



Construction and characterization of a genomic BAC library for *Quercus robur*



- Characteristics of the *Q. robur* BAC library
- A BAC end view of the *Q. robur* genome
- Comparative genomic
- Prospects



Genomics of Forest and Ecosystem Health in the Fagaceae (Beech Family)
November 10-13, 2009
Research Triangle Park, North Carolina, USA

Construction of a large insert DNA library from *Q. robur* (BAC library)

Individual 3P = female parent of one model family

-Genetic maps : - Barreneche et al, 1998, *Theor Appl. Genet.* 97:1090-1103
- Saintagne et al, 2004, *heredity* 92:20-30

-QTL for adaptative traits :

-Bud burst, growth :

Scotti-Saintagne et al, 2004, *Theor Appl. Genet.* 109:1648-1659

Derory et al, 2009, *Heredity*

-Water-logging:

Parelle et al, 2007, *Plant cell and Environment* 30:422-434

-Water use efficiency:

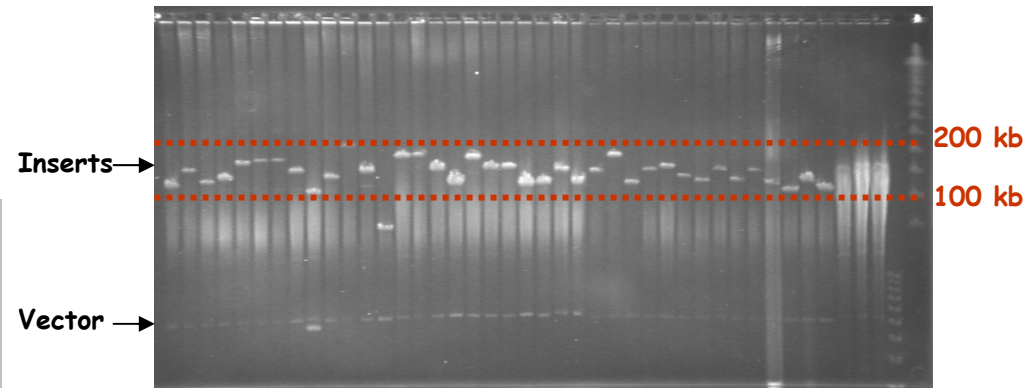
Brendel et al, 2008; *Tree Genet. and Genome* 4:263-278

-Comparative genetic mapping in Fagaceae : Bodenes et al, 2009, this meeting

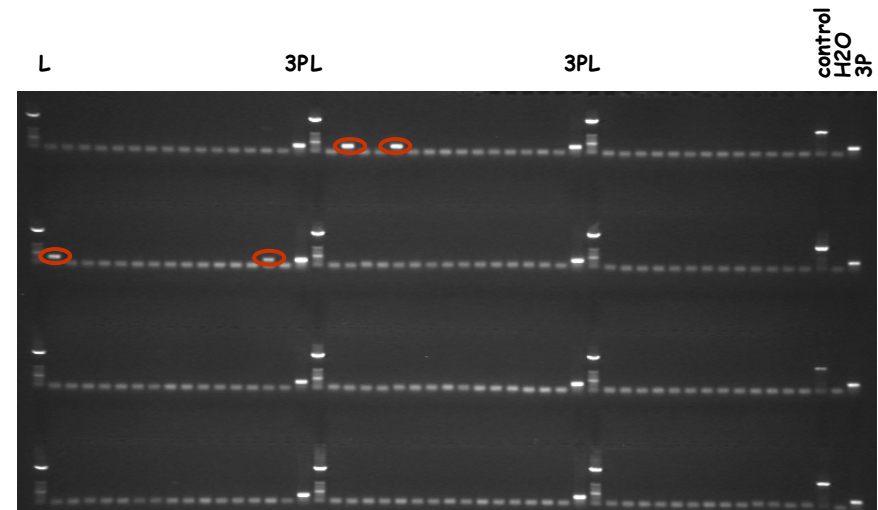
I-Characteristics of the *Q. robur* BAC library

Vector	pIndigoBAC 536
Partial digest enzyme	HindIII
Numer of clones	92 160
Number of 384 well plates	240
Missed wells	~7%
Mean insert size	136 kb
Minimum insert size	50 kb
Maximum insert size	250 kb
Chloroplastic contamination	2.2%
Mitochondrial contamination	-
Number of genome equivalent	12 x

Analysis of insert size on a pulsed-field gel

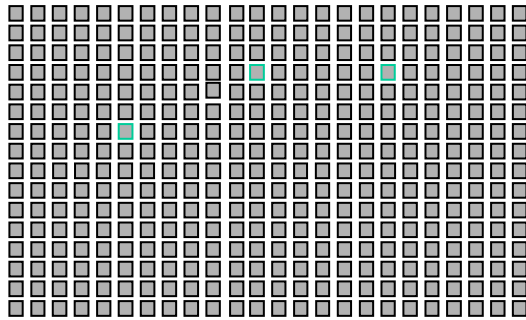


PCR screening for chloroplastic DNA



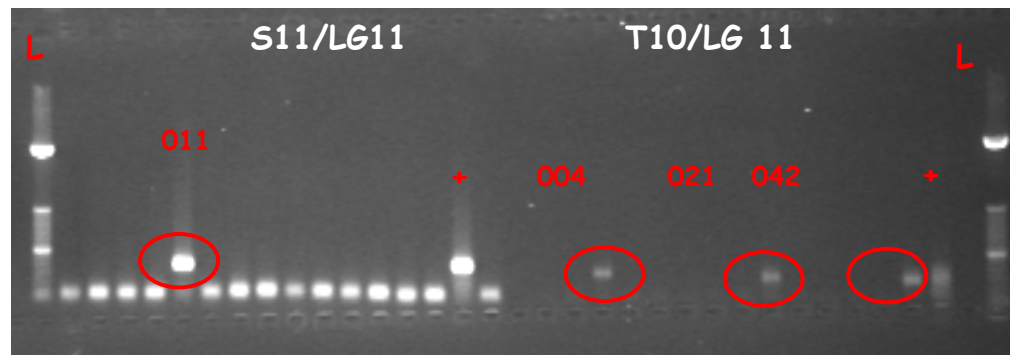


Genomic representation on ~7 genome equivalent

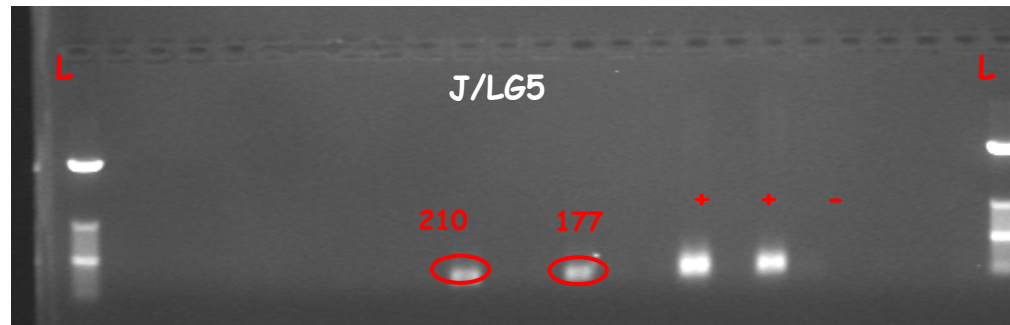


127 x 384 well plates

127 plate pools
1 plate pool = 384 clones
1 plate pool ~ 0.7 x



PCR on plate pools with genetic markers



PCR based screening results with markers from the oak genetic map on 127 plate pools



Linkage group	Mean n° of hits	Min-Max n° of hits
1	7.2	3-14
2	7.4	3-12
3	6.6	0-17
4	6.2	0-12
5	6.8	5-12
6	10.2	3-20
7	9.6	6-14
8	3	0-7
9	6.2	2-8
10	6.6	1-10
11	7.2	2-14
12	7	4-9
all	6.9	

II- A BAC end sequences view of the *Q. robur* genome

Preliminary results



Total useful sequences	23 737
Total base count	14 242 734
Minimum length	65bp
Maximum length	967bp
average length	623bp
GC content	35%
Chloroplastic matches	
Oak	2.5%
Poplar	2.11%
Grape	2.09%
Mitochondrion matches	
Grape	0.99%

< expected value Zoldo et al, 1998
Genome 41:162-168

Repetitive sequence composition of *Q. robur* BES Repeat masker v3.2.8/ Repbase viridiplantae

Class, subclass, group	N° of elements	N° of bases (bp)	% of BES
Retroelements	2409	711 334	4.81
LINES	348	84 881	0.57
LTR elements	2061	626 453	4.24
Copia	1261	389 349	2.63
Gypsy	778	233 901	1.58
DNA transposons	244	49 702	0.34
Unclassified	3	207	
Total interspersed repeat		761 243	5.1
Small RNA	232	94 816	0.64





Distribution and frequency of SSR

Sputnik

-2-5 nucleotides

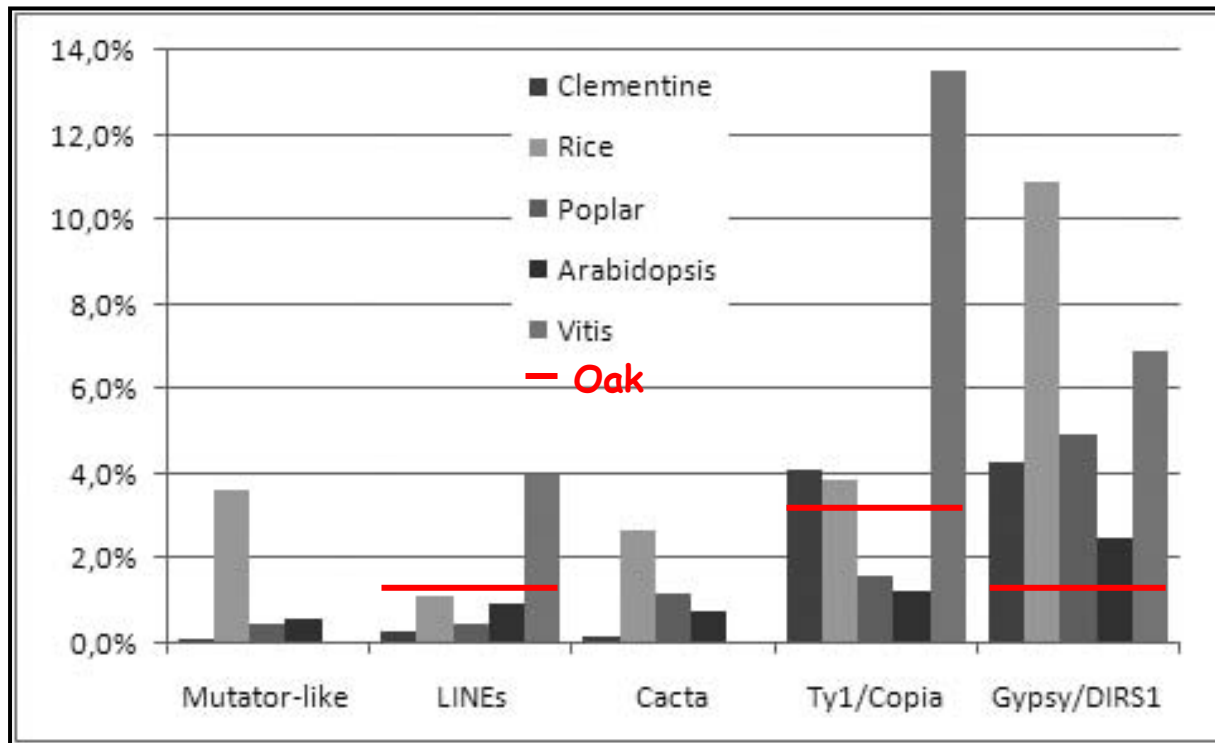
-> 15 nucleotides



	n° SSR (%)
Dinucleotides	
Total	1280 (37.8)
AT/TA	307
TC/GA	333
AG/CT	283
TG/CA	184
AC/GT	169
CG/GC	4
Trinucleotides	
Total	790 (23.3)
CAA/AAC/ACA/GTT/TTG	129
AAG/AGA/GAA/CTT/TTC/TCT	246
AAT/ATA/TAA/ATT/TTA/TAT	249
ATC/TCA/CAT/GAT/ATG/TGA	75
others	91
Tetranucleotides	
Total	600 (17.7)
AGAA/AAAG/AAGA/TTCT/TTT	88
TAAA/AAAT/TTTA/ATTT	141
TTGT/TTTG/TGTT/AAAC	69
TATT/ATAA/AATA/TTAT	73
others	229
Pentanucleotides	
Total	719 (21.2)
TTTTG/AAAAC	41
AAAAT/TTTTA/ATTTT	83
AAAG/TTTTC	116
others	479

III-Comparative genomic - Preliminary results

Comparative analysis of transposable elements from Oak and data from Terol et al, 2008 BMC Genomics 9:423





Frequency of SSR in BAC ends of 11 plant species

data from Cavagnaro et al, 2009 *Mol. Genet. Genomics* 281: 273

	Carrot	Arabidopsis	Soybean	Potato	Tomato	Medicago	Grapevine	Brassica	Papaya	Poplar	Oak
Dimers	17.1	17	23.4	20.4	23.0	19.5	24.8	31.5	41.8	38.0	37.7
Trimers	43.4	38.3	32.3	29.8	23.8	30.7	22.3	28.1	21.1	29.1	23.3
Tetramers	24.6	31.2	30.5	32.0	41.5	32.6	35.3	28.0	26.5	21.5	17.7
Pentamers	14.9	13.4	13.8	17.7	11.7	17.2	17.6	12.4	10.6	26.6	21.2
Most frequent SSR	AAG	AAG	AT	AT	AT	AAAT	AAAT	AT	AT	AT	TC&AT





BLASTN Alignment of oak BAC ends onto the poplar and grape genome

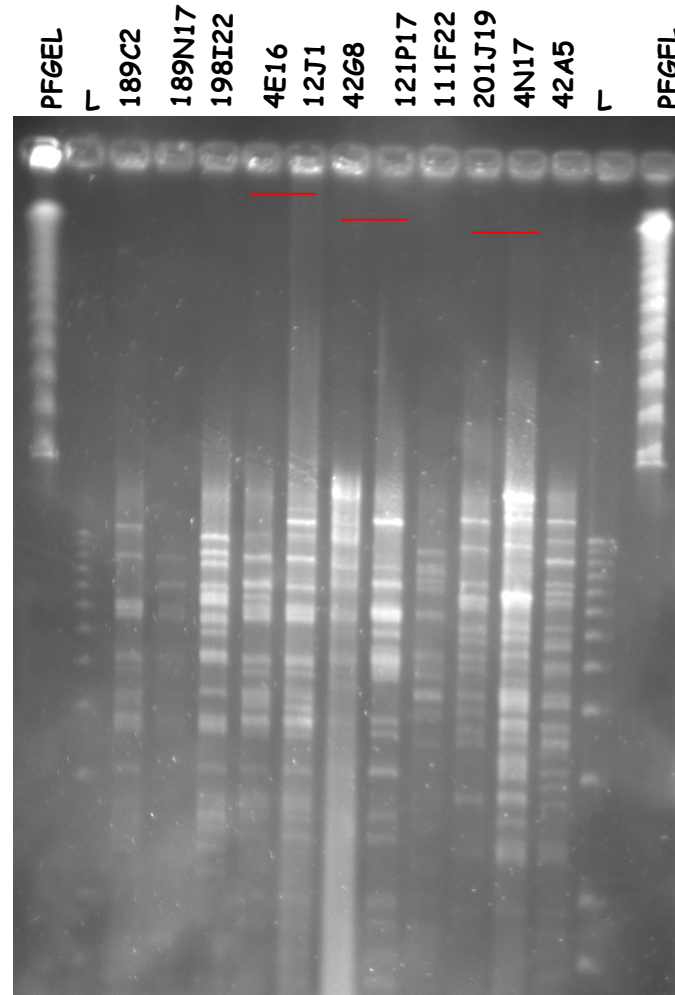
P. trichocarpa LG	n° BES mapped	V. Vinifera LG	n° BES mapped
I	57	VVI01	77
II	43	VVI02	39
III	34	VVI03	40
IV	24	VVI04	54
V	37	VVI05	72
VI	30	VVI06	65
VII	28	VVI07	62
VIII	51	VVI08	63
IX	42	VVI09	47
X	49	VVI10	29
XI	17	VVI11	48
XII	24	VVI12	52
XIII	25	VVI13	55
XIV	36	VVI14	73
XV	28	VVI15	45
XVI	10	VVI16	42
XVII	8	VVI17	54
XVIII	18	VVI18	67
XIX	12	VVI19	52
Scaffolds	120	VVIR	50
Total	693		1086

Comparative mapping of paired BAC ends to poplar and grape genomes

Plant species	Paired Oak Bac ends	Chromosomal location	Span (bp)
<i>P. trichocarpa</i>	11017	LG X	14 466
	25G18	Scaffold 86	79 286
	27D09	LG VII	47 389
	33YD16	LG V	11 129
	33K20	LG II	53 566
<i>V. vinifera</i>	11E12	VVI03	147 997
	15H10	VVI06	75 596
	16L18	VVI17	60 099
	18E05	VVI12	211 719
	20LD21	VVI13	37 353
	20O15	VVI02	30 193
	24B08	VVI13	76 215
	27M06	VVI02	50 469

IV-Proepects

-1- Sequencing of homologous BAC to estimate heterozygoty

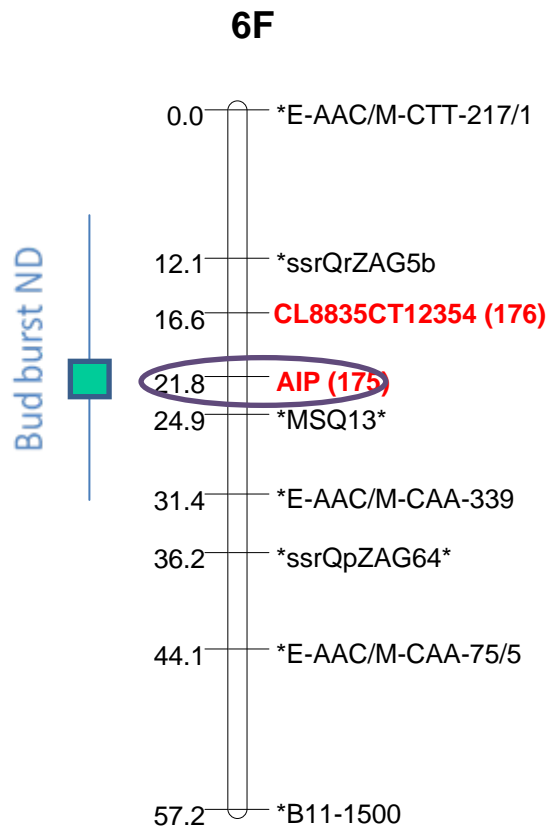


Fingerprint HindIII of allelic BAC

-2-PCR based screening for candidate genes linked with QTL for adaptative traits

-Creation of super row and column plates to optimize PCR

-PCR screening with specific primer pairs from candidate gene



Growth

CL8543: function in various aspects of photoperiodism, including flowering.

CL4: unknown protein

Epinasty

C2H4: Ethylene Responsive Element

Bud burst

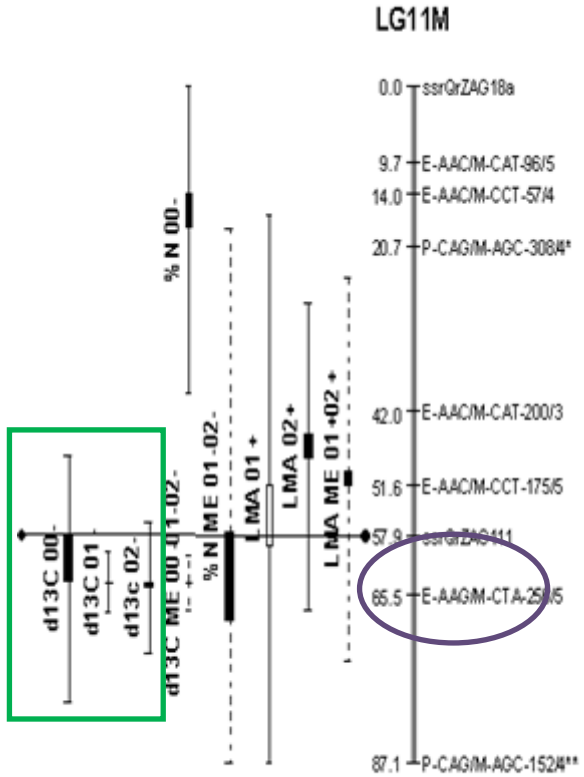
EST_RASI: inhibit α -amylase from germinating in rice

EST_COMT: Catechol-O-methyltransferase

CL753: Auxilin-Like J-Domain Protein

AIP: Auxin Induced protein

-3-Construction of local physical map for a QTL linked with water used efficiency by concomitant approach of physical and genetic mapping



QTL d13: carbon stable isotope composition

-4-Comparative genomics in fagaceae species





Acknowledgements



C. Boussardon

F. Bitton

C. Bodenes

A. Kremer

C. Plomion

J.M. Frigerio

G. Le Provost

J. Durand



UMR- EEF

O. Brendel



C. Cabane



F. Gavory

CUGI

C. Sasaki

Thank you for your attention
