

Bioinformatic Resources for *Populus* Genome Editing

Background

- CRISPR genome editing is being used in short-rotation bioenergy woody crops, such as *Populus*, to generate targeted null mutations.
- A major challenge in efficient genome editing of outcrossing species is the frequent occurrence of sequence polymorphisms that can render CRISPR/Cas unproductive.
- Reliable detection of on-target and off-target editing requires deep sequencing.

Approach

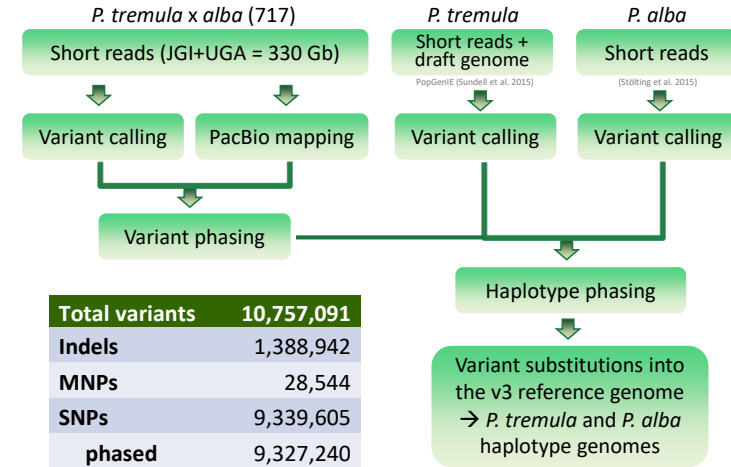
- An improved variant-calling pipeline for *P. tremula x alba* INRA 717-1B4 (717) was developed, using JGI genome sequencing data and community resources.
- The Variant DB was redesigned to incorporate confidence ratings and haplotype phasing information for the identified sequence polymorphisms.
- Web dissemination of the database and tools to benefit the research community is at <http://aspendb.uga.edu/index.php/databases/spta-717-genome>.

Outcome

- We identified >10M high-confidence variants with haplotype resolution for poplar clone 717, at an average frequency of one per 39 bp.
- The improved Variant DB facilitates variant-free gRNA designs for efficient and precision gene editing in heterozygous taxa.
- The Variant DB has >385 users worldwide, with >2200 sessions in the last 1.5 years.
- The Variant DB also supports *P. trichocarpa* Nisqually-1 and *P. deltoides* WV-94.
- We developed a stand-alone, user-friendly program, **AGEseq** for analysis of genome editting by amplicon sequencing. AGEseq is also available on Galaxy.

Significance

- The computational resources greatly facilitate genome editing research in *Populus*, enabling variant-free gRNA design and efficient analysis of editing patterns from high-throughput sequencing of multiplexed samples.



Database selected sPta717_v2

Target 1: Chr02:22837135 Strand: - Mismatch No.: 0
Gene:Potri.002G235200 Feature:CDS

Alignment: Q(Query) T(tremula) A(alba) C(Coverage) P(Phased) V(VN)
Coverage Range | 0.0, A:1-10, B:11-50, C:51-100, D:101-500, E:501+

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Q CATGCTTTGAAAATCCCTTTGG
T CATGCTTTGAAAATCCCTTTGG
A CATGCTTTGAAAATCCCTTTGG
C *****
P 000000000000000000000000
F -----

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Target 2: Chr01:43204416 Strand: - Mismatch No.: 2
Gene:Potri.001G409500-Potri.001G409500 Feature:intergenic

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Q CATGCTTTGAAAATCCCTTTGG
T CATGCTTTGAAAATCCCTTTGG
A TGTGCTTTGAAAAGCCTTTTG
C *****
P EEEEEEEEEEEEEEEEEEEEE
YY-----Y-----

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Tsai C-J, and Xue L-J (2015). *GM Crops & Food* 6: 206-215. DOI: 10.1080/21645698.2015.1091553.

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