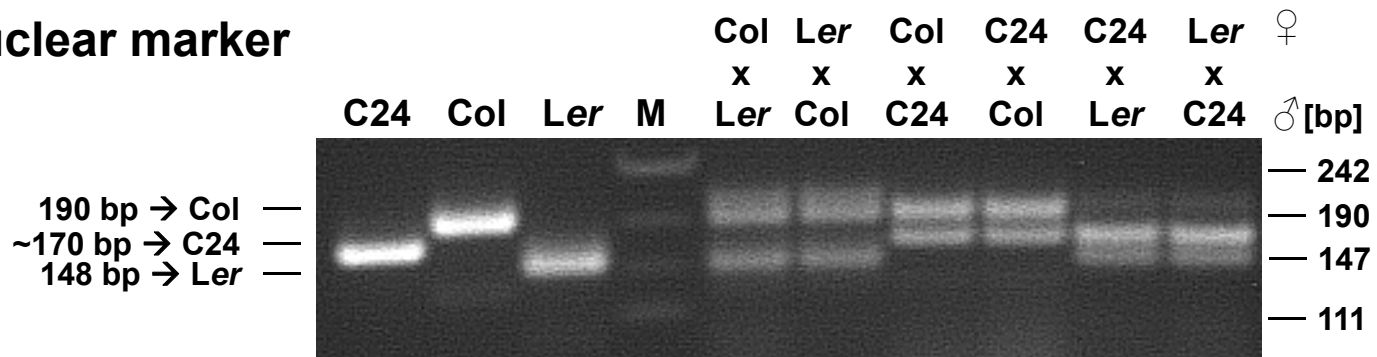
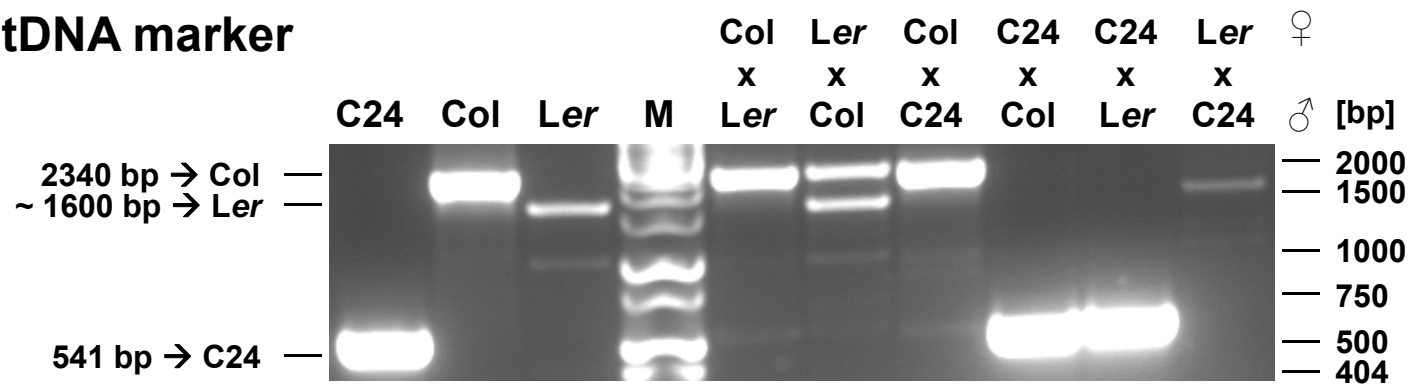
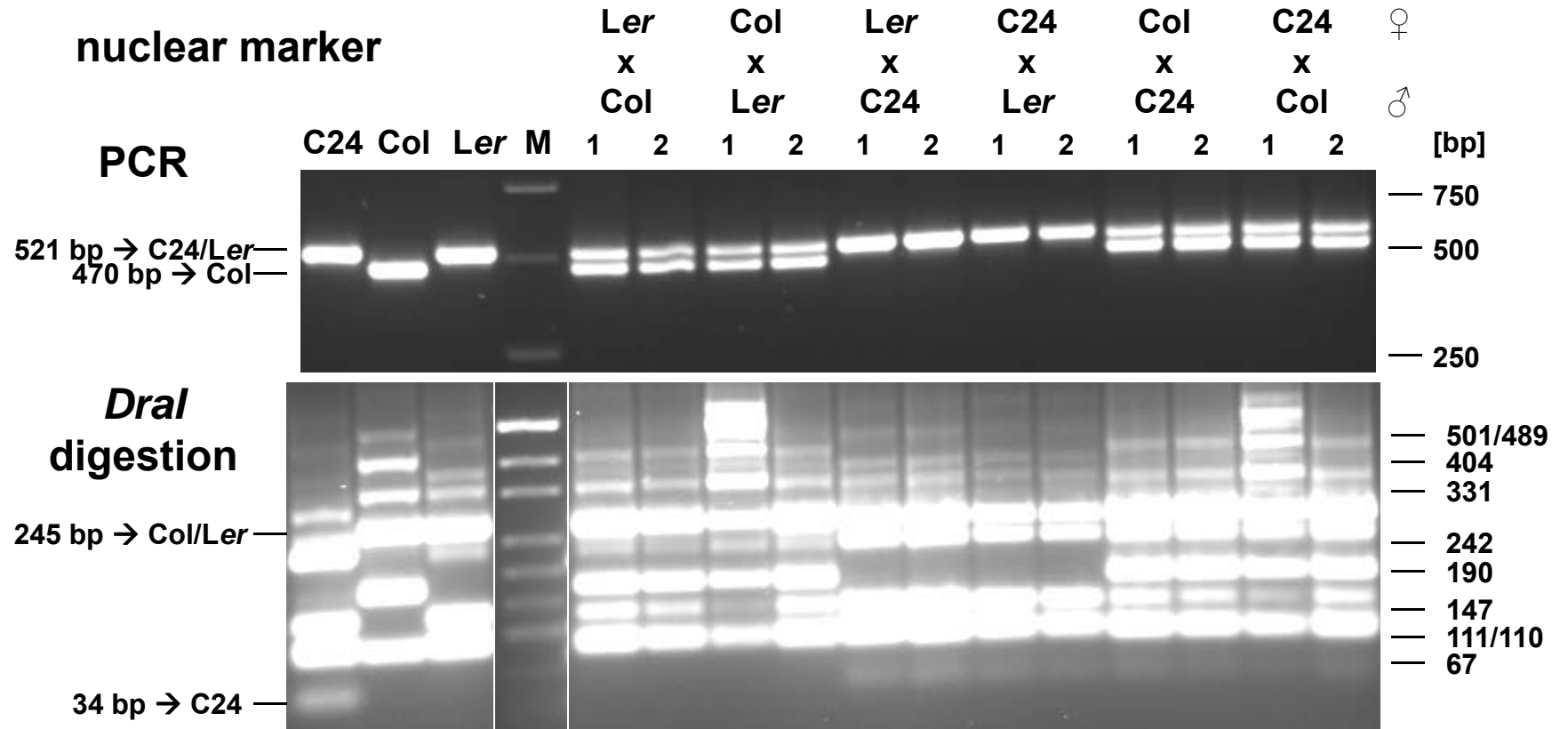
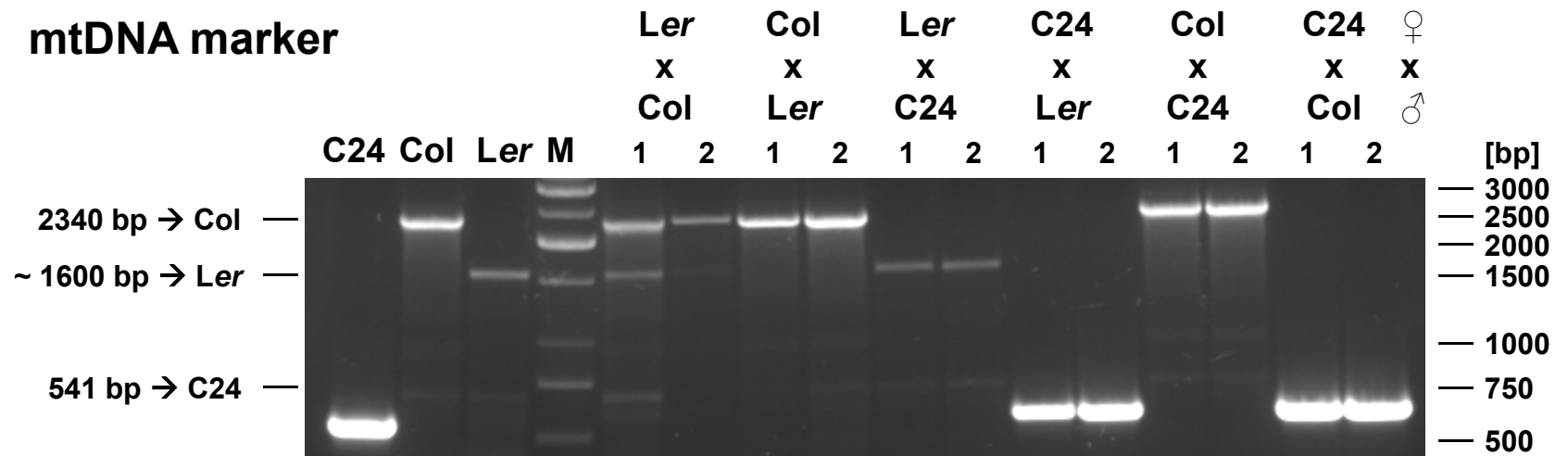


**A****nuclear marker****B****mtDNA marker**

**C****nuclear marker**

**D****mtDNA marker**

## Genotyping of F<sub>1</sub> plants and parental lines.

The genetic status of the F<sub>1</sub> hybrid plants and the parental lines. F<sub>1</sub> hybrids used for segregation analysis of the *nad9*(-202), *ccmB*(-200/-231), *rpl5*(-459:-406), *ccmC* (-484/2 and -391/0), *atp6-2* [(A) and (B)] and *nad4*(-228) [(C) and (D)] transcript polymorphisms, respectively, were checked for mitochondrial and nuclear marker.

(A) Nuclear marker CER479928 (Jander et al., 2002). In *Ler*, the nucleotides located between positions 20,610,105 and 20,610,148 on chromosome 1 (database entry nc\_003070.5) in *Col* are deleted. This region was amplified with primers CER479928.H 5'-cagttccaatgagcaatgtacacatgg-3' and CER479928.R 5'-ccttgttgatgtaaaatagtttcgggtcc-3' giving rise to PCR products of 190 bp, ~170 bp and 148 bp in *Col*, C24 and *Ler*, respectively. All hybrids investigated display two products each of the expected sizes, which are also found in the respective parental lines. This confirms the expected genetic status of the parental lines as well as of the hybrid plants. Template: residual DNA in total RNA preparations. M: marker (sizes indicated at the right margin).

(B) To confirm that the F<sub>1</sub> hybrids were indeed derived from reciprocal crosses, the origin of the mitochondrial DNA was investigated using the polymorphic mtDNA region upstream of the *cox3* gene (Forner et al., 2005). PCR was carried out with primers Atcox3-3 5'-tagttctggggaggttggtc-3' and ATB2174-1 5'-gtagcccaggatccatccc-3'. Expected sizes of the PCR products are 2340 bp in *Col*, 541 bp in C24 and appr. 1600 bp in *Ler*. All PCRs yielded the expected products confirming their expected maternal origin of the mtDNA. The presence of the *Col*-specific product in the *Ler* x *Col* hybrid is due to the mtDNA insertion in chromosome 2 in *Col*. Template as in A.

(C) Nuclear marker MASC04686 (Törjék et al., 2003) located on chromosome 4. PCR was performed on total DNA with primers MASC04686.H 5'-cggctctgattcactcaacg-3' and MASC04686.R 5'-ttcccactcgggattcgtag-3'. PCR product sizes were 470 bp in *Col* and 521 bp in C24 and *Ler* (upper panel). As the indel polymorphism cannot discriminate between the C24 and *Ler* alleles, PCR products were subsequently purified and digested with *Dra*I (lower panel). The C24-specific 34 bp digestion product and the 245 bp digestion product absent in this accession together with the PCR product size difference allow to unambiguously distinguish between the three parental lines and the distinct F<sub>1</sub> hybrids.

(D) Analysis of the origin of the mitochondrial DNA in the F<sub>1</sub> plants used for investigation of the *nad4* transcripts. For details see (B). Template: total DNA

Exact sizes of the PCR products calculated from the DNA sequences are given in bp.