**Supplementary Materials for**

**Cloning and physical localization of male-biased repetitive DNA sequences in spinach**

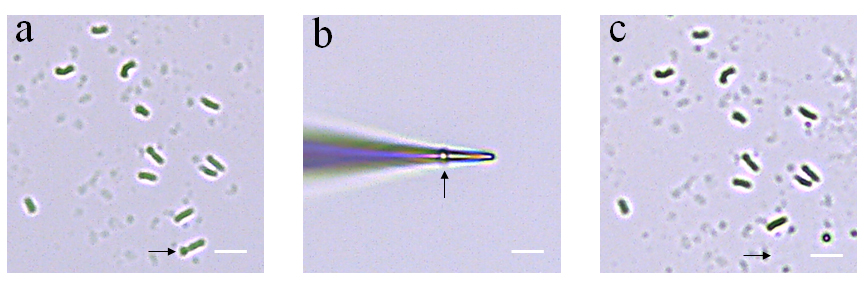
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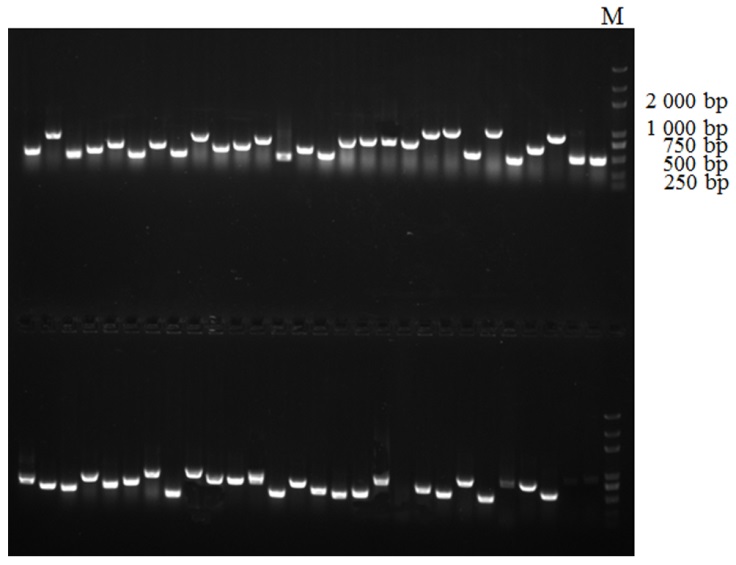
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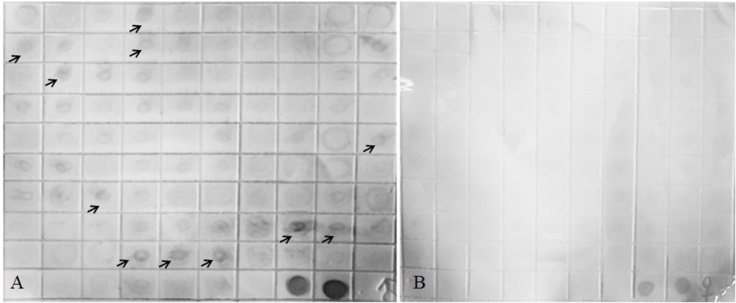
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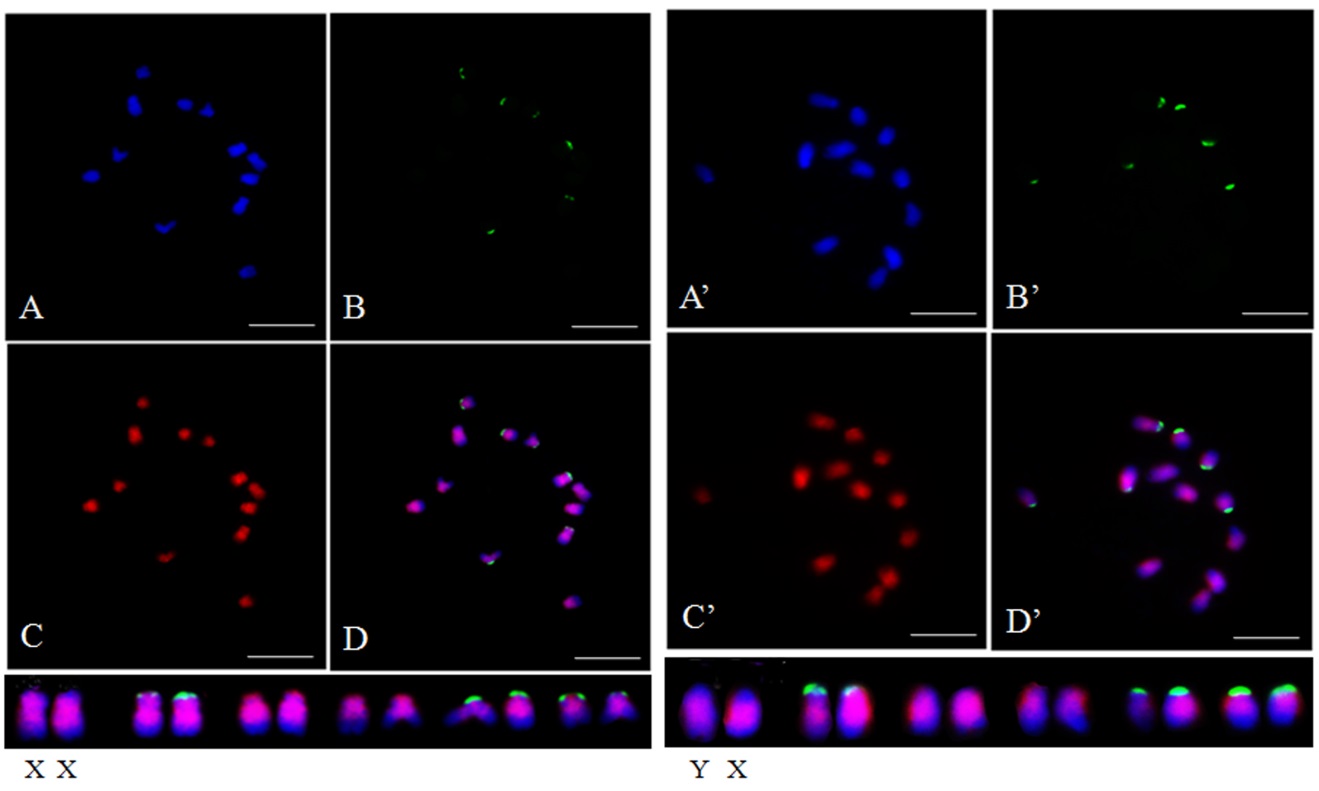
\*\*Co-first author

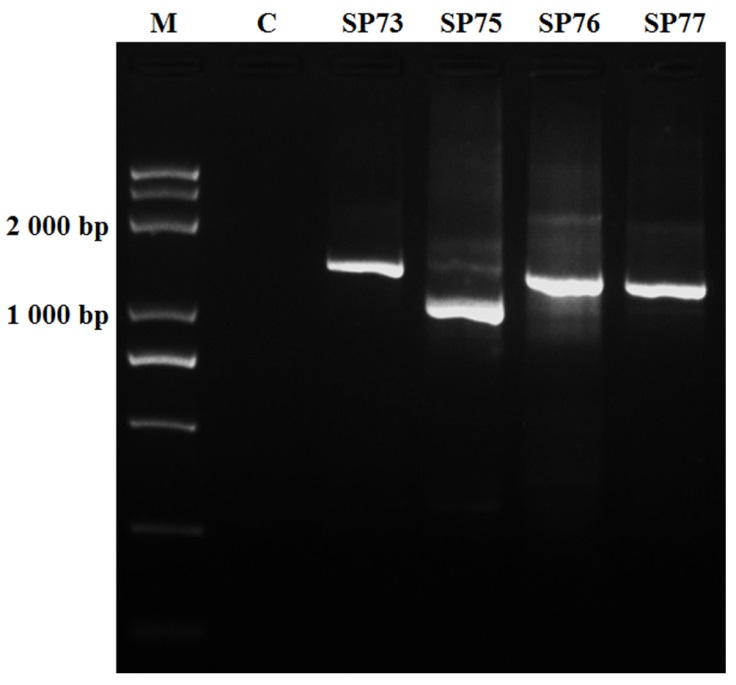


**Supplemental Figure 1 Procedure of isolation of biggest chromosome in spinach by micromanipulator.** a Mitosis metaphase chromosomes of a root tip cell before chromosome isolation (Arrow showed the biggest chromosome), b the target chromosome adhering to the tip of a glass needle, c metaphase chromosomes after isolation of the chromosome indicated by arrow in a by a glass needle. Bar 10 μm

**Supplemental Figure 2 Partial PCR products of recombinant clones using M13R and M13F as primers.** M: Trans 2K Plus DNA Marker.

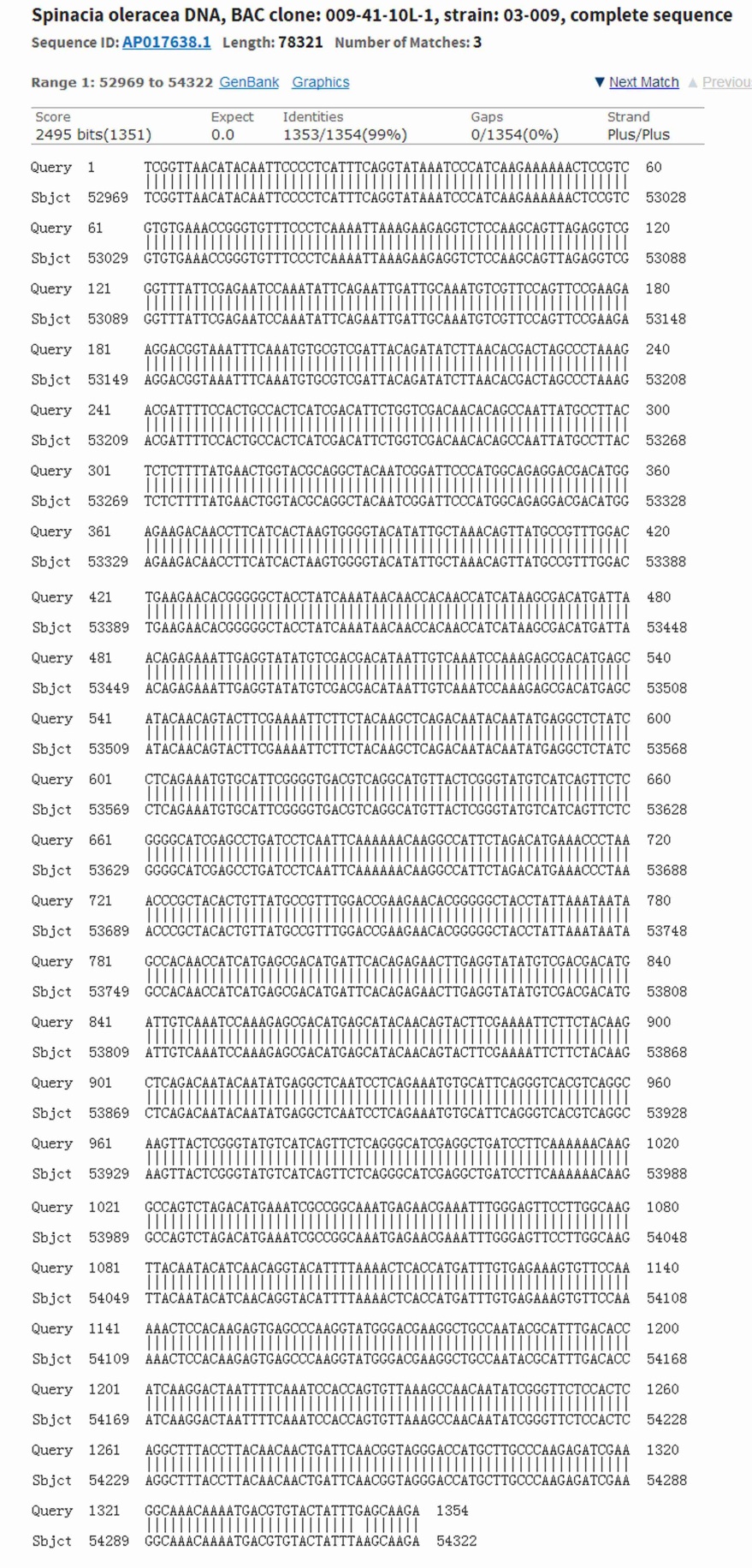
**Supplemental Figure 3 Dot blot hybridization results of partial subtractive hybridization clones.** Dot blot hybridization with DIG-labeled the genome DNA of male spinach (A) and the genome DNA of female spinach (B); Arrows showed male biased dots.

**Supplemental Figure 4 Distribution patterns of hybridization signals from female and male spinach using 45S rDNA (green) and SP1-86 (red) as probes.** A (A’), DAPI; B (B’), 45S rDNA (green) as probe; C (C’), SP1-86 (red) as probe; D (D’), The merged figure of A (A’), B (B’) and C (C’). (Bar=10 μm)

**Supplemental Figure 5 PCR amplification result of SP73, SP75, SP76 and SP77 marker.** M, Ttrans 2K Plus DNA Marker; C, negative control.



**Supplemental Figure** **6 Pair-wise alignment between SP73 and BAC clone 009-41-10L-1.**



**Supplemental Figure 7 Pair-wise alignment between SP75 and BAC clone 009-41-10L-1.**

**Supplemental Table 1 Primer sequences for repetitive DNA sequences.**

|  |  |  |
| --- | --- | --- |
| Primer | 5’→3’ | Annealing temperature |
| 73-F | GTATTTCCAGCTAAATATGTTGT | 51℃ |
| 73-R | CTAATCAAAACGGATGTGGT | 51℃ |
| 75-F | CTTGCTCAAATAGTACACGTC | 54℃ |
| 75-R | TCGGTTAACATACAATTCCC | 51℃ |
| 76-F | ATTTCAAAACACGATGCTC | 52℃ |
| 76-R | TAGAGGCCATATATAGACTCA | 52℃ |
| 77-F | TATACTGGCATCTGTATTCCC | 52℃ |
| 77-R | TGGTATTAAATACAGACAACCGA | 54℃ |

