Genomic and Mapping Resources for the Genetic Improvement of Shrub Willow Feedstock Crops

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WILLOWPEDIA

http://willow.cals.cornell.edu/

Crop and forest biomass is a feedstock for biopower, liquid biofuels, and heat

Lyonsdale Biomass Power Plant, Lyons Falls, NY
Photo: Catalyst Renewables

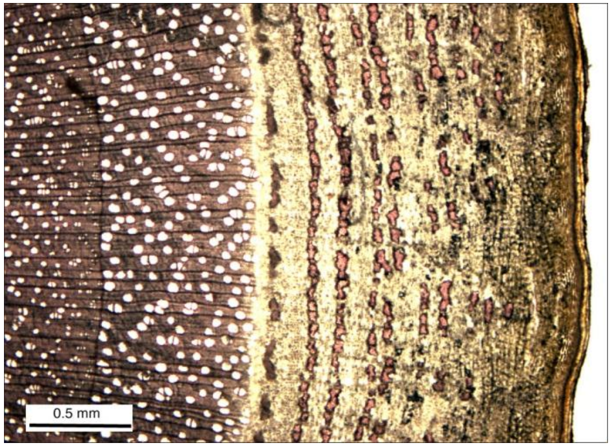
VeraSun Station, Brookings, SD

ACT Bioenergy Wood Boiler
NYSAES, Geneva, NY

Breeding Objectives

Marginal Land Adaptability

Biomass Quality Traits



Potato Leaf Hopper Resistance

Rust Resistance

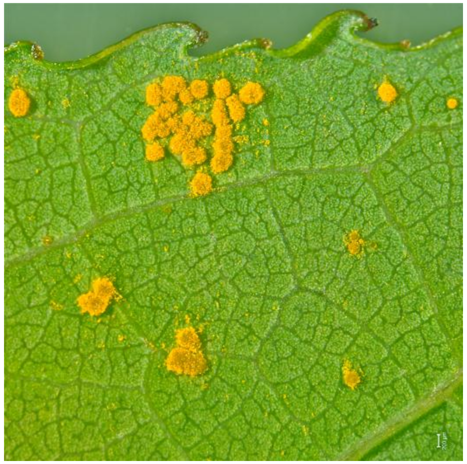


Photo by Shawn Kenaley

Intra- and Inter-specific Hybridizations

F_1

S. integra
S. viminalis
S. cordata
S. nigra
S. sachalinensis
S. cordata x *S. eriocephala*
S. purpurea x *S. eriocephala*
S. purpurea x *S. viminalis*
S. purpurea x *S. sachalinensis*
S. purpurea x *S. gilgiana*
S. koriyanagi x *S. purpurea*
S. viminalis x *S. miyabeana*
S. viminalis x *S. eriocephala*
S. x *dasyclados* x *S. miyabeana*
S. x *dasyclados* x *S. eriocephala*
S. x *dasyclados* x *S. viminalis*
S. eriocephala x *S. purpurea*
S. matsudana x *S. alba*
S. sachalinensis x *S. eriocephala*
S. sericea x *S. purpurea*
S. sericea x *S. eriocephala*
S. sericea x *S. sachalinensis*
S. discolor x *S. cinerea*
S. discolor x *S. eriocephala*

F_1 and *F_2*

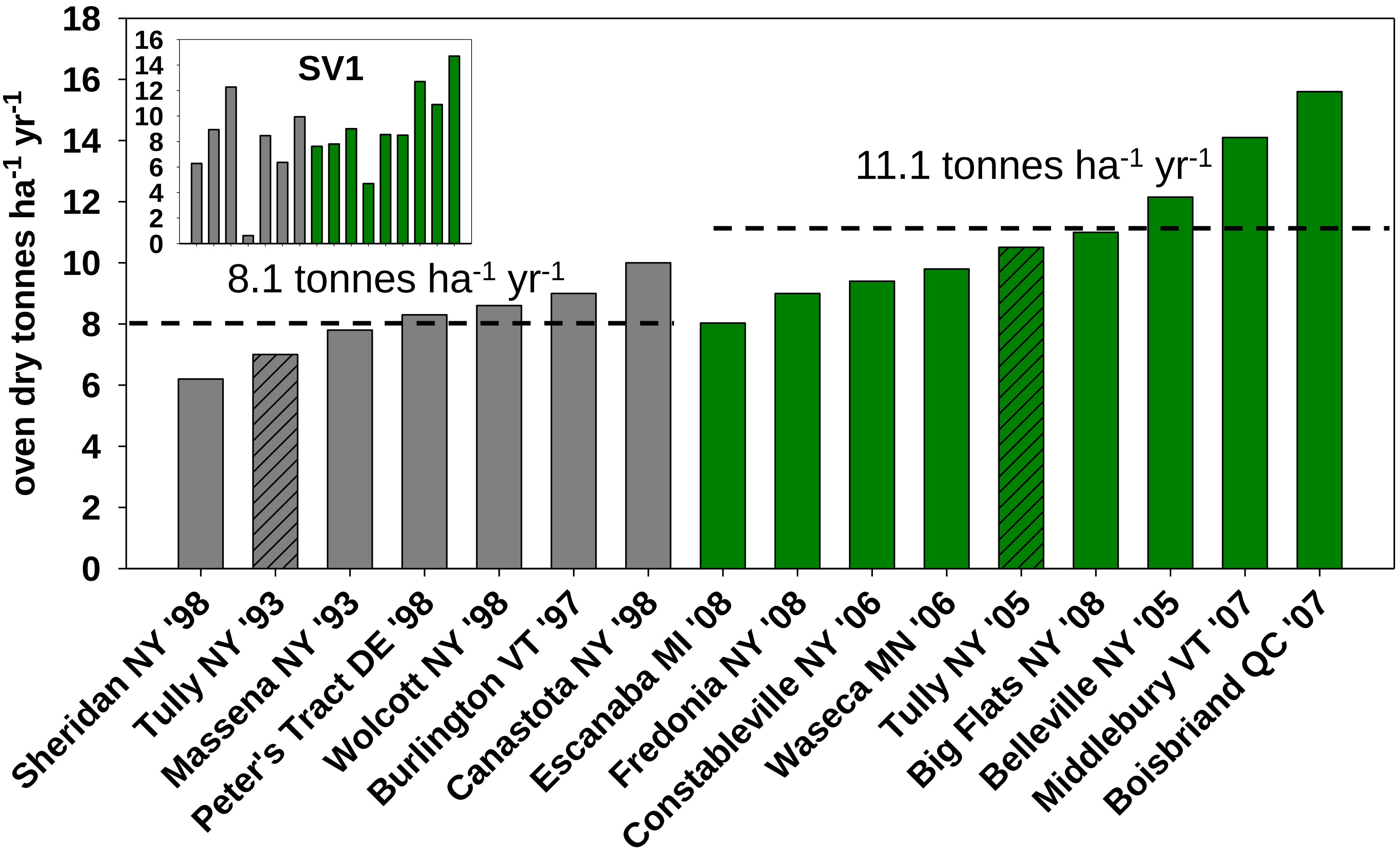
S. purpurea
S. eriocephala
S. miyabeana
S. sachalinensis x *S. miyabeana*
S. purpurea x *S. integra*

Multi-species hybrids

S. viminalis x (*S. sachalinensis* x *S. miyabeana*)
S. viminalis x (*S. purpurea* x *S. miyabeana*)
S. viminalis x (*S. viminalis* x *S. miyabeana*)
S. purpurea x (*S. sachalinensis* x *S. miyabeana*)
S. purpurea x (*S. purpurea* x *S. miyabeana*)
S. purpurea x (*S. viminalis* x *S. miyabeana*)
(*S. sachalinensis* x *S. miyabeana*) x *S. purpurea*
(*S. sachalinensis* x *S. miyabeana*) x *S. miyabeana*
(*S. sachalinensis* x *S. miyabeana*) x *S. viminalis*
(*S. viminalis* x *S. schwerinii*) x *S. cinerea*
(*S. sachalinensis* x *S. miyabeana*) x (*S. purpurea* x *S. miyabeana*)
(*S. sach* x *S. miya*) x (*S. viminalis* x (*S. schwerinii* x *S. viminalis*))

Mean Yield of Top Five Genotypes in Yield Trials

- New cultivars contribute to 36% increase in yield of top five

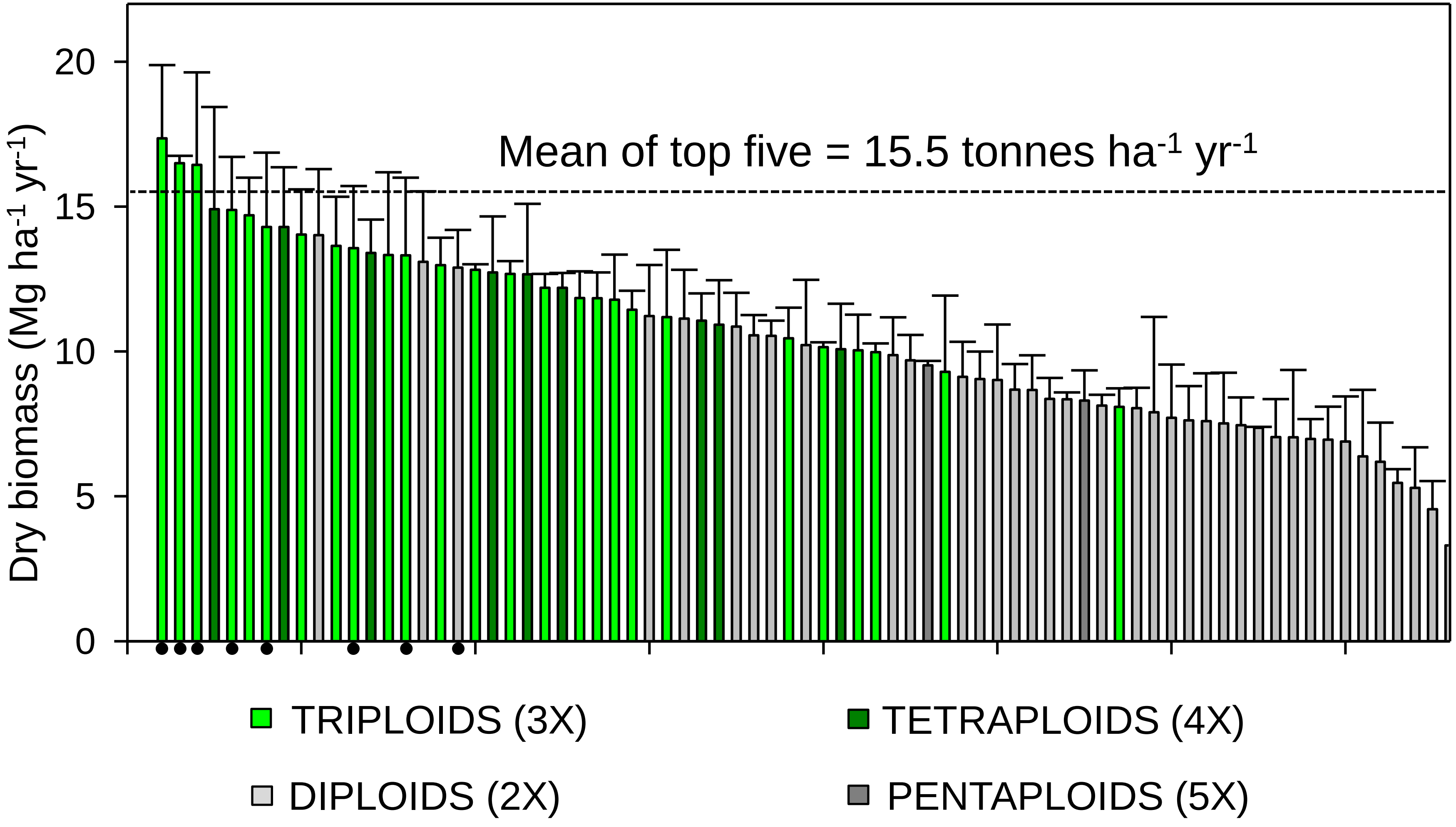


Flow Cytometric Estimation of Nuclear DNA Content

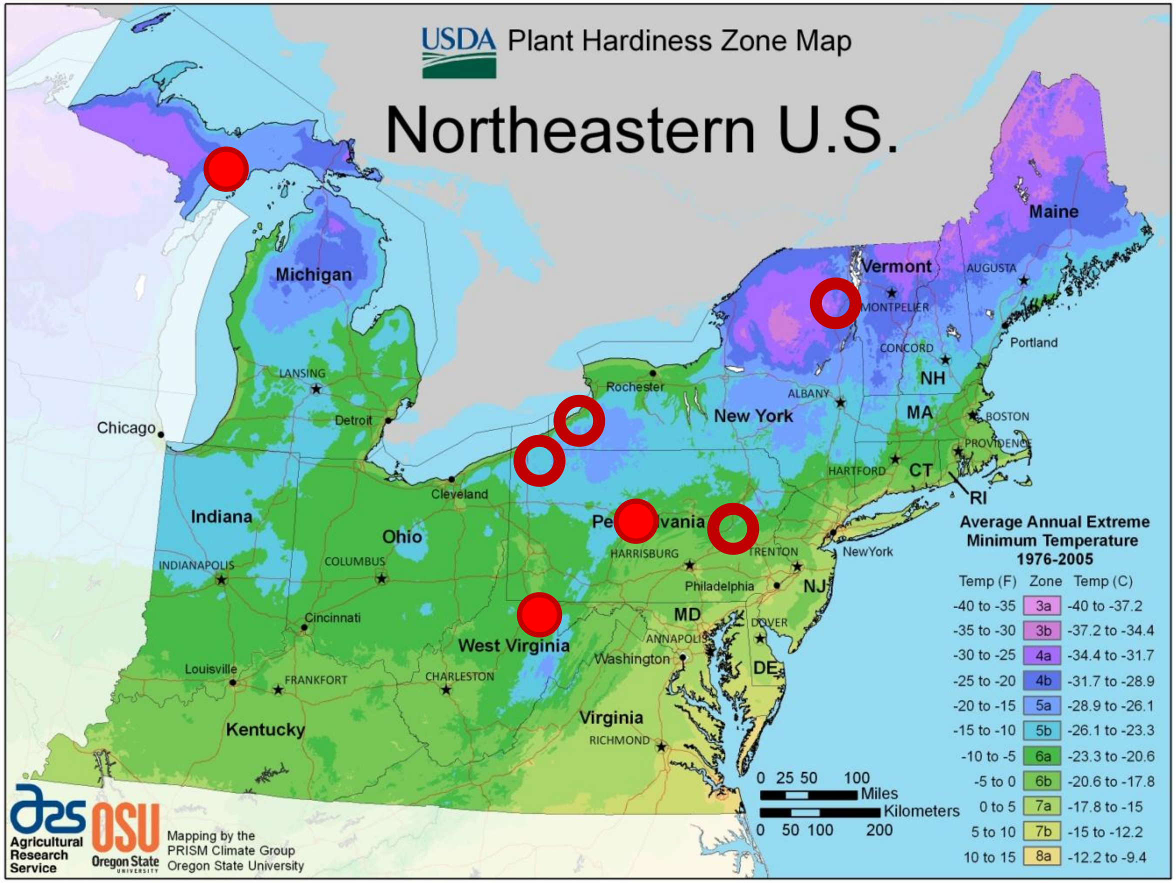
Cultivar	Species	(pg/2C)
Diploid		
Björn	*S. schwerinii* x *S. viminalis*	0.76
Olof	*S. viminalis* x (*S. schwerinii* x *S. viminalis*)	0.80
Jorr	*S. viminalis*	0.83
P63	*S. integra*	0.93
94006	*S. purpurea*	0.94
SV1	*S.* x *dasyclados*	0.94
Triploid		
Nimrod	(*S. schwerinii* x *S. viminalis*) x *S. udensis*	1.15
Terra Nova	(*S. triandra* x *S. viminalis*) x *S. udensis*	1.11
Tully Champion	*S. viminalis* x *S. miyabeana*	1.31
Oneida	*S. purpurea* x *S. miyabeana*	1.33
01X-266-001	*S. viminalis* x (*S. viminalis* x *S. miyabeana*)	1.26
Tetraploid		
SX64	*S. miyabeana*	1.65
SX61	*S. sachalinensis* (*S. udensis*)	1.65
Canastota	*S. sachalinensis* x *S. miyabeana*	1.67
05X-284-001	*S. purpurea* x (*S. purpurea* x *S. miyabeana*)	1.87
Pentaploid?		
05X-286-001	*S. miyabeana* x (*S. purpurea* x *S. miyabeana*)	2.17

2008 Genetic Selection Trial - Geneva, NY

- 24-plant plots, 3 replicates, 76 genotypes
- Biomass harvested from middle 8 plants in Dec. 2011



NE Sun Grant - NEWBio Regional Trials

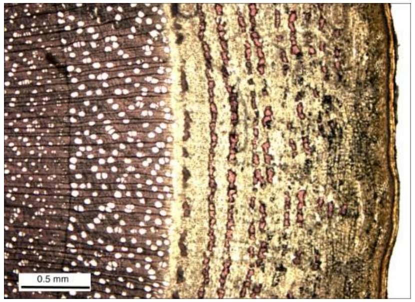


- ● Yield Trials (24 genotypes, 48 plant plots)
- ○ Yield Trial Sites for 2013

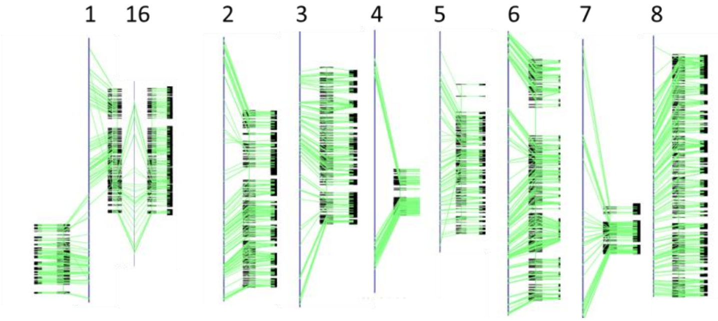
USDA NIFA NEWBio

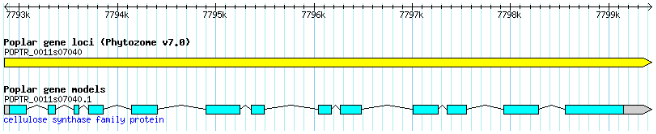
Genomic Tools for Genetic Improvement

Quantitative Traits



High-density Linkage Map
QTL Mapping





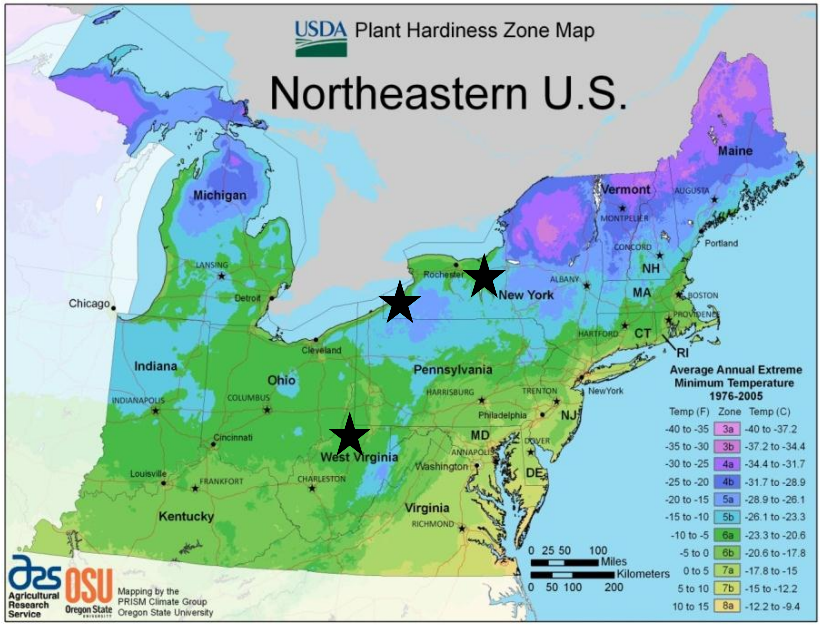
Candidate Gene
Association Mapping

Genome
Sequence

TTGGCA
GTCAAC
TCCCTA
AGCTCC
TTCATG

Association Mapping Population

- Candidate gene:trait association mapping
  - 114 *S. purpurea* natural accessions
  - 16 *S. purpurea* hybrids, *S. koriyanagi*, *S. integra*, and current hybrid cultivars



Trait mapping populations

Salix purpurea mapping population

94006 x 94001

F_1

Family 82 (~440 progeny)

9882-41 x 9882-34

F_2

Family 317 (~500 progeny)

***S. purpurea* 94006 x *S. viminalis* ‘Jorr’**
[Willow beetle; potato leafhopper resistance]

***S. koriyanagi* ‘SH3’ x *S. purpurea* 94001**
[Biomass chemical composition; Stem number]

***S. purpurea* 94006 x *S. integra* P63**

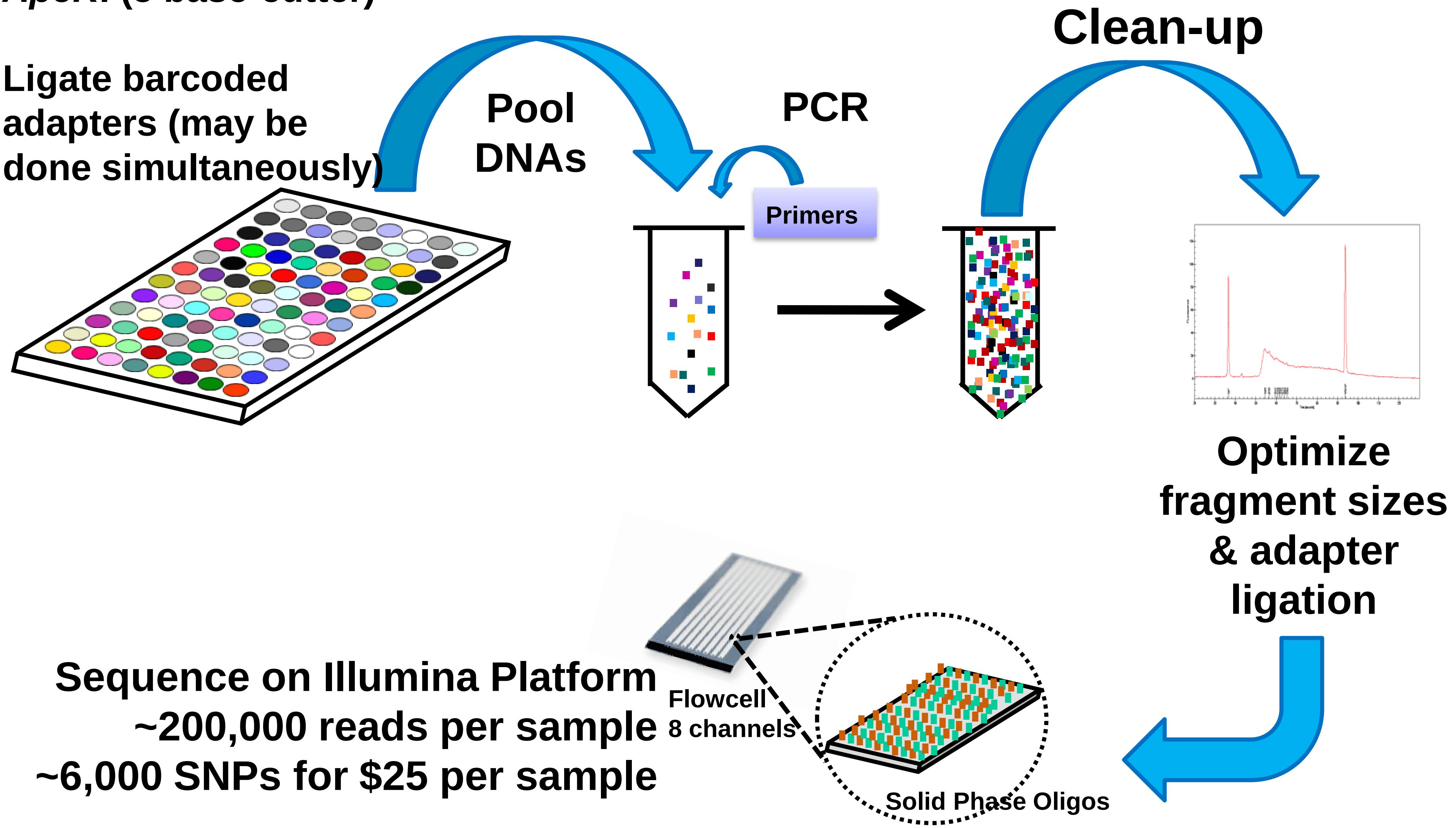
[Stem diameter; Wood density & composition; Rust resistance]

High-density markers: GBS 96-plex protocol

Rob Elshire, Sharon Mitchell, Ed Buckler

Elshire et al. (2011) PLoS One

Digest DNA with *EcoT22*I (6-base) or *Apek*I (5 base-cutter)

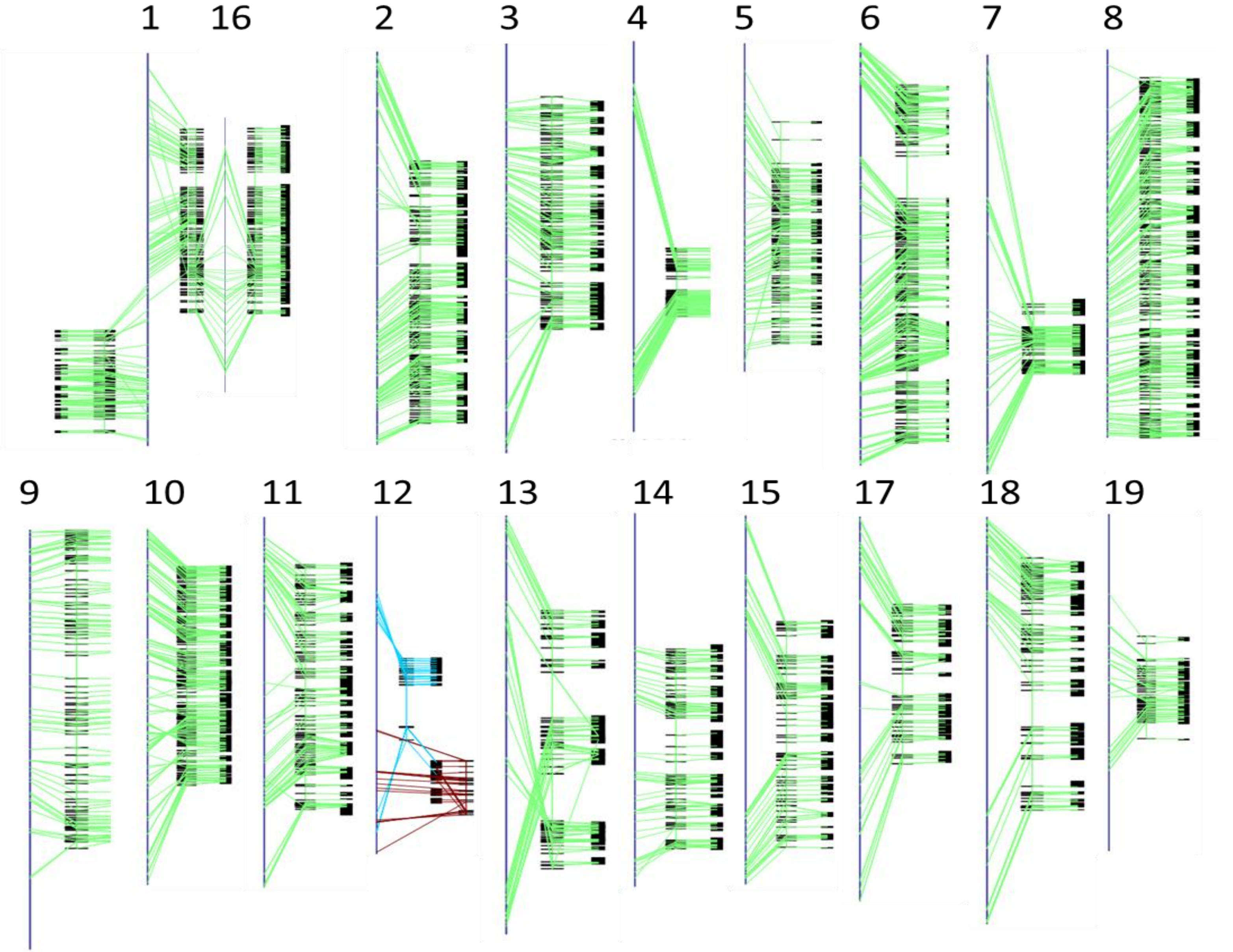


Ligate barcoded adapters (may be done simultaneously)

Optimize fragment sizes & adapter ligation

Sequence on Illumina Platform
~200,000 reads per sample
~6,000 SNPs for $25 per sample

Preliminary Genetic Map of *S. purpurea*



Steve DiFazio and Eli Rodgers-Melnick

Evidence for Divergence of Two Genera

- *Salix* > 300 species vs. *Populus* = 32 species
- Shrub vs. Tree Growth habit
- Multiple ploidy levels (2n=38)
- No terminal bud in *Salix*

Sequencing of the *Salix purpurea* L. genome

- Whole genome shotgun sequencing of clone ID 94006
  - diploid female (*n* = 19, ~485 Mb)
  - conducted at DOE JGI
  (PIs: Tuskan, Smart, Town)

- ALLPATHS genome assembly:
- 140X Illumina coverage:
  - Two lanes of 250bp PE
  - 4.5kb, 5.3kb, and 6.5Kb MP
- Total bases = 349 Mb (~85%)
- Max contig size = 784 kb
- Contig L50 = 46 kb

- Two 5X BAC libraries constructed

Transcriptome sequencing

- *Salix purpurea* transcriptome data to date:
  - 5 lanes Illumina 2x76 from 8 tissue libraries done at JGI
  - 84.6% of 201,926 transcripts aligned to genome assembly

Shoot tip
44.0 M reads

Leaves
Day: 16.4 M
Nite: 15.3 M
Drought: 13.6

Stem
Node:18 M reads
Internode:21.5 M

Roots
51.7 M reads

Catkins
46.1M reads

Genomic and Mapping Resources for the Genetic Improvement of Shrub Willow

Summary

- Excellent hybrid pedigrees have been produced and new hybrid combinations are being explored
- Trials have been planted on a wide range of sites to estimate regional yield potentials
- Many new high-yielding hybrids are triploid
- Candidate gene association mapping
- Developing a high-density genetic map using SNPs generated from GBS
- Draft sequence of the *S. purpurea* genome and transcriptome

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