Linkage group-specific BAC clones developed to identify corresponding sunflower chromosomes

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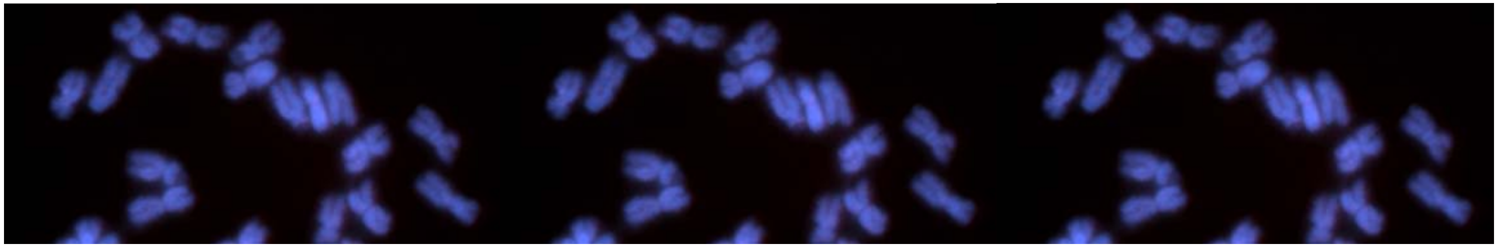
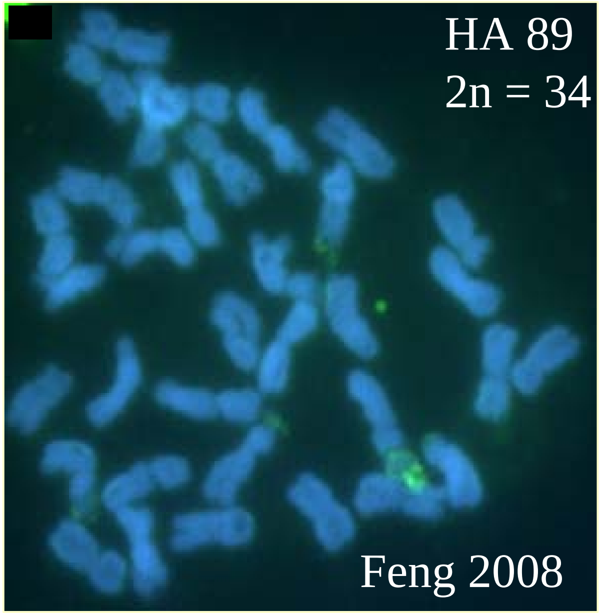


OUTLINE

- ♦ Background
  - -- Linkage Group vs. Chromosome
- ♦ Identification of Linkage Group-specific BAC Clones
  - --Construction of BAC Libraries
  - --Screening of BAC Libraries
- ♦ Development of Sunflower Cytogenetic Map
  - --FISH Mapping by Anchored BAC Clones
  - --Integrating the RFLP and SSR Linkage Groups
- ♦ Summary and Outlook

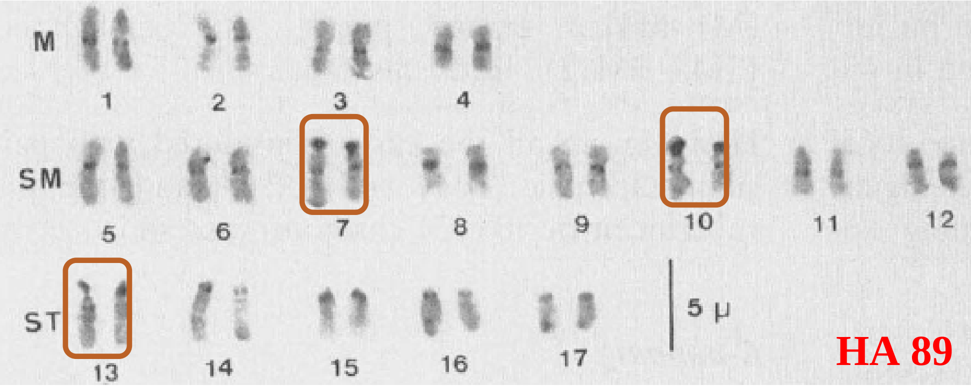
Background



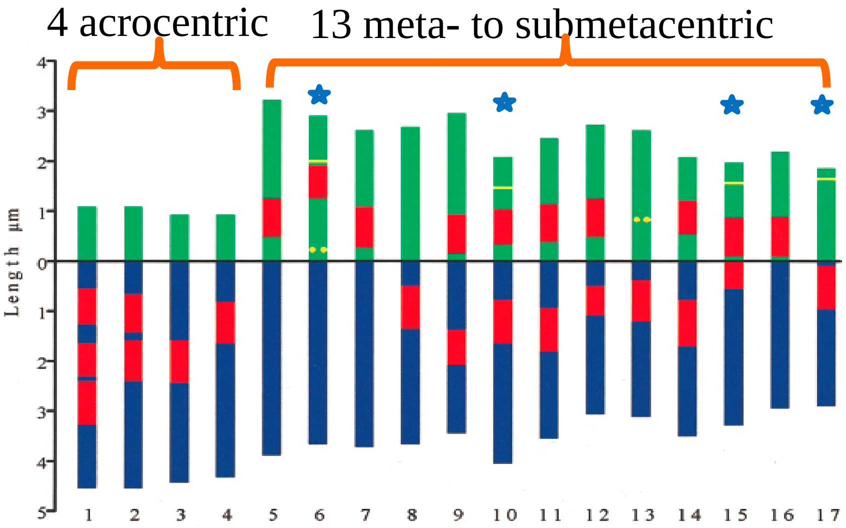
Sunflower genome size ~3500Mb

--**8 times** of rice (430Mb)

--**28 times** of *Arabidopsis* (125Mb)



Cuellar et al 1996



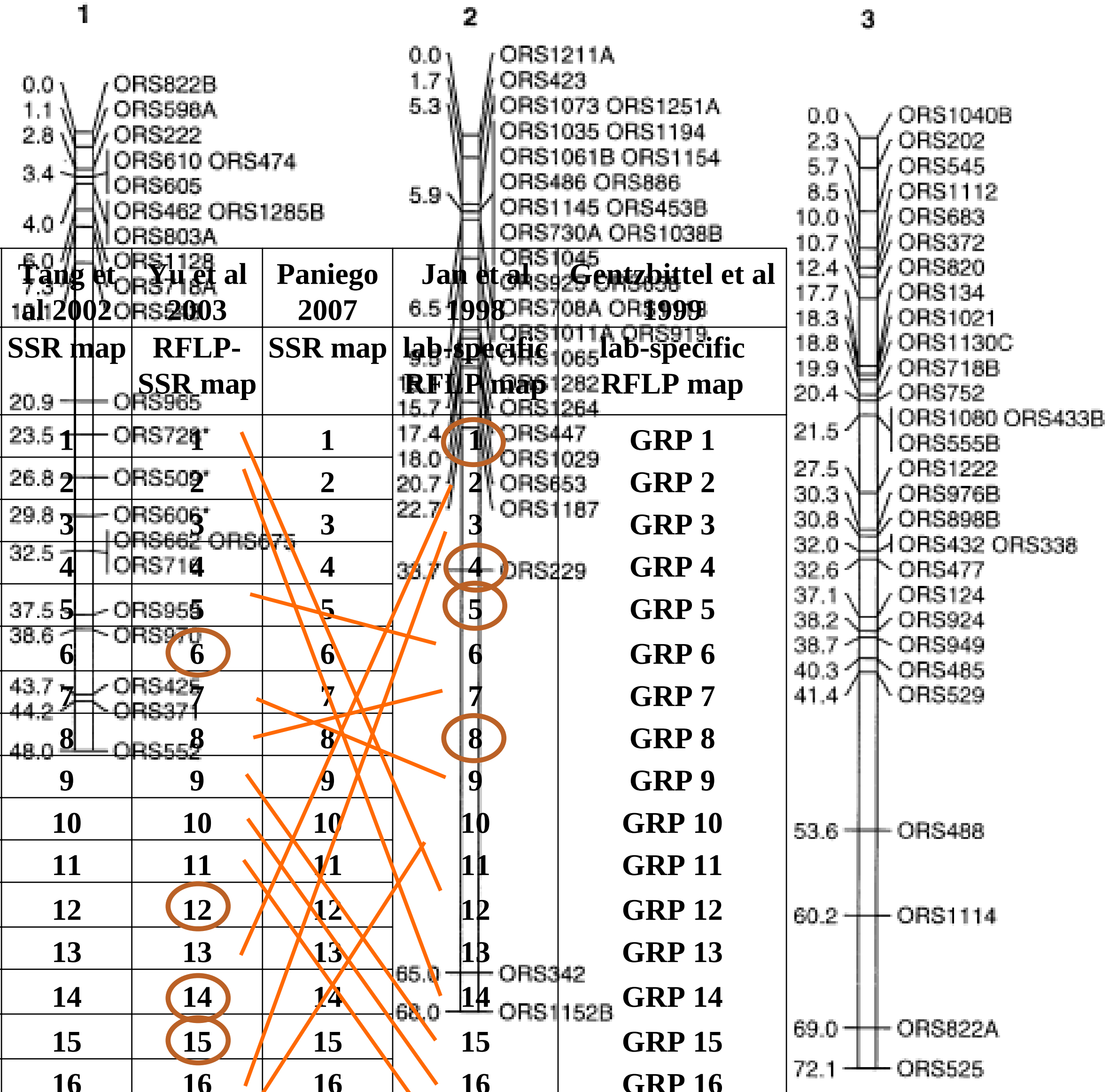
Schrader et al 1997

HA 89

Partial references reported on chromosome karyotypes and secondary constructions in sunflower

	Chromosome type			Secondary constriction			Techniques and FISH probes used if applicable	Materials
References	**meta-**	**submeta-**	**acro-**	**satellite**	**45s rDNA**	**5s rDNA**		
Raicu *et al*.1976	**10**	**3**	**4**	**3**				
Al-Allaf *et al.* 1979	**4**	**8**	**5**	**3**				**HI, HB, HR, HX**
Cuellar *et al.* 1996	**4**	**8**	**5**	**3**				**HA89, hybrid**
Schrader *et al.* 1997	**13**		**4**	**3**	**4**	**2**	**C-banding; 45s rDNA: VER17 5s rDNA**	**HA89**
Cuellar *et al.* 1999	**4**	**8**	**5^{a}**	**3**	**3**	**2**	**C-banding; 45s rDNA: pTa71 5s rDNA: 36pBG13**	***H. argophyllus*; *H. annuus***
Vanzela *et al.* 2002				**2,3,4 (2x) 4 (4x) 6 (6x)**	**2,3,4 (2x) 4 (4x) 6 (6x)**		**C-banding C-CMA banding 45s rDNA: pTa71**	**diploid (2x) tetraploid (4x) hexaploid (6x)**
Ceccarelli *et al.* 2007	**13**		**4**	**3**	**4**		**tandem repeats 45s rDNA: pTa71**	**HA89, RA20031, HOR**
Talia *et al.* 2010	**12**	**1**	**4^{a}**	**3**	**3**		**repetitive retrotransposon-like sequences; homologous rDNA**	**HA89**

Linkage Group vs. Chromosome



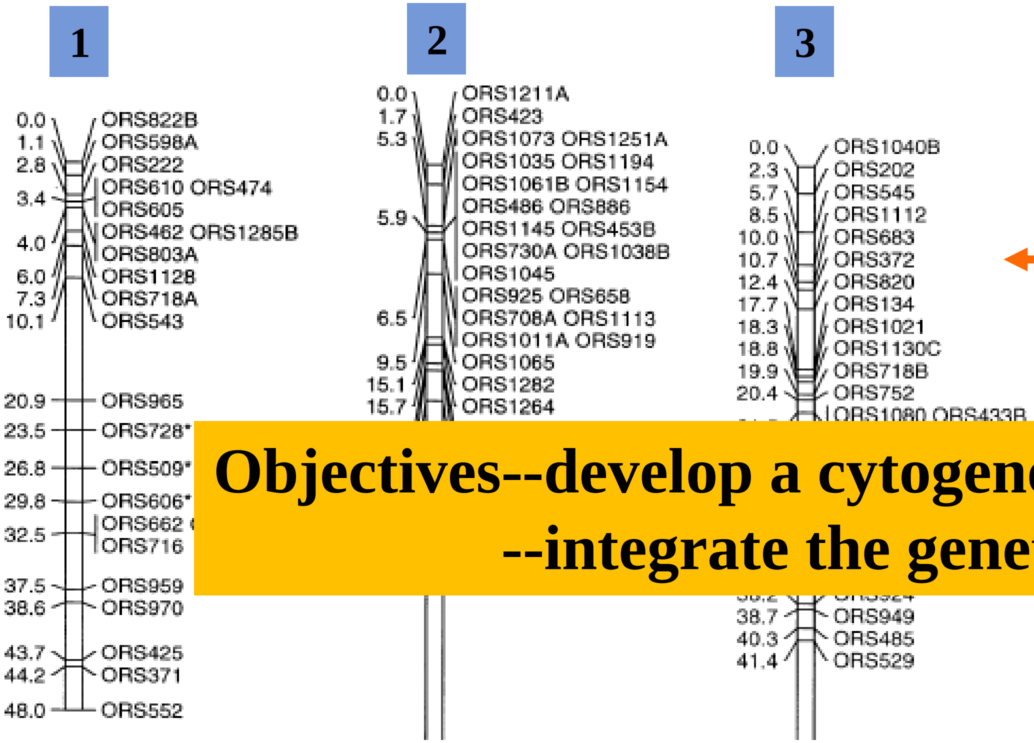
Berry et al 1995	Berry et al 1997	Gedil et al 2001	Tang et al 2002	Yu et al 2003	Paniego 2007	Jan et al 1998	Gentzbittel et al 1999
lab-specific RFLP map	reference RFLP map	RFLP-AFLP map	SSR map	RFLP-SSR map	SSR map	lab-specific RFLP map	lab-specific RFLP map
A	1	1	1	1	1	1	GRP 1
B	2	2	2	2	2	2	GRP 2
C	3	3	3	3	3	3	GRP 3
D	4	4	4	4	4	4	GRP 4
E	5	5	5	5	5	5	GRP 5
F	6	6	6	6	6	6	GRP 6
G	7	7	7	7	7	7	GRP 7
H	8	8	8	8	8	8	GRP 8
I	9	9	9	9	9	9	GRP 9
J	10	10	10	10	10	10	GRP 10
K	11	11	11	11	11	11	GRP 11
L	12	12	12	12	12	12	GRP 12
M	13	13	13	13	13	13	GRP 13
N	14	14	14	14	14	14	GRP 14
O	15	15	15	15	15	15	GRP 15
P	16	16	16	16	16	16	GRP 16
Q	17	17	17	17	17	17	GRP 17

?

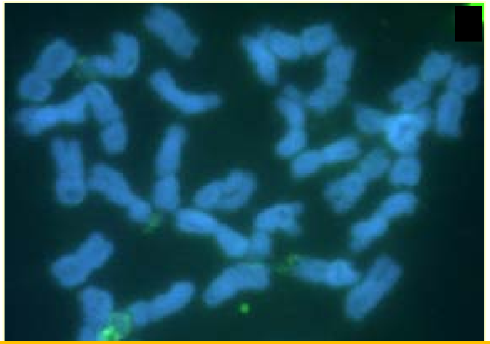
Tang et al (2002)

Linkage Group vs. Chromosome

17 Linkage groups ⟷ 17 Chromosome pairs



⟷



Objectives--develop a cytogenetic map (FISH)
--integrate the genetic and cytogenetic map

A linkage group shows the relative locations of DNA markers along the chromosome

BAC-FISH: the use of genomic DNA cloned in large-insert vectors such as BAC in combination with FISH, called BAC-FISH

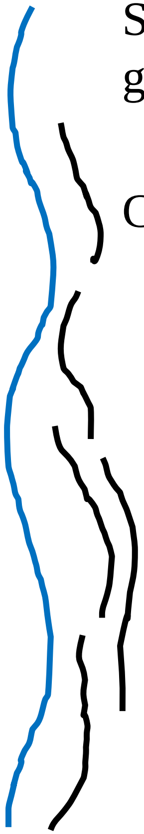
BAC/BIBAC Libraries

BAC–bacterial artificial chromosome

BIBAC–transformation-competent binary BAC

-- Collection of hundreds or thousands of **BAC clones**

-- Maintained in labeled arrays



Sunflower total genome DNA

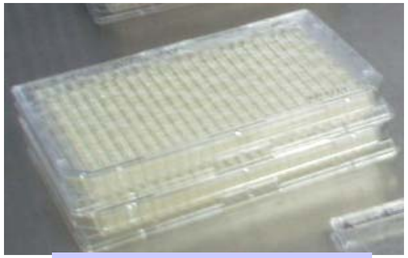
↓

Cut into fragments

↓

BAC Clones

↓



384-well plates

(~750 bp/each sequencing)

Construction of BAC Libraries

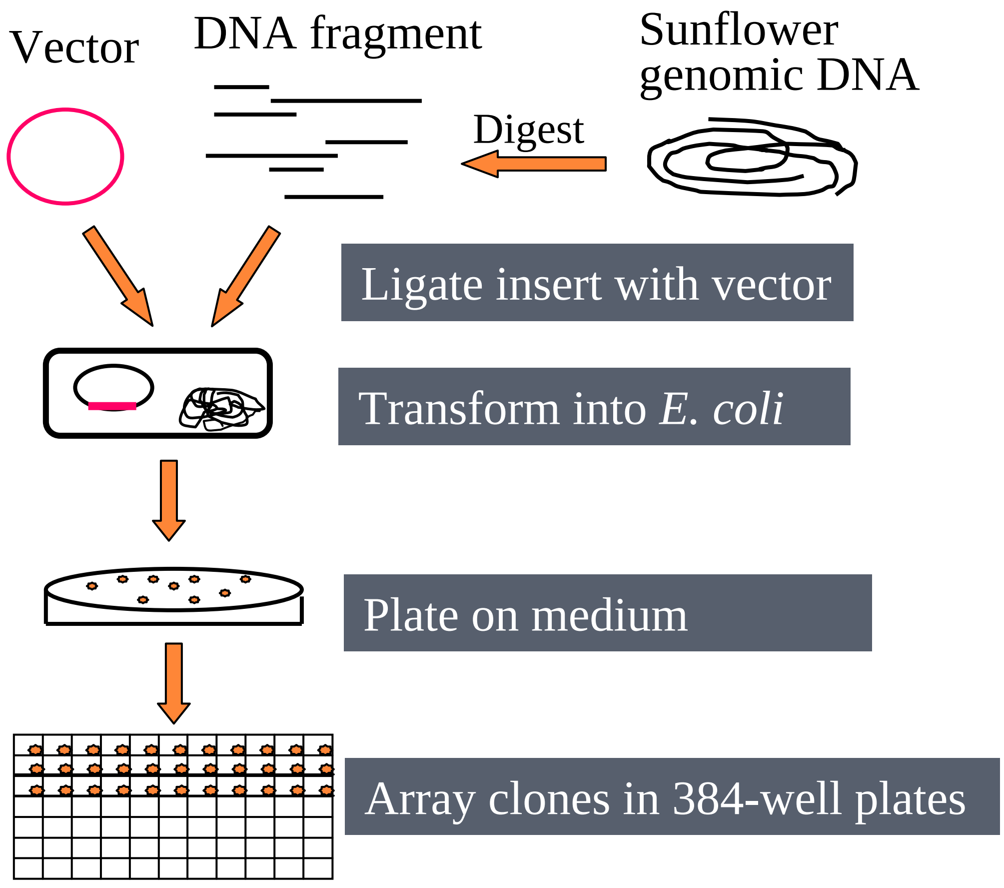
The limitations:
a single enzyme
low genome coverage
smaller insert sizes

Sunflower BAC libraries reported

Author	Enzyme	Coverage	Insert size	Material
Gentzbittl et al. 2002 (France)	*Hind*III	4-5 fold	80 kb	HA 821
Özdemir et al.2004 (Turkey)	*Hind*III	1.9 fold	60 kb	RHA 325
Bouzidi et al. 2006 (France)	*Hind*III	5 fold	118kb	YDQ
CUGI (USA)	*Hind*III	8.3 fold	125kb	HA 383
CNRGV (France)	*Hind*III *Bam*HI *Eco*RI	9.0 × 2.6× 2.2×	132kb 114kb 93kb	HA412 HA412 HA412

Construction of BAC Libraries

A general scheme for construction of a BAC library



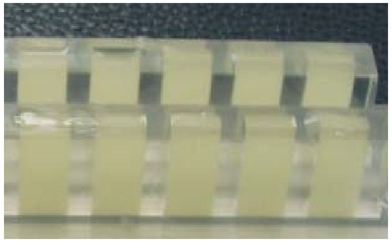
Ligate insert with vector

Transform into *E. coli*

Plate on medium

Array clones in 384-well plates

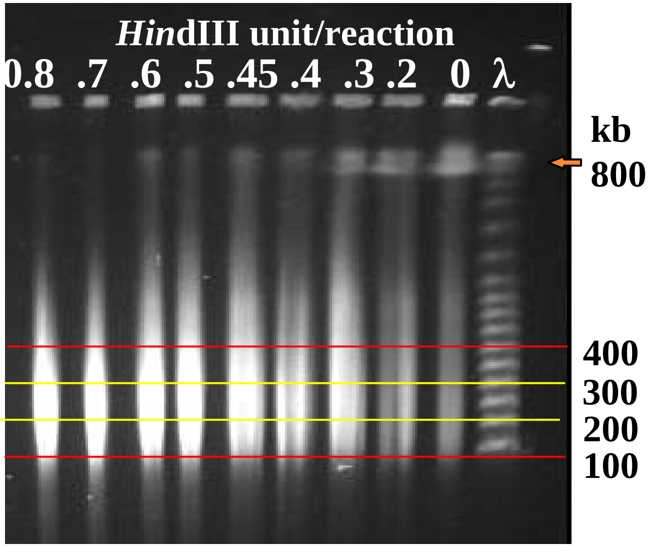
An ordered BAC library



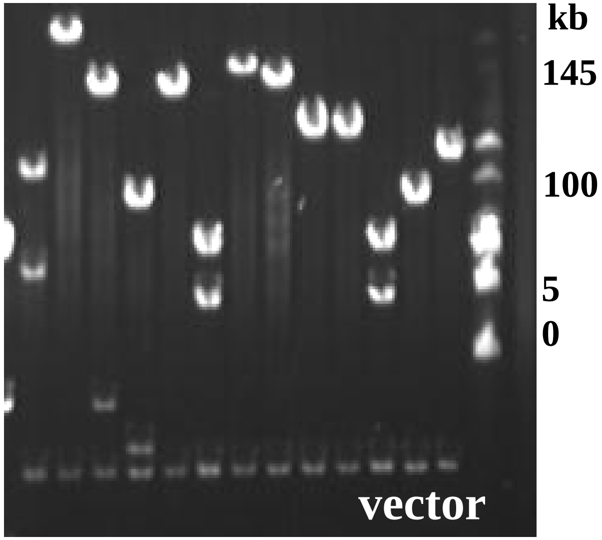
agarose plugs

Construction of BAC Libraries

Partial enzyme digest



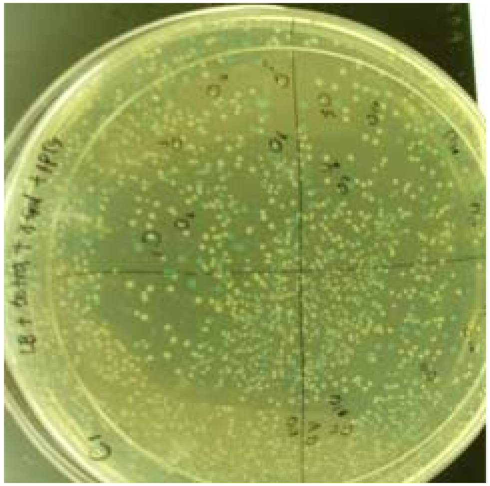
pECBAC1/*Bam*H1



Plasmid with insert

Jackson et al. 1999, 17:581-587

Positive colonies



Manually picking colonies

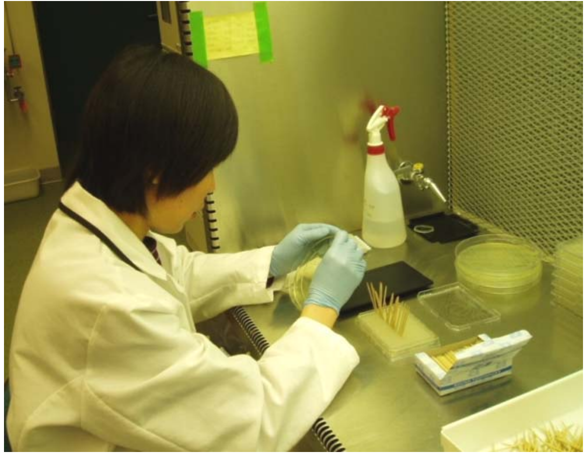


Plate duplication

Construction of BAC Libraries

Features	BAC library	BIBAC library	Combined
Cloning vector	pECBAC1	pCLD04541	2
Restriction enzyme	*Bam*HI	*Hind*III	2
No. of clones arrayed	107,136	84,864	192,000
No. of 384-well microtitres	279	221	500
Average insert size (kb)	140	137	139
Insert-empty clones (%)	0.44%	0.0	<0.5%
cpDNA clones (%)	2.10%	2.59%	2.35%
mtDNA clones (%)	0.03%	0.04%	0.04%
Genome equivalents	5.0 ×	3.9 ×	8.9 ×

<u>Major Features:</u>

Two enzyme/ vector combinations

Larger insert size

Deeper coverage

1st sunflower BIBAC library

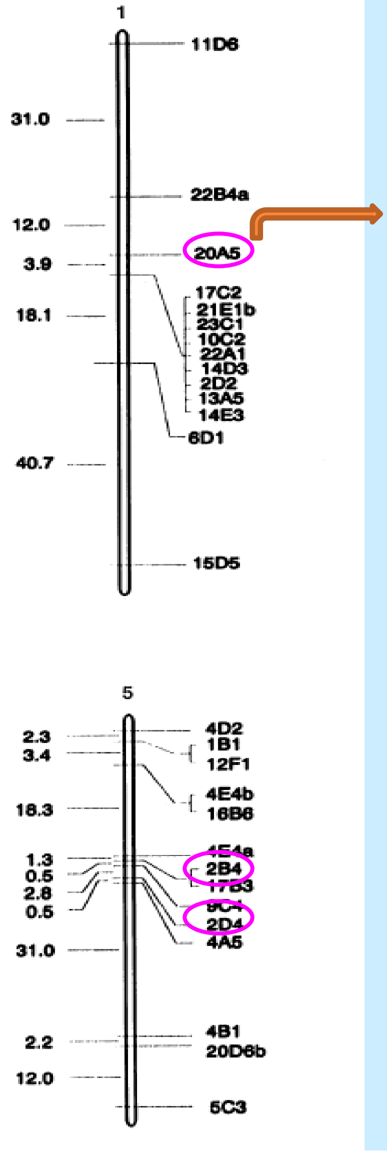
Giving a 99 % probability Of finding any gene of interest

Sunflower whole BAC libraries

Which one do I want?

Screening the BAC Libraries

20 RFLP linkage groups (Jan et al. 1998)
Choosing 36 RFLP markers



Overgo probe- Overlapping oligonucleotides

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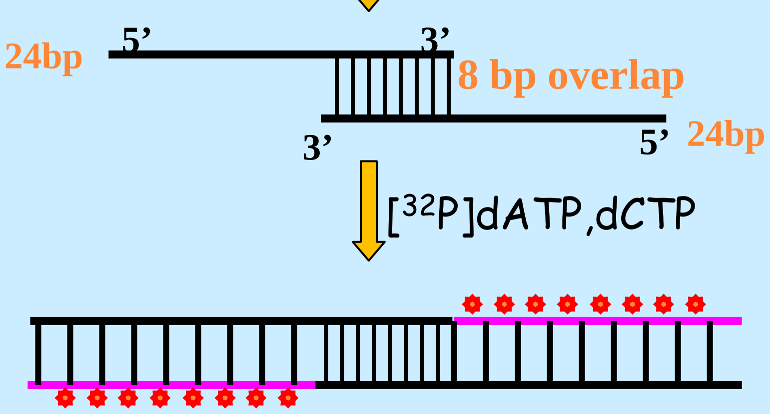
AAACTGTAACCAACGATGAGATTGYCGCTGATCTTAAAGAGCATGTGATCAAGCCTGTTGTTCCAGCCAATT
ACCTCGACGAGAAGACCATCTTCCACTTGAACCCATCTGGTCGTTTCGTCATTGGTGGACCTCACGGTGACGC
TGGACTCACCGGACGTAAGATCATCATTGATACTTATGGTGGTTGGGGTGCACACGGTGGTGGTGCTTTTTCA
GGGAAAGACCCCACCAAGGTGGACAGAAGTGGTGCCTATATTGTGAGACAAGCCGCAAAGAGCATTGTGGC
TTGTGGGCTAGCGAGAAGAGCGATTGTGCAAGTGTCTTACGCTATTGGTGTCCCTGAACCGTTGTCCGTCTTT
GTTGACACGTATGGAACCGGAAAGATCCCCGACAAGGAGATCTTGAAGATTGTTAAGGAGAACTTTGATTTC
ANGCCGGGAATGATATCGATCAATCTTGATTTGAAGAGGGGTGGGAACAAGAGGTTCTTGAAGACAGCTGC
ATATGGGCATTTTGGTAGAACAGACCCTGATTTCACCTGGGAGGTGGTGAAGGAACTCAAGTGGGAGAAAGC
ATAAGAAGGGTGGTCTGTCTGTGTTAGTAACCTAAATCATATATTATGCTTGCCTGCATGTTTGGCTTGGTA
TAATGATGATAATGTGTTTTTTTTTTTTTCCTTTGTNAGTTTGATATGACCTAATTTAAGNGTTGGTTTCTTTAT
ATCTTTAAAGAG
```

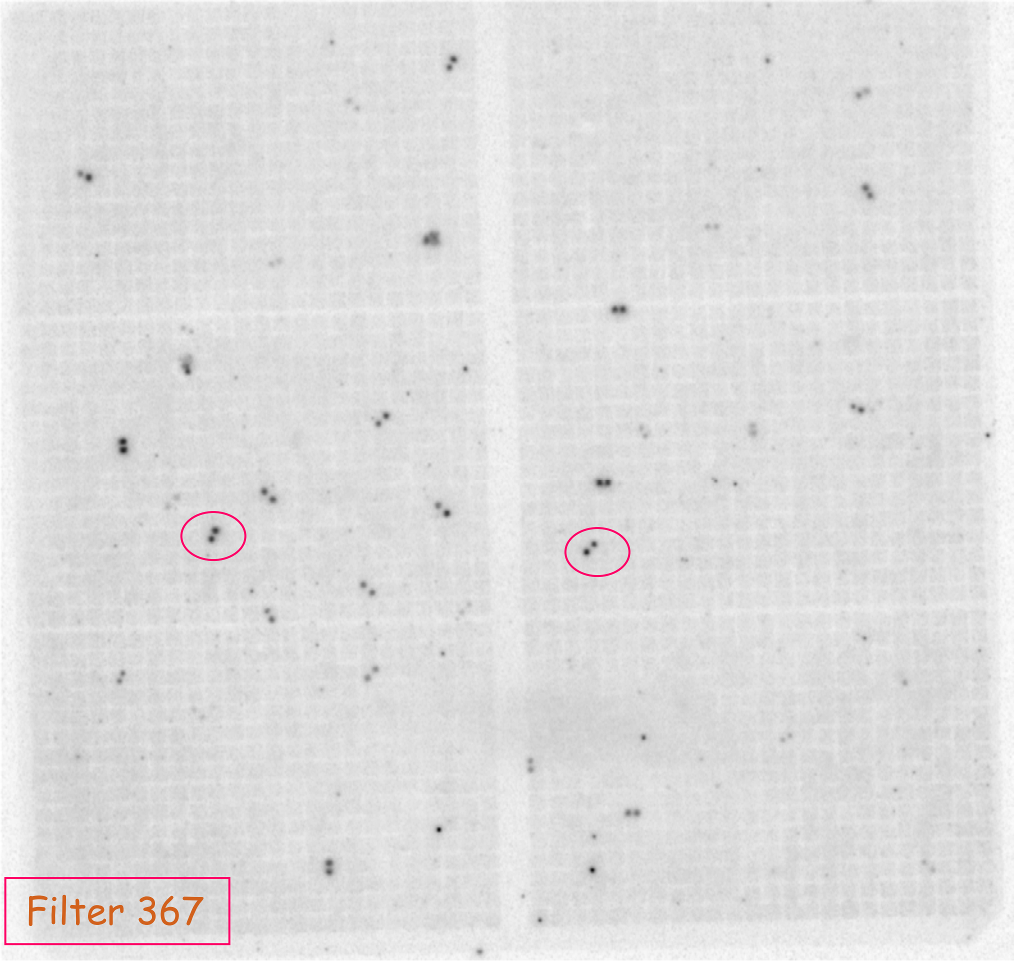


$[^{32}P]$dATP,dCTP

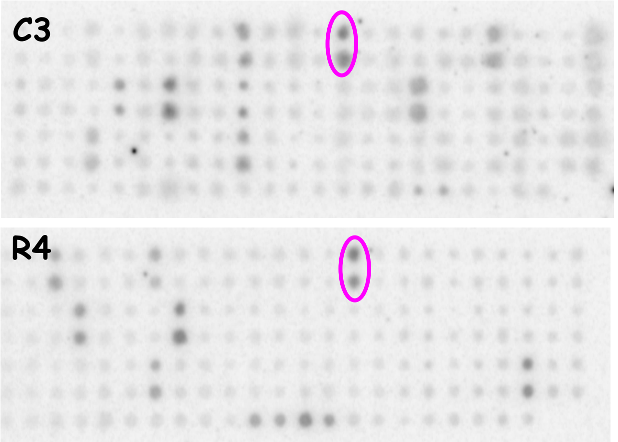
Span ~40 bp in length and
have an 8-bp overlapping region

Identify the positive clones by two-step screening

1st pool hybridization

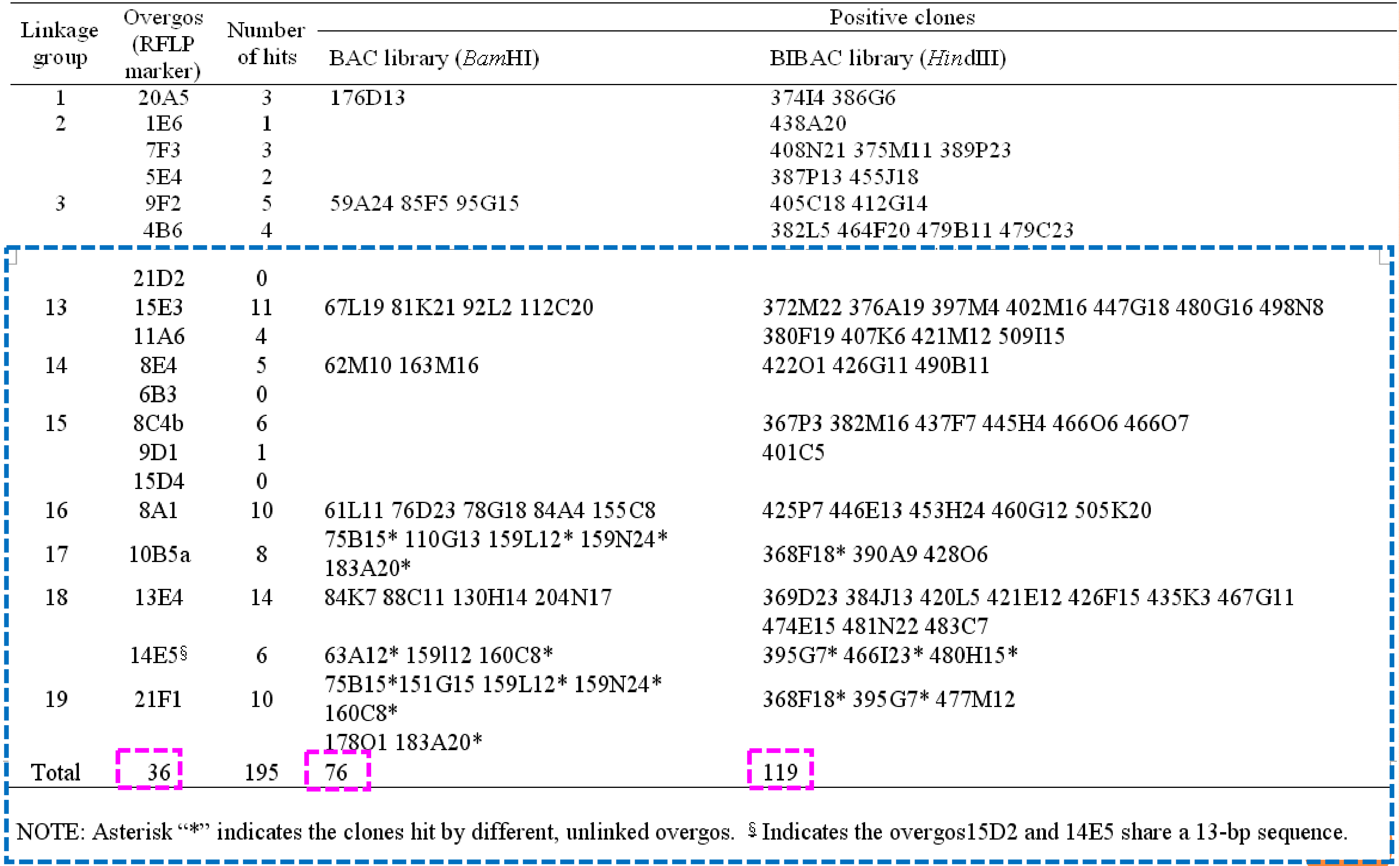


2nd pool hybridization



	C1	C2	C3	C4
R1	overgo1	overgo2	overgo3	overgo4
R2	overgo1	overgo2	overgo3	overgo4
R3	overgo1	overgo2	overgo3	overgo4
R4	overgo1	overgo2	**overgo3**	overgo4

Screening the BAC Libraries



Linkage group	Overgos (RFLP marker)	Number of hits	Positive clones	
			BAC library (*Bam*HI)	BIBAC library (*Hin*dIII)
1	20A5	3	176D13	374I4 386G6
2	1E6	1		438A20
	7F3	3		408N21 375M11 389P23
	5E4	2		387P13 455J18
3	9F2	5	59A24 85F5 95G15	405C18 412G14
	4B6	4		382L5 464F20 479B11 479C23
	21D2	0		
13	15E3	11	67L19 81K21 92L2 112C20	372M22 376A19 397M4 402M16 447G18 480G16 498N8
	11A6	4		380F19 407K6 421M12 509I15
14	8E4	5	62M10 163M16	422O1 426G11 490B11
	6B3	0		
15	8C4b	6		367P3 382M16 437F7 445H4 466O6 466O7
	9D1	1		401C5
	15D4	0		
16	8A1	10	61L11 76D23 78G18 84A4 155C8	425P7 446E13 453H24 460G12 505K20
17	10B5a	8	75B15* 110G13 159L12* 159N24* 183A20*	368F18* 390A9 428O6
18	13E4	14	84K7 88C11 130H14 204N17	369D23 384J13 420L5 421E12 426F15 435K3 467G11 474E15 481N22 483C7
	14E5§	6	63A12* 159I12 160C8*	395G7* 466I23* 480H15*
19	21F1	10	75B15*151G15 159L12* 159N24* 160C8* 178O1 183A20*	368F18* 395G7* 477M12
Total	36	195	76	119

NOTE: Asterisk "*" indicates the clones hit by different, unlinked overgos. § Indicates the overgos15D2 and 14E5 share a 13-bp sequence.

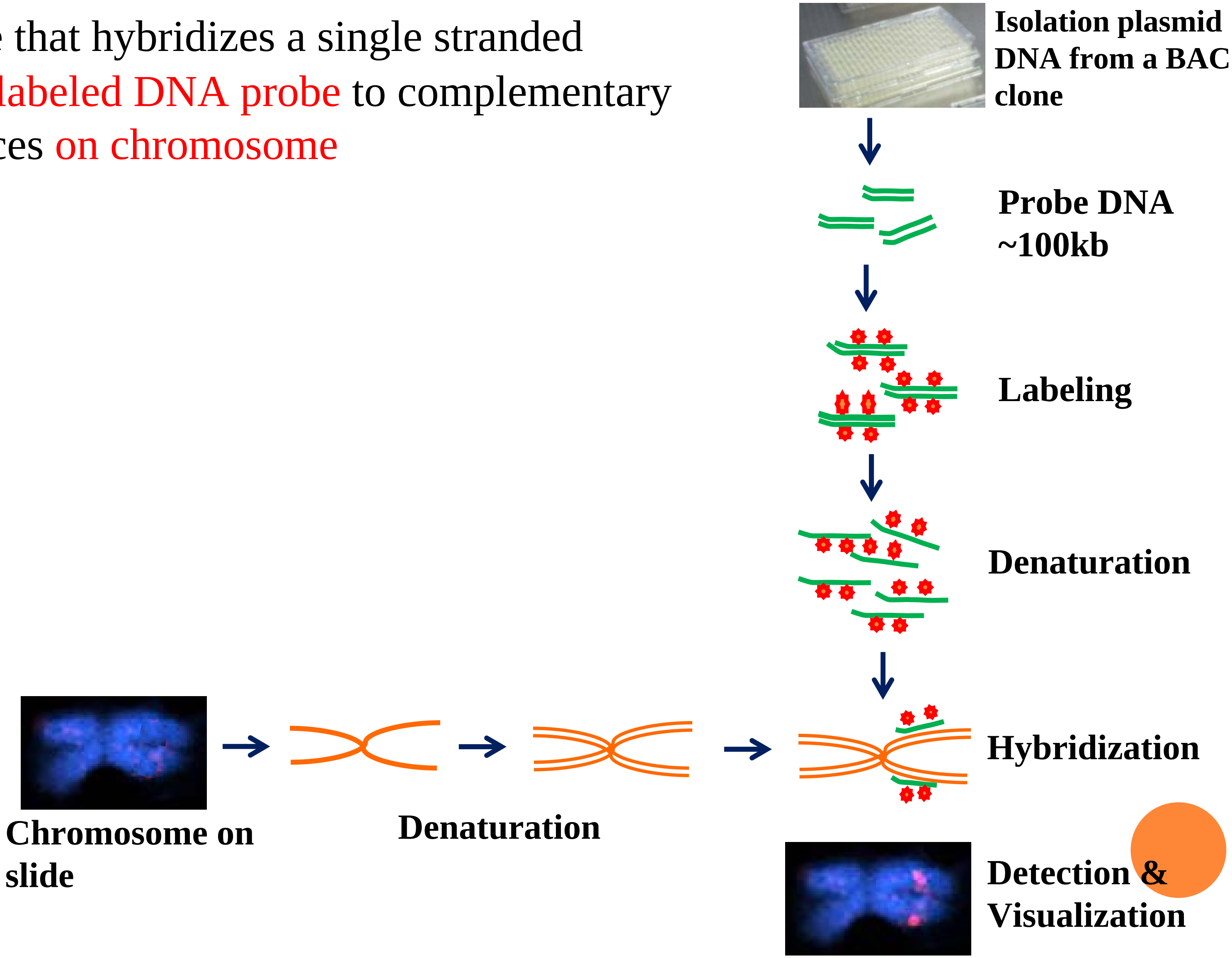
19 linkage group → 36 RFLP markers → 195 positive clones

FISH Mapping

195 positive clones

Fluorescence *in situ* hybridization(FISH) by BAC clones

--A technique that hybridizes a single stranded fluorescently labeled DNA probe to complementary target sequences on chromosome



Isolation plasmid DNA from a BAC clone

Probe DNA ~100kb

Labeling

Denaturation

Chromosome on slide

Denaturation

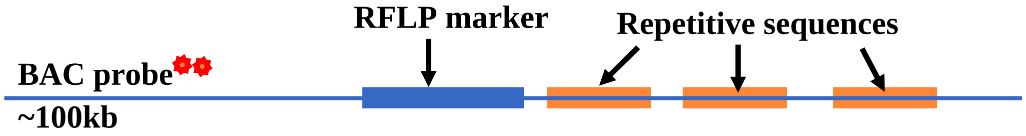
Hybridization

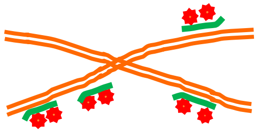
Detection & Visualization

FISH Mapping

Cross-hybridization

--Due to repetitive DNA sequences of the sunflower genome, non-specific hybridization is a common problem in FISH

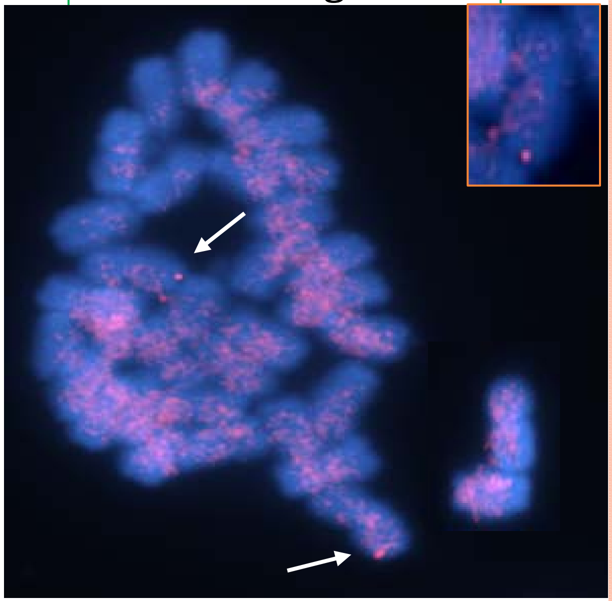


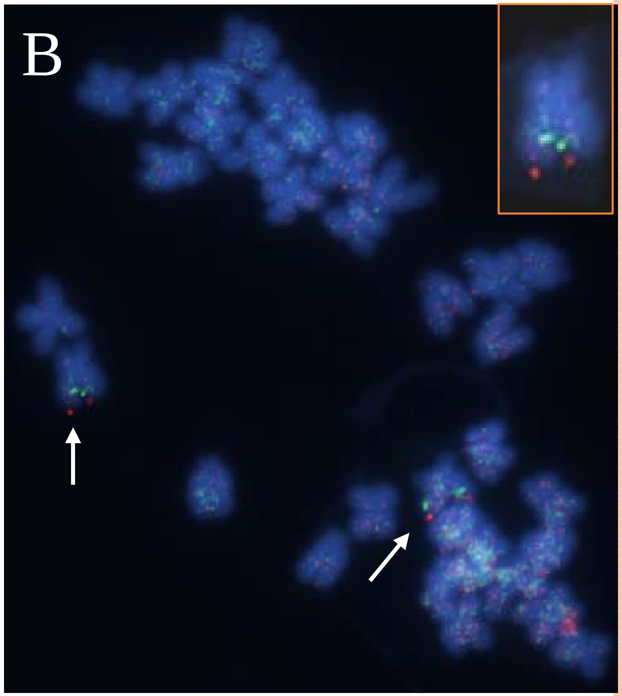


- ◆ Blocking DNA: Randomly sheared genomic DNA blocks repetitive DNA sequences and prevents non-specific hybridization
- ◆ Stringency washes

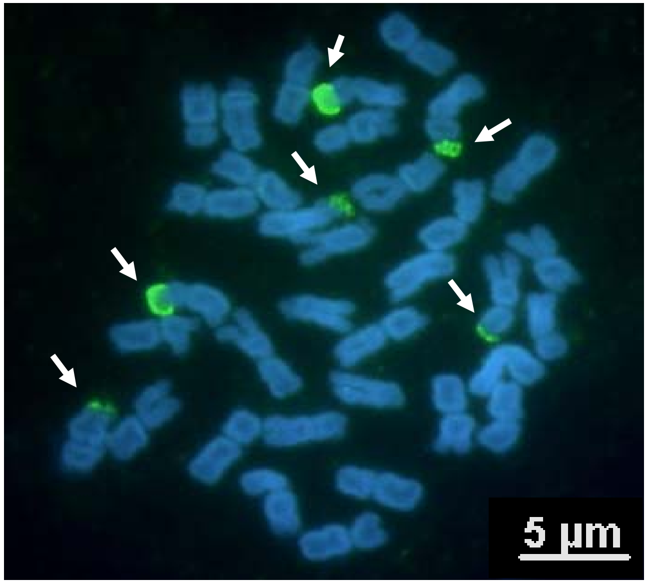
Species	% of repetitive sequences
Arabidopsis	10
Rice	35
Soybean	59
Maize	66
Sunflower	78

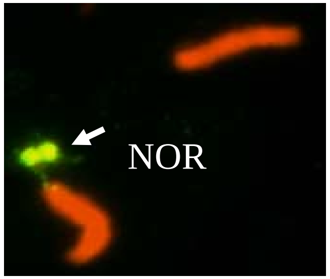
100× blocking DNA



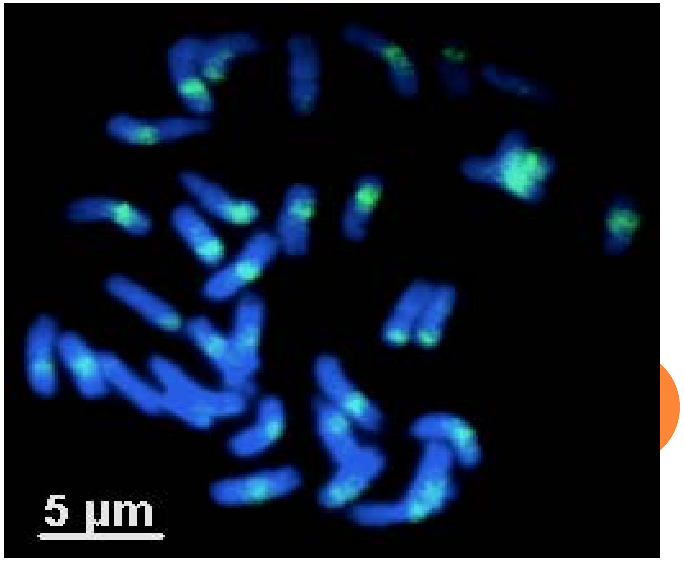


Repetitive DNA sequences





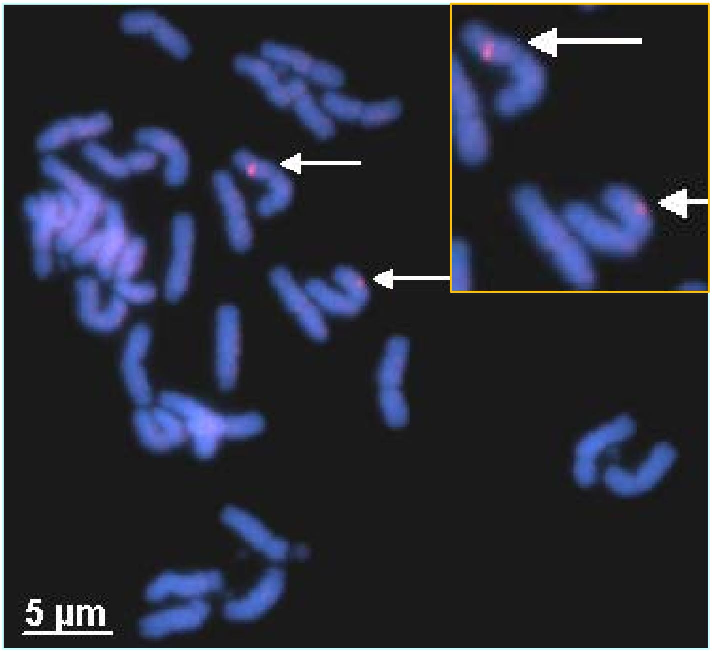
FISH image of a BAC clone with 45S rDNA sequences on nucleolus organizing region (NOR). The chromosomes were counterstained with DAPI (blue) and PI (red).

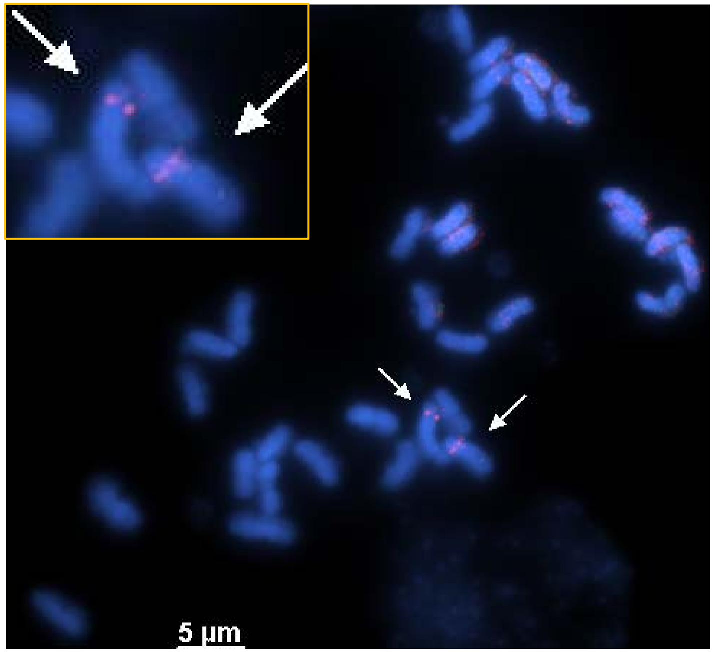


FISH signals on the pericentromeric heterochromatic region of almost all sunflower chromosomes, and reflecting the distribution of repetitive DNA

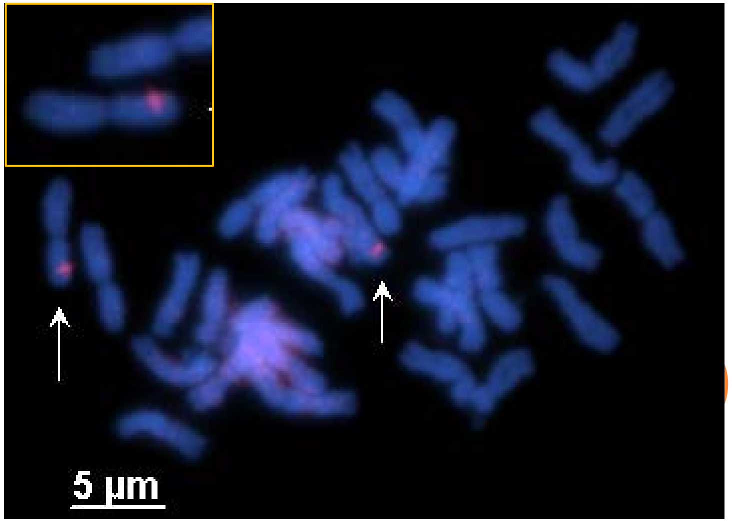
FISH Mapping

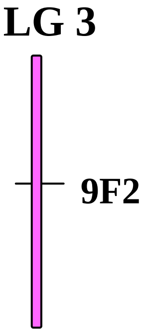
Single/low-copy DNA sequences





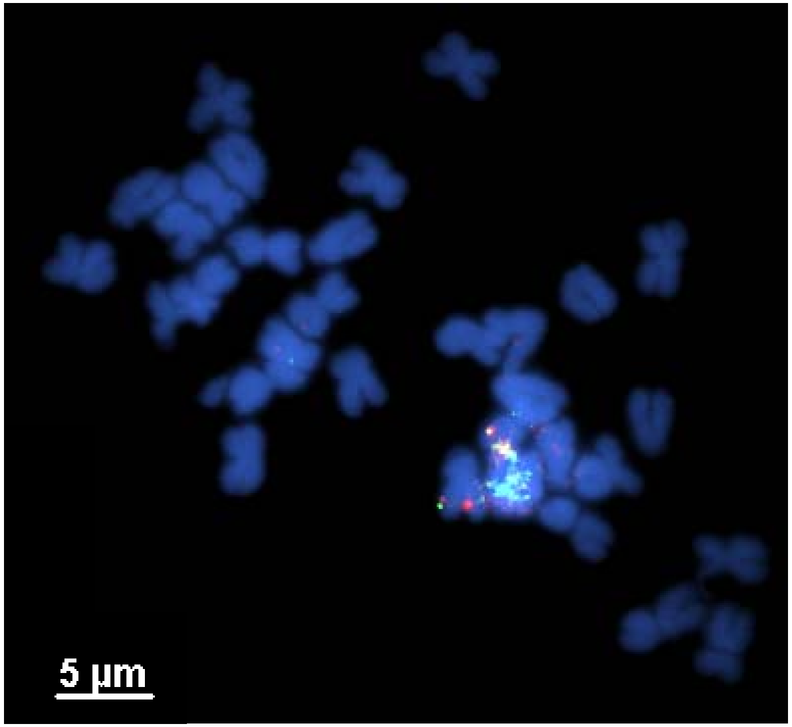
BAC clone 59A4 generated a unambiguous FISH signal (arrow) on a chromosome pair with 50×blocking DNA

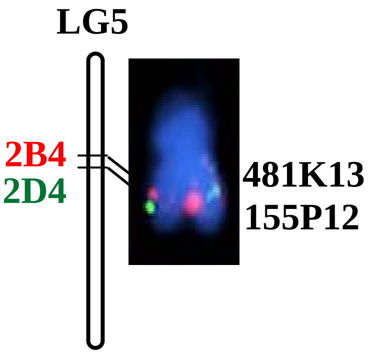




FISH Mapping

Bi-color FISH



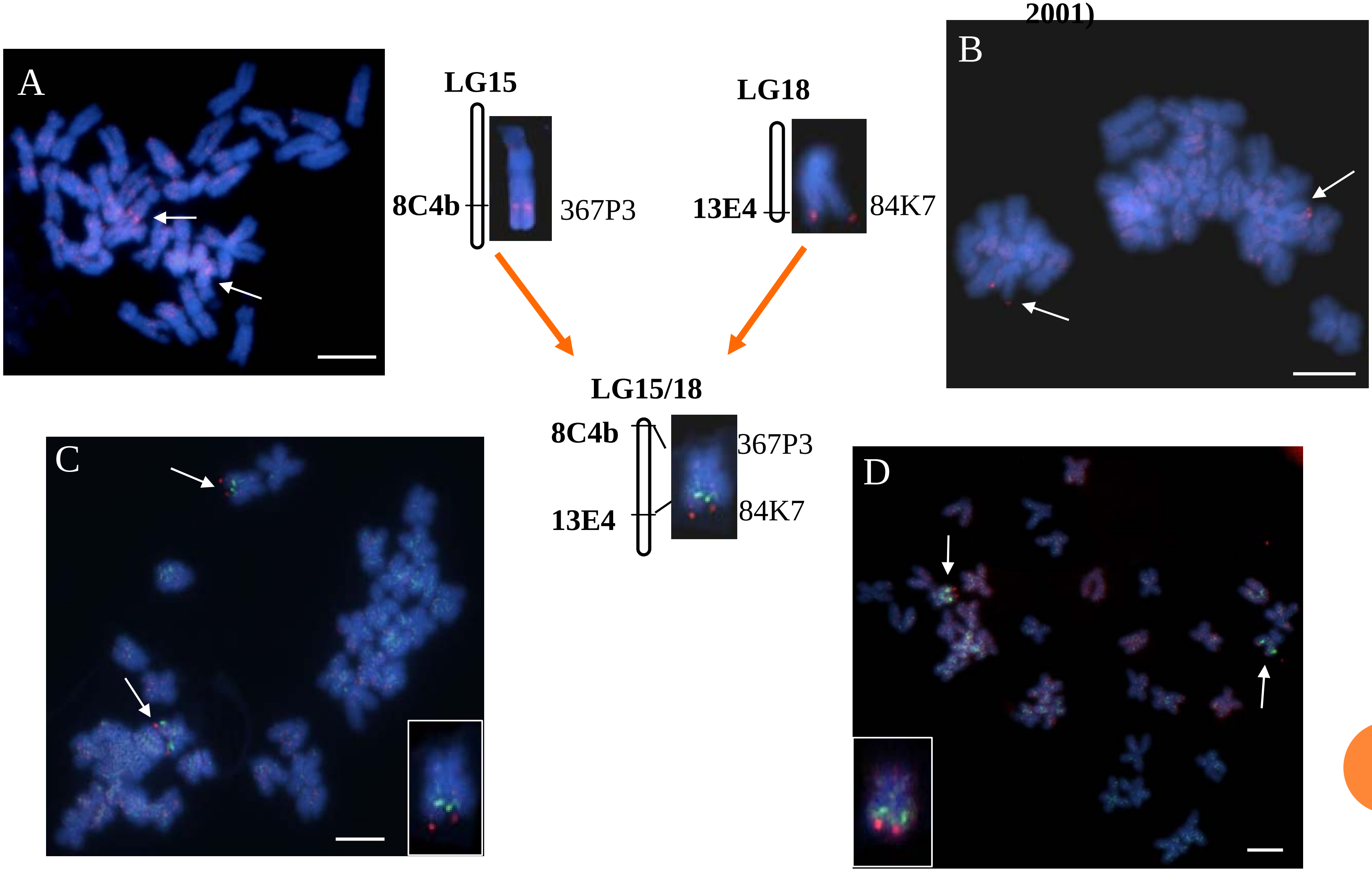


Bi-color FISH simultaneously determined the physical localizations of two BACs, 481K13 (red) and 155P12 (green), which correspond to RFLP markers 2B4 and 2D4, respectively, on LG5.

FISH Mapping

Rectify previous linkage maps

- **Two RFLP markers from LG15 and LG18 of Jan et al (1998) mapped to SSR-LG9 (Yu et al. 2003; Gedil et al 2001)**

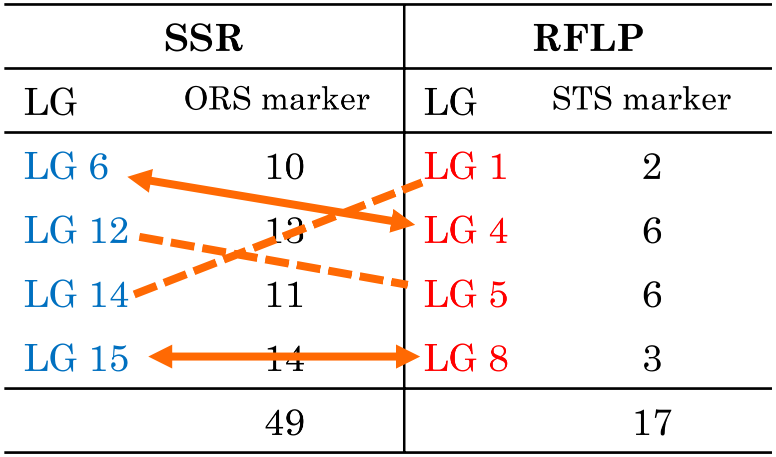


Integrate RFLP and SSR Maps

Coalescence of an independently developed RFLP map with SSR map

--Of 17 LGs, 13 LGs were cross-referenced by 40 shared RFLP marker loci; 4 RFLP LGs have not yet been unified into the SSR genetic map (Yu *et al*. 2003; Gedil *et al*. 2001).

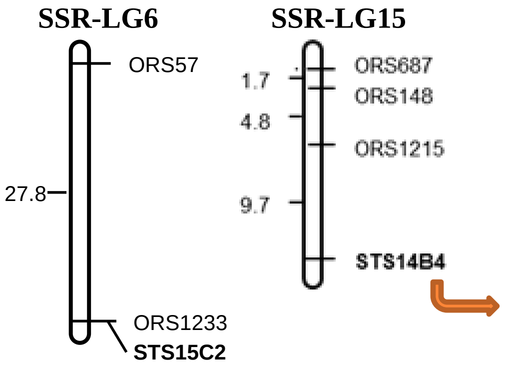
--Genetic analysis of a F2 population from the cross of CMS HA89 x RHA280 to integrate the SSR and RFLP map.



SSR		RFLP	
LG	ORS marker	LG	STS marker
LG 6	10	LG 1	2
LG 12	13	LG 4	6
LG 14	11	LG 5	6
LG 15	14	LG 8	3
	49		17

SSR-LG (Yu et al)	RFLP-LG (Jan et al)
1	12
2	14
3	11
4	13
5	6
6	4 ?
7	9
8	7
9	15
10	16
11	17
12	5 ?
13	2
14	1 ?
15	8 ?
16	3
17	10

Partial linkage map of LG6 and LG15 (Tang et al 2002) with linked RFLP markers (Jan et al 1998)

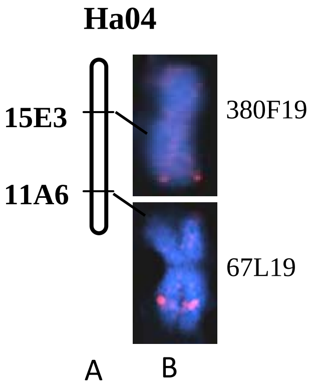


RFLP-derived markers

Integrate Linkage Groups and Chromosomes

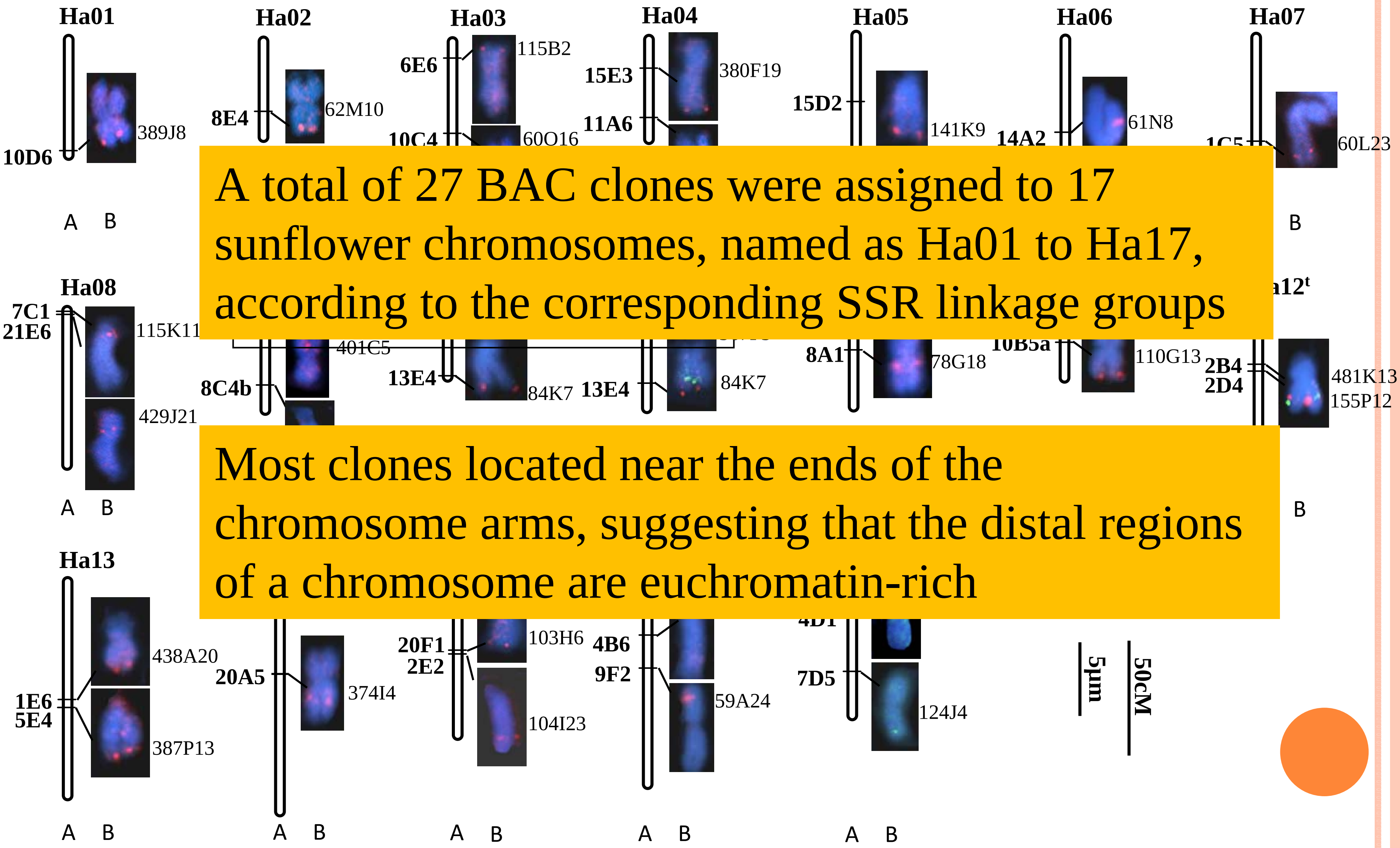
RFLP Markers selected for screening the libraries and the BAC/BIBAC clones used as FISH probes

Chrom. no	SSR-LG	RFLP-LG	RFLP marker	BAC clone (No. cells examined for FISH image)	BIBAC clone (No. cells examined for FISH image)
Ha01	1	LG12	10D6		**389J8** (64), 438D10 (12)
Ha02	2	LG14	8E4	**62M10** (14)	
Ha03	3	LG11	10C4	**60O16** (26), 150N10 ^{b} (21)	
			6E6	**115B2** (22)	
Ha04	4	LG13	15E3	**67L19** (22)	402M16 (6)
			11A6		**380F19** (5), 407K6 (6)
Ha05	5	LG6	15D2	**141K9** (29), 126N9 (10), 63A12 ^{a} (21)	
Ha06	6	LG4	14A2	**61N8** (31)	
Ha07	7	LG9	1C5	**60L23** (15), 183P19 (14), 184P8 (9)	
Ha08	8	LG7	7C1	**115K11** (8)	
			21E6		**429J21** (12)
Ha09	9	LG15	8C4b		**367P3** ^{b} (9), 437F7 (19), 445H4 (18)
			9D1		**401C5** (5)
		LG18	13E4	**84K7** (17)	
Ha10	10	LG16	8A1	**78G18** (51)	
Ha11	11	LG17	10B5a	**110G13** (10), 159N24 (23)	368F18 (12), 428O6 (5)
Ha12^{t}	12	LG5	2B4		**481K13** (18)
			2D4	**155P12** (19)	
Ha13	13	LG2	1E6		**438A20** (8)
			5E4		**387P13** (18)
Ha14^{t}	14	LG1	20A5		374I4 (7), 386G6 (5)
Ha15	15	LG8	2E2	**104I23** (7)	
			20F1	**103H6** (27), 124A11 (9)	470I10 ^{b} (10)
Ha16	16	LG3	9F2	**59A24** (45)	
			4B6		**464F20** (15)
Ha17	17	LG10	4D1		**381J20** ^{b} (16)
			7D5	**124J4** (12), 135J2 ^{a} (12)	
Total			**27**	**24** (474)	**20** (270)



Sunflower Cytogenetic (FISH) Map

Assignment of RFLP linkage groups to individual chromosomes



A total of 27 BAC clones were assigned to 17 sunflower chromosomes, named as Ha01 to Ha17, according to the corresponding SSR linkage groups

Most clones located near the ends of the chromosome arms, suggesting that the distal regions of a chromosome are euchromatin-rich

Summary and Outlook

Using the mapped cDNA-derived RFLP markers, 195 linkage group-specific BAC/BIBAC clones were identified

Of 60 BAC clones analyzed, 44 was used for FISH mapping

Of these, 27 BAC clones, as chromosome-specific markers, were placed on the cytogenetic map of cultivated sunflower

This allowed us to assign each genetic linkage group to a specific sunflower chromosome

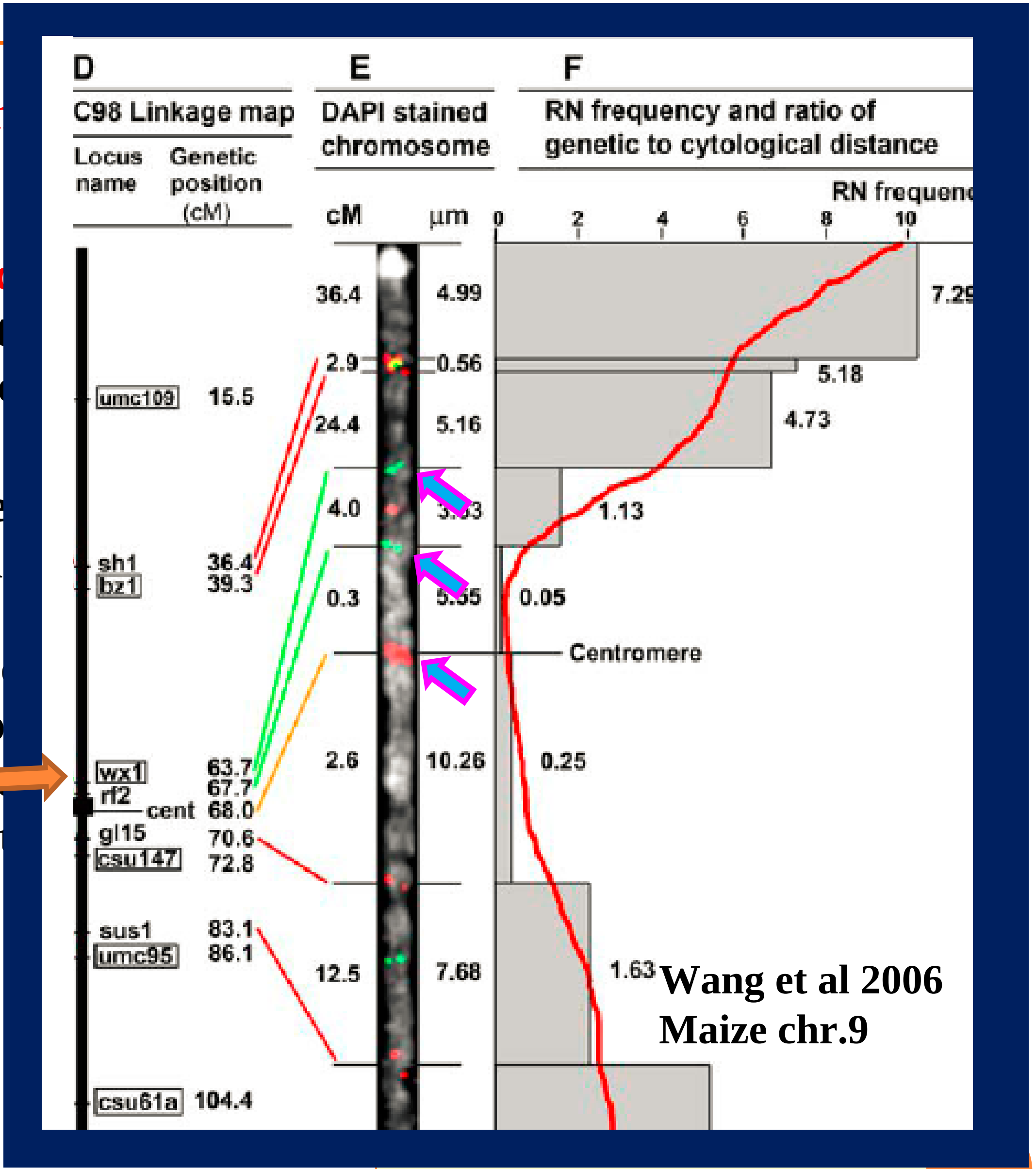
Summary and Outlook

- The established BAC-FISH techn
more BAC clones and to develop a

- Chromosome-specific cytogenetic
identifying sunflower cytogenetic st
chromosomes in interspecific crosse

- BAC libraries provide resource e
BAC-end sequencing and fingerpri

- With the sunflower genome sequ
cytogenetic map will facilitate to co
repetitive sequences, validate discr
map and fill the gaps in recombinat



Wang et al 2006
Maize chr.9

Map-based gene cloning

BAC and BIBAC Libraries

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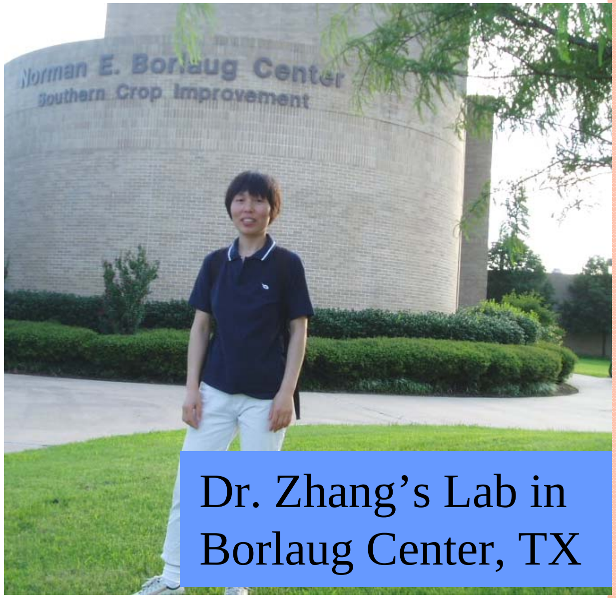
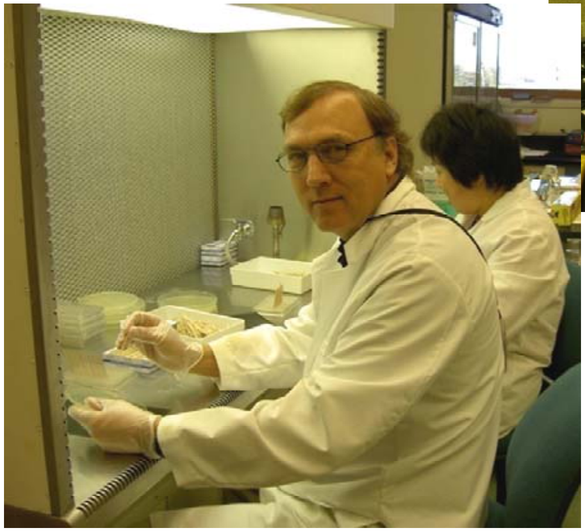


Dr. Zhang’s Lab in Borlaug Center, TX



Thank you !