PineRefSeq: Sequencing Strategies in Conifer

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PineRefSeq

Goal

To provide the benefits of conifer reference genome sequences to the research, management and policy communities.

Specific Objectives

- *Provide a high-quality reference genome sequence of loblolly pine looking toward sugar pine and Douglas-fir.*
- Provide a complete transcriptome resource for gene discovery, reference building, and aids to genome assembly
- Provide annotation, data integration, and data distribution through Dendrome and TreeGenes databases.

The Large, Complex Conifer Genomes Present a Formidable Challenge

- Challenges
  - The estimated 24 Gigabase loblolly pine genome is 8 times larger than the human genome, and far exceeds any genome sequenced to date.
  - Conifer genomes generally possess large gene families (duplicated and divergent copies of a gene), and abundant pseudo-genes.
  - The vast majority of the genome appears to be moderately or highly repetitive DNA
- Approaches to Resolving Challenges
  - Complementary sequencing strategies that seek to simplify the process through use of actual or functional haploid genomes and reduced size of individual assemblies.

Plant Genome Size Comparisons

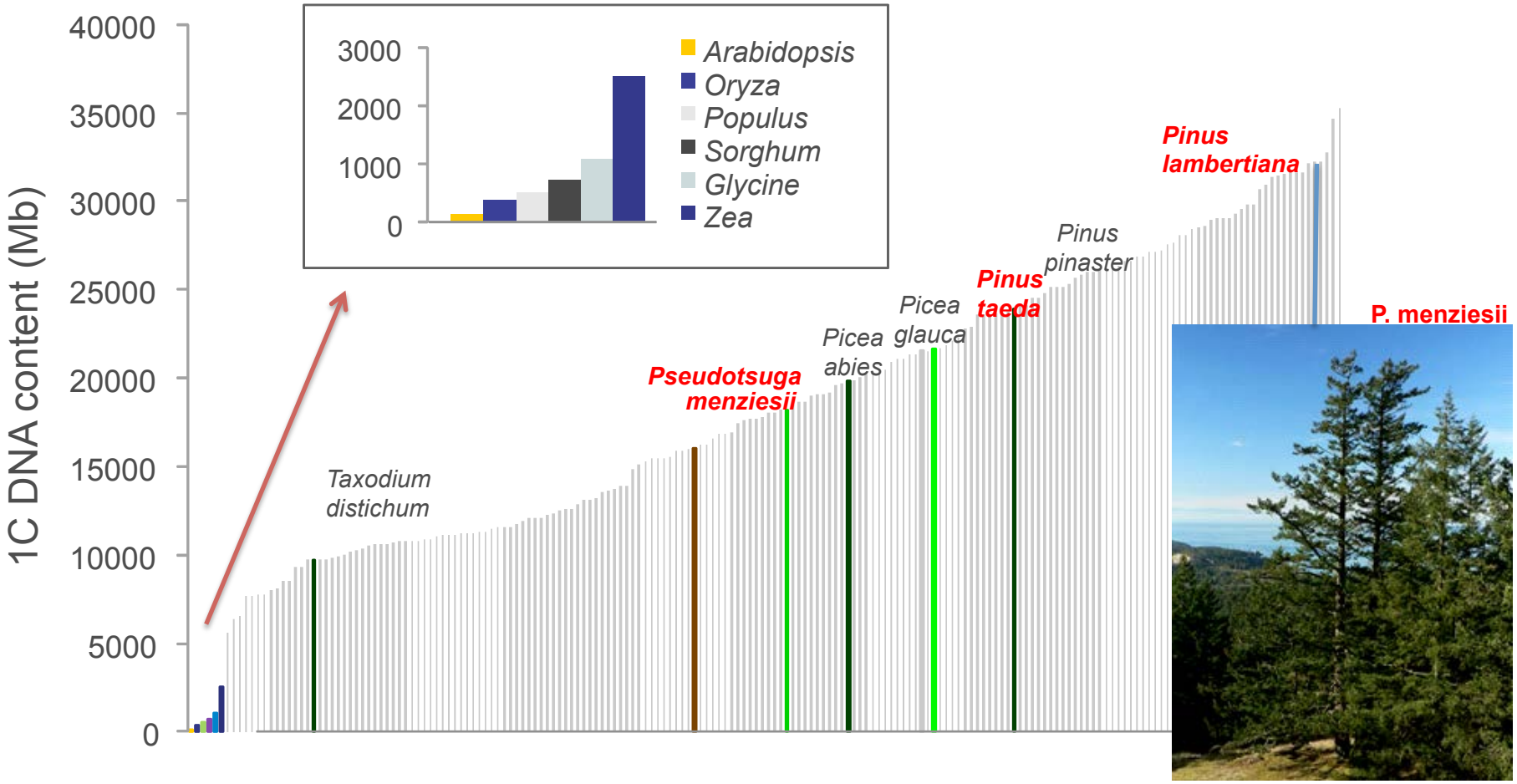


Image Credit: Modified from Daniel Peterson, Mississippi State University

Existing and Planned Angiosperm Tree Genome Sequences

Species		Genome Size[1]	Number of Genes[2]	Status[3]
In Progress With Draft Assemblies				
Populus trichocarpa	Black Cottonwood	500 Mbp	~ 40,000	2.0 / 2.2
Eucalyptus grandis	Rose Gum	691 Mbp	~36,000	1.0 / 1.1
Malus domestica	Apple	881 Mbp	~26,000	1.0 / 1.0
Prunus persica	Peach	227 Mbp	~28,000	1.0 / 1.0
Citrus sinensis	Sweet Orange	319 Mbp	~ 25,000	1.0 / 1.0
Carica papaya	Papaya	372 Mbp	-	
Amborella trichopoda	Amborella	870 Mbp	-	
In Progress Or Planned – No Published Assemblies				
Castanea mollissima	Chinese Chestnut	800 Mbp	-	
Salix purpurea	Purple Willow	327 Mbp	-	
Quercus robur	Pedunculate Oak	740 Mbp	-	
Populus spp and ecotypes	Various	various	-	
Azadirachta indica	Neem	384 Mbp	-	

1) Genome size: Approximate total size, not completely assembled.
2) Number of Genes: Approximate number of loci containing protein coding sequence.
3) Status: Assembly / Annotation versions; http://www.phytozome.net/ ; http://asgpb.mhpcc.hawaii.edu/papaya/ ; http://www.amborella.org ;

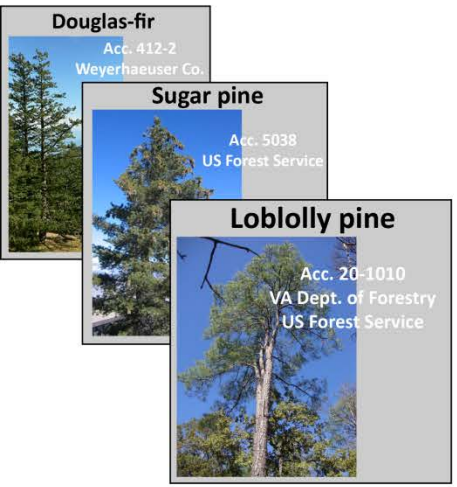
(purple willow – Http://www.poplar.ca/pdf/edomonton11smart.pdf ; Neem - (http://www.strandls.com/viewnews.php?param=5&param1=68

Existing and Planned Gymnosperm Tree Genome Sequences

Species		Genome Size[1]	Number of Genes[2]	Status[3]
Gymnosperms				
Picea abies	Norway Spruce	20,000 Mbp	?	Pending
Picea glauca	White Spruce	22,000 Mbp	?	Pending
Pinus taeda	Loblolly Pine	24,000 Mbp	?	Pending
Pinus lambertiana	Sugar Pine	33,500 Mbp	?	Pending
Pseudotsuga menziesii	Douglas-fir	18,700 Mbp	?	Pending
Larix sibirica	Siberian Larch	12,030 Mbp	?	Pending
Pinus pinaster	Maritime Pine	23,810 Mbp	?	Pending
Pinus sylvestris	Scots Pine	~23,000 Mbp	?	Pending

1) Genome size: Approximate total size, not completely assembled.
2) Number of Genes: Approximate number of loci containing protein coding sequence.
3) Status: Assembly / Annotation versions; See http://www.phytozome.net for all publically released tree genomes. Conifer genomes will also be posted here as they are completed.

Elements of the Conifer Genome Sequencing Project



WGS (1N & 2N)

2N

1N

Sequencing

UC Davis — UC DAVIS Evolution & Ecology
-Libraries: short/long inserts
-Sequencing
= GAIIX (overlapping reads)
= HiSeq (125 bp reads)
-Data curation and fosmid assembly

Fosmid Cloning

CHORI BAC/PAC
- 37 kb clones
- >2X coverage
- Pool size ≥ 500
- diTags
- Illumina library construction

Assembly

Maryland-JHU Genome Assembly Group
-Assembler development (MSR-CA)
-Assembly
=WGS
=Fosmid
=Meta

Genetic Maps

UC Davis
-SNP calls from scaffolds and genetic mapping to order scaffolds

Transcriptome

IU-TAMU-UC Davis
-Multiple tissues/conditions
-Library development
-Sequencing (Roche 454/Illumina)
-Assembly

Annotation

UC Davis-WSU
-Functional Annotation
-Comparative genomics
-Anchoring to existing genetic maps
-Integration of genome and transcriptome resources

Database-Dendrome

UC Davis
-Data organization and distribution
-Ontology Development (PO&TO)
-Community Annotation (GenSAS)
-Web Services (SSWAP)
-Analysis Pipeline Integration (iPlant)

Two Approaches to Conifer Sequencing

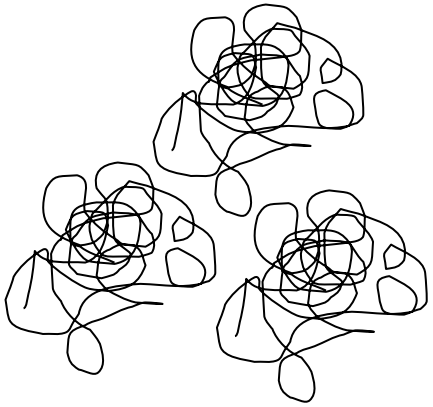
- Whole Genome Shotgun (WGS) sequencing of
  - Haploid Megagametophyte: Goal – deep representative short insert libraries from a single haploid ($1N$) segregant. Haploid genome significantly improves sequence assembly
  - Diploid Parental Genotype 20-1010
    (Released to public for this project)

botit.botany.wisc.edu

- Direct Sequencing of Pooled Fosmid libraries
  - Pools of multiple 38.5 kbp *P.taeda* fosmid clones that are well within available assembler's specs.
  - Key is to tune complexity of individual pools for both economy and assembly quality
  - Small enough to be haploid, facilitating assembly ($2N$ library but $1N$ pool).

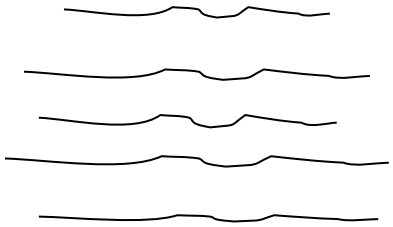
Whole Genome Shotgun Reads

Genomic DNA



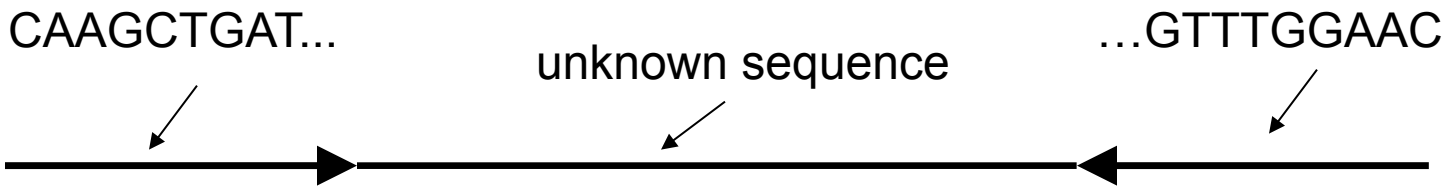
fragmented & size selected

fragments of 200 - 10,000 bases



sequence the ends

pairs of reads of 100 – 250 bases each



Technology for De Novo Sequencing of the Conifer Genomes

Parallel and Complementary Approaches

Whole Genome Shotgun Sequencing (Haploid/Diploid)

- Illumina GAIIx
- Illumina HiSeq

Sequencing of Pooled Fosmid Clones (Diploid)[1]

- Illumina GAIIx
  - Max Output: 95 Gigabases
  - Max. paired end reads - 640 million
  - Max. Read Length – 2 x 150 bp
- Illumina HiSeq
  - Max Output: 300 Gigabases
  - Max. paired end reads - 3 billion
  - Max. Read Length – 2 x 100 bp

[1] Effectively haploid

Data current as of 3/2012 (Illumina)

Megagametophyte Whole Genome Shotgun (M-WGS)

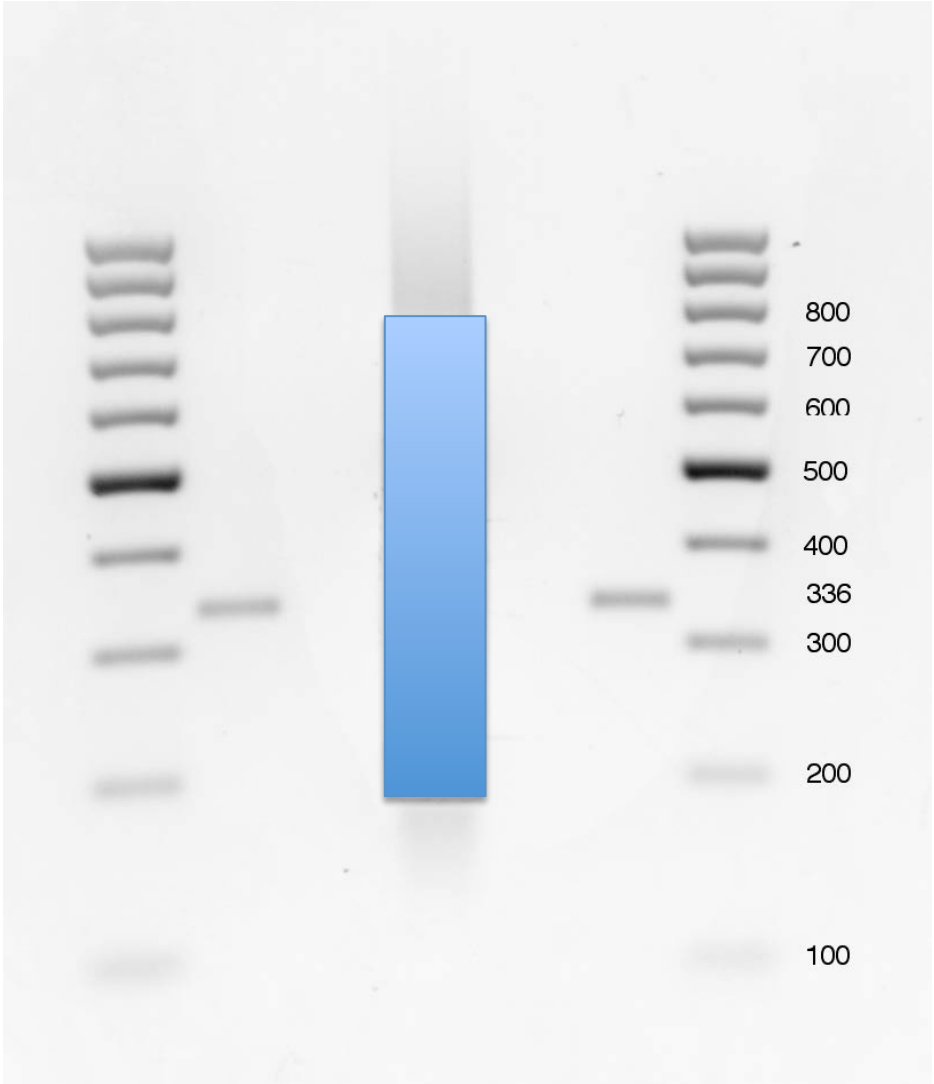
- Not enough haploid DNA in a megagametophyte to implement a complete list of WGS ingredients.
  - Obtain DNA for longer insert linking libraries (> 1kbp) from diploid needle tissue.
  - Prepare only short insert Illumina libraries from megagametophye tissue.
  - Library complexity is still a challenge
    - Many short libraries will be needed

***P. taeda* 2011 crop**

N	54
mean	1361 ng
st. dev.	675 ng
min	580 ng
max	3560 ng

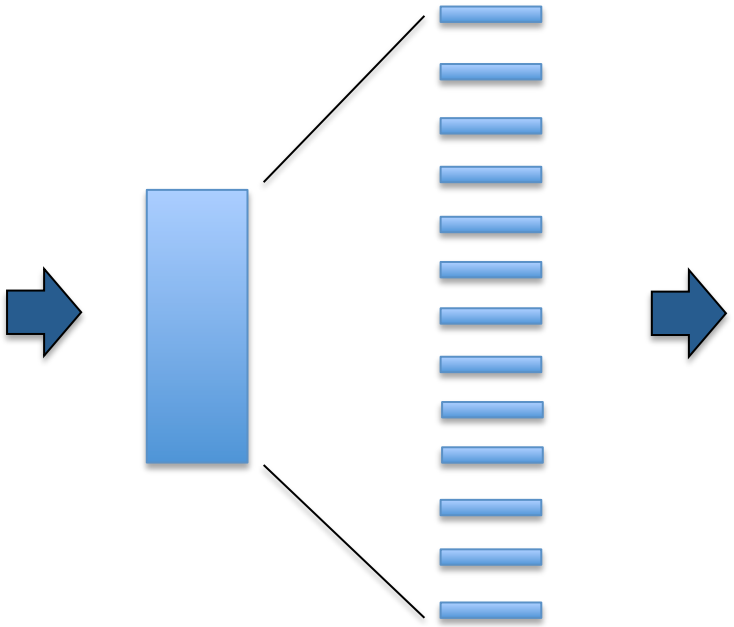
M-WGS Short Insert Libraries

DNA Fragmentation and Partitioning



[Left] The fragmented meg DNA sample is run on an agarose gel.

[Right] A target size range is extracted and partitioned for tight c.v. on the insert size distribution.

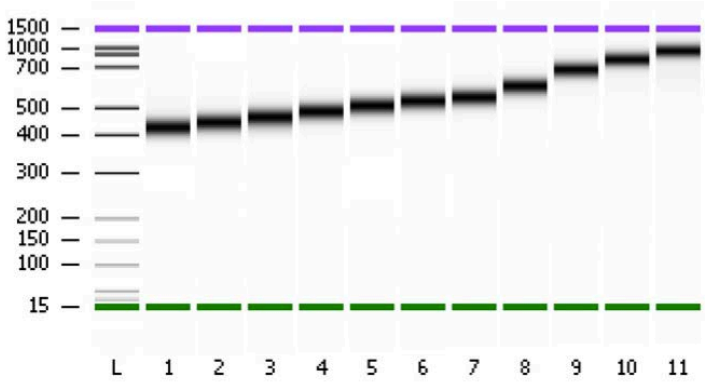


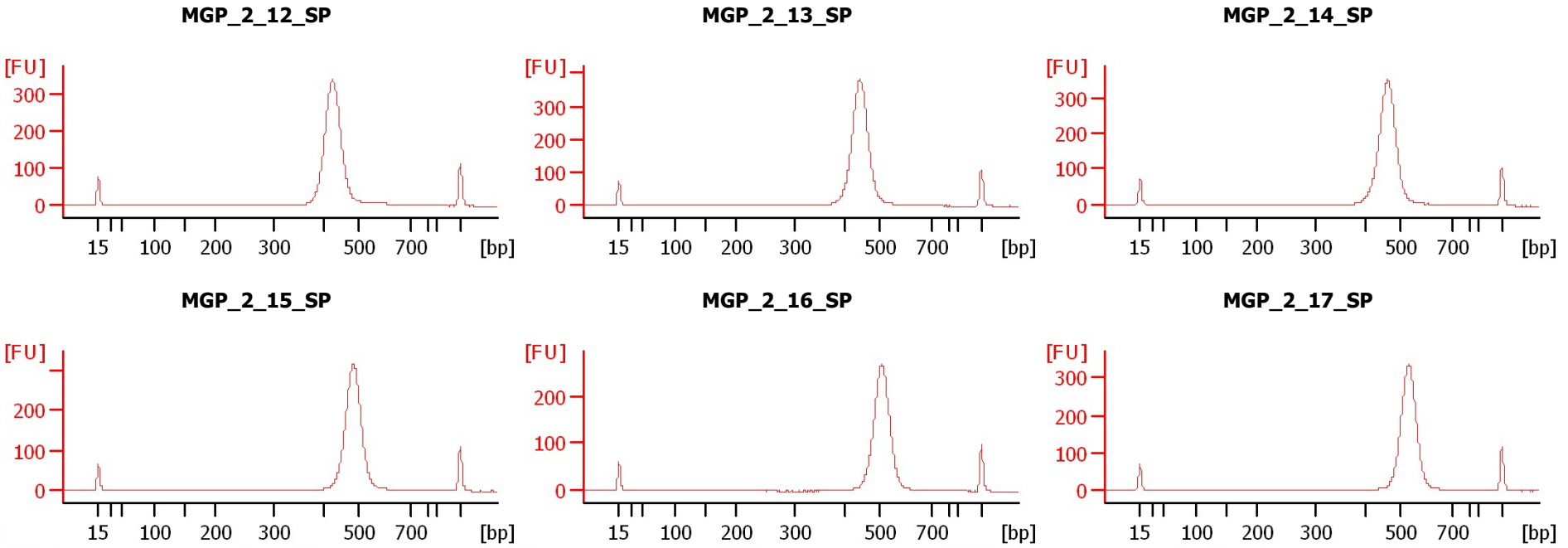
M-WGS Short Insert Libraries

Preliminary QC and Size Selection

Each DNA sample is then run on an Agilent Bioanalyzer to determine a preliminary estimate of insert size and coefficient of variation.

If within spec, selected DNA samples are converted into Illumina libraries





M-WGS Short Insert Libraries

Library QC and Titration

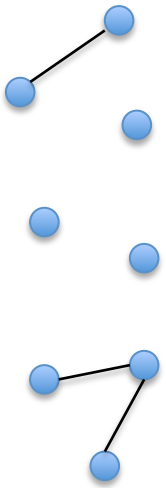
- Libraries are subsequently QCed on a MiSeq

Library ID	ecies	Median	C.V.
MGP_2_3_SP	*Pinus lambertiana*	180	0.05
MGP_2_4_SP	*Pinus lambertiana*	191	0.05
MGP_2_5_SP	*Pinus lambertiana*	201	0.04
MGP_2_6_SP	*Pinus lambertiana*	212	0.05
MGP_2_7_SP	*Pinus lambertiana*	226	0.05
MGP_2_8_SP	*Pinus lambertiana*	240	0.04
MGP_2_9_SP	*Pinus lambertiana*	254	0.04
MGP_2_10_SP	*Pinus lambertiana*	268	0.04
MGP_2_11_SP	*Pinus lambertiana*	281	0.04
MGP_2_12_SP	*Pinus lambertiana*	296	0.04
MGP_2_13_SP	*Pinus lambertiana*	313	0.04
MGP_2_14_SP	*Pinus lambertiana*	329	0.04
MGP_2_15_SP	*Pinus lambertiana*	347	0.04
MGP_2_16_SP	*Pinus lambertiana*	365	0.04
MGP_2_17_SP	*Pinus lambertiana*	380	0.04
MGP_2_18_SP	*Pinus lambertiana*	395	0.04
MGP_2_500_SP	*Pinus lambertiana*	447	0.04
MGP_2_600_SP	*Pinus lambertiana*	546	0.05
MGP_2_700_SP	*Pinus lambertiana*	633	0.05
MGP_2_800_SP	*Pinus lambertiana*	714	0.07

Metric	Definition
Sample Name	Sample name from the sample sheet
Clusters	Number of clusters sequenced for this sample
Clusters %	Percentage of successfully indexed clusters from this sample
% PF	Percentage of clusters for this sample that passed filters
% Aligned R1	Percentage of clusters for which read 1 successfully aligned
% Aligned R2	Percentage of clusters for which read 2 successfully aligned
Length Median	Median fragment length for this sample
Length Min	Low percentile (corresponding to 3 standard deviations from the median) of fragment lengths for this sample
Length Max	High percentile (corresponding to 3 standard deviations from the median) of fragment lengths for this sample
Mismatch R1	Mismatch rate for this sample in read 1
Mismatch R2	Mismatch rate for this sample in read 2

M-WGS Libraries

How deep to sequence a library?



- Determine vertices
  - k length prefixes of each DNA molecule (paired end read) are concatenated
- Determine edges
  - An edge between two vertices if they differ by one or fewer sites
- Identify and count connected components.
  - a.k.a single linkage clustering
- Compute average multiplicity
  - Number of paired end reads / number of clusters
  - Target < 1.25X

Notable Assemblers for Illumina Data

- MaSuRCA
- Allpaths-LG
- SOAPdenovo
- ABySS
- Velvet
- Contrail
- SGA

k-mers

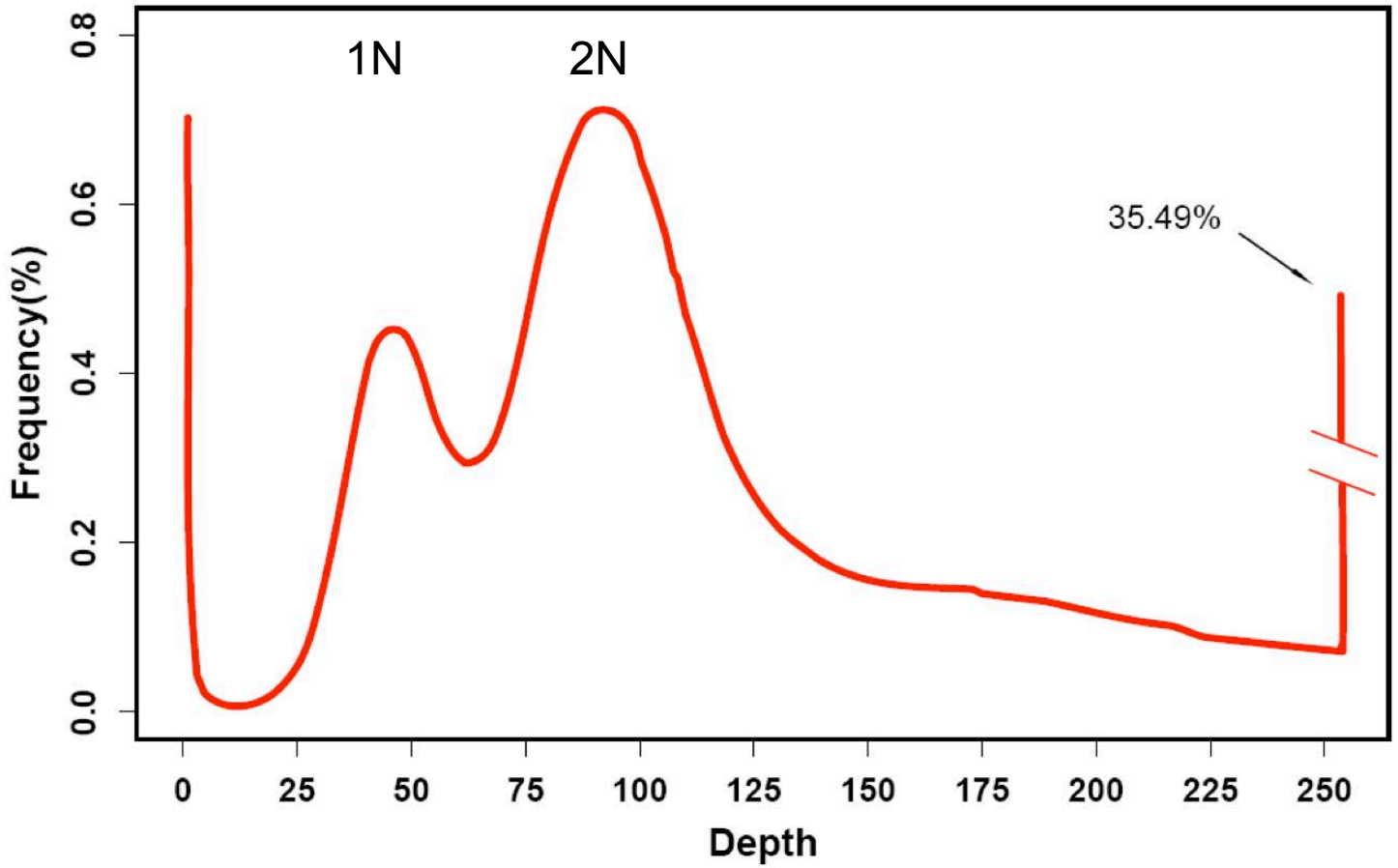
Query: Does a distinct length *k* string occur in the genome?

- Experimentally, this is often answered with hybridization
- To answer it of the WGS reads we will first chop the sequence up into all substrings of length *k*
- Each length *r* read becomes *r – k + 1* strings of size *k*

- We choose a *k* that best supports the query above allowing specific locations on the genome to be queried.
- From *k*-mers alone we can estimate genome size, repeat content, and even assemble whole genomes (SBH).

17-Mer Complexity Analysis

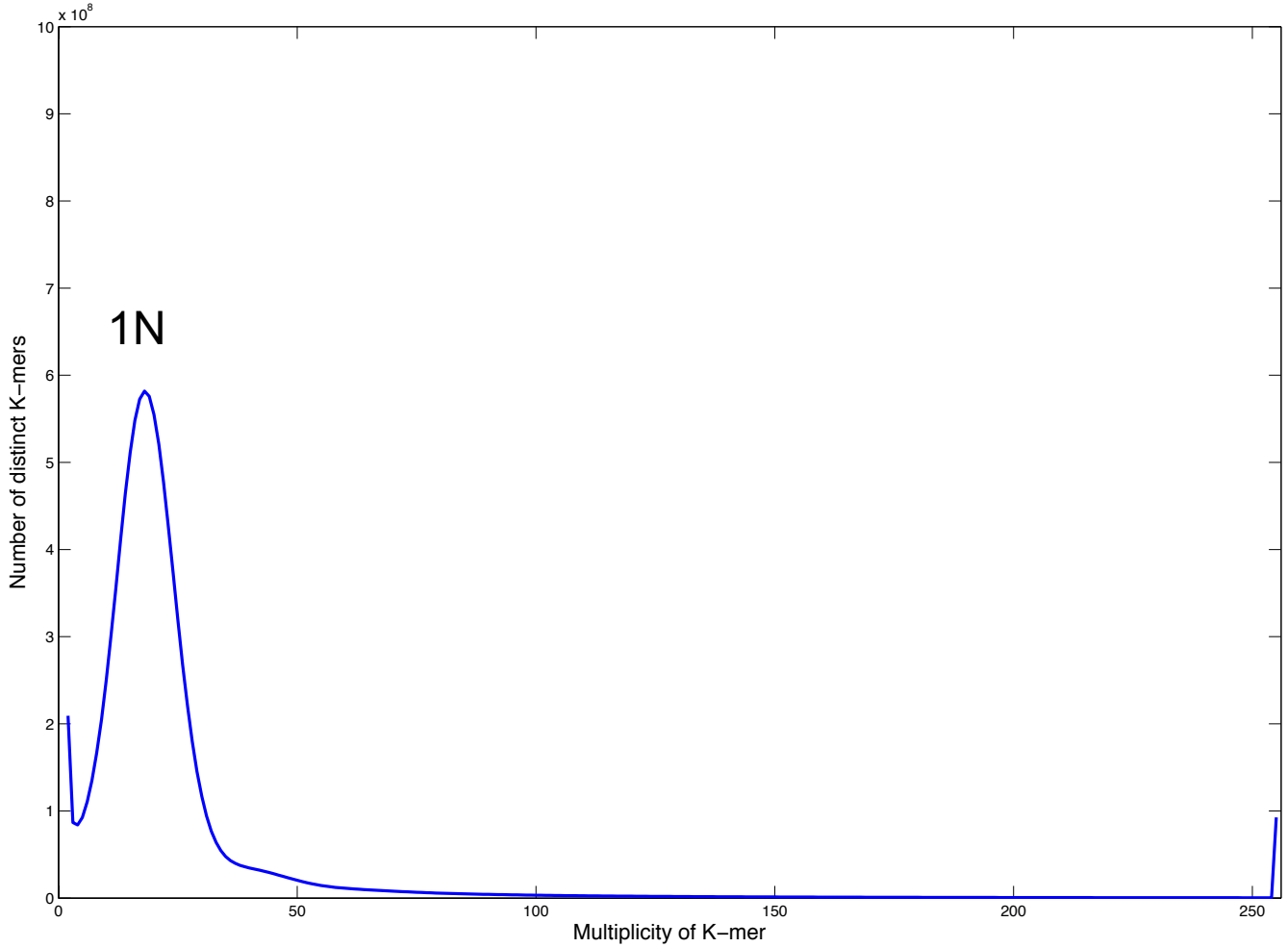
Pacific oyster - *Crassostrea gigas*



(Zhang et al. 2012)

31-Mer Complexity Analysis

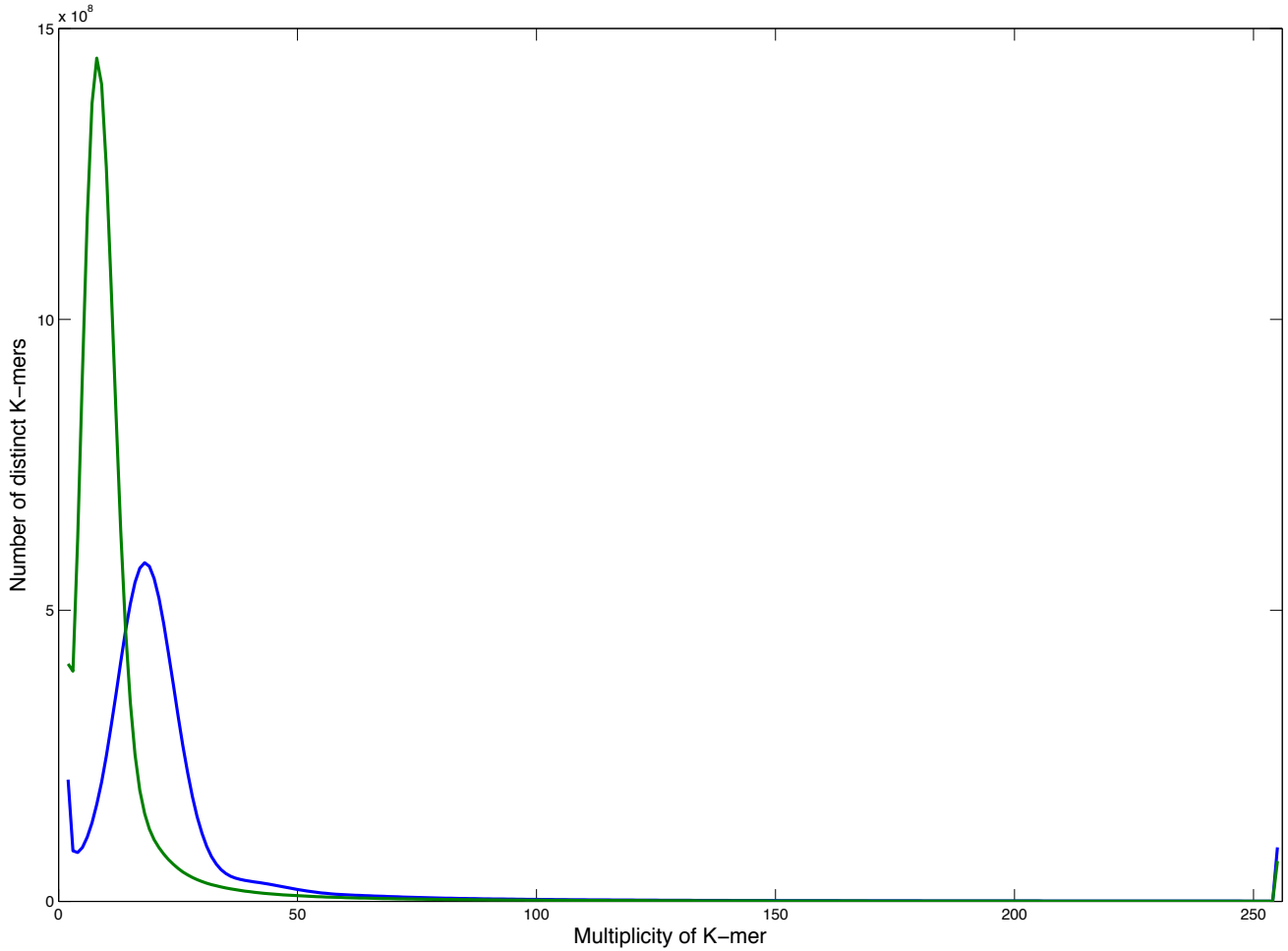
Loblolly pine – *Pinus taeda* – *25x HiSeq*



31-Mer Complexity Analysis

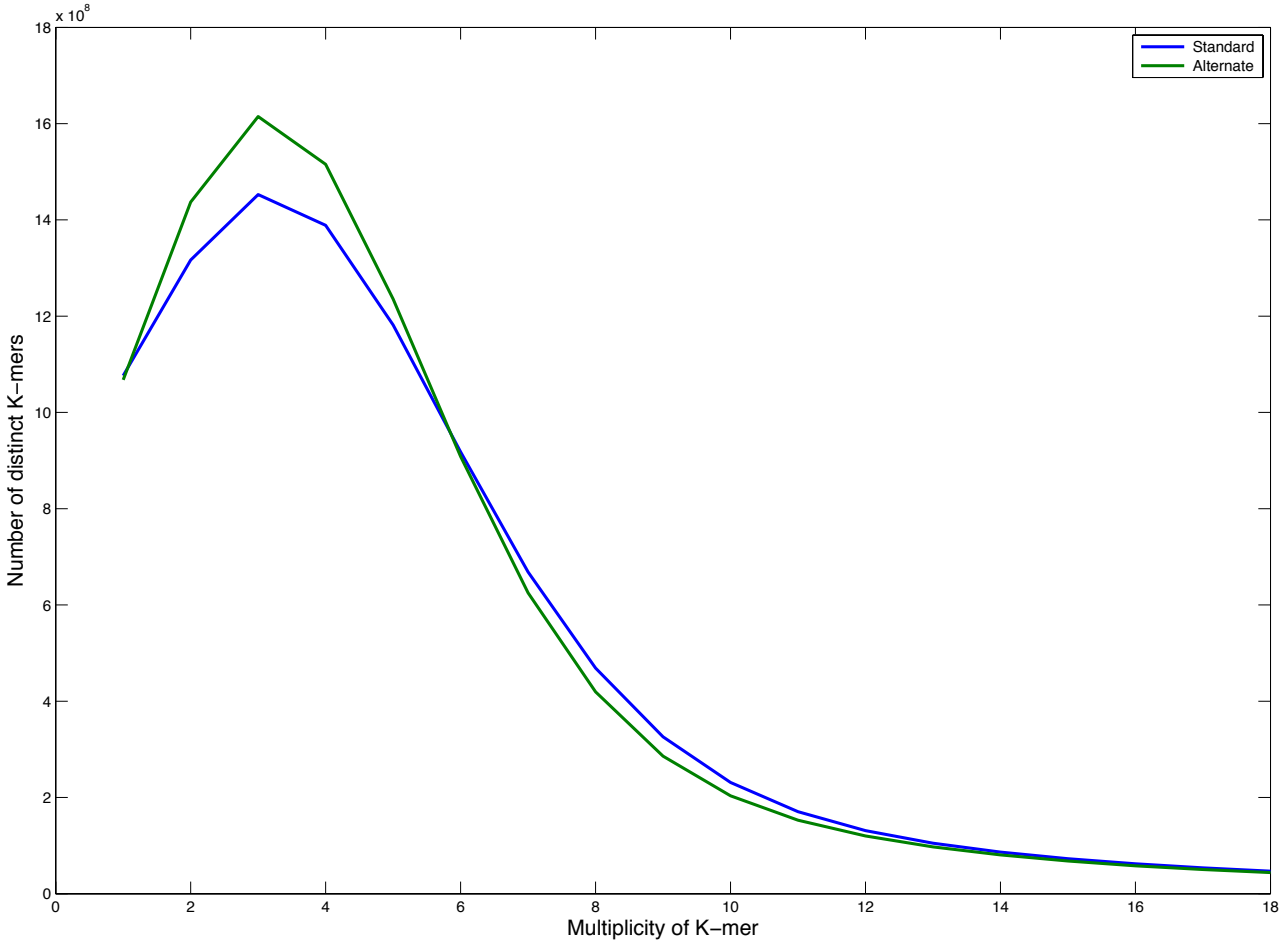
Loblolly pine – *Pinus taeda – 25x HiSeq*

Sugar pine – *Pinus lambertiana – 10x HiSeq + MiSeq + GA2x*



31-Mer Library Construction Analysis

Sugar pine – *Pinus lambertiana – HiSeq + MiSeq + GA2x*



A k-mer Genome Size Estimate

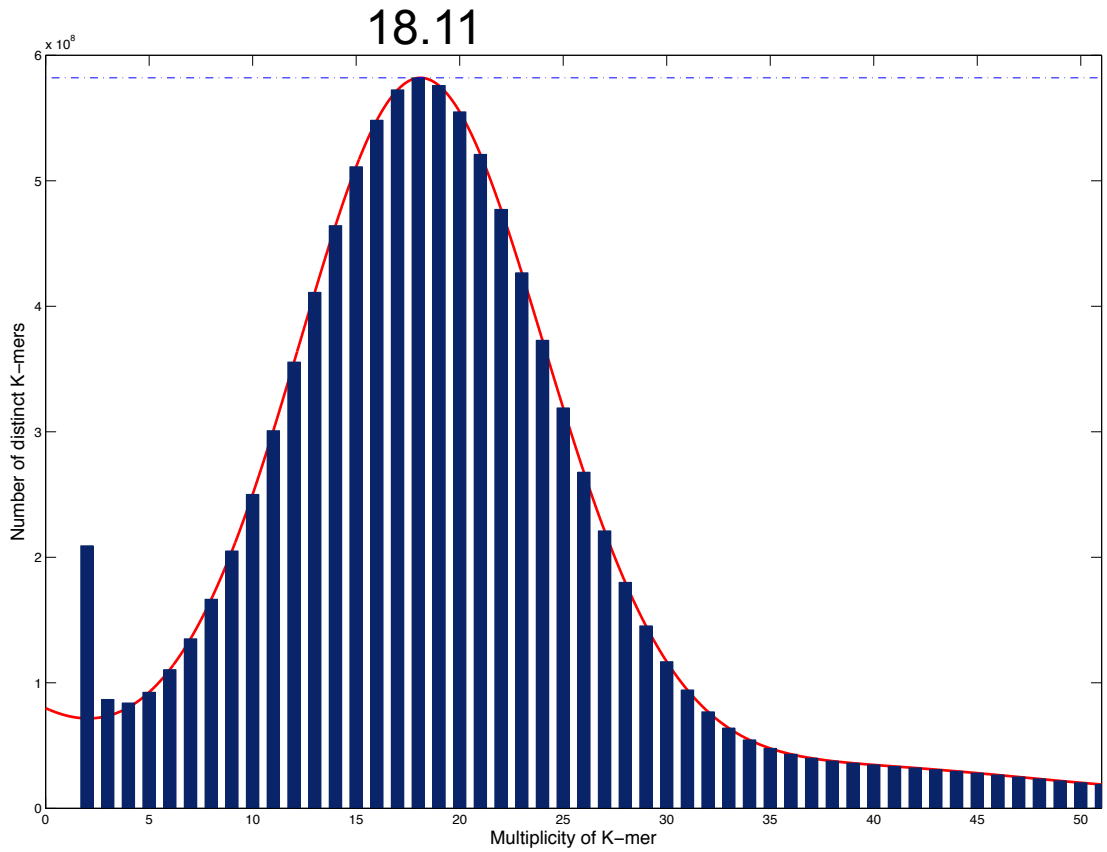
P. taeda genome size ≅ total k-mers in genome

total k-mers in *P. taeda* genome ≅

$$\frac{\text{total k-mers in } \textit{P. taeda} \text{ reads}}{\text{expected number of times a genomically unique k-mer is observed in the reads}}$$

Expected unique k-Mer multiplicity

We will use the mode of a fitted PDF



k-mer Genome Size Estimates

Loblolly pine *Pinus taeda:*

31-mers total: 3.736×10^{11}

Expected k-mer depth: 18.11

Estimated genome size: 20.63 GB

High Copy 31-mers

1.09% of distinct 31-mers

33% of all 31-mers

24-mers total: : 4.092×10^{11}

Expected k-mer depth: 19.79

Estimated genome size: 20.68 GB

Sugar pine *Pinus lambertiana:*

31-mers total: 2.776×10^{11}

Expected k-mer depth: 8.12

Estimated genome size: 34.19 GB

High Copy 31-mers

0.35% of distinct 31-mers

33% of all 31-mers

24-mers total: 3.031×10^{11}

Expected k-mer depth: 8.89

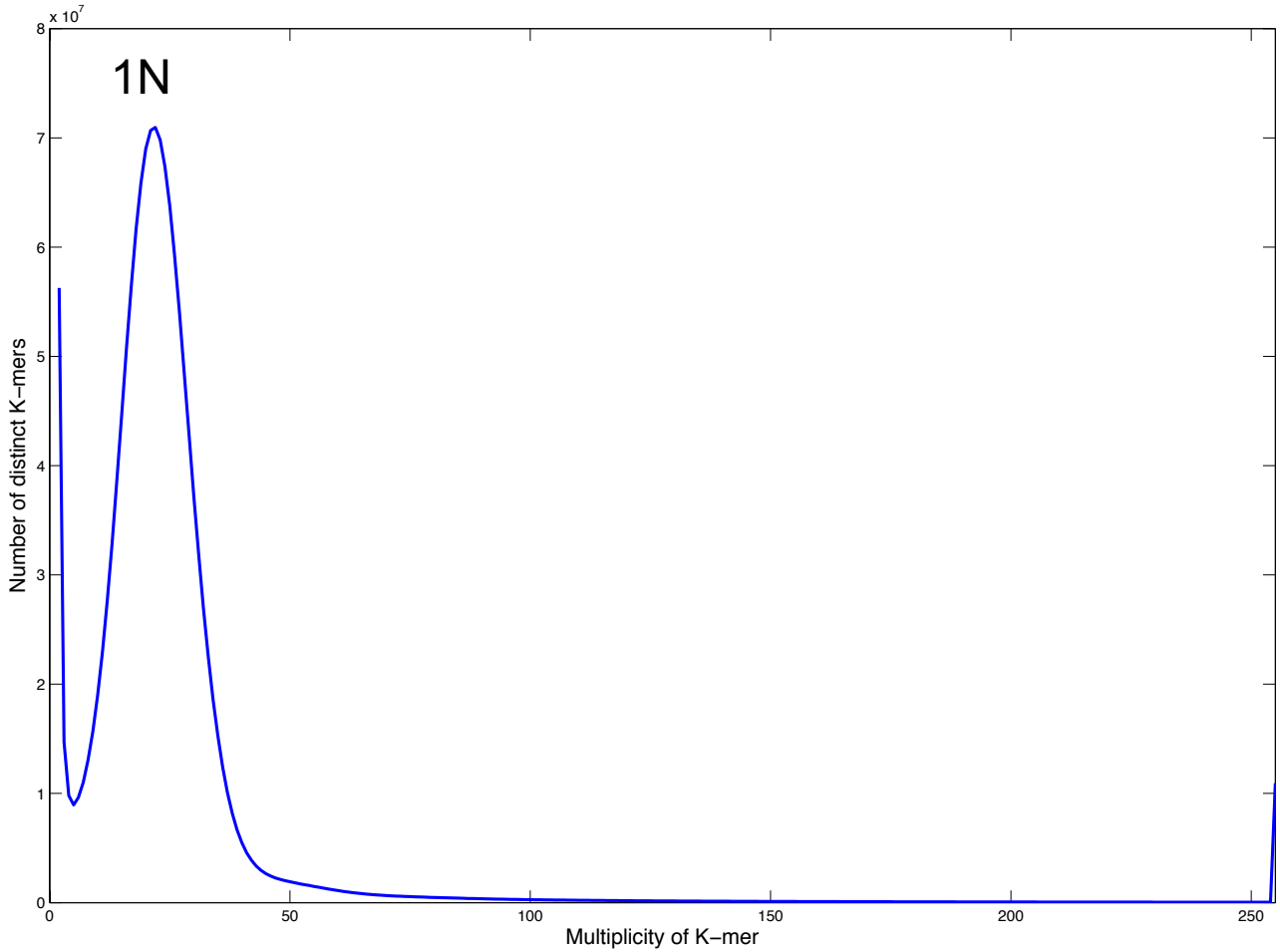
Estimated genome size: 33.98 GB

P. taeda Version 0.8 Library Statistics

- Haploid short insert libraries
  - 10 short insert libraries 200 - 640bp
  - 1.4Tbp raw total sequence
  - 60 fold coverage
- Diploid jumping libraries
  - 47 jumping libraries 1300 – 5500bp
  - 280Gbp raw total sequence
  - 12 fold coverage
- 13 Fosmid DiTag Libraries 38,500bp

79-mer Complexity Analysis

Loblolly pine – *Pinus taeda* – (62x *HiSeq + MiSeq + GA2x* coverage)



Early Access *P. taeda WGS V0.6*

- Approximately 35X coverage
- Total Sequence: 18,321,727,393 bp
- Total contig sequence: 14,606,783,345 bp
- N50 1199bp (9.16 Gbp is contained in contigs of 1199 bp or longer)
- Total scaffold sequence (with imputed gaps): 18,428,460,141bp
- N50 1230bp (9.21 Gbp is contained in scaffolds of 1230 bp or longer)
- Degenerate contig sequence 3.8Gb

Fosmid Pools

A Molecular Approach to Complexity Reduction

Reduced Complexity from E.coli clone pools

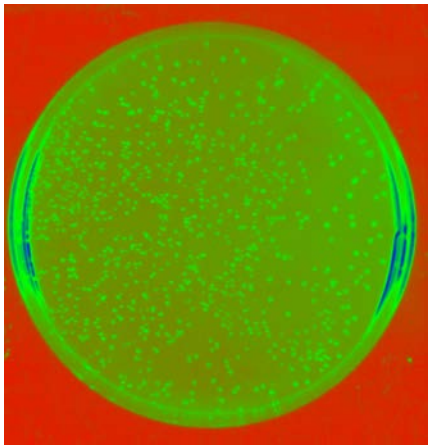
- Traditionally clone tiling paths were used to create good assemblies.
- Recently clone pools have been used to derive long-haplotype information.
- Ideally: larger clones (BACs) are more desirable, more likely to span repeats
- Economically: much cheaper to create large fosmid libraries using sheared DNA

Fosmid Pooling:
Genome partitioning for reduced assembly complexity

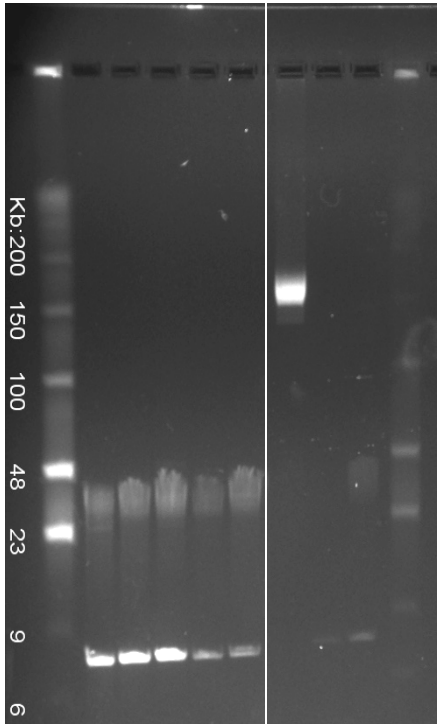
- The immense and complex diploid pine genome can be *economically* and *efficiently* partitioned into smaller, functionally haploid, pieces using pools of fosmid clones.
- Fosmids in a pool should have a combined insert size far less than a haploid genome size; This assures a haploid genome representation.
- The sequence data obtained from a single fosmid pool may be up to 80 X deep.
- The sequence data obtained from a pool must be screened for vector and E. coli contamination

Fosmid Pool DNA Preparation

- Genomic DNA extraction
- Shearing (to average ~40 kbp)
- Size-purification (pulsed-field gel electrophoresis)
- Ligation to excess vector (dephosphorylated ends)
- Packaging (extracts from mcrA, B, C strains)
- Determine titer of the "particle library"
- Create E.coli colonies
- Isolate DNA from colonies
- Quality Assessment: Quantitation & Q-PCR (TAQMAN assays) to determine E.coli & fosmid vector contamination



Fosmid colonies: Documented & counted prior to pooling



Fosmid pools: DNA digested (PI-SceI); Lane 6: Undigested pool

Fosmid Pool Sequencing Pipeline

- Fosmid pool DNA received from CHORI
- Quantify DNA

Short Insert Library

- Aliquot 5 ul DNA
- Sonicate to fragment DNA
- End repair fragments
- A-tail fragments
- Ligate Illumina multiplex adapter
- Size select adapter-ligated fragments by agarose gel electrophoresis
- QC and quantitate using Agilent Bioanalyzer
- Enrich 10 ng size selected fragments using 10 cycles PCR
- QC and quantitate enriched library using Agilent Bioanalyzer
- Sequence

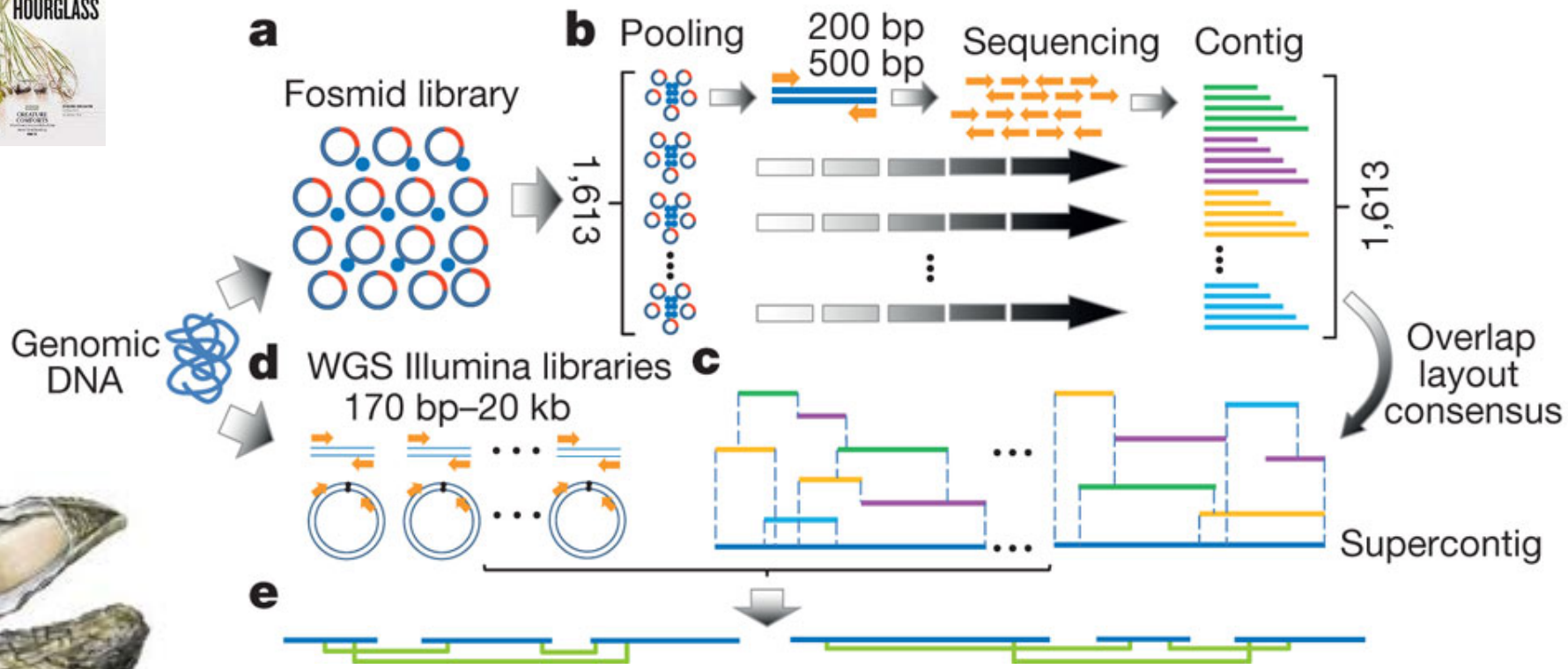
Long Insert Library

- Pool DNAs to create pool of pools
- Aliquot 10 ul DNA
- Fragment DNA using HydroShear
- End repair and biotinylate fragments
- Size select by agarose gel electrophoresis
- QC and quantitate using Agilent Bioanalyzer
- Circularize size selected fragments
- Digest un-circularized DNA
- Sonicate to fragment circularized DNA
- Bind biotinylated fragments to streptavidin beads
- End repair fragments
- A-tail fragments
- Ligate Illumina multiplex adapter
- Enrich adapter-ligated fragments using 18 cycles PCR
- Remove enriched library from beads
- Size select enriched library by agarose gel electrophoresis
- Sequence

Fosmid Pools

Pacific oyster - *Crassostrea gigas*

(Zhang et al. Oct 2012)

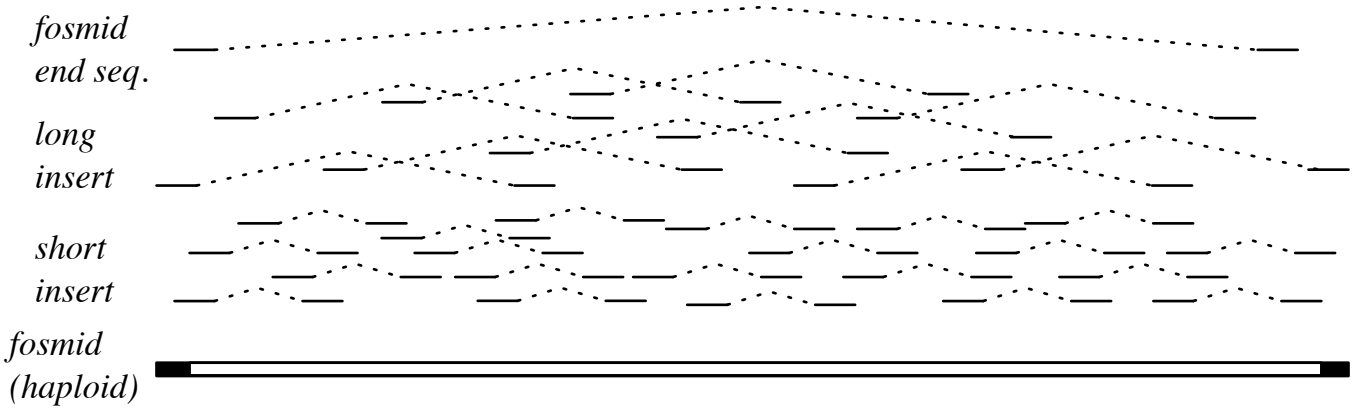


Fosmid Pools

Strategy made possible by cheap sequencing.

- Pacific Oyster 0.56 Gbp Genome
  - Fosmid Pool Size: 90
  - Number of Pools: 1,613
  - Pool Coverage: 10x
  - Sequence Coverage per Pool 60x
  - Total fragment library coverage: 360Gbp (600x)
- Loblolly Pine 24Gbp Genome
  - Number of pools with 90 fosmids per pool: 69,000
  - Total fragment library coverage: 14,400Gbp

Fosmid Sequence Components



- Haploid fosmids with vector tagged ends
- Primary coverage from short insert libraries
- Additional coverage from long insert libraries from equi-molar pool of pools.
- Fosmid end sequences (diTags) link ends of the assembly and count fosmids in a pool

Fosmid Pools

Determining the Best Assembler for the Job

Assembly results for a relatively large pool of approximately 500 *P. taeda* fosmids

			quartiles				
Assembler	Stat	Count	Q1	Q2	Q3	N50	Sum
Allpaths-LG	scf	987	2499	7781	30271	26298	14×10^6
	ctg	1524	2355	6031	12509	10324	14×10^6
	scf30K+	248	33595	35682	38361	30114	9×10^6
MSR-CA	scf	2162	506	1375	9224	14753	15×10^6
	ctg	3519	503	1339	5000	6826	14×10^6
	scf30K+	136	32603	35087	38119	30147	5×10^6
SOAP	scf	3251	123	185	495	33389	15×10^6
	ctg	23873	76	175	348	1515	15×10^6
	scf30K+	322	33907	35766	38683	33389	12×10^6

Fosmid Pools

Increasing the Pool Size

- Five nested fosmid pools
  - 1, 2, 4, 6, and 8 unit pools of approx 600 fosmids
- Assembled with SOAP denovo
- Scaffolds larger than 30kbp are reported below

estimated pool size	elem	min	max	mean	sum	end verified	coverage
600	567	30030	60754	35513	20135877	343	87.2%
1200	1094	30029	66421	35567	38910427	694	84.2%
2400	1465	30001	78520	35093.85	51412495	740	55.6%
3600	2598	30003	74253	35330.12	91787645	1343	66.2%
4800	3305	30015	73986	35309.59	116698186	1676	63.1%

Fosmid Pools

Increasing the Pool Size

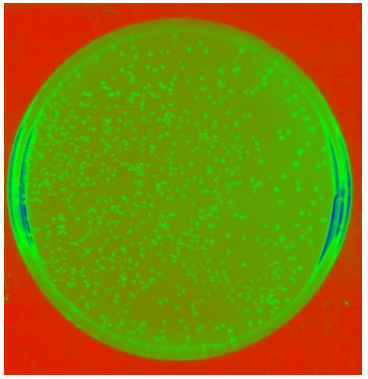
- 4800 fosmid pool
- Assembled with SOAP denovo
- Iterative gap closing applied

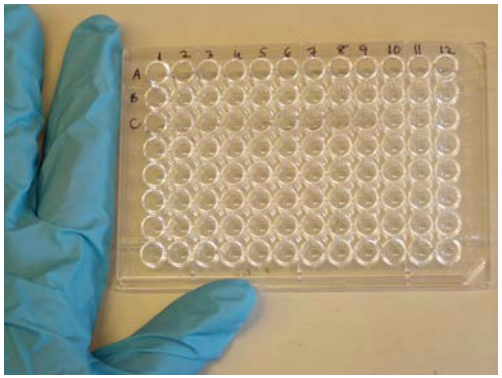
	elem	min	max	mean	sum	end verified	coverage
scf20K+	5323	20013	75791	32284.95	171852787	2001	93%
scf30K+	3719	30010	75791	35426.96	131752852	1911	71%
scf40K+	338	40000	75791	43007.42	14536509	181	8%

- 20K+ scaffolds for largest pool exceeds target coverage
- This assembly appears in later

Components of a 96 Well Production Format

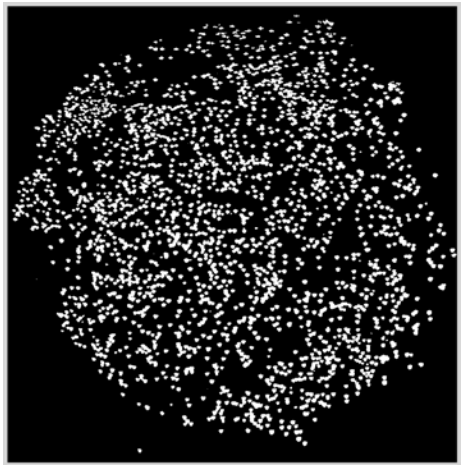
- Harvest 96 bacterial pools of colonies with one fosmid per colony.
- Amplify the harvested colonies as “pools”
- Purify fosmid pool DNA
- Digest DNA with the homing endonuclease PI-SceI
- *Capture fosmid inserts, using a biotinylated DNA Triple Helix motif, washing away the fosmid vector backbone.*
- Convert to 96-well format
- Shear the individual pools for short insert libraries in a 96-well micro-tube plate.
- Illumina’s 96-well Tru-Seq protocol.
- MiSeq QC step for more efficient use of HiSeq
- Superpooling to create long insert libraries

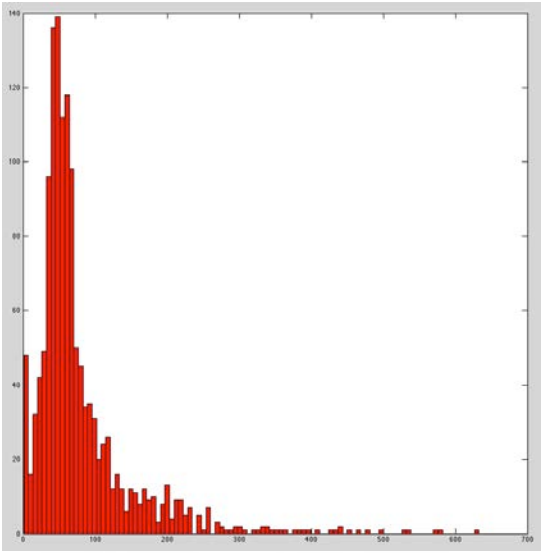




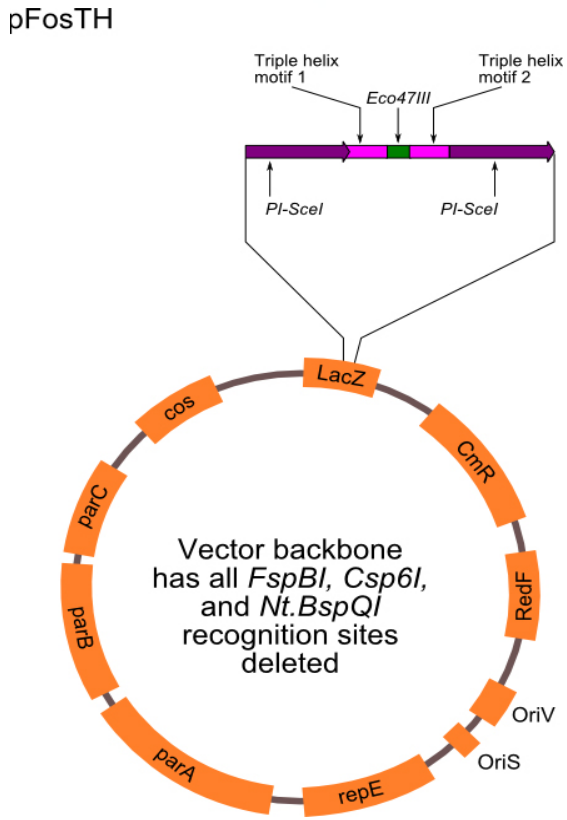
Fosmid Pool Size Estimation

- Threshold image using minimum intra-class variance
- Compute connected components *C* based on adjacency.
- Estimate the expected component size *s*.
- Estimated pool size = $|C|\ s^{-1}$





Triple_Helix Purification of fosmid inserts*



- Removal of vector & E.coli contamination
- Scalable
- Simple Purification

*** Rapid isolation of cosmid insert DNA by triple-helix-mediated affinity capture.**
Ji H, Smith LM, Guilfoyle RA. Genet Anal Tech Appl. 1994;11(2):43-7.

*** Rapid restriction mapping of cosmids by sequence-specific triple-helix-mediated affinity capture.** Ji H, Francisco T, Smith LM, Guilfoyle RA. Genomics. 1996 Jan 15;31(2):185-92.

Results from Triple Helix Purification

Comparison of fosmid pools with and without TH

Scale
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0

Fosmid Pool Library	% E. coli + Vector
LFP_500_336L	17.8
LFP_1000_336L	17.8
LFP_2000_336L	16.3
LFP_3K_3	17.7
LFP_4K_3	17.6
LFP_5500 (jumping)	15.9
Median	17.6

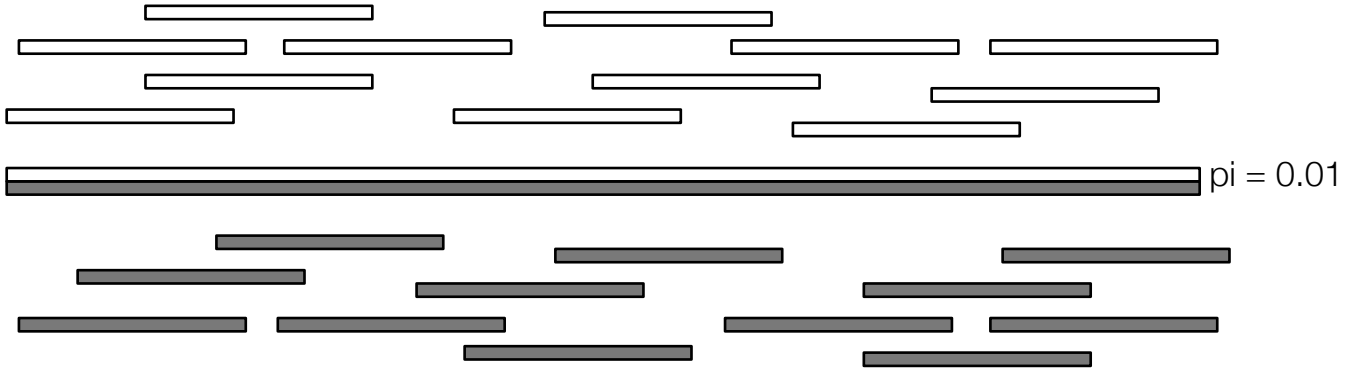
94.7% Reduction in E. coli + Vector

16.3% Reduction in sequencing costs

CHORI2820_LP1 % E. coli + Vector

	1	2	3	4	5	6	7	8	9	10	11	12
A	4.0	2.2	1.6	1.1	1.5	2.4	2.5	1.8	2.1	2.2	1.8	6.5
B	1.0	1.3	2.3	1.1	1.5	1.7	1.3	2.2	1.4	1.4	1.3	1.6
C	0.0	0.0	1.8	1.4	1.1	1.2	1.3	1.2	2.6	2.1	1.1	1.8
D	1.8	0.8	0.5	1.8	1.2	1.1	1.7	1.9	1.2	1.8	1.3	1.8
E	0.4	0.6	0.8	1.3	1.0	0.9	1.1	1.1	1.1	1.0	1.0	1.2
F	1.9	1.4	1.1	2.4	1.0	1.4	2.3	1.5	2.1	1.0	1.6	1.0
G	1.3	1.1	1.6	1.2	1.2	1.1	2.5	1.2	1.1	1.1	1.0	0.9
H	1.6	1.7	1.4	4.8	1.2	1.1	2.1	1.1	2.4	1.1	1.3	0.9
Median			1.3									

A Strategy for Fosmid Based Assembly



- Approx 4000 fosmids per pool (approx 150 Mbp)
- 2.5 – 3.8x fosmid clone coverage (4 - 6 plates)
  - pilot plate + 3 additional plates
- Assemble fosmid scaffolds into larger scaffolds
  - OLC Approach (e.g. MSR-CA, CABOG)
  - Use existing WGS scaffolds with MUMmer/nucmer

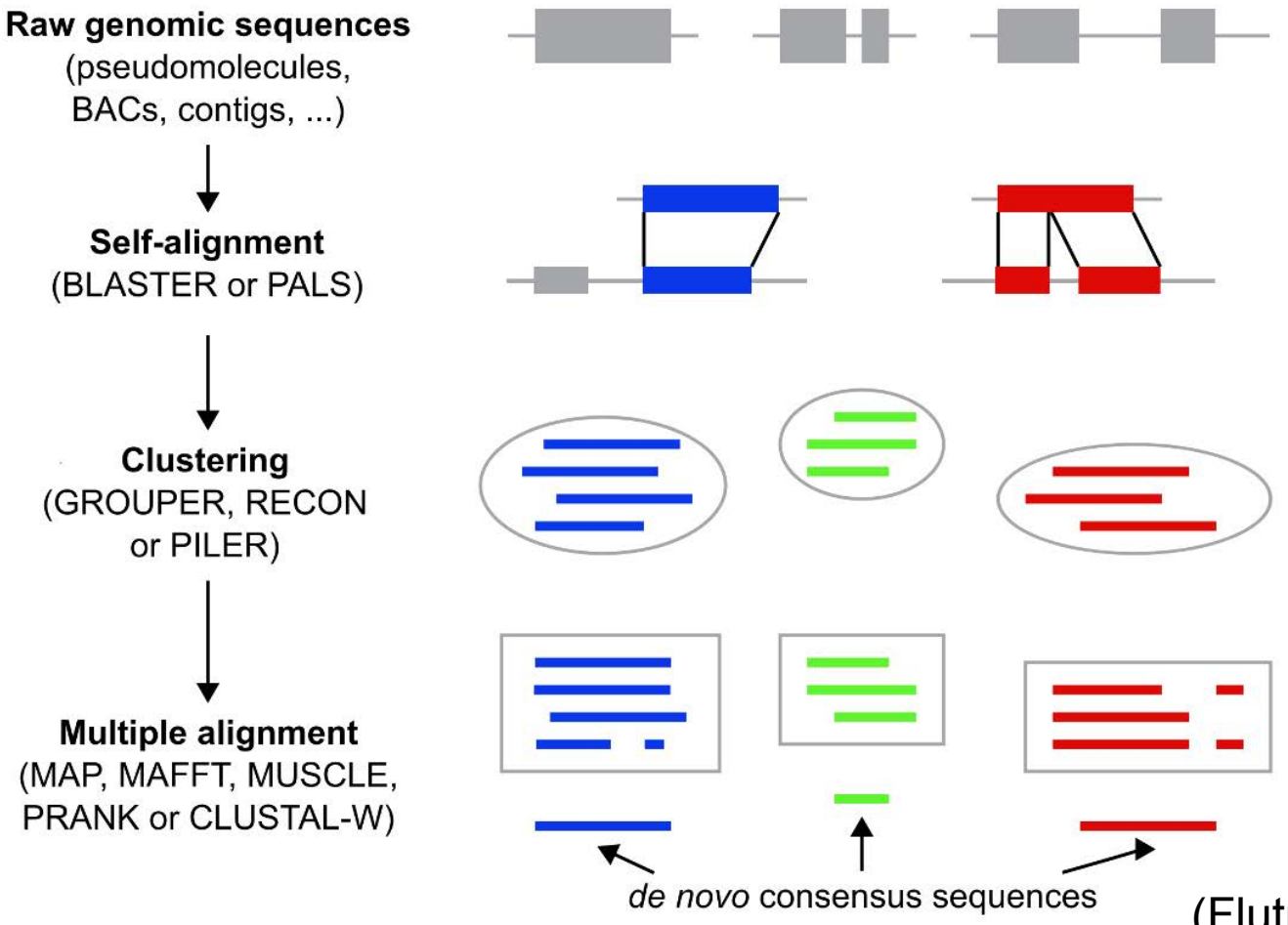
Additional Use Cases for Fosmid Pools

- Assembler Evaluation
- Repeat Library Construction
- SNP Discovery

Repeat Discovery in Fosmid Pools

- For similar repeats methods utilizing k-mers, suffix trees, suffix arrays, or De Bruijn graphs work well.
- Very long contigs allow us to use a “top-down” strategy to find more divergent repeats.
- We used the REPET pipeline (Flutre et. al. 2011) developed at URGI - Unité de Recherche Génomique Info.
  Authors are well known for TE annotation.

REPET *TEdenovo* Pipeline



(Flutre et al, 2011)

SNP Discovery in 20-1010 Aligned Fosmid Consensus Results

- Contigs from the 4800 fosmid pool were aligned to genome using MUMmer/nucmer package developed in the Salzberg lab.
- SNPs per contig:

(bi-modal)

Number of contigs	Min	Max
11	50	60
58	40	49
190	30	39
221	20	29
384	10	19
391	6	10
2,543	0	5

- Estimated heterozygosity of 1.5%

SNP Discovery in 20-1010

- Established paradigm for NGS sequence
  - Align reads to reference sequence
    - MAQ
    - Bowtie, Bowtie2
    - BWA
    - SOAP
  - Determine alleles
    - MAQ
    - Samtools
    - GATK

Only BWA works with genomes larger than 2^{32}

20-1010 SNPs: Aligned Fosmid Read Results

- Reads from 4800 fosmid pool were aligned to the genome with bowtie2 from the Salzberg lab.
- Genomic target recruited using reciprocal best hit
- Use samtools for allele calling.
- 262,290 snps were identified as high quality in 66,685 contigs
- Alignments span approximately 67 Mbp
- SNP Rate of 0.4%
- Estimated heterozygosity of 0.8%
- This implies approx 70-140 million SNPs from 20-1010

Thank You

Up Next:

The Loblolly Pine WGS *V0.8*