range associations. Ligation events are indicated with red asterisks. The initially distant ends of the original fragment (A) are brought into close proximity by crosslinking (B) and physical non-linear crosslinks are created between proximal ends via ligation (E). F: Crosslinks are reversed, DNA purified, and after marker pulldown ligation-containing fragments provide more information than the original Illumina-fragment assembly. Library preparation is performed via conventional sequencing. Froenicke reported impressive assembly metrics for assembly of the lettuce genome (Michelmore lab). The assembly pipeline agreed with the original Illumina assembly and enabled the generation of a high-density genetic map that could be validated. After HiRise (two lanes) about 87% of lettuce scaffolds were validated. Only about 1% of the super scaffolds were chimeric. The number of scaffolds on the genetic map increased from about 1000 to 3000. The largest scaffold was 9.6 Mbp. The error rate was 3.7%, but Froenicke believed 1% error was possible. No work has been done on the tetraploid peanut genome due to lack of high quality DNA. **Ozias-Akins stated that about one-half of the original amount of high-quality DNA extracted at the University of Arizona was still available, and agreed to have a useful portion sent to Froenicke/Michelmore.**

Progress on the Hudson-Alpha project: Schmutz reported that data collection was completed for 13 tetraploid libraries (160X coverage of 400bp-800bp fragments, and 28X coverage of 3kb to 8kb pairs); 130X coverage of 12 *A. duranensis* diploid libraries; and 217X coverage of *A. ipaensis* diploid libraries. Libraries were assembled with AllPaths, Abyss, SSPACE, and Meraculous technology. Abyss generated a 2.7 Gb assembly with 32 kb scaffold N50 in 15 to 20 days runtime. AllPaths generated a 1.6 Gb assembly with 54 kb scaffold N50 in 40 to 50 days runtime. Meraculous generated a 1.9 Gb assembly with 78kb scaffold N50 in 12 days.

These assemblies were evaluated against diploid gene sets containing about 34,800 genes (A-genome) and 39,500 genes (B-genome). The proportion of A- and B-genome genes aligned by a given assembler were similar; but increased from AllPaths (ca 54%) to Abyss (63%) to Meraculous (75%). Analysis of each assembly revealed: 1) AllPaths and Abyss tends to collapse subgenomes; 2) Meraculous appeared to keep subgenomes separated, but there were a significant number of regions where a diploid-A and diploid-B gene alignment overlapped on a tetraploid-B scaffold (unexpected recombination). About 46% of a Meraculous 1011 Mb assembly could be assigned to an A- or B-subgenome; while 8% were found in both subgenomes and the remainder of the assembly contained no genes.

Next steps include integration of Meraculous scaffolds with tetraploid Moleculo scaffolds. Longer (10kb) pair tetraploid sequence would be helpful for finished scaffolding and patching In addition, 5 Mb of accurate tetraploid sequence from clones of 10X BAC libraries from Tifrunner (recently purchased from BIOCOMPARE) would be useful as a benchmark. Short pair diploid sequence from BGI is not suitable for Meraculous assembly.

Plans for Improving the Tetraploid Genome Assembly

D. Bertioli expressed need for improved high-density genetic maps of Tifrunner to help overcome problems is gene alignment due to segregation distortion among subgenomes. Three approaches toward that goal were prioritized in order of urgency:

1) ***DoveTail.*** High-quality tetraploid DNA held at the University of Arizona will be made available to Michelmore (DoveTail). Work on the tetraploid genome will take precedent over work on the diploid genomes. No peanut data is available at this time, but cost of raw data from preliminary tests was quoted at $17,000/library (two libraries are required). TPF funding for this work was allocated to UC-Davis in 2015. Pending receipt of high-quality DNA, work should proceed in 30 days.

2) ***PacBio***. New PacBio chemistry available this year reportedly enables 2 to 3-fold increases in yield with assemblies averaging 13 kb. Such long reads would significantly improve the tetraploid peanut assembly. 70X coverage was recommended for de novo assembly, development of an ordered map and filling gaps in tetraploid subgenome alignment. The cost was estimated at $180,000. Current PacBio technology may require 80 days run time, with no guarantee of the outcome with tetraploid peanut. Schmutz suggested options: 1) to go forward with current technology or 2) wait for the April 2016 release of PacBio SEQUEL technology which should generate 10 kb libraries for 50% of current technology cost. A proposal for funding work with PacBio technology from Schmutz and Scheffler was available but not shared with or discussed by the PGC.

3) ***Sequence clones of BAC libraries (from BIOCOMPARE).*** PacBio technology was proposed to sequence 100 clones to use in comparisons for sequencing and assembly efforts. The estimated cost was $15,000.

Update on Diploid Sequence Publication

D. Bertioli reported that the Editor of Nature Genetics had requested a third revision of the paper entitled, 'The genomes of the ancestral species of peanut-a remarkable case of living archaeology'. The revision was submitted on December 3. The Editor is expected to make a decision in a few weeks.

TPF Business

Brown reported that research progress reports from TPF projects funded in 2015 were scheduled to be held from 8:00 AM to 12:30 PM on December 8 at the W-Atlanta Midtown hotel. These reports plus presentations made at APRES-2015 and AAGB-2015 would be used to develop the 2015 to 2015 annual IPGI research accomplishment report, which will be presented to TPF Board in July 2016. In addition, TPF Technical Review committee is scheduled to consider Peer Panel evaluations of 2016 TPF research proposals for funding from 1:00 PM to 5:00 PM on December 8 at the W-Atlanta Midtown hotel.

Development of the IPGI Strategic Plan 2017-2021:

Wilson provided handouts of updated worksheets representing a near finished draft of the IPGI Strategic Plan (2017-2021). The outline represented a compilation of input received from AAGB-2014, APRES-2015, AAGB-2015 and a number of ad hoc meetings with various industry segments. Electronic copies have been distributed and are available on request. Wilson stated that suggestions for change are welcome and appreciated at the earliest convenience. A final draft plan will be presented to the Peanut Foundation Board in June 2016. Pending acceptance, the new plan would be implemented after July 2016.

Update on AAGB-2015 (November 5-7, 2015):

There were no additions to the subject report previously presented by Wright on AAGB-2015 logistics at the Rydges Hotel, Brisbane, Queensland, Australia.

Next Meetings: No formal meeting will be held during PAG XXIV in San Diego CA (January 9-13, 2016). A less formal gathering is planned with DoveTail and PacBio reps. Wilson will survey PGC members for a convenient time/date and make arrangements.

American Peanut Research Education Society, July 11, 2016; 1:00 PM to 2:30 PM, Hilton Clearwater Beach Hotel, Clearwater Beach, FL

Tentative date for AAGB-2017 in Cordoba, Argentina: March 2017

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