

Structure and Evolution of Wheat Centromeres and Pericentromeres

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2006 5 25

Table 1 Southern hybridization results of RAPD profile by cloned genome-specific RAPD markers

RAPD Markers	Genome or species													
	A ^u	S	D	E ^b	E ^e	St	P	Ns	R ^m	H	I	W	<i>Th.int</i> Z1141	<i>Th.pont</i> R431
St-OPN-01 ₈₁₇	-	-	-	-	-	+++	-	-	-	-	-	-	+++	+++
St-OPN-05 ₄₄₄	-	-	-	-	-	+++	-	-	-	-	-	-	+++ ^a	++ ^a
St-OPB-08 ₅₂₅	-	-	-	+	-	+++	+	-	-	+	-	-	+++	+++
St-OPD-15 ₅₀₄	-	+ ^b	-	+ ^b	+	+++	+ ^b	+++ ^a	+ ^b	+ ^b	+ ^a	+	+++	+++
St-OPN-03 ₇₈₀	+	+ ^b	+	+	+	+++	+	+++	+	+	+	++	+++	+



Table 1 Southern hybridization results of RAPD profile by cloned genome-specific RAPD markers

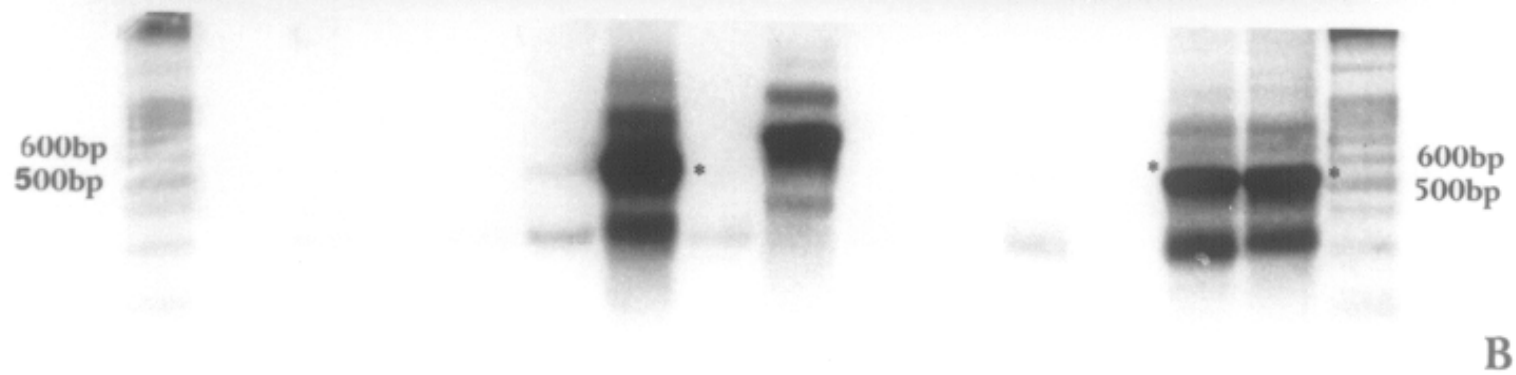
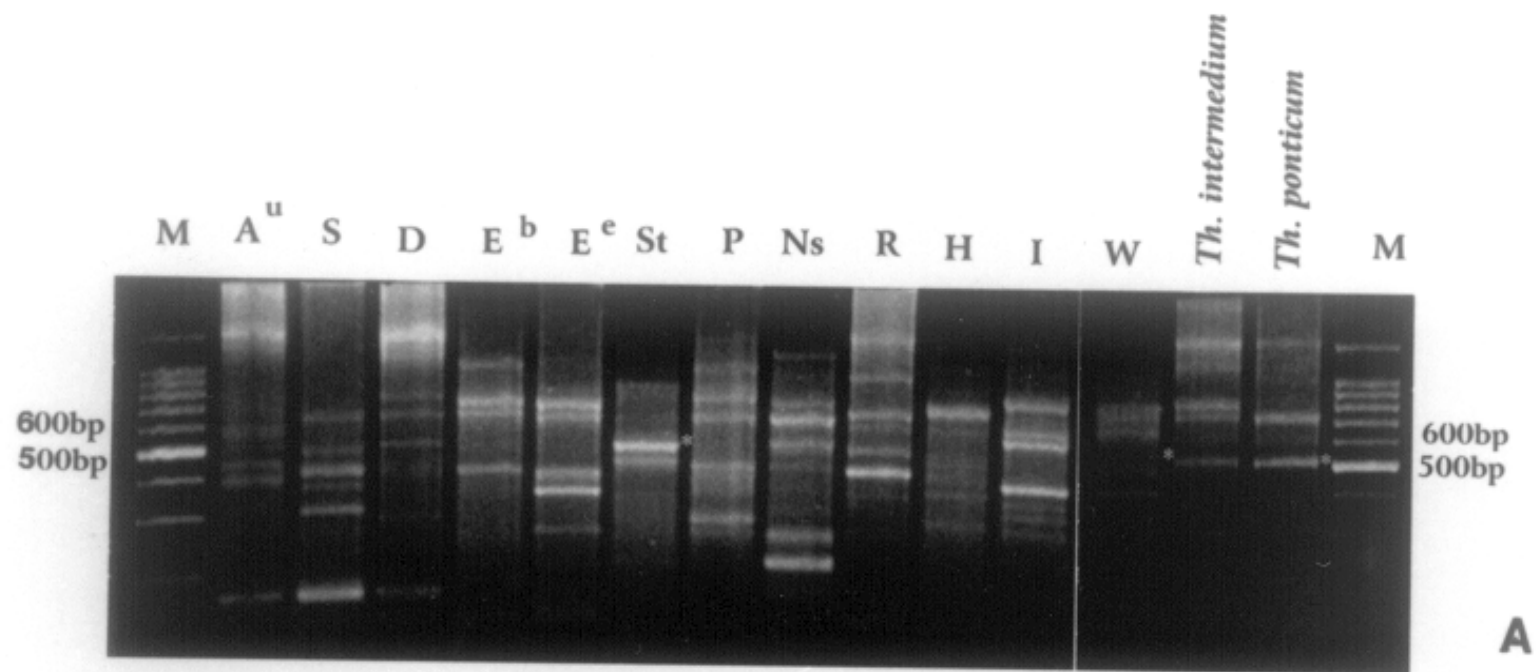
RAPD Markers	Genome or species													
	A ^u	S	D	E ^b	E ^e	St	P	Ns	R ^m	H	I	W	<i>Th.int</i> Z1141	<i>Th.pont</i> R431
E ^b -OPF-03 ₁₂₉₆	-	-	-	+++	+ ^b	-	+	-	++	-	-	-	++	++
E ^b -OPN-01 ₂₆₉	-	-	-	+++	+ ^a	-	+ ^a	+ ^a	+ ^a	+	-	-	+++	+++
E ^b -OPC-03 ₃₃₂	+	+	+	+++	++	+	++	+	++ ^a	++ ^a	+	-	++	++
E ^b -OPL-05 ₆₁₃	-	-	-	+++	++	+	-	+ ^b	-	-	-	-	+	+
E ^e -OPN-03 ₈₇₀	-	-	+	+	+++	-	-	-	+	-	-	-	+	-
E ^e -OPR-05 ₇₀₀	-	-	+ ^b	+	+++	+ ^b	+	-	+	+ ^b	-	-	-	+
E--OPC-15 ₂₉₁	-	+ ^a	+ ^a	+++	+++	+ ^a	+ ^a	-	-	-	-	-	+ ^a	+++
E--OPF-03 ₃₇₃	+	+	+	+++	+++	-	-	-	-	-	-	+	+	+++

"-"No hybridization signal; "+" Weak hybridization signals;

"+++ " Strong hybridization signals; "a" Fragment size increased;

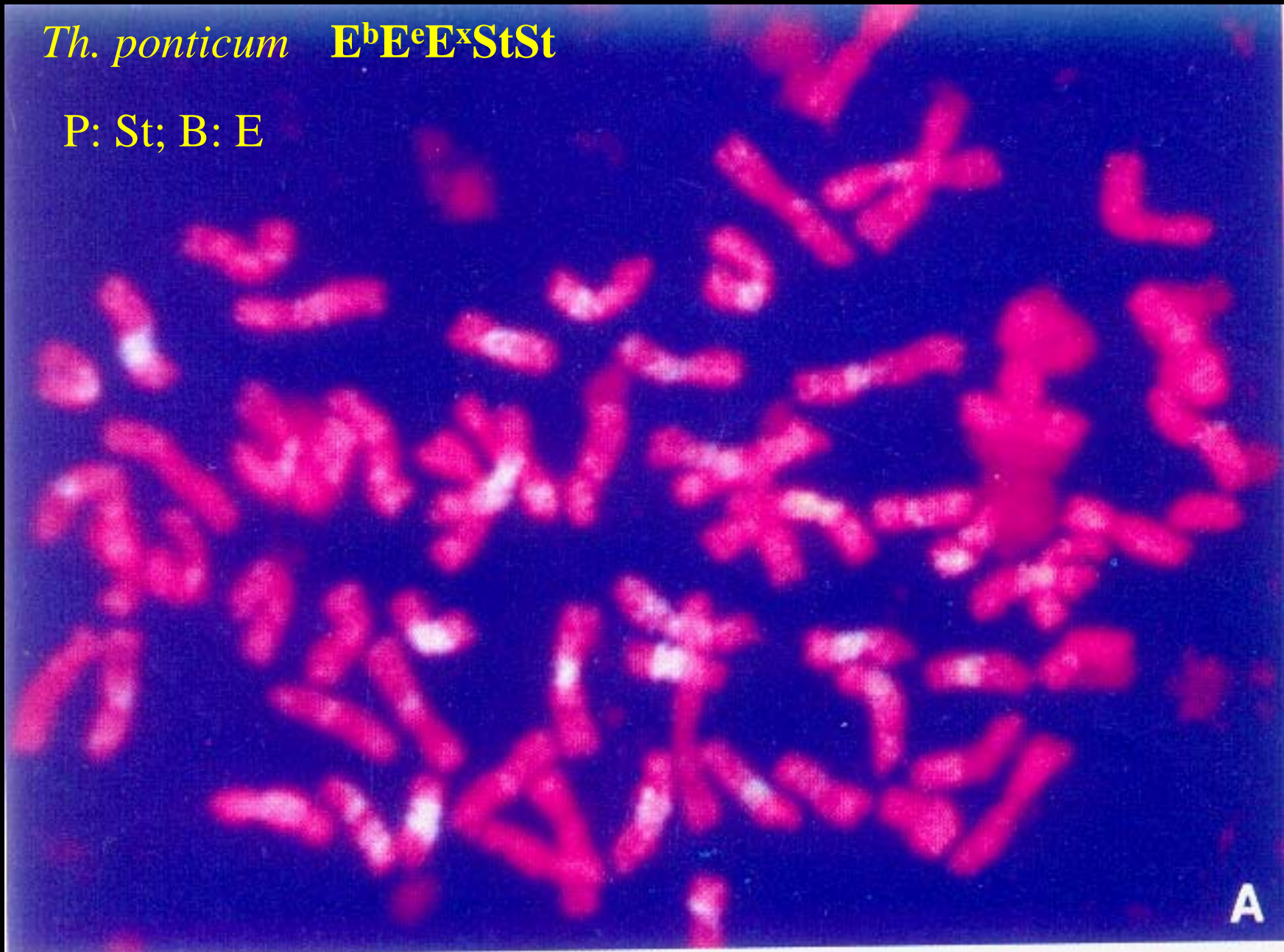
"b" Fragment size decreased.





Th. ponticum **E^bE^eE^xStSt**

P: St; B: E



Th.ponticum

P: E; B: St



B

Centromeric and pericentromeric region may be the critical area that discriminates the St genome from the E genome in *Thinopyrum ponticum* (Tall wheatgrass).

Zhang XY et al. 1996, GENOME, 39:1062-1071

Is this truth or false?

Structure and Localization of the Centromere and Kinetochore

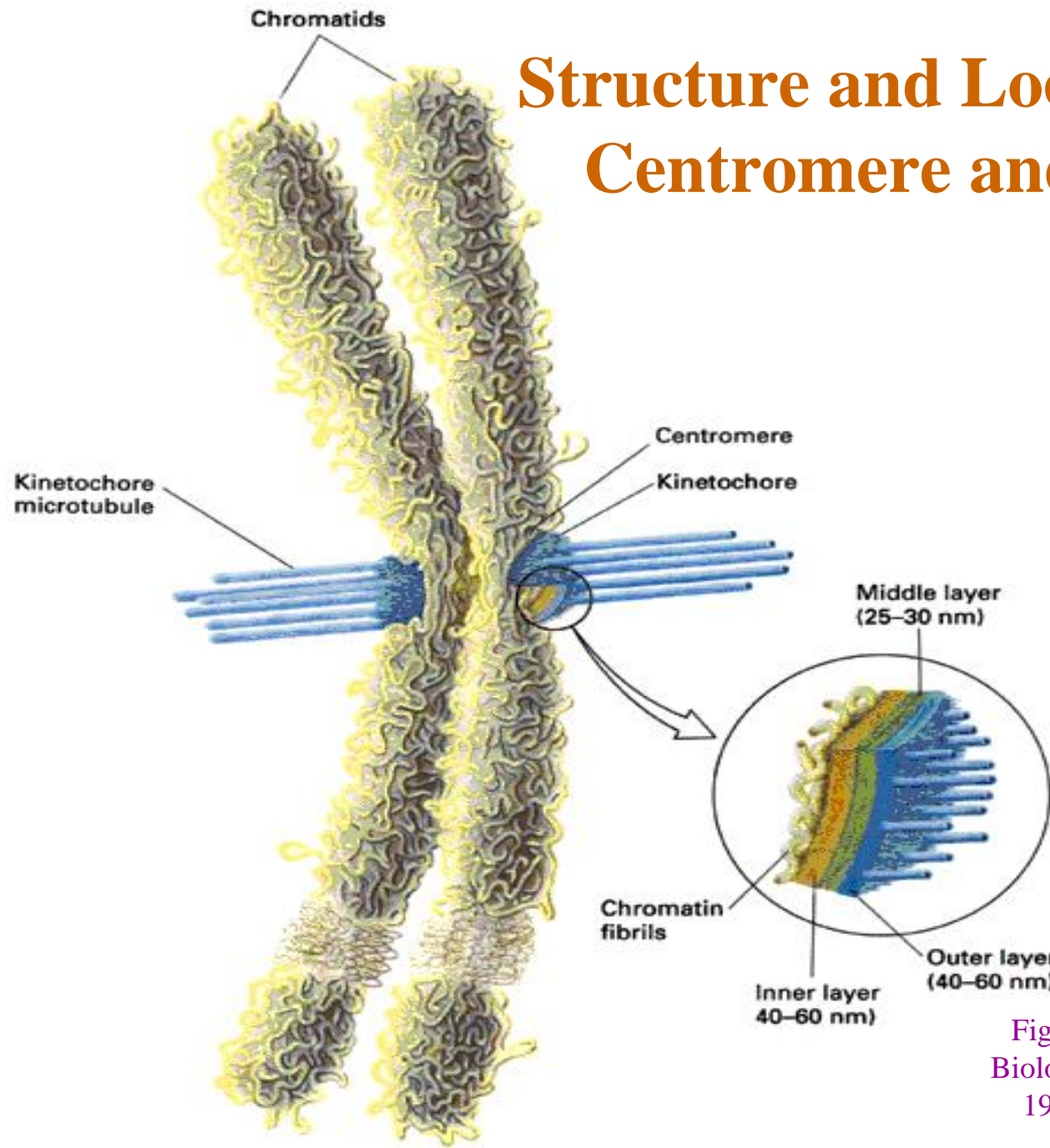


Figure 23-38, p. 1094, Molecular Cell Biology, 3rd ed., Lodish, et al., copyright 1995, W.H. Freeman and Company, reprinted with permission.

Functions of centromeres:

Responsible for the proper segregation of chromosomes during cell division.

Sites for sister chromatids association.

Sites for microtubules of spindle to attach chromosomes.

Size:

Point centromere: 125 bp

Regional centromere: several Kb to Mb

Structure Components of Centromeres

- repetitive sequences:
 - centromeric satellite arrays
 - centromere-specific retrotransposons
- CENPs: [CENH3](#), CENP-B, CENP-C, etc.
- Genes
- Heterochromatin proteins

Centromeric-satellites on centromeres

They **diverged rapidly**, most of them are specific to only closely related species.

Serve as the **core** of the centomere, such as in rice, maize and *Arabidopsis*.

Partial of them are associated with the **CENH3** proteins proved by ChIP technology, in rice, maize and *Arabidopsis*.

Retrotransposons on centromeres

Ty1/copia:



Ty3/gypsy:



Centromeric retrotransposons (CR) family

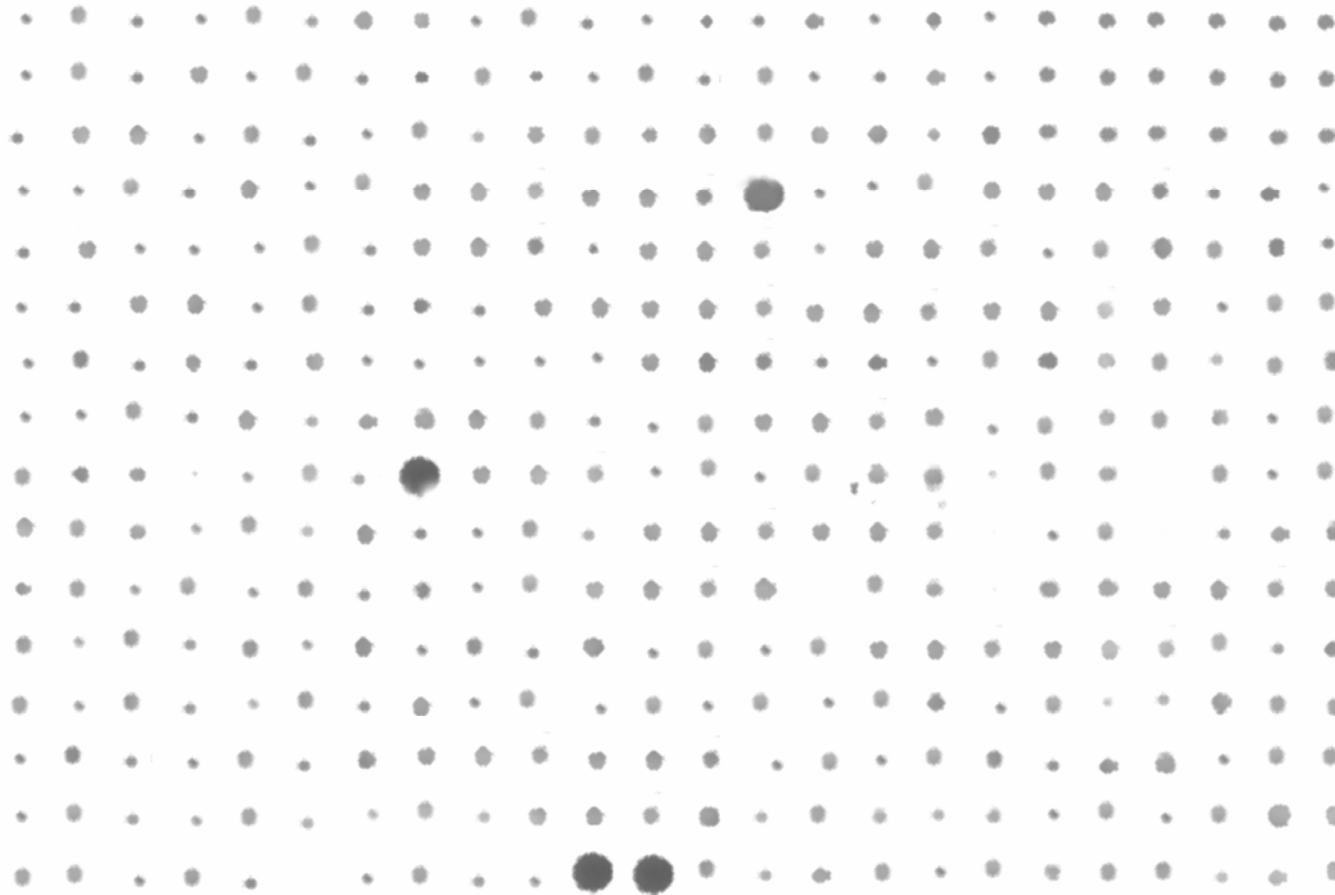
Found in the centromere regions of **all grass species** studied, such as sorghum, maize, rice, barley, wheat.

Derived from **Ty3/gypsy** retrotransposons, frequently inserted into centromeric satellites, or into each other.

Partial of them are associated with the **CENH3** proteins proved by ChIP technology, in rice, maize and *Arabidopsis*.

I. Full Sequence of Two BAC Clones
Associating with Centromeres and
Isolation of **C**entromeric **R**etrotransposon
of **W**heat (**CRW**)

Fig. Screening the BAC library with cpDNA sequence psbA gene and mtDNA sequence atp6. The pollution is less than 1%.



***T. boeoticum* BAC library Chen et al. 2002**

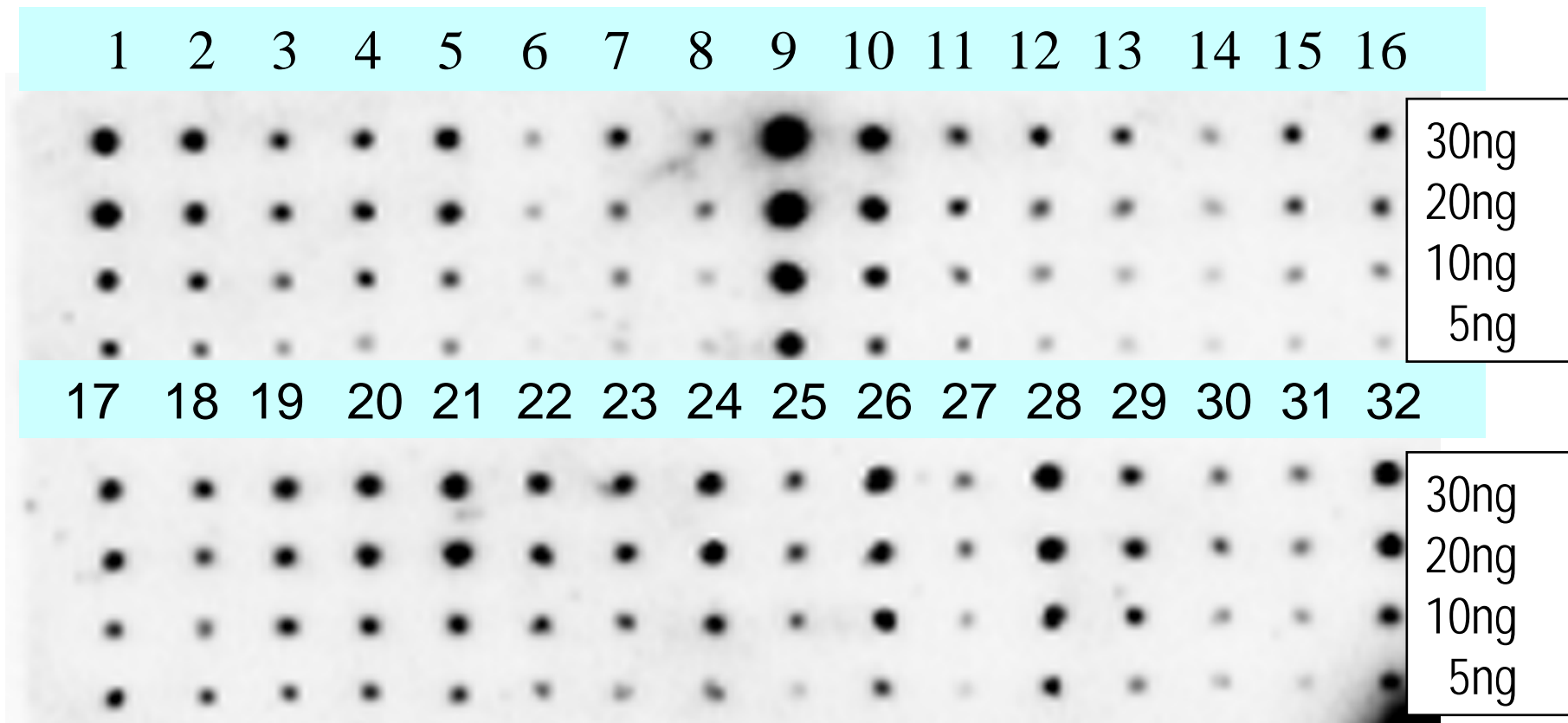
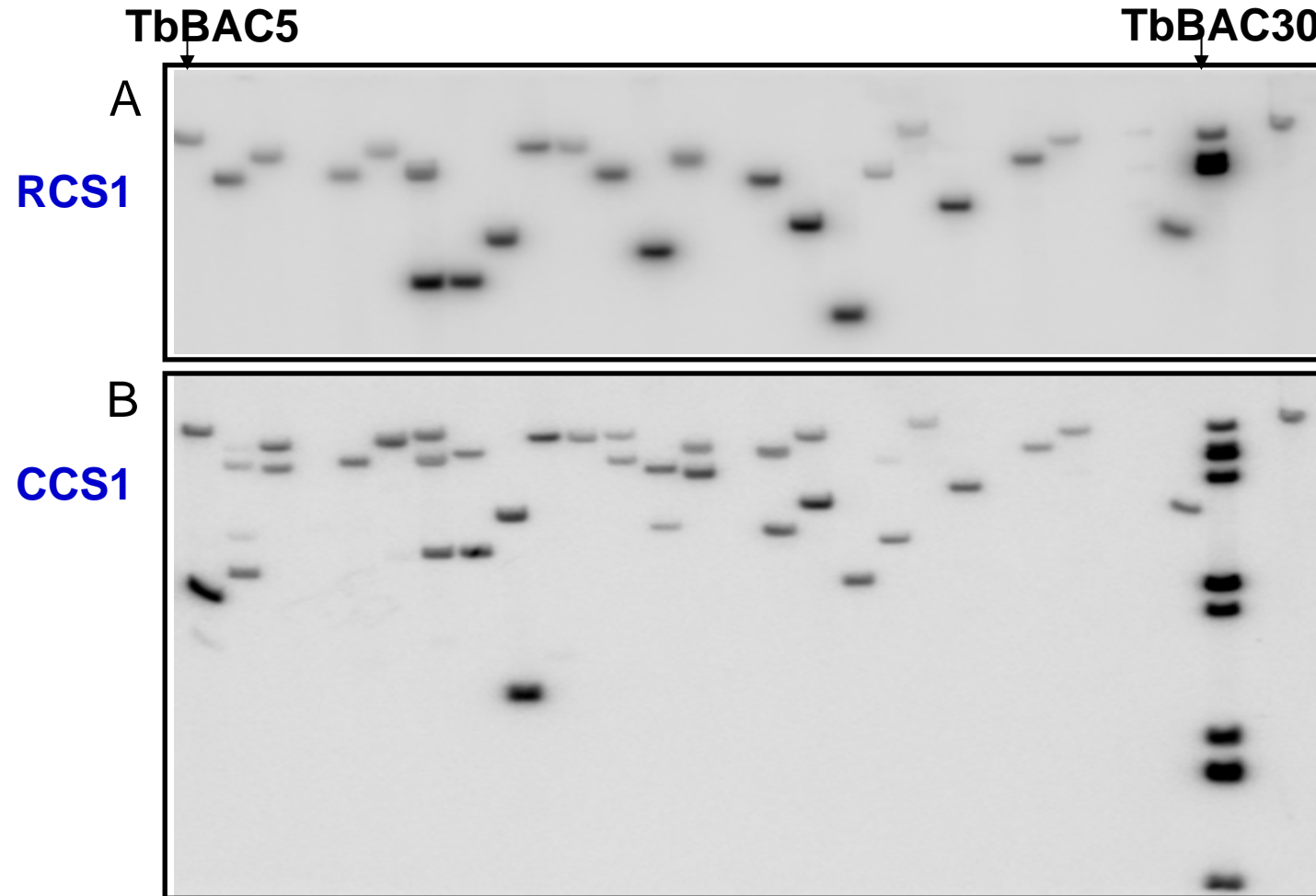
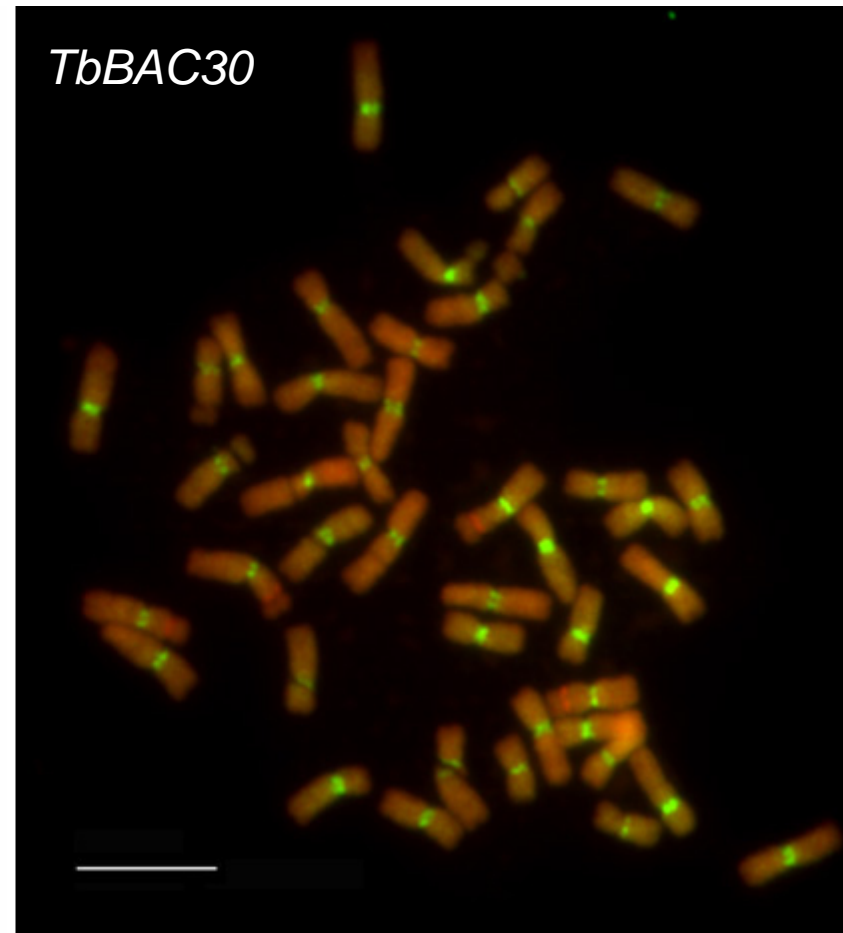
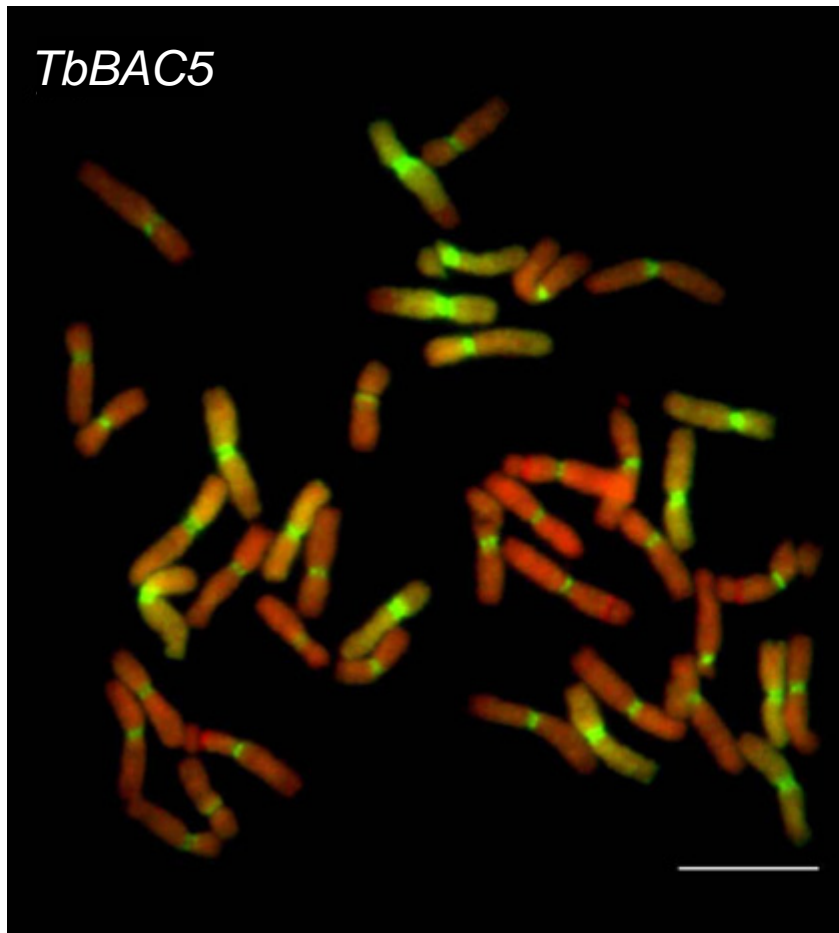


Fig. Certification with *Tsc250*, A wheat centromeric repeat (Chen et al, 2003, Genetics, 164:665).

Southern hybridization of the candidate clones with **RCS1** and **CCS1**





Chinese Spring probed by *TbBAC5* and *TbBAC30*

1. Schematic organisation of the 90kb and 84kb contigs of BAC clones *TbBAC5* and *TbBAC30*

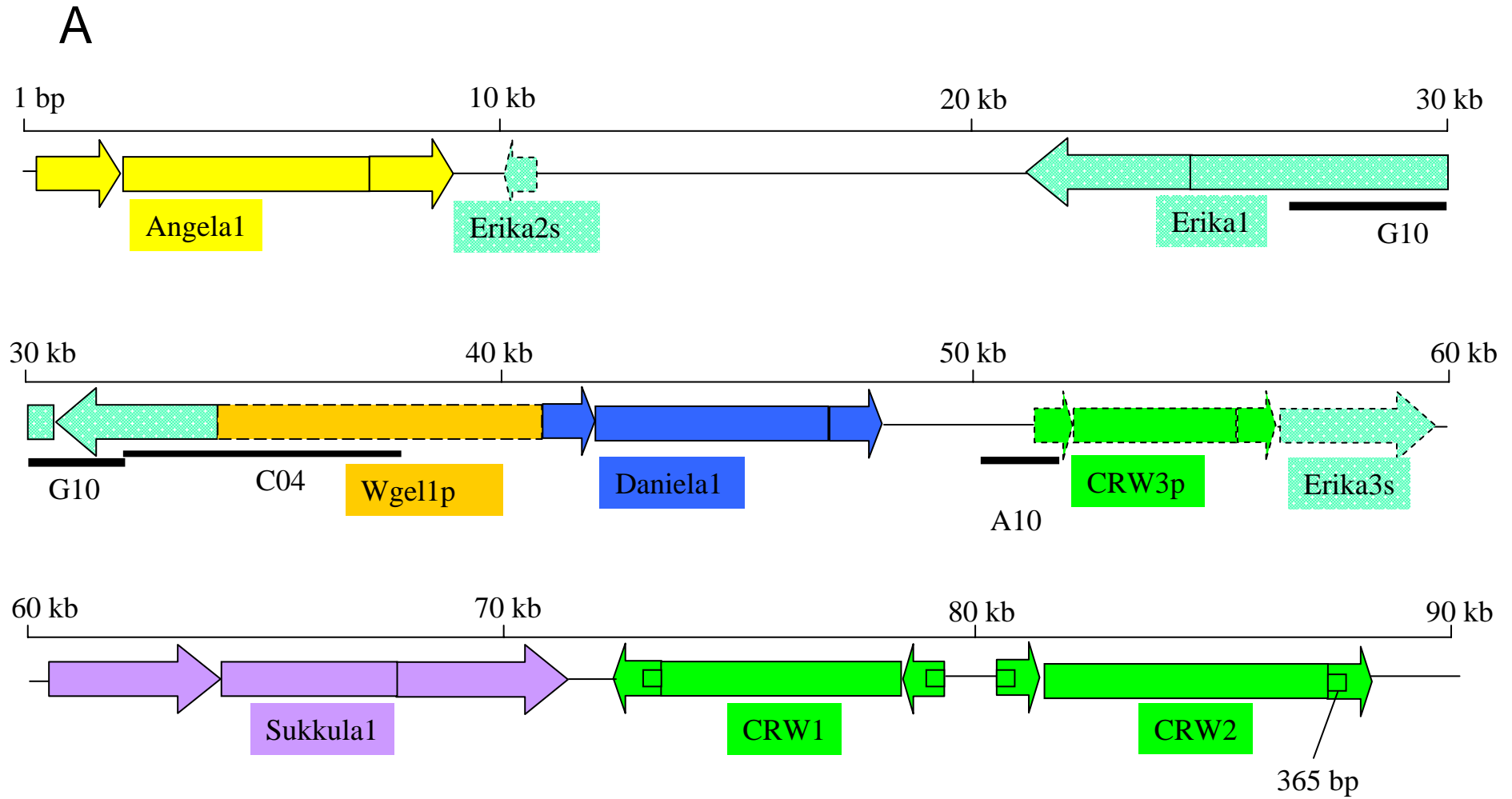


Figure 1. Schematic organisation of the 90kb and 84kb contigs of BAC clones **TbBAC5** (A) and (B) TbBAC30.

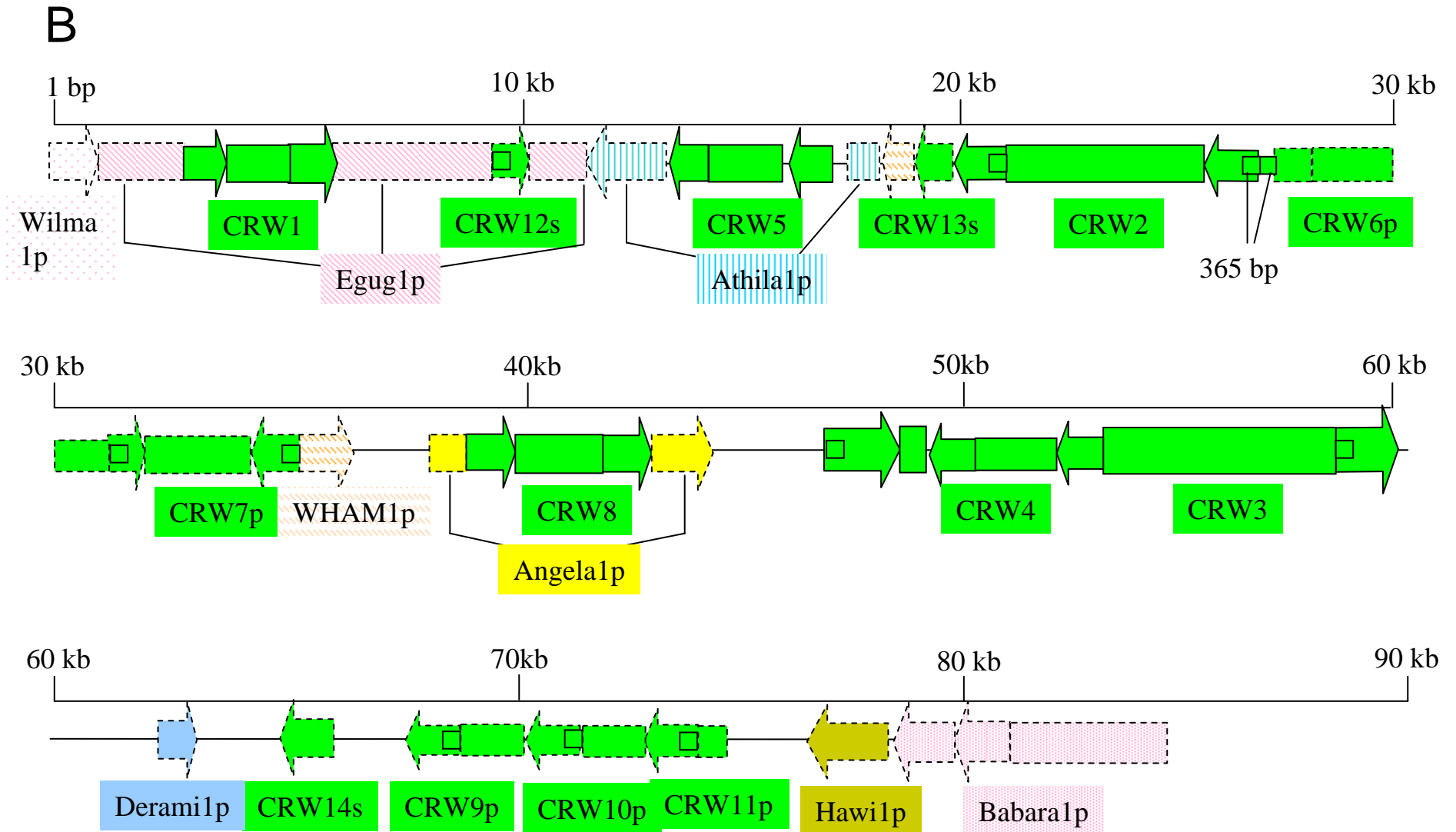
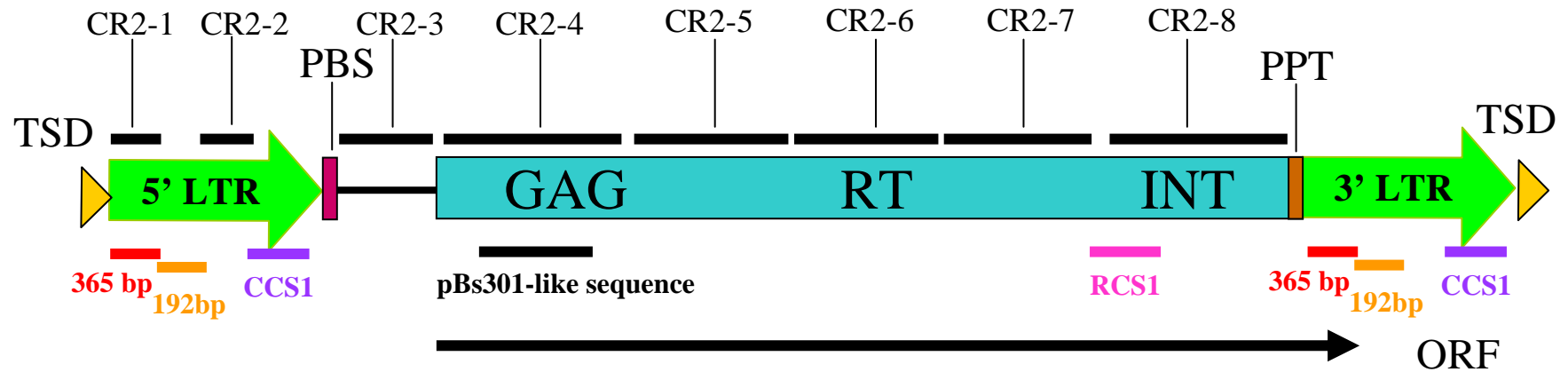


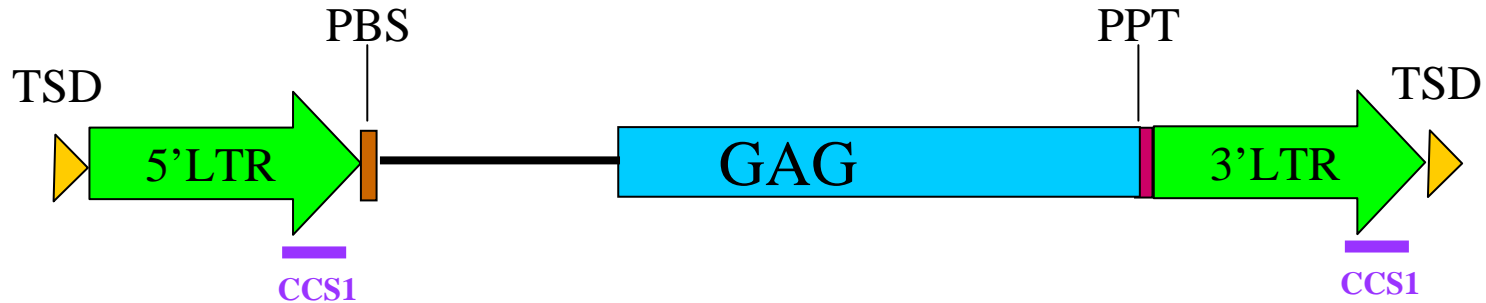
Figure 1. Schematic organisation of the 90kb and 84kb contigs of BAC clones TbBAC5 (A) and **TbBAC30 (B)**.

2. Schematic organisation of the autonomous and non-autonomous CRWs

A



B



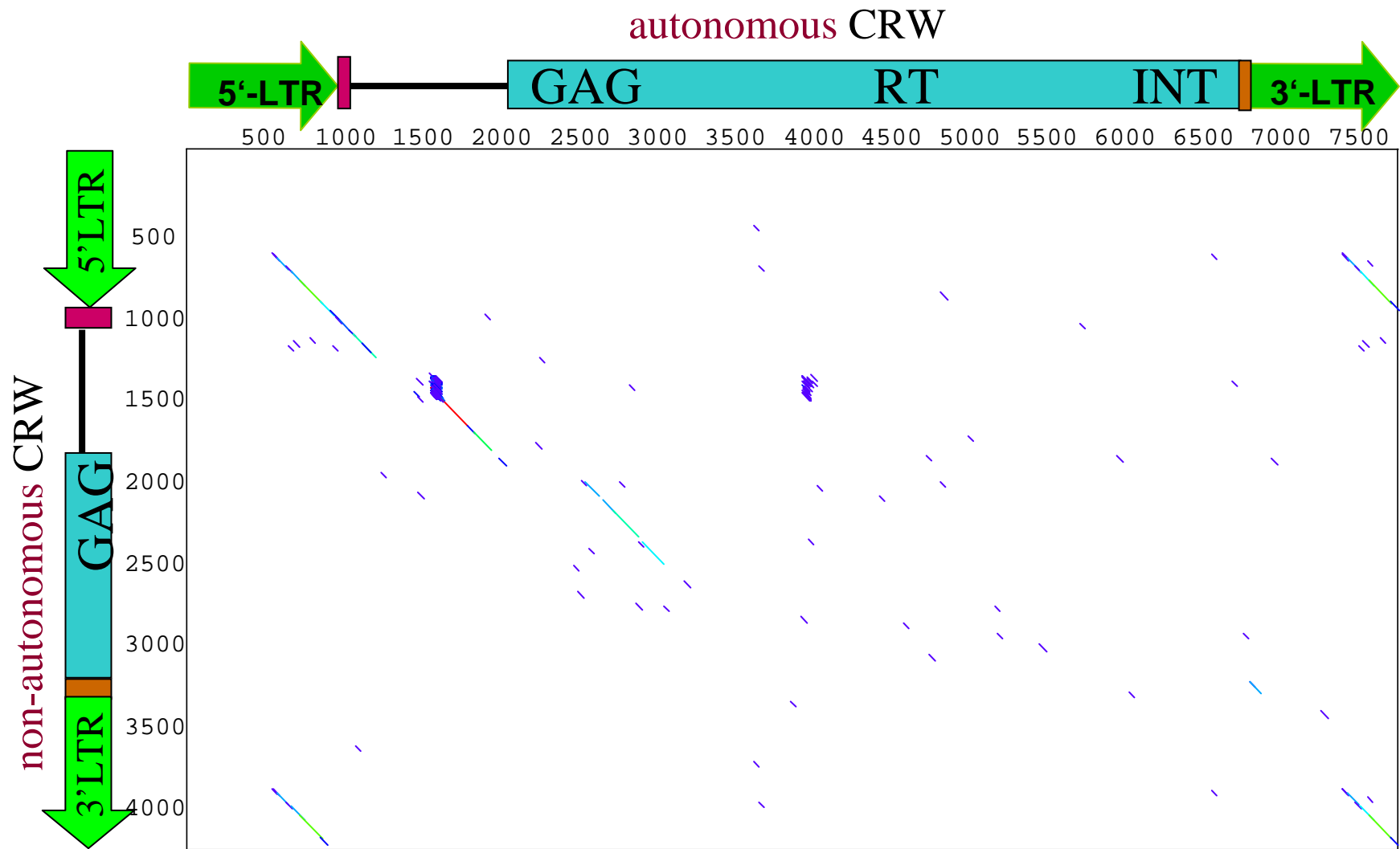


Fig. Dotplot between *autonomous* and *nonautonomous* CRWs

3. CRWs from different genomes

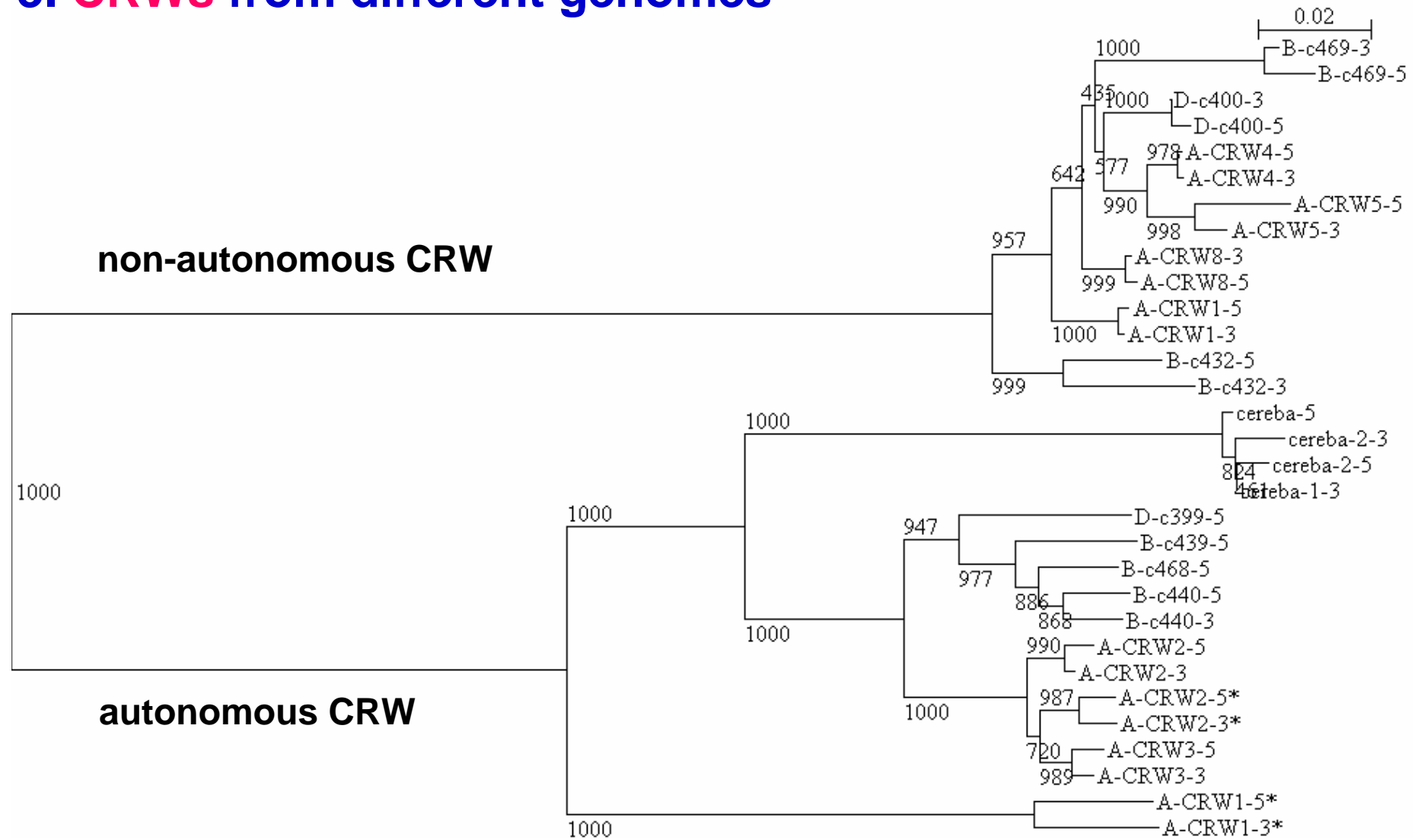
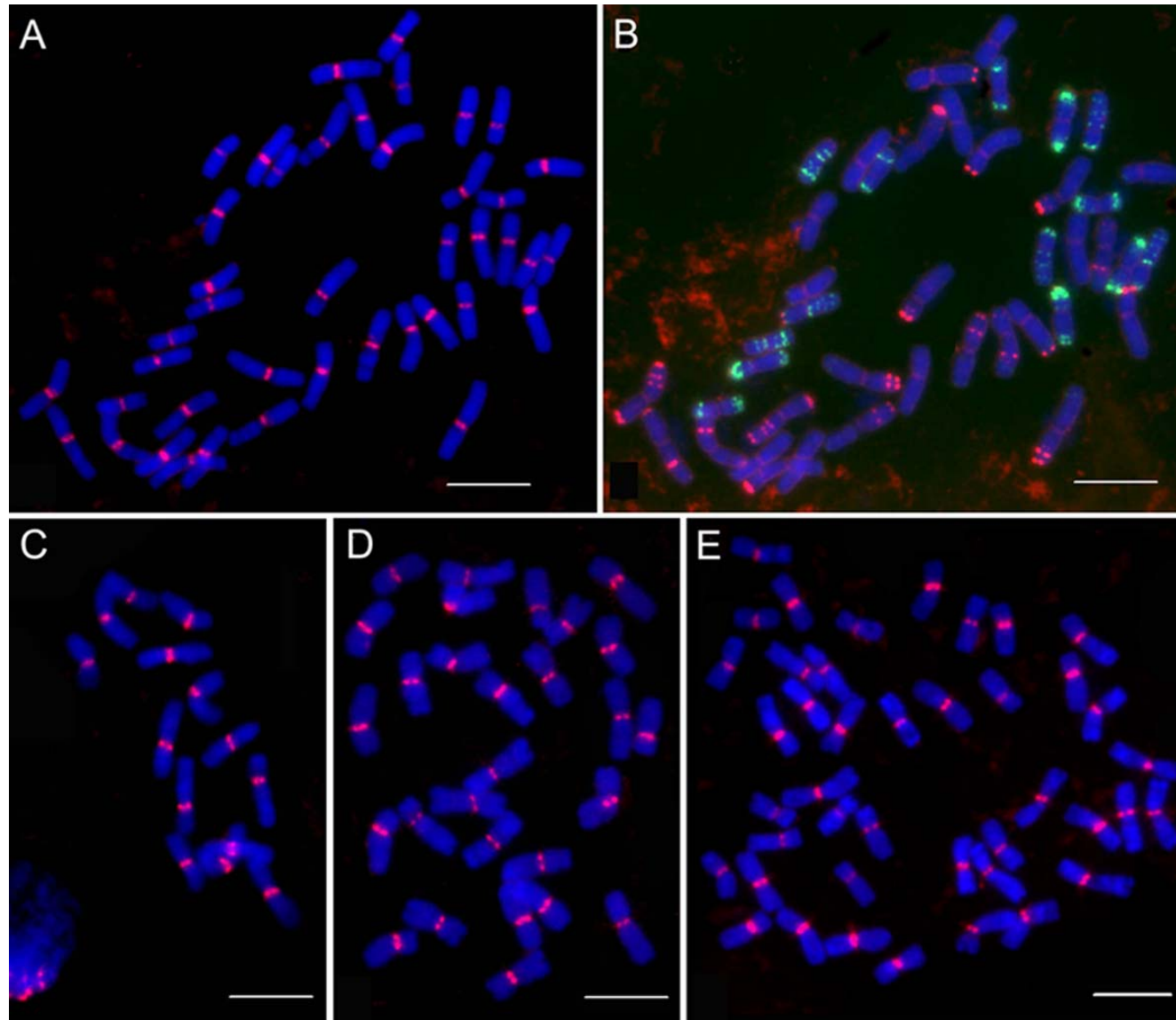


Fig. Phylogenetic tree based on the LTR regions

II. Distributional and Functional Characterization of *CRWs*

1. Chromosomal localization of CRWs in *Triticum* species



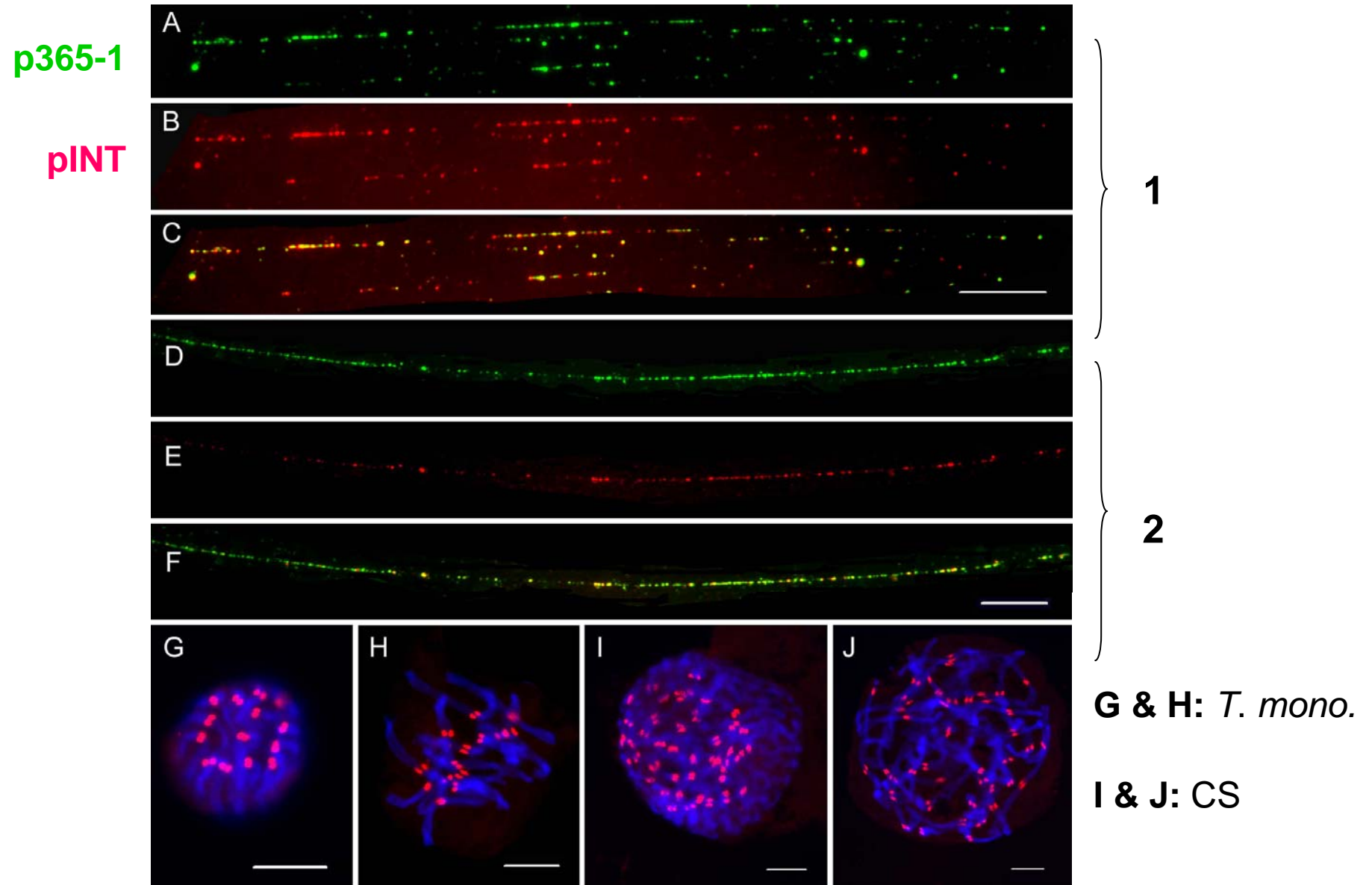
A, B & E: CS

C: *T. monoccocum*

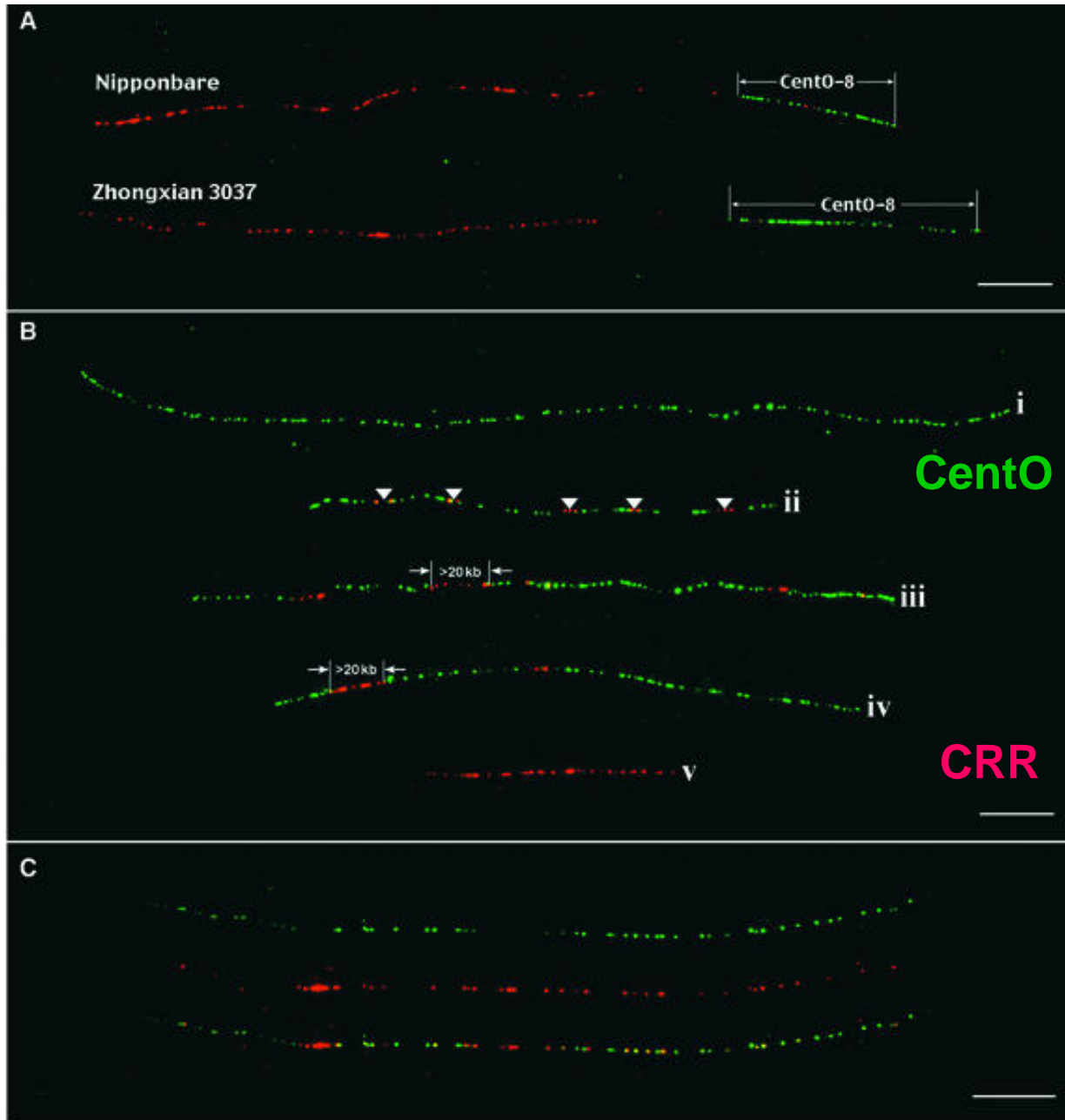
D: *T. dicoccoides*

A, B: **autonomous**; C-E: **non-autonomous**

2. Distribution patterns of CRWs on stretched centromeric DNA



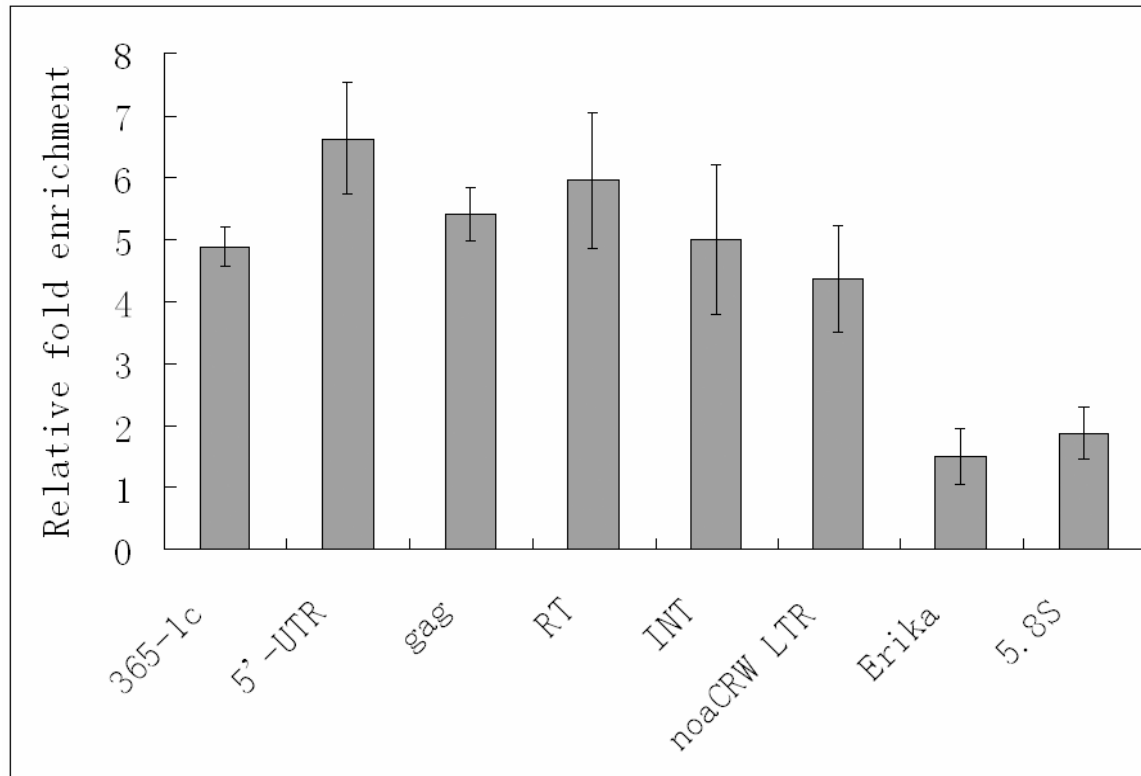
Immunosignals generated by rice anti-CENH3 antibody



DNA Components at Rice Centromeres

Cheng et al., 2002,
Plant Cell. 14: 1691–
1704

3. Certification of CRWs for Function of Centromeres



P1=0.0001; P2=0.0005; P3=0.0001; P4=0.0026; P5=0.0095; P6=0.0056; P7=0.0922

Fig. Relative fold enrichment obtained following **ChIP analysis of seven wheat repetitive elements.**

4. Distribution of CRWs in wheat and its relatives

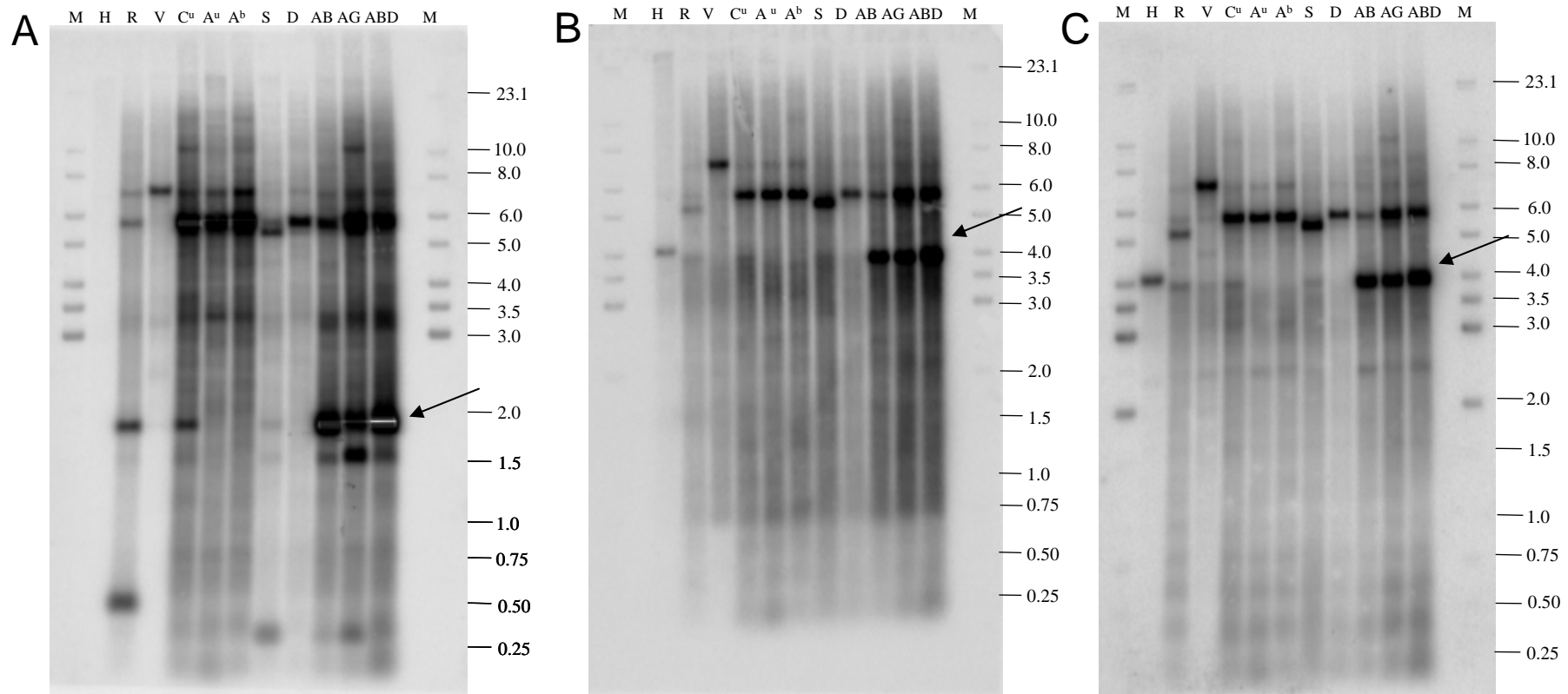
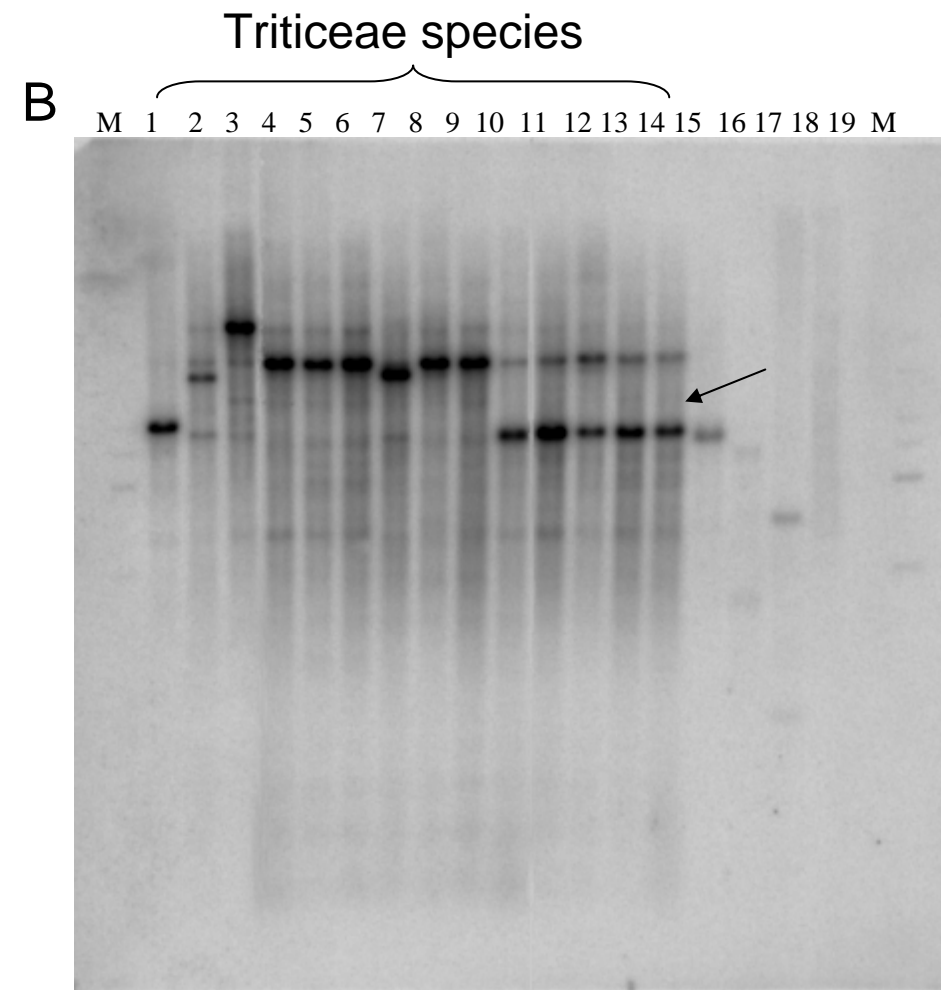
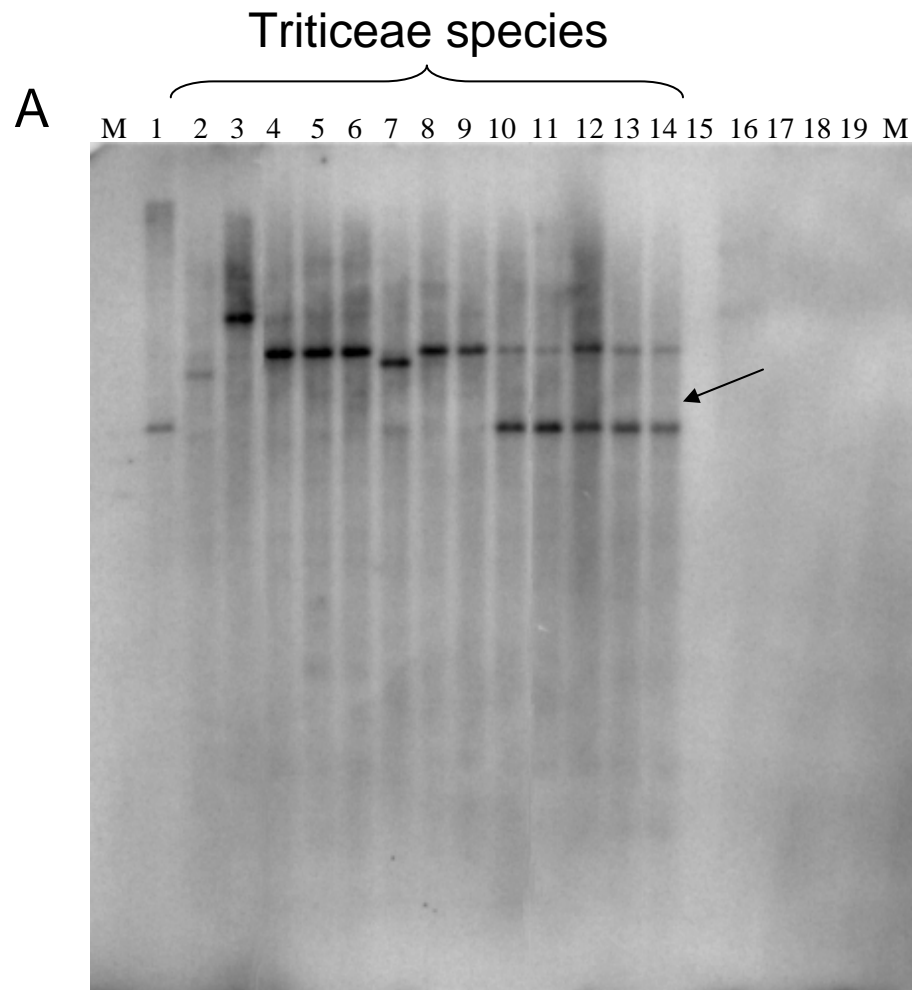


Fig. Southern hybridisation profiles generated by probing with (A) gag, (B) LTR, (C) integrase sequences of an autonomous CRW.

Tab. Nineteen species used in Southern hybridization

No.	Species	No.	Species
1	<i>Hordeum vulgare</i> L.	11	<i>T. orientale</i> Percival
2	<i>Secale cereale</i> L.	12	<i>T. araraticum</i> Jakubz.
3	<i>Dasypyrum villosum</i> (L.) Candargy	13	<i>T. aestivum</i> L. (中国春)
4	<i>Triticum urartu</i> Thum. et Gandil	14	<i>T. aestivum</i> L. (偃展1号)
5	<i>T. boeoticum</i> Boiss.	15	<i>Avena sativa</i> L.
6	<i>T. monococcum</i> L.	16	<i>Oryza sativa</i> L. ssp. <i>japonica</i>
7	<i>Aegelops speltoides</i> Tansch	17	<i>Zea mays</i> L.
8	<i>Ae. tauschii</i> Coss	18	Millet
9	AD artificial amphiploid	19	<i>Arabidopsis</i>
10	<i>T. dicoccoides</i> Korn, ex Schweinf.		



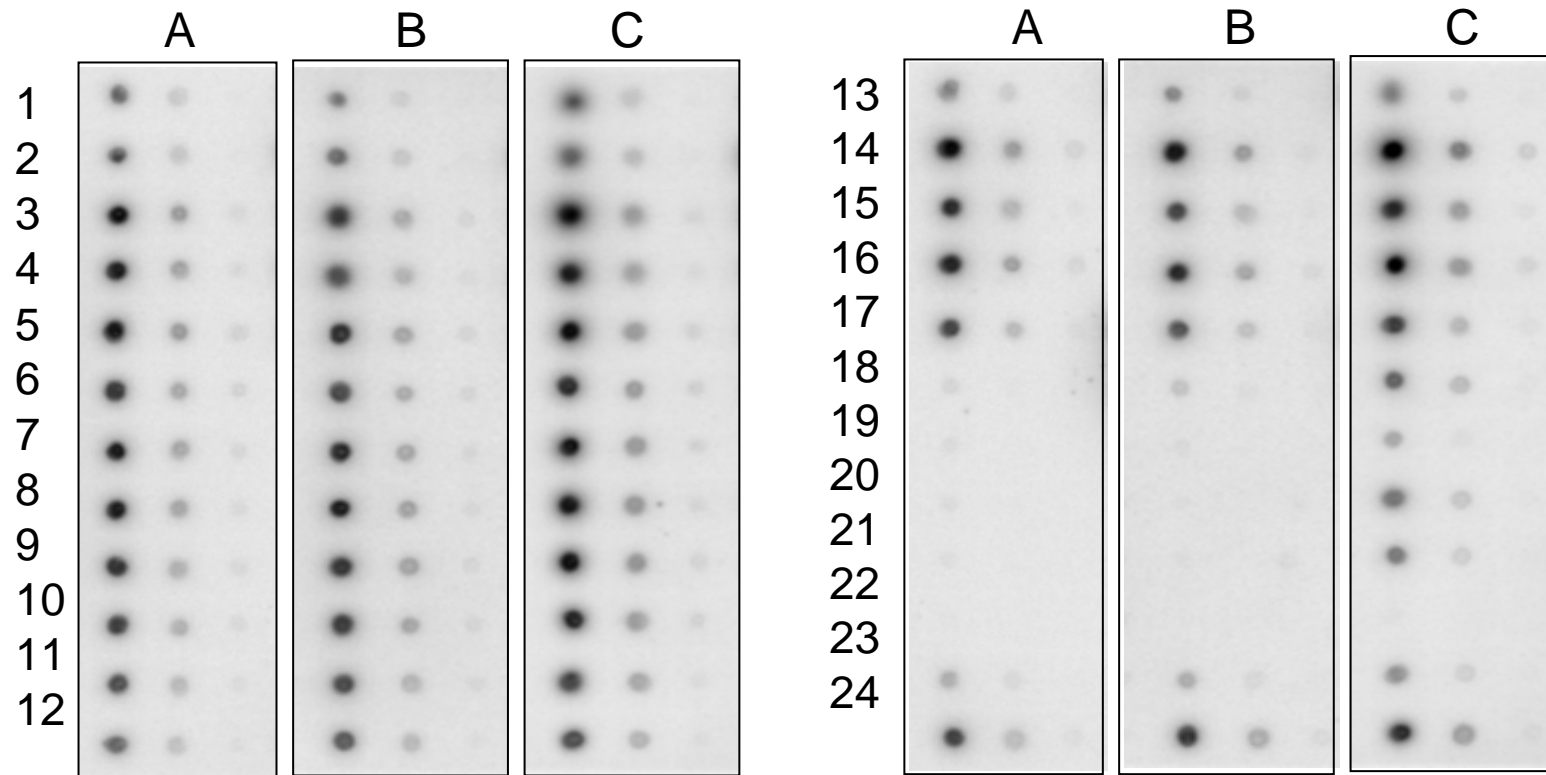
A: 365bp (LTR) ;

B: INT (integrase)

Fig. Southern hybridization of CRW with Gramine species (*HindIII*)

Tab. 24 Species used in dot blotting hybridization

No.	100ng	10ng	1ng	No.	100ng	10ng	1ng
1	<i>Hordeum vulgare</i> L.			13	<i>T. durum</i>		
2	<i>Secale cereale</i> L.			14	<i>T. orientale</i> Percival		
3	<i>Dasypyrum villosum</i> (L.) Candargy			15	<i>T. araraticum</i> Jakubz.		
4	<i>Ae.umbellulata</i> Zhuk.			16	<i>T. aestivum</i> L. (中国春)		
5	<i>Triticum urartu</i> Thum. et Gandil			17	<i>T. aestivum</i> L. (偃展1号)		
6	<i>T. boeoticum</i> Boiss.			18	<i>Avena sativa</i> L.		
7	<i>T. monococcum</i> L.			19	<i>Oryza sativa</i> L. ssp. <i>japonica</i>		
8	<i>Aegelops speltoides</i> Tansch			20	<i>Zea mays</i> L.		
9	<i>Aegelops speltoides</i> Tansch			21	Millet		
10	<i>Ae. tauschii</i> Coss			22	<i>Arabidopsis</i>		
11	Artificial AD tetraploid			23	St95		
12	<i>T. dicoccoides</i> Korn, ex Schweinf.			24	<i>Ae. comosa</i> Sibth. And Sm.		



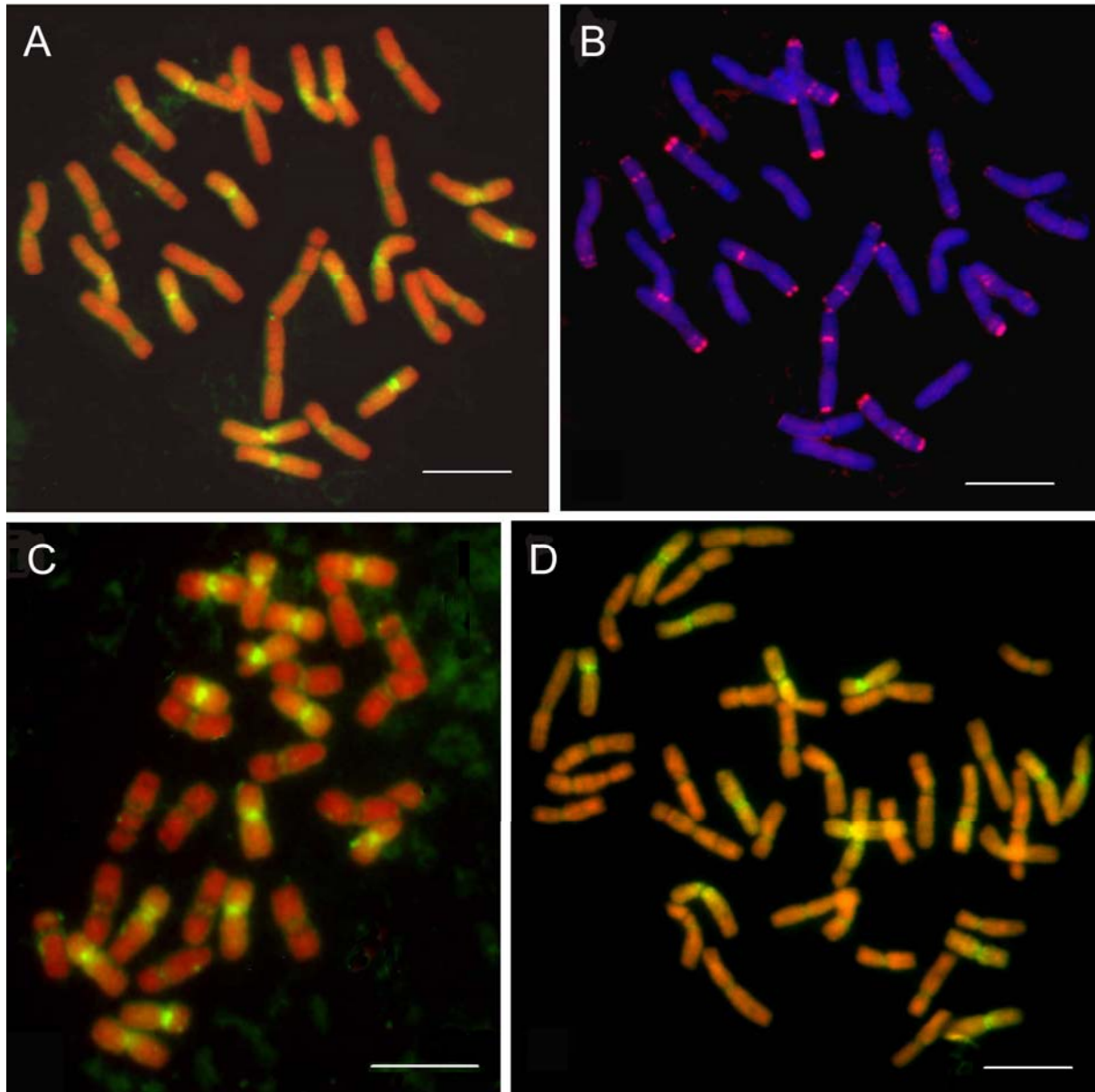
A:365 bp (LTR)

B:192bp (LTR);

C:INT

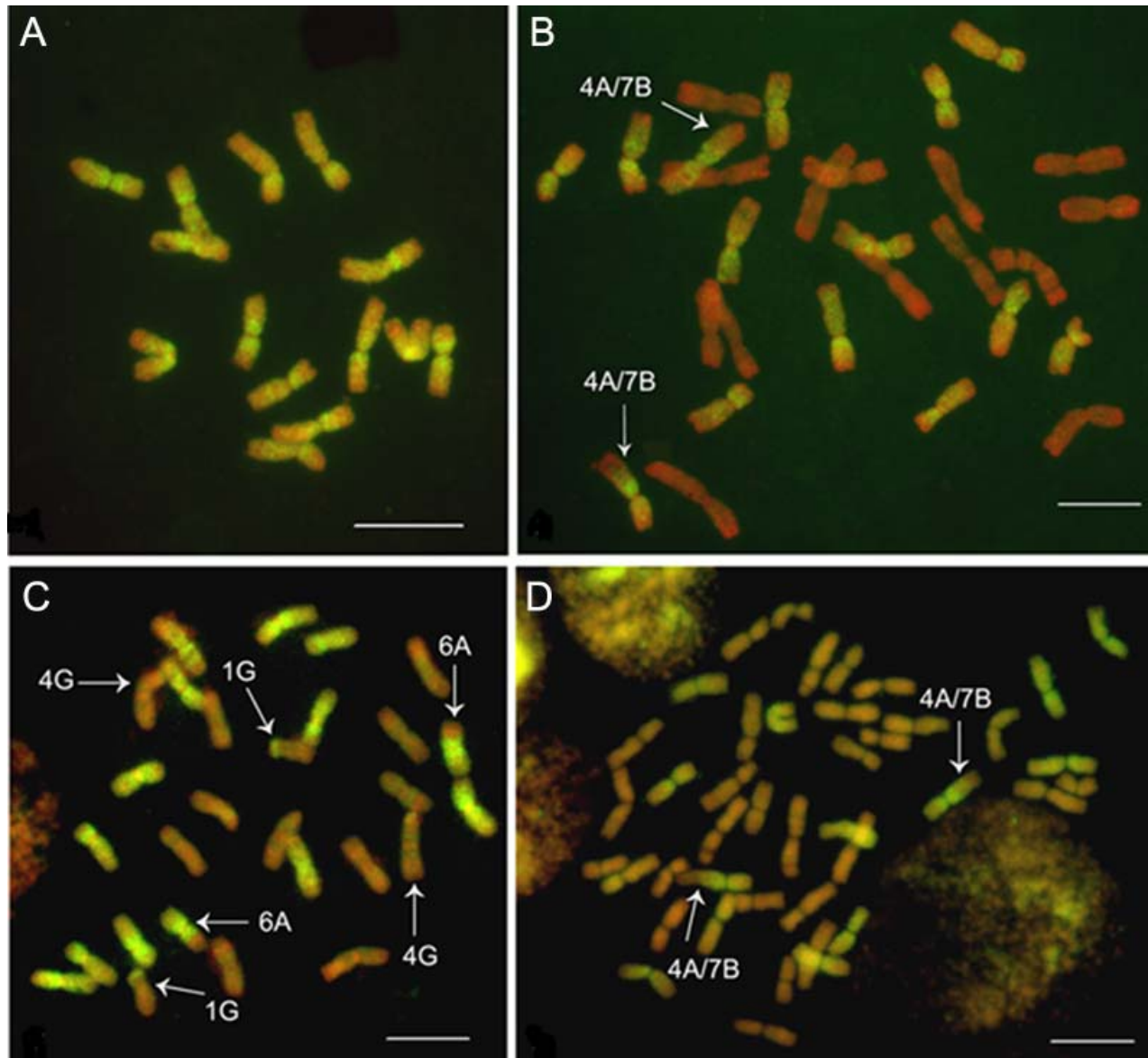
**III. Other retrotransposons
present in both the centromeric and
pericentromeric regions of the A
genome chromosomes**

1. Chromosome localisation of sub-clone C04 in *Triticum* species



A,B: *T. orientalis*
C: *T. araraticum*
D: *T. aestivum*

2. The **A** genome specific dispersed retrotransposon



A: *T. urartu*;
B: *T. dicoccoides*;
C: *T. araraticum*;
D: *T. aestivum*

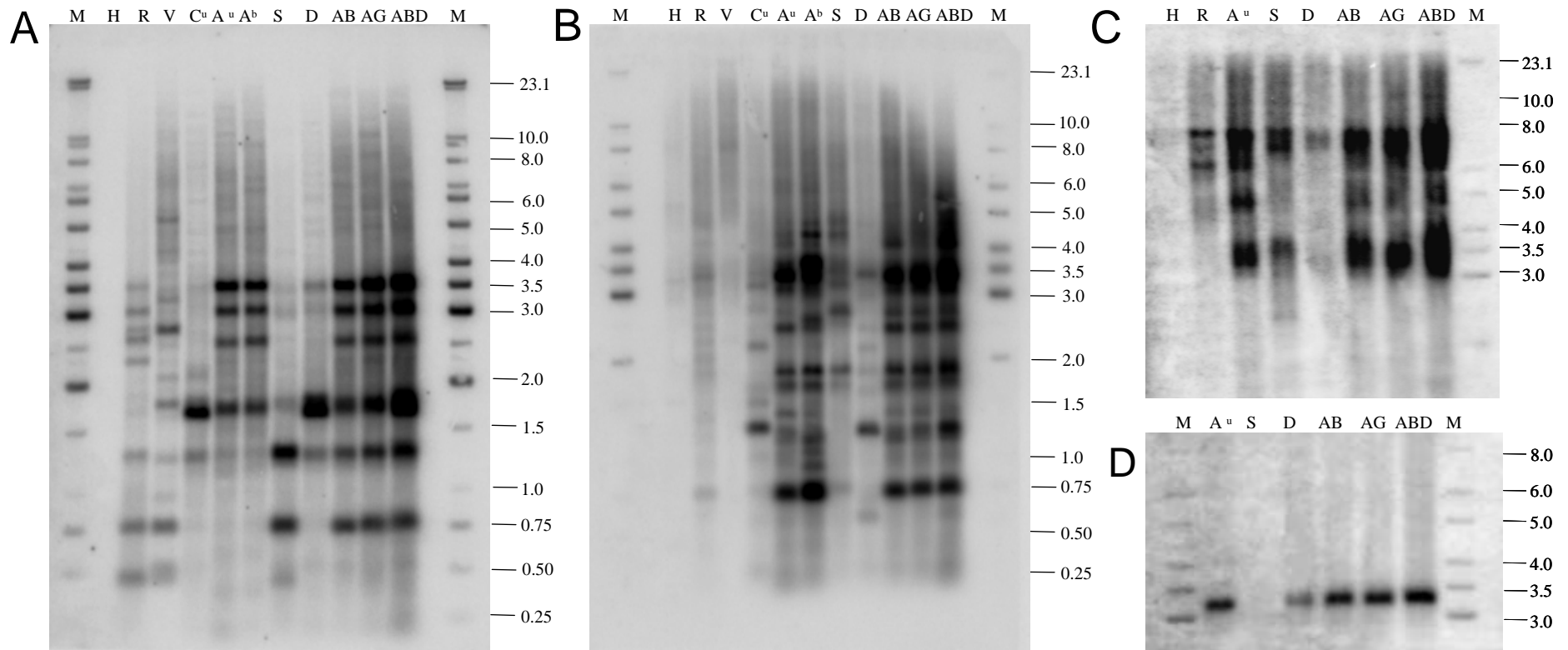


Fig. Distribution of A genome specific dispersed retrotransposons in wheat and its relatives.

A: Wgel_TbBAC5-1

B: Erika_TbBAC5-1

C: Sukkula_TbBAC5-1

D, E: Daniela_TbBAC5-1

F: CCS1

A, B, D-F: *HindIII* ,

C: *BamHI*

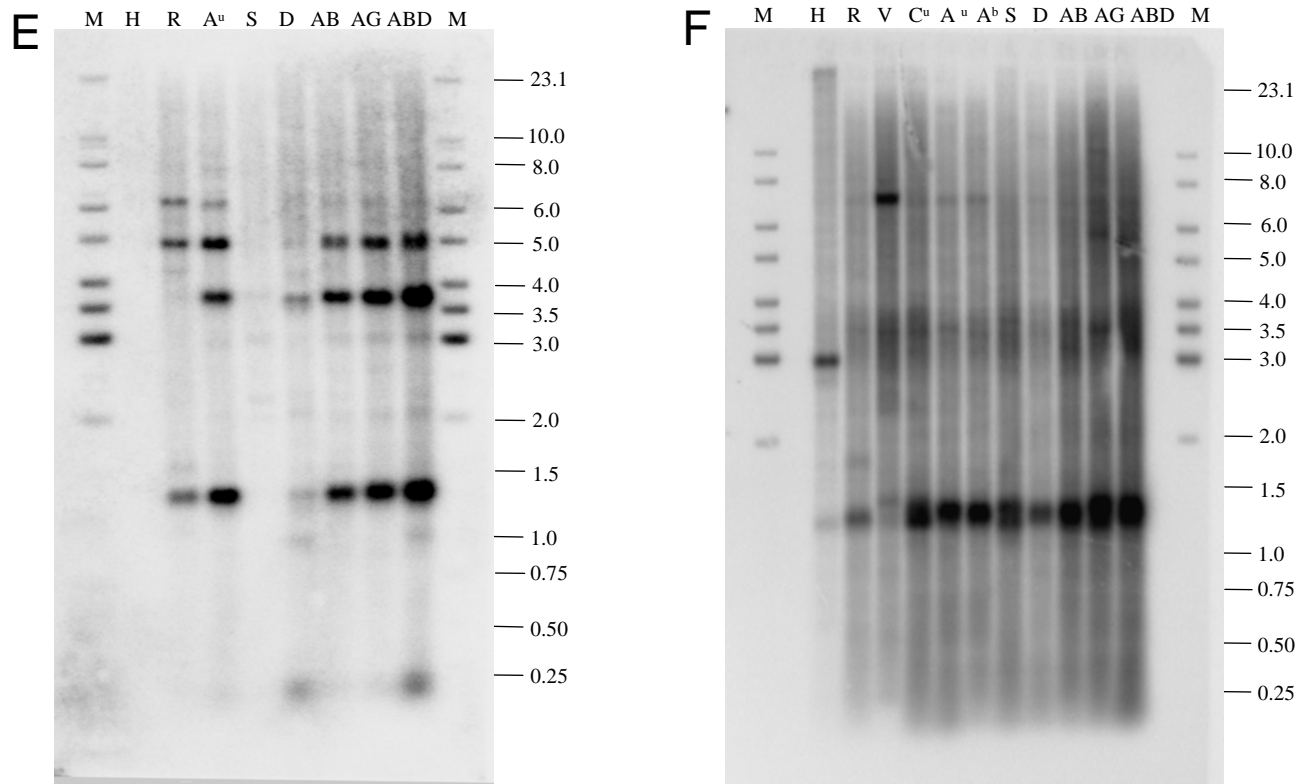


Fig. Distribution of A genome specific dispersed retrotransposons in wheat and its relatives.

A: Wgel_TbBAC5-1

B: Erika_TbBAC5-1

C: Sukkula_TbBAC5-1

D, E: Daniela_TbBAC5-1

F: CCS1

A, B, D-F: *HindIII* ,

C: *BamHI*

VI. Estimation of Invasion Time
of Retrotransposons Found
in *TbBAC5* & *TbBAC30*

The eight retrotransposons in BAC5 insert

Table 3.13 Retrotransposons in the BAC5 insert.

Code	Name	Location (bp)	Length (bp)	TSD	Time (MYA)
1	Angela-like-1	261-8,818	8,558	TAAAT	2.2
2	Erika-like-1	20,450-34,545	14,096	TTGGT	1.2
3	Wgel-2-like-1	34,546-41,429	6,884	-	-
4	Daniela-like-1	41,429-48,616	7,188	ATTCC	2.8
5	Cereba-like-3	51,798-56,422	4,625	-	-
6	Sukkula-like-1	60,278-71,439	11,162	GGCGG/T	2.0
7	Cereba-like-1	72,453-79,385	6,933	GATAC	3.5
8	Cereba-like-2	80,463-88,338	7,876	<u>ATCC(A)G</u>	1.1
Total			67,322		

1.1-3.5 MYA

Intact CRW in *TbBAC30* and *TbBAC5**

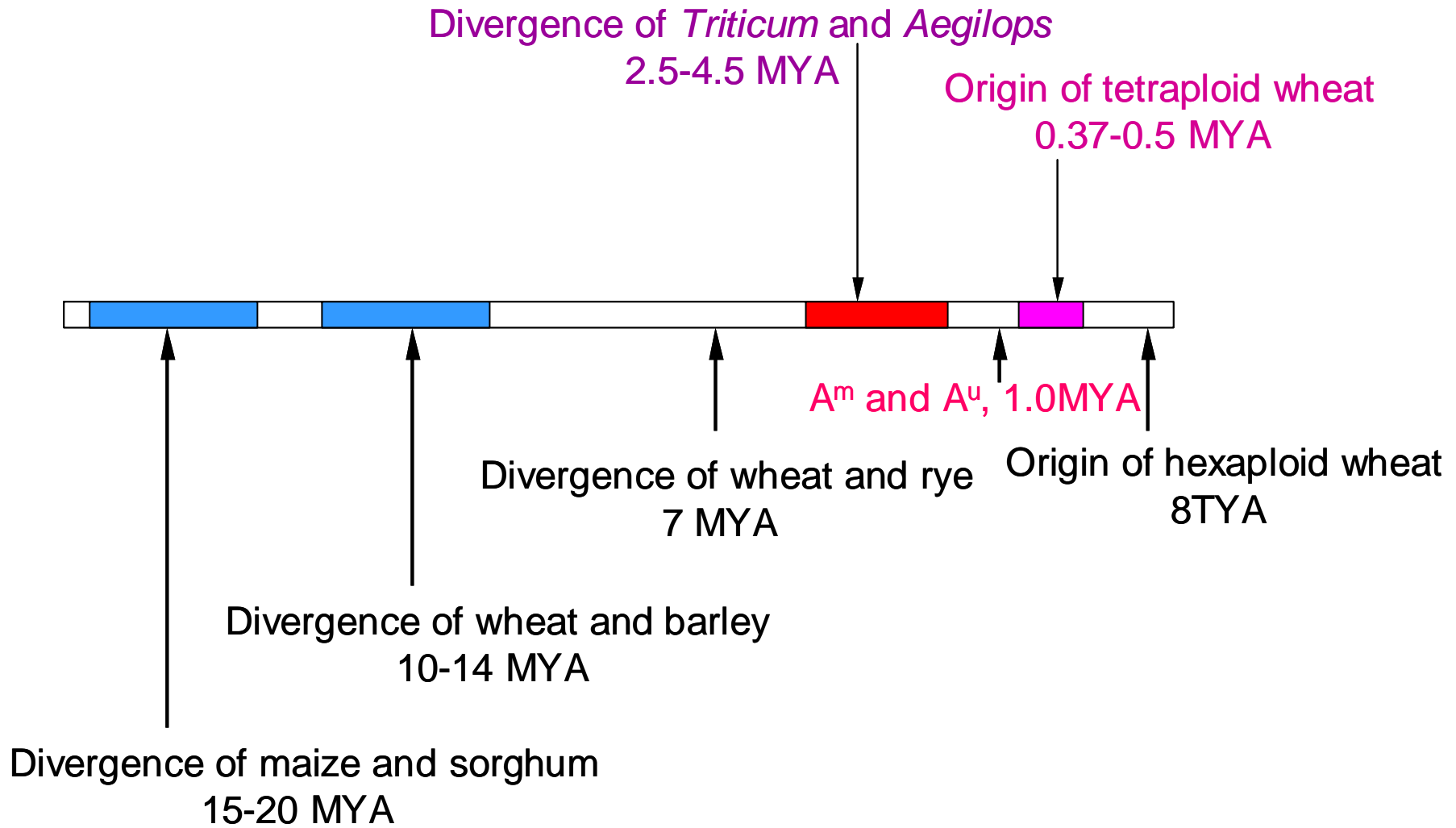
CRW	Length (bp)	LTR length (bp)	TSD	PBS	PPT	Insertion times (MYA)	Types
1	4305	981/981	CCTTC	AGTGGTATCAG-TTTTC	AAGAAGGGGAGGA	0.24	nonautonomous
2	7762	919/918	CCTAT	AGTGGTATCAGA-TTTC	AAGAAGGGGAGGA	0.58	autonomous
3	7861	918/918	CCTTA/G	AGTGGTATCAGATTT-C	AAGAAGGAGAGGA	0.75	autonomous
4	4776	982/982	ATTTG	AGTGGTATCA-ATTTTC	AAAAAGGGGAGGA	0.16	nonautonomous
5	4540	749/982	TAGCT	AGTGGTATCAG-TTTTC	AAAAAGGGGAGGA	0.86	nonautonomous
8	4769	982/983	TAGTC	AGTGGTATCAG-TTTTC	AAGAAGGGGAGGA	0.31	nonautonomous
2- <i>TbBAC5</i> *	7865	919/920	ATCC(A)G	AGTGGTATCAGA-TTTC	AAGAAGGGGAGGA	1.09	autonomous

TSD: Target site duplication, **PBS:** Primer binding site, **PPT:** Polypurine tract

MYA: means million years ago.

* Personal communication.

Figure for wheat genome differentiation



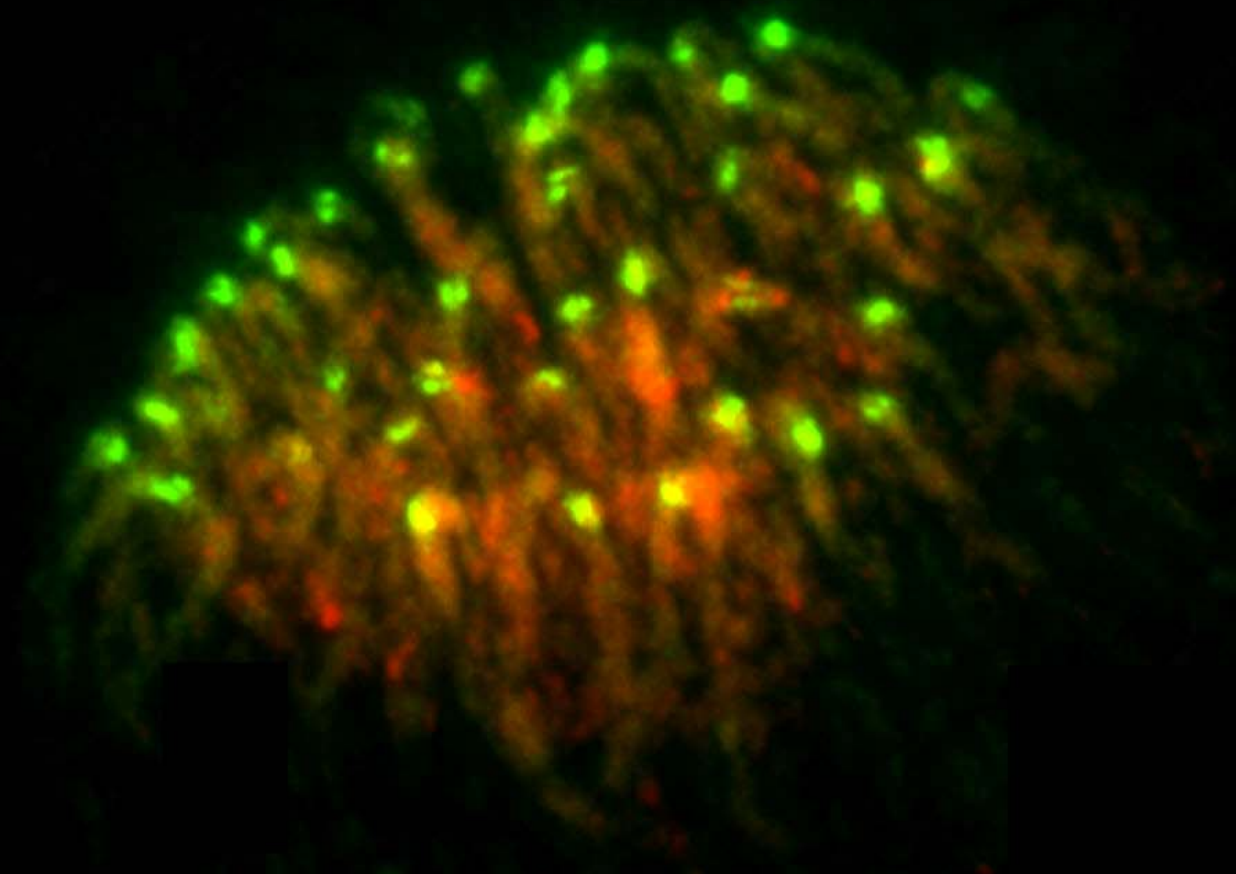
Huang et al., 2002; Gill et al., 2004; Dvorak et al., 2004

Conclusions

- The dominant role of the **CRWs** in determining the structure and function of the centromere was confirmed by fiber-FISH and CHIP.
- The **CRWs** were probably amplified during the polyploidization process leading to the formation of hexaploid bread wheat.
- **Wgel** retrotransposon is more abundant in the pericentromeric regions of the **A**, than in those of either the **B** or **D** genome chromosomes, and this variation may contribute to the inhibition of homoeologous chromosome pairing.







Thank you for your attention!