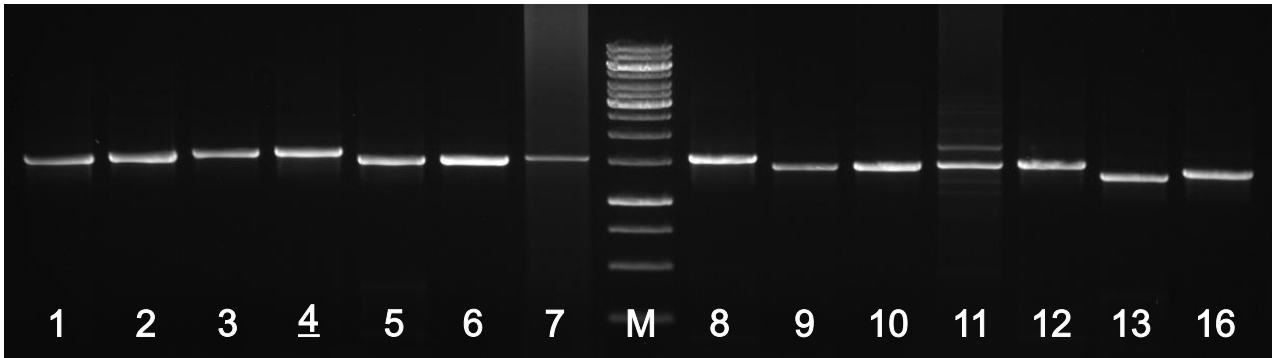
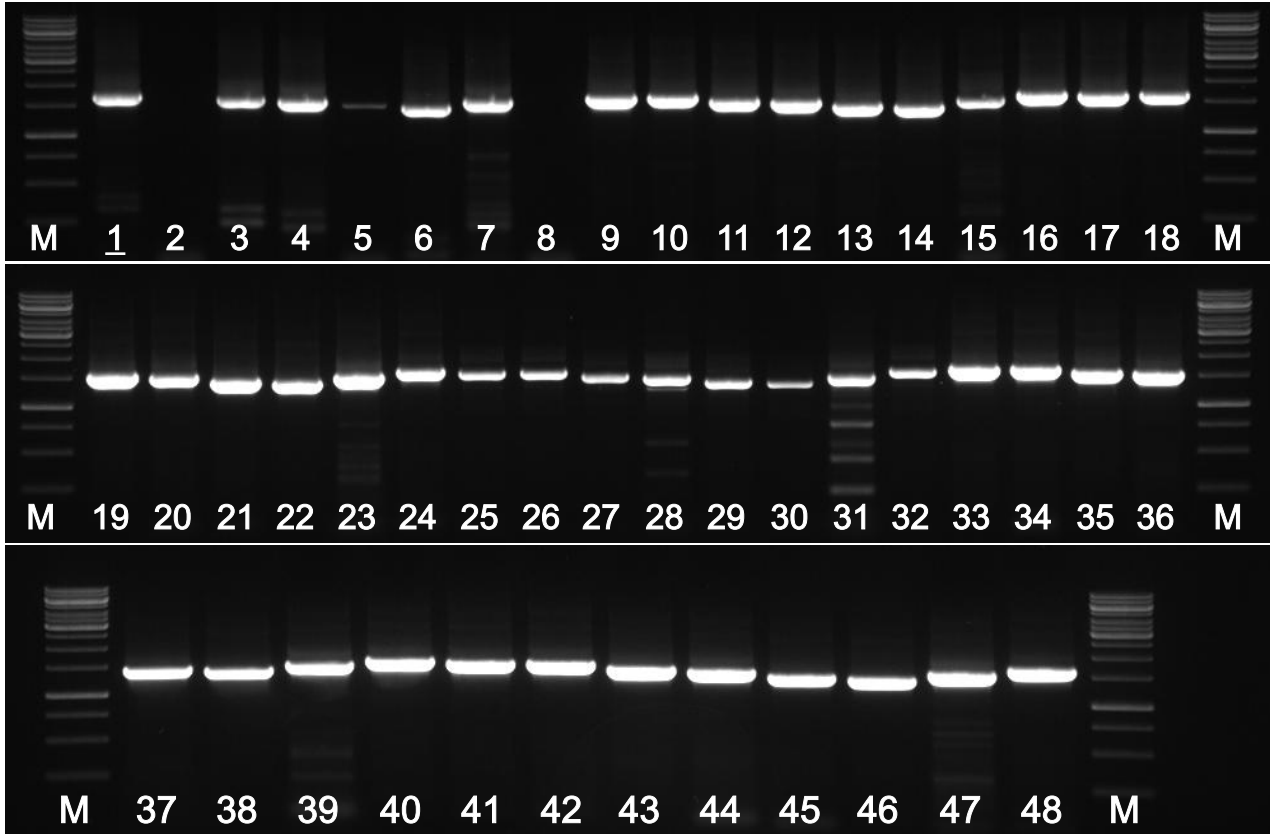


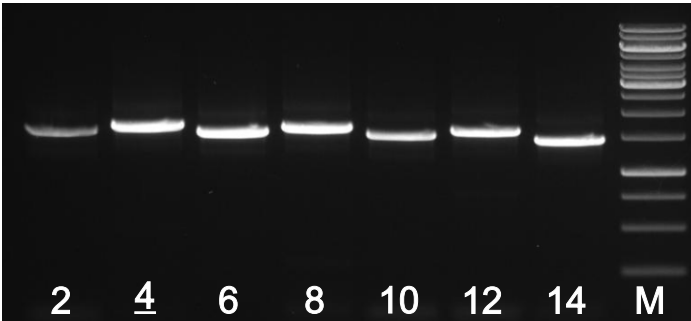
a)



b)



c)



d)

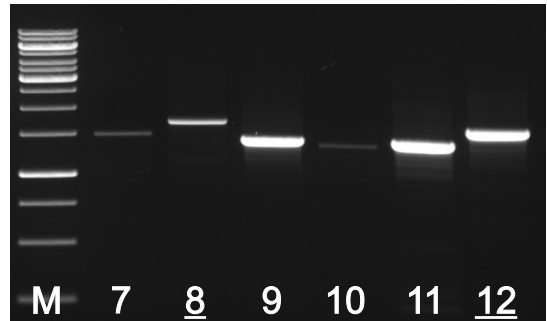


Figure S1. Representative cropped agarose gels of CR1/CR2 amplicons obtained for *Struthio camelus* (a), *Rhea pennata* (b), *Rhea americana* (c) and *Crypturellus tataupa* (d). The numbering of amplicons separated in the agarose gel corresponds to the reaction numbered in Table S1. The numbers of PCR fragments, which were finally sequenced, are underlined. Lane M - GeneRuler 1 kb DNA Ladder (Thermo Scientific). Original uncropped and unprocessed gels are presented in Fig. S13a-d.

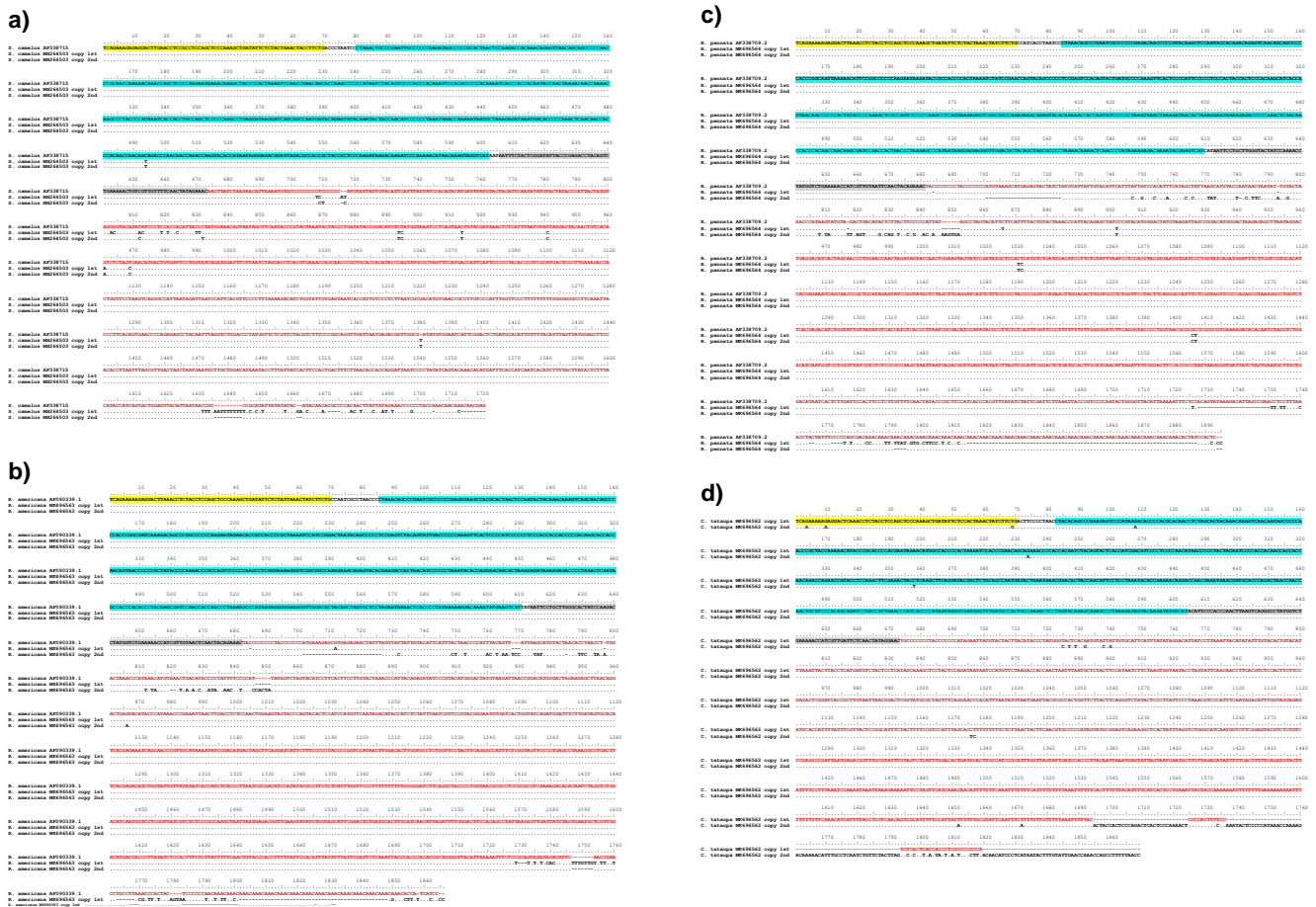


Figure S2. Sequence comparison of two copies of *tRNA-Pro*, *ND6*, *tRNA-Glu* genes and control region, found in this study as duplicated, with appropriate sequences of mitogenomes previously deposited in GenBank and showing a typical avian gene order. The alignments are shown for *Struthio camelus* (a), *Rhea americana* (b), *Rhea pennata* (c) and *Crypturellus tataupa* (d). Dots indicate residues identical in the obtained copies with those in the single sequence previously published. **Genes for *tRNA-Pro* are marked in yellow coloring, *ND6* dehydrogenase subunit 6 in cyan coloring, *tRNA-Glu* in grey shading, and control regions in red fonts.** In the case of *Crypturellus tataupa* only two obtained copies are compared to each other because the previously published mitogenome of these taxa is incomplete and it does not contain any of the analyzed genes or the control region. The sequences were aligned with MUSCLE [125] in MEGA [124].

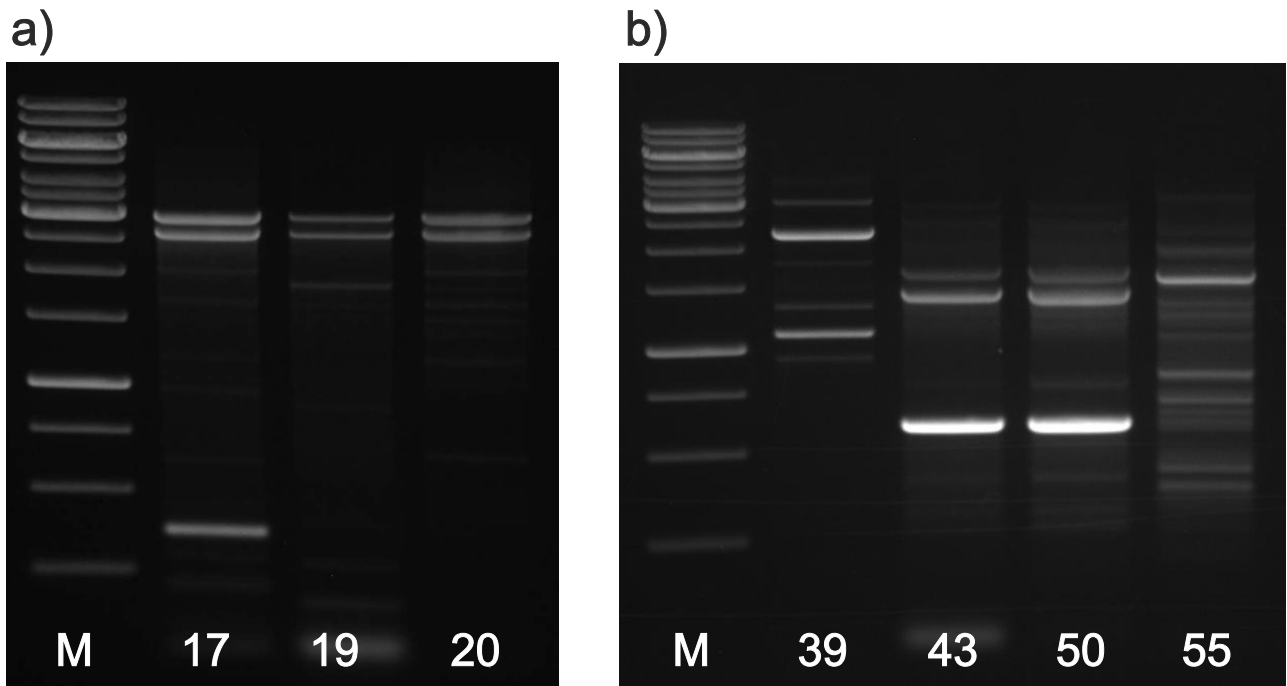


Figure S4. Cropped agarose gels of potential NUMTs amplified for *Dromaius novaehollandiae* (a) and *Casuarius casuarius* (b) with the use of primers designed for detection of CR1/CR2 fragments. The numbering of lanes corresponds to the reaction numbered in Table S1. Lane M - GeneRuler 1 kb DNA Ladder (Thermo Scientific). Original uncropped and unprocessed gels are presented in Fig. S13e and f.

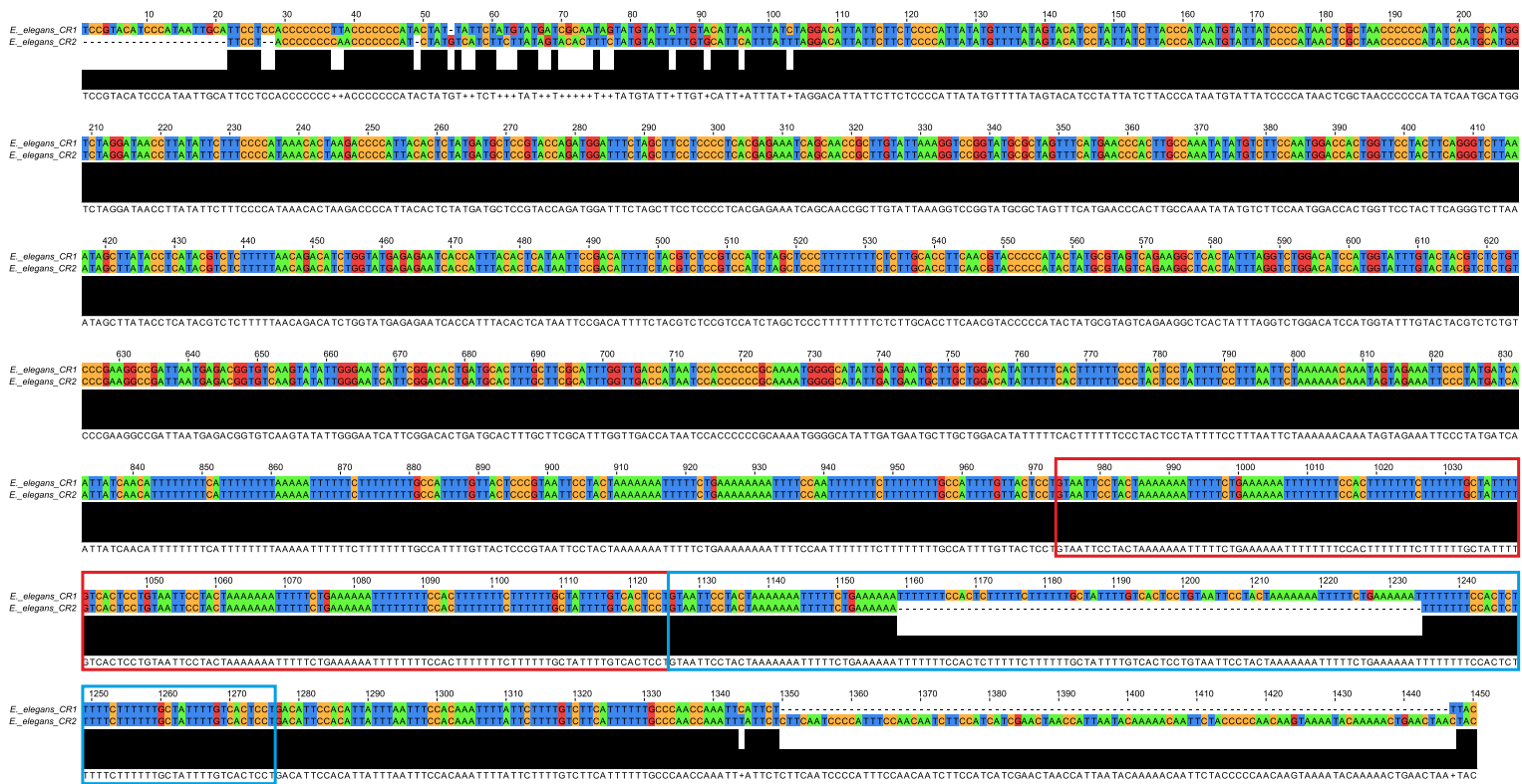


Figure S6. Sequence comparison of two *Eudromia elegans* control regions. Dots indicate residues identical in the compared copies. Dots indicate residues identical in the compared sequences. Sequence repeats present in both control regions are marked in red and blue boxes. The sequences were aligned with MUSCLE [125] in MEGA [124].

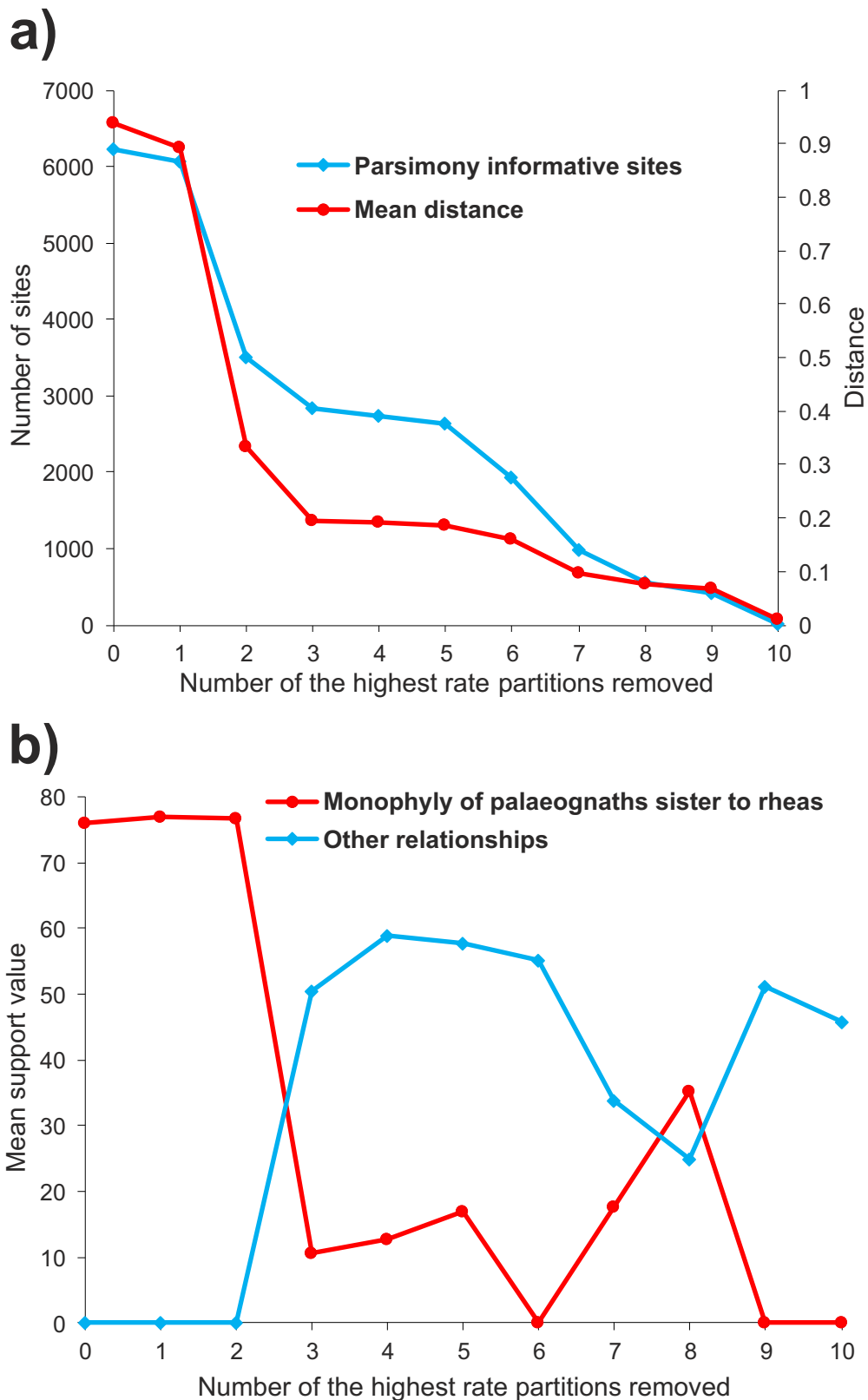


Figure S7. Influence of removing partitions with the highest substitution rate on alignment and tree parameters: the number of parsimony informative sites and mean distance (a) as well as mean support values (b). The mean phylogenetic distance was obtained from MrBayes tree. The mean support values were calculated from posterior probabilities received in MrBayes and PhyloBayes as well as SH-aLRT and non-parametric bootstrap percentages obtained in IQ-TREE. The posterior probabilities were scaled to 100%.

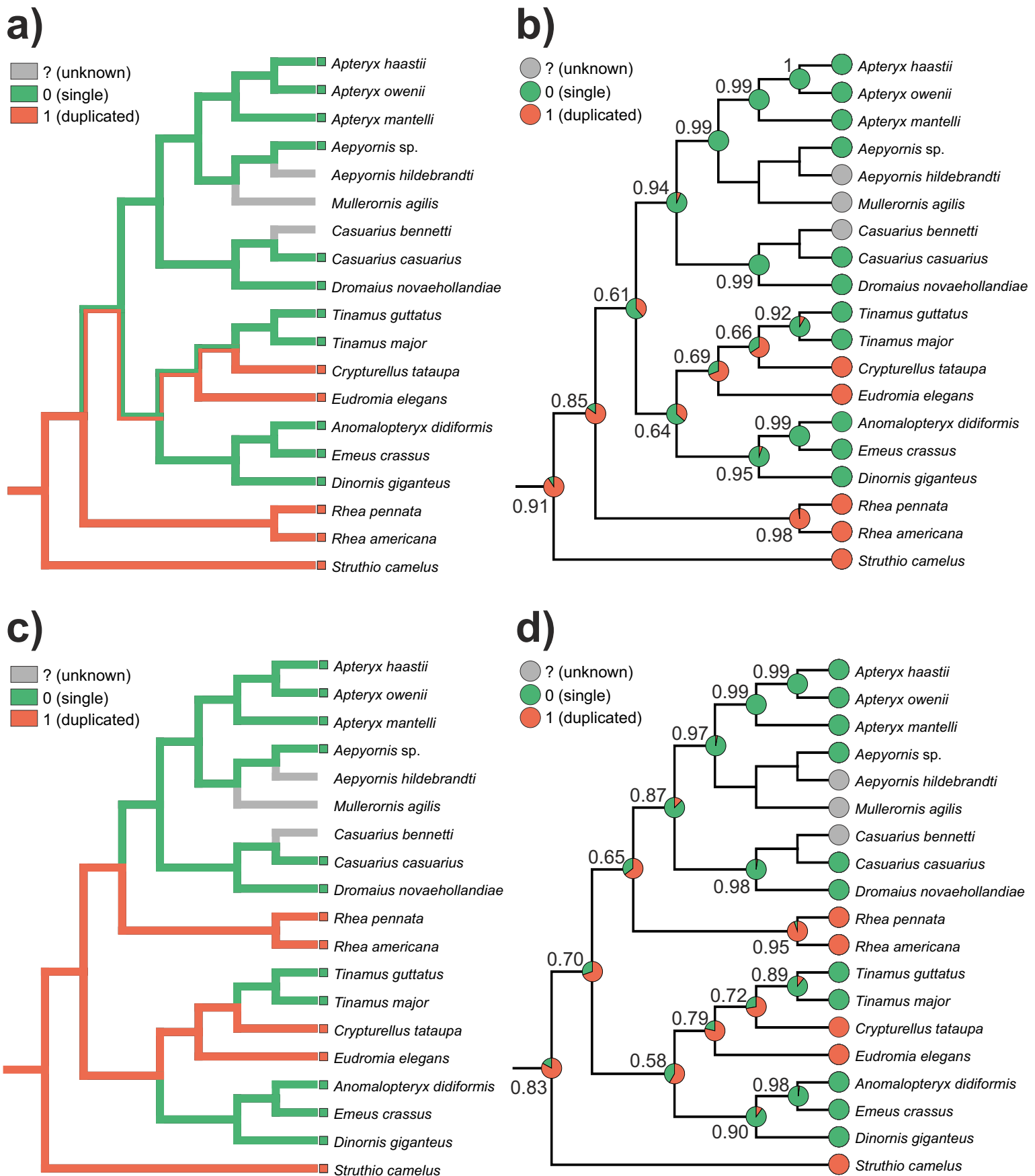


Figure S8. Maximum parsimony (a, c) and maximum likelihood (b, d) reconstruction of ancestral states and mapping of mitogenomic duplications onto the Palaeognathae tree topology t1 based on sequences of mitochondrial genes (a, b) and the Palaeognathae tree topology t2 supported by nuclear markers in coalescent-based approaches (c, d). In contrast to Fig. 6, this approach assumes that *Anomalopteryx didiformis*, *Emeus crassus* and *Dinornis giganteus* already lost mitogenomic duplication. The area of colors at nodes in b and d corresponds to the probability of the given state, single or duplicated region. Two-colored branches correspond to the equal probability of two states, single or duplicated region. The probability value for a more likely state was also given at these nodes. Mk1 model was applied for ML approach.

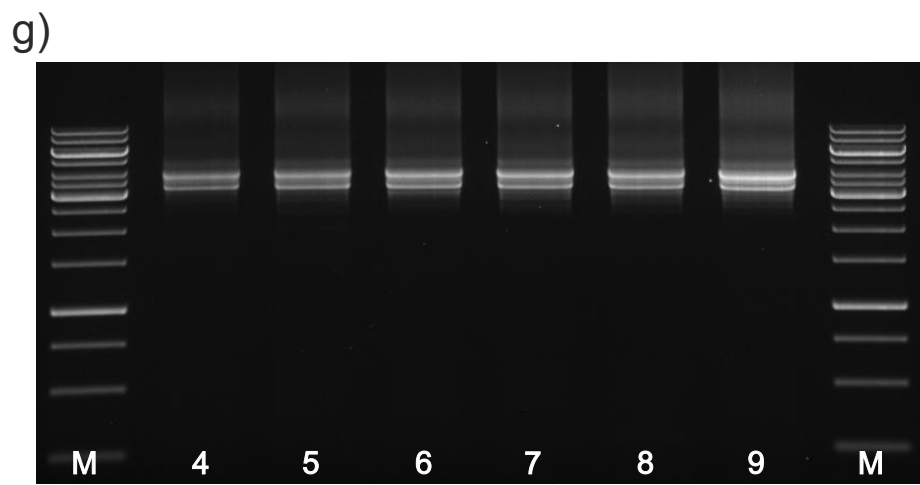
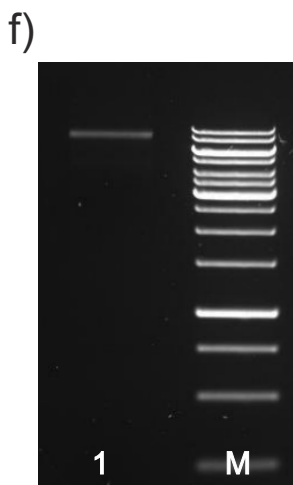
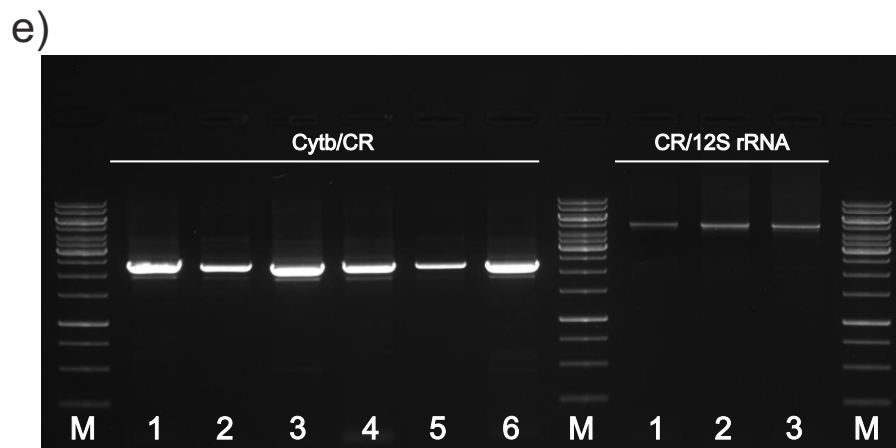
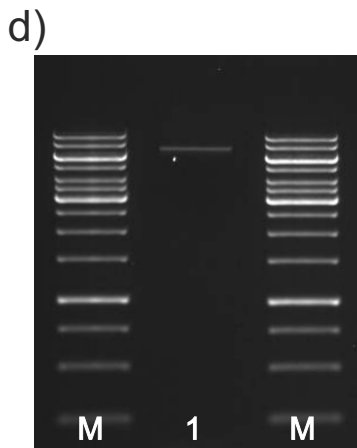
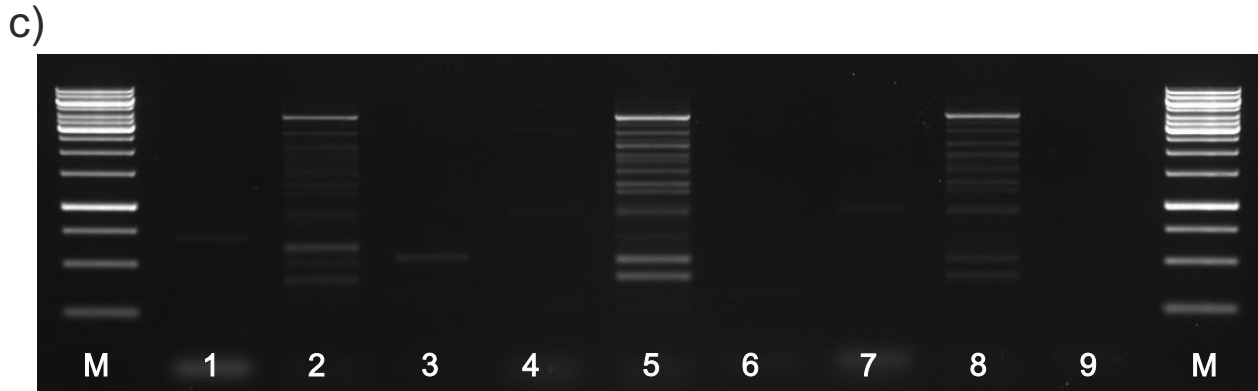
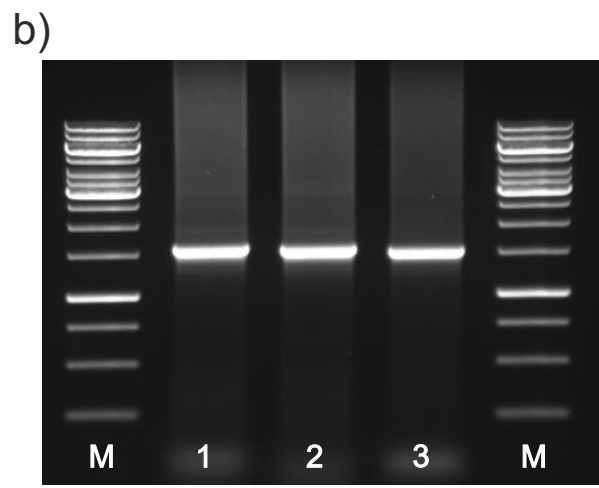
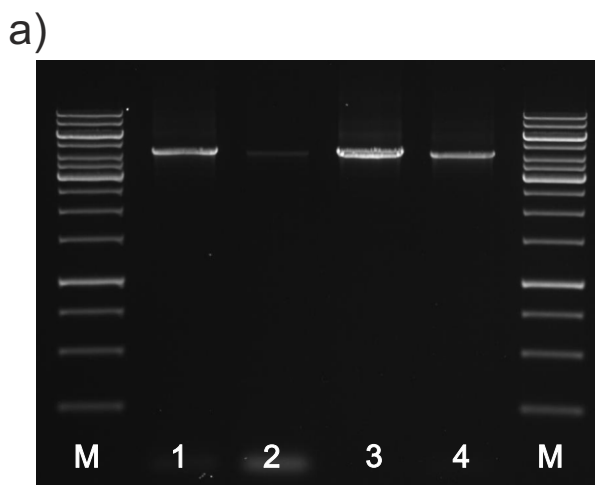
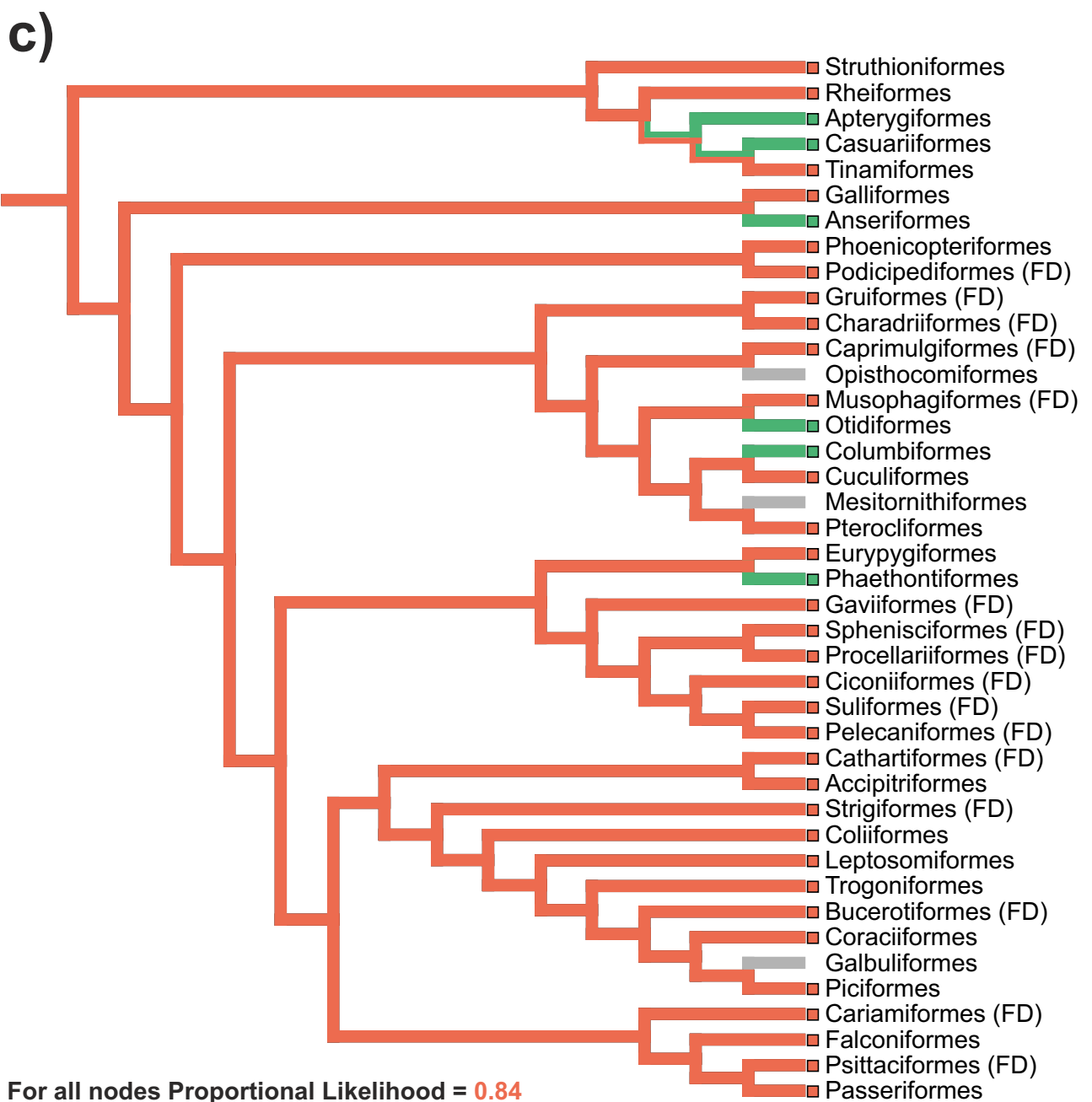
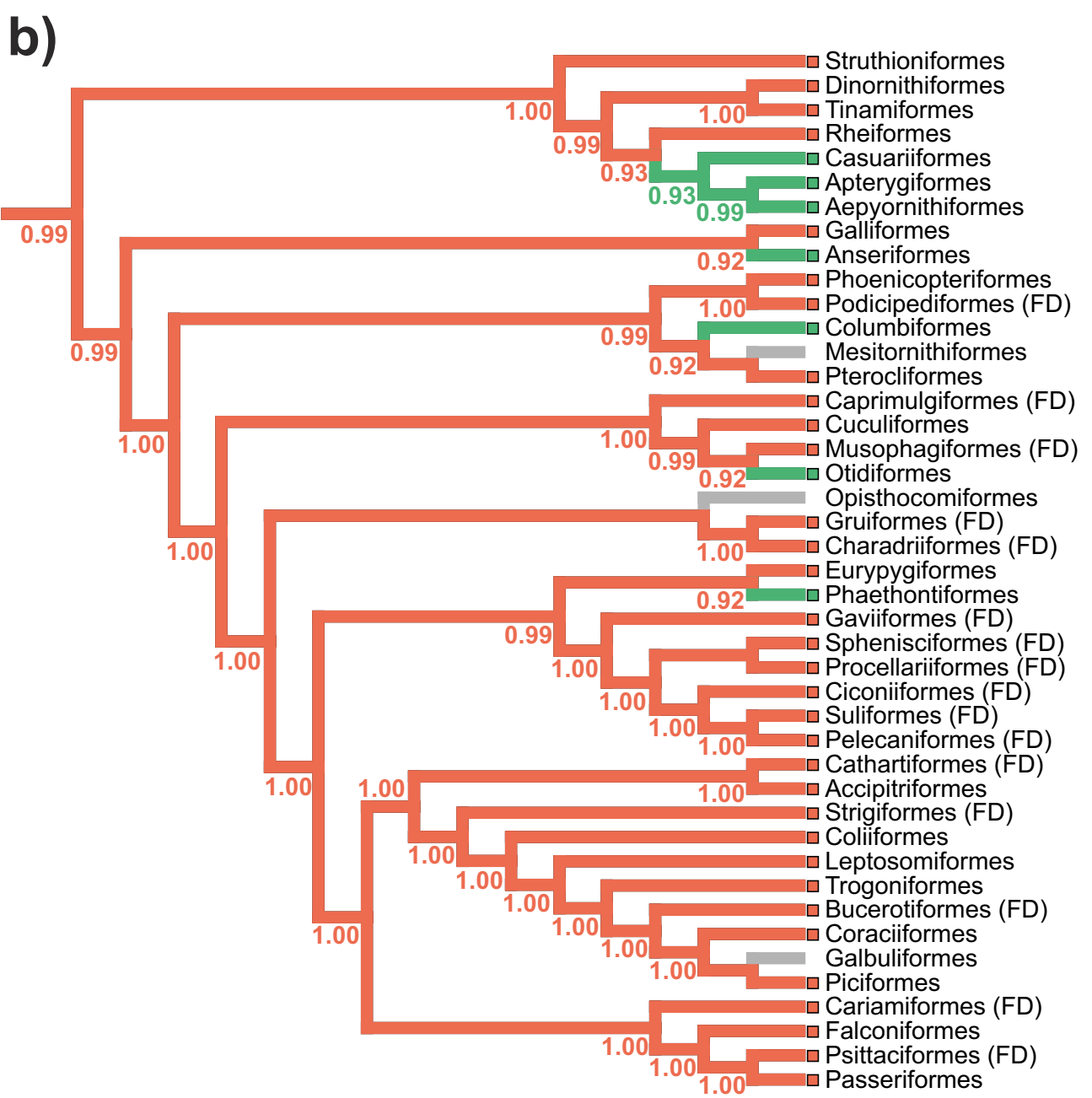
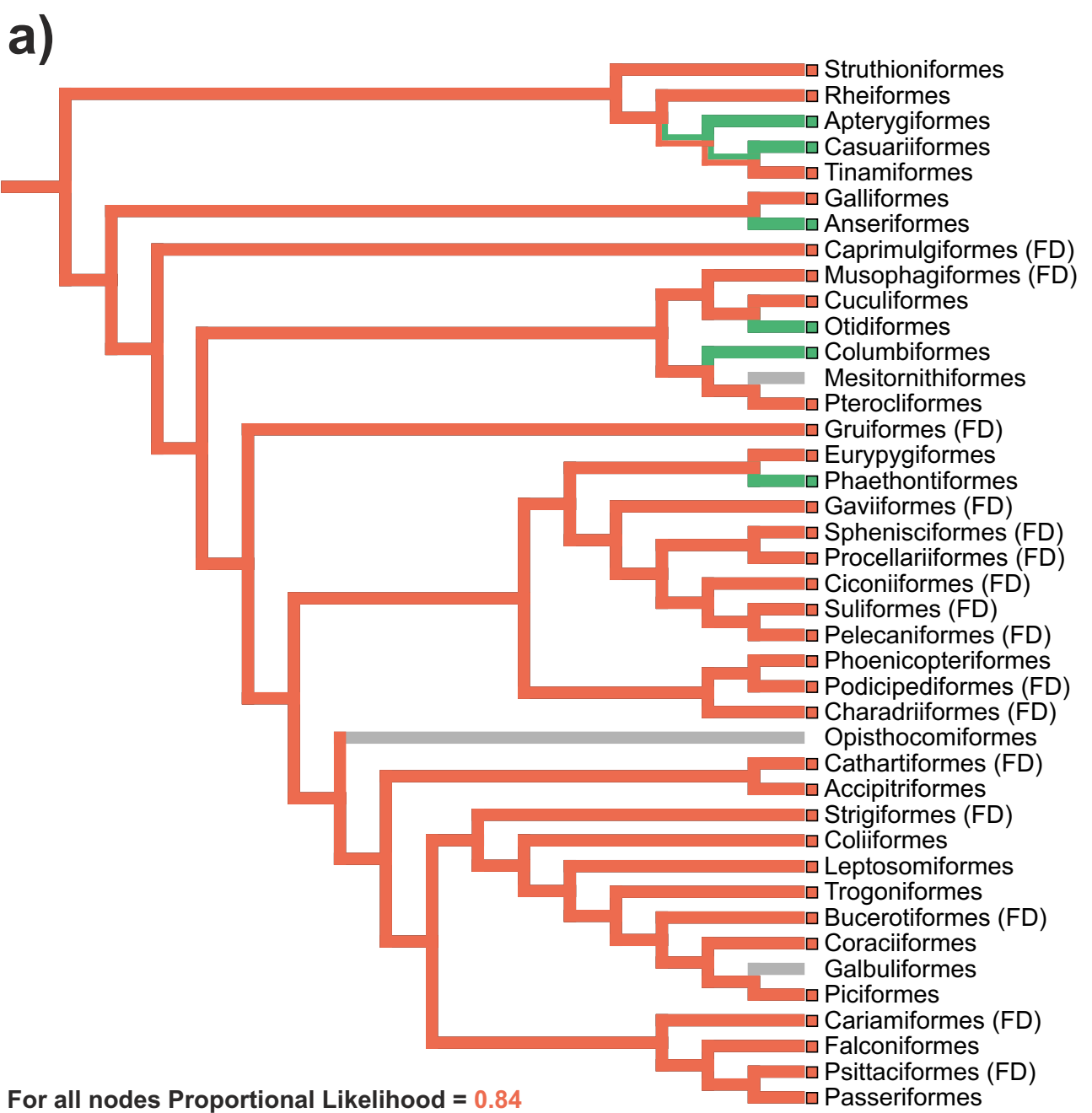


Figure S11. Representative cropped agarose gels of the following amplicons: *cytb*/12S rRNA obtained for *Rhynochetos jubatus* (a), *Eurypyga helias* (d) and *Trogon collaris* (f); CR1/CR2 obtained for *Rhynochetos jubatus* (c) and *Trogon collaris* (g); CR/12S rRNA obtained for *Rhynochetos jubatus* (b) and *Eurypyga helias* (e); *cytb*/CR obtained for *Eurypyga helias* (e). The numbering of amplicons separated in the agarose gel corresponds to the reaction numbered in Table S9. Lane M - GeneRuler 1 kb DNA Ladder (Thermo Scientific).

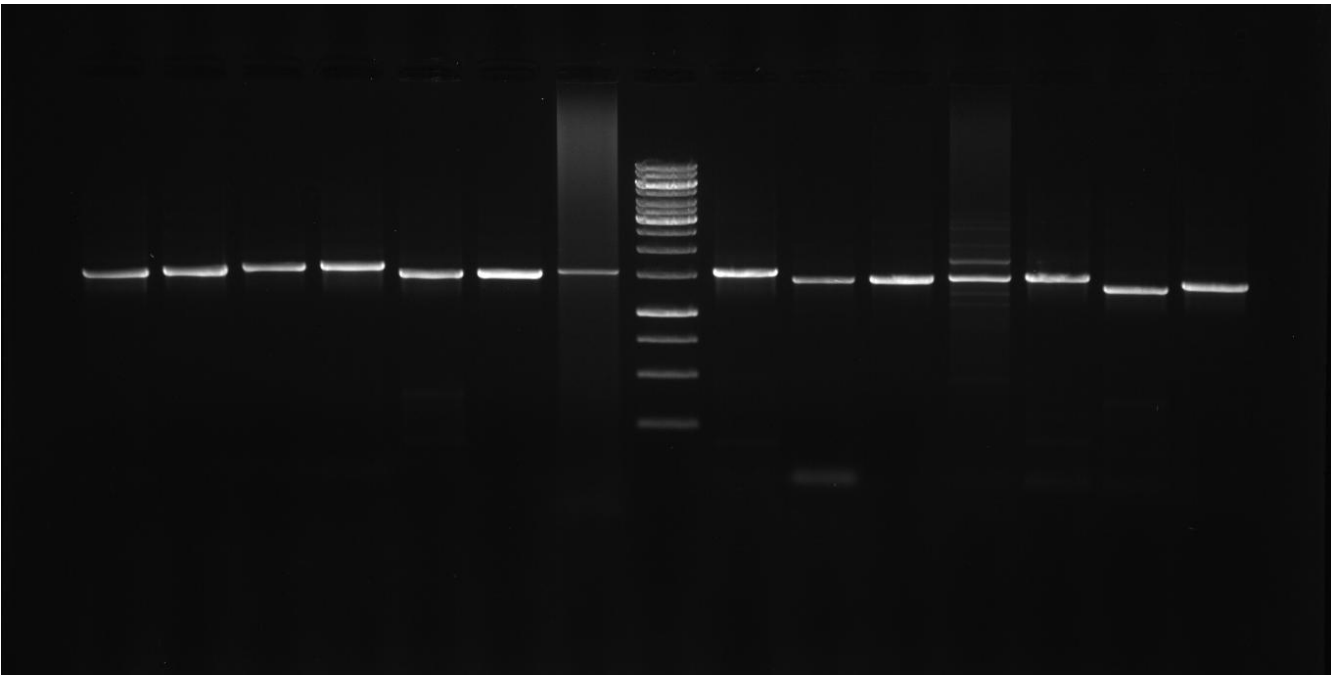


■ ? (unknown state) ■ 0 (single version) ■ 1 (duplicated region)

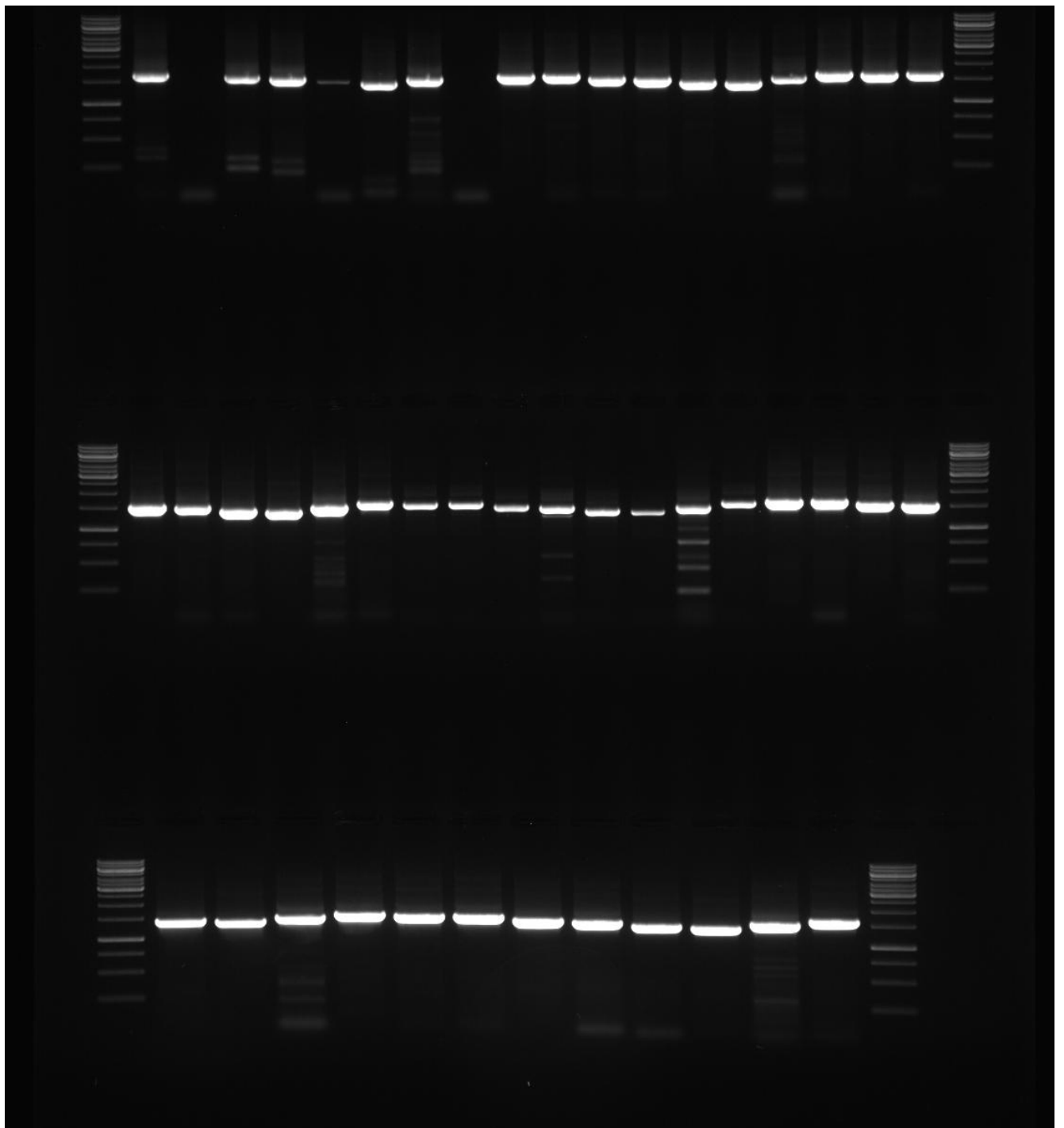
Figure S12. Reconstruction of ancestral states and mapping of mitogenomic duplications onto the Aves three tree topologies under a more liberal assumption for assignment of duplication states. The colored thick lines of the trees correspond to maximum parsimony reconstruction, whereas values at nodes mean the probability of a more likely ancestral state (proportional likelihood) provided by maximum likelihood reconstruction. The trees obtained by Prum, et al. [45] are in a, by Kimball, et al. [47] in b, whereas by Kuhl, et al. [120] in c. The duplication state was assumed for a given order if a duplication was reported in at least one species from this order. FD indicates the presence of the most fully duplicated avian region. In ML approach, AsymmMk model was applied for a and, c whereas Mk1 for b.

Figure S13. Original uncropped and unprocessed agarose gels of: CR1/CR2 amplicons obtained for *Struthio camelus* (a), which were also shown in the Fig. S1a; CR1/CR2 amplicons obtained for *Rhea pennata* (b) which were also shown in the Fig. S1b; CR1/CR2 amplicons obtained for *Rhea americana* (7 lanes to the left of DNA Ladder) (c), which were also shown in the Fig. S1c; CR1/CR2 amplicons obtained for *Crypturellus tataupa* (6 lanes to the right of the DNA Ladder) (d), which were also shown in the Fig. S1d; potential NUMTs amplified for *Dromaius novaehollandiae* (e), which were also shown in the Fig. S4a; potential NUMTs amplified for *Casuarius casuarius* (f), which were also shown in the Fig. S4b; *cytb/12S rRNA* amplicons obtained for *Rhynchotos jubatus* (g), which were shown in the Fig. S11a; *CR/12S rRNA* amplicons obtained for *Rhynchotos jubatus* (h), which were shown in the Fig. S11b; CR1/CR2 amplicons (potential NUMTs) obtained for *Rhynchotos jubatus* (i), which were shown in the Fig. S11c; *cytb/12S rRNA* amplicon obtained for *Eurypyga helias* (j), which was shown in the Fig. S11d; *cytb/CR* and *CR/12S rRNA* amplicons obtained for *Eurypyga helias* (k), which were shown in the Fig. S11e; *cytb/12S rRNA* amplicon obtained for *Trogon collaris* (the first lane prior the first DNA Ladder) (l), which was shown in the Fig. S11f; CR1/CR2 amplicons obtained for *Trogon collaris* (m), which were shown in the Fig. S11g.

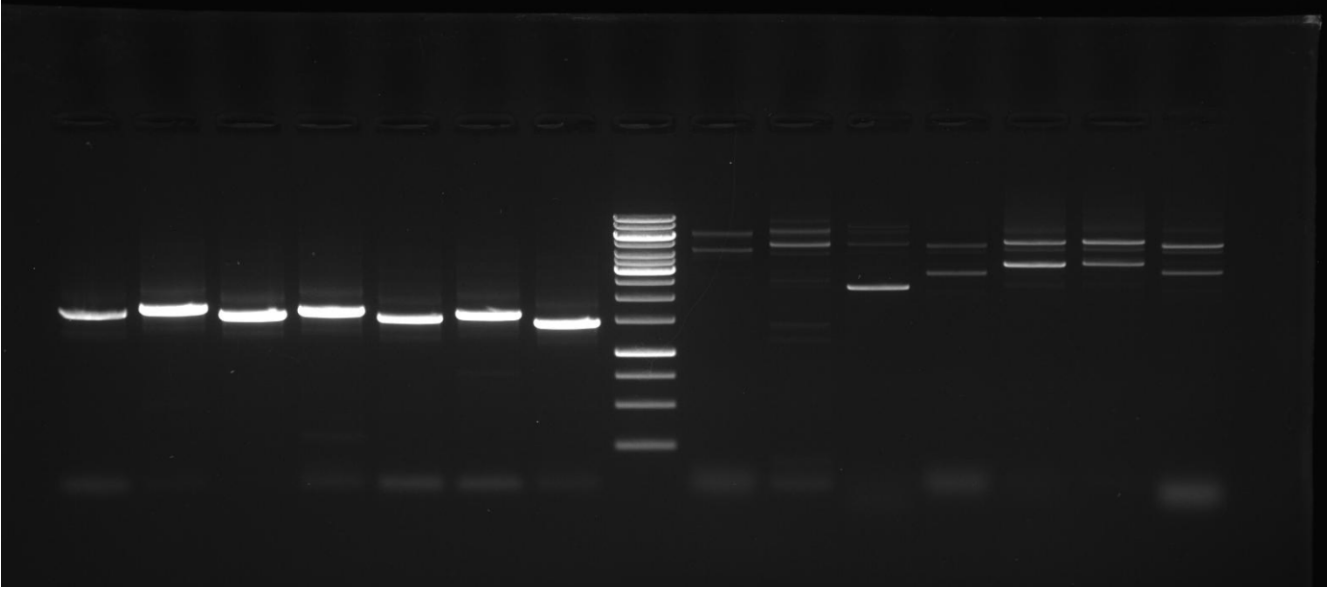
a)



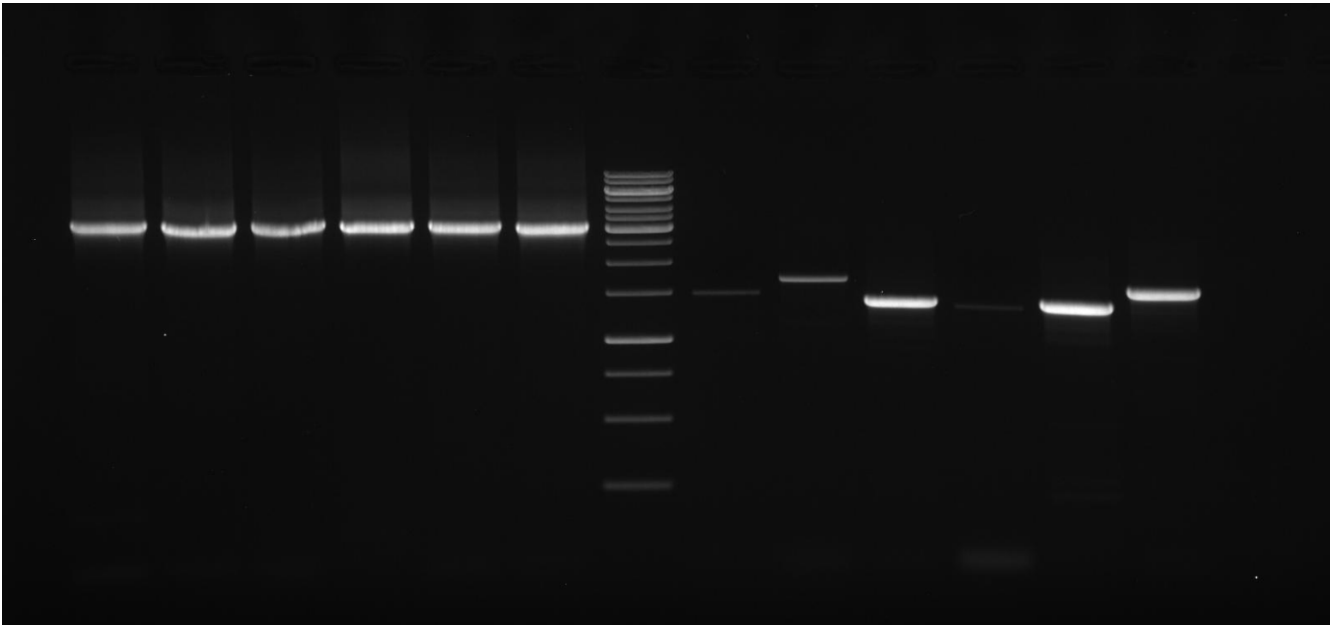
b)



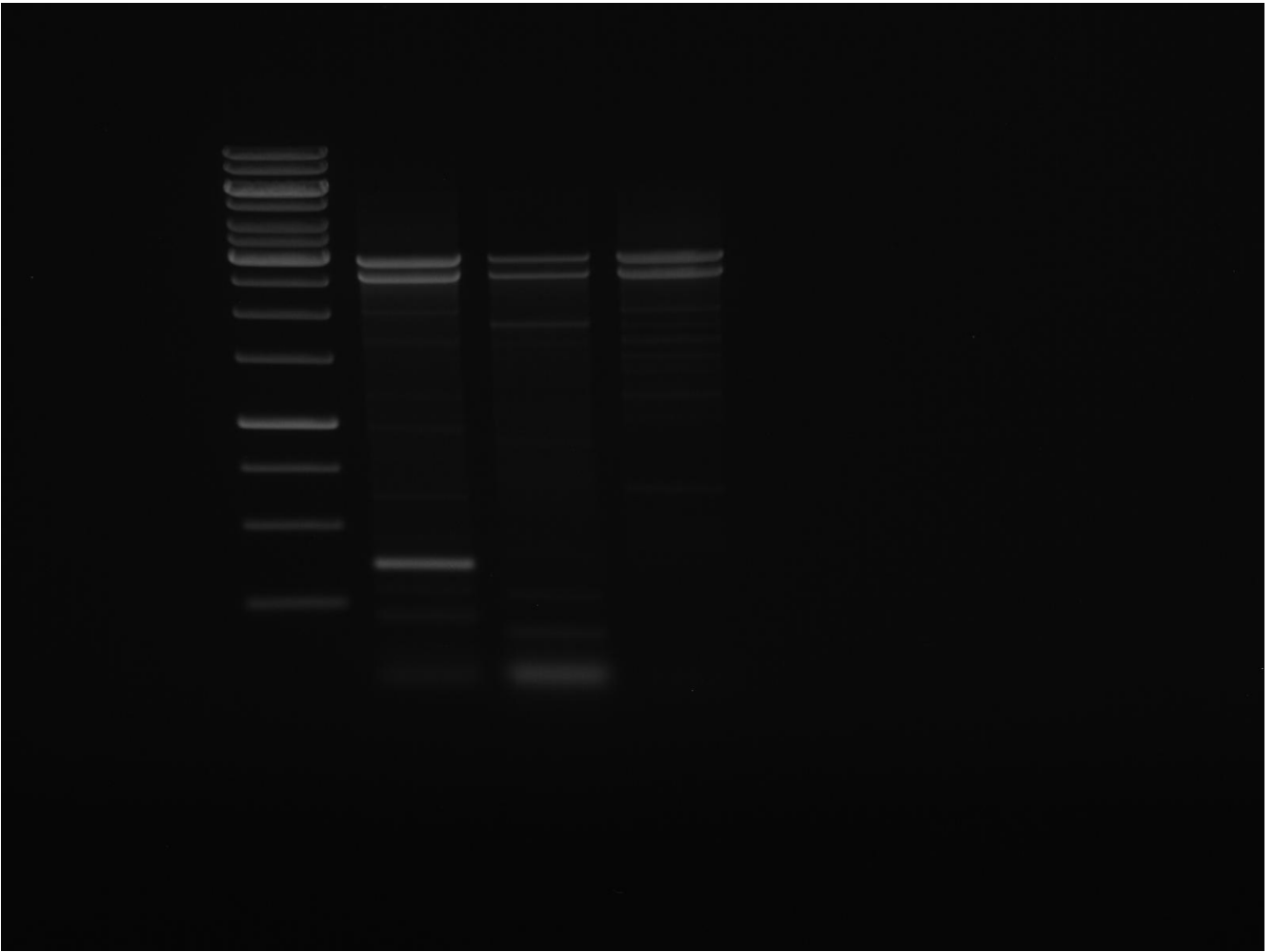
c)



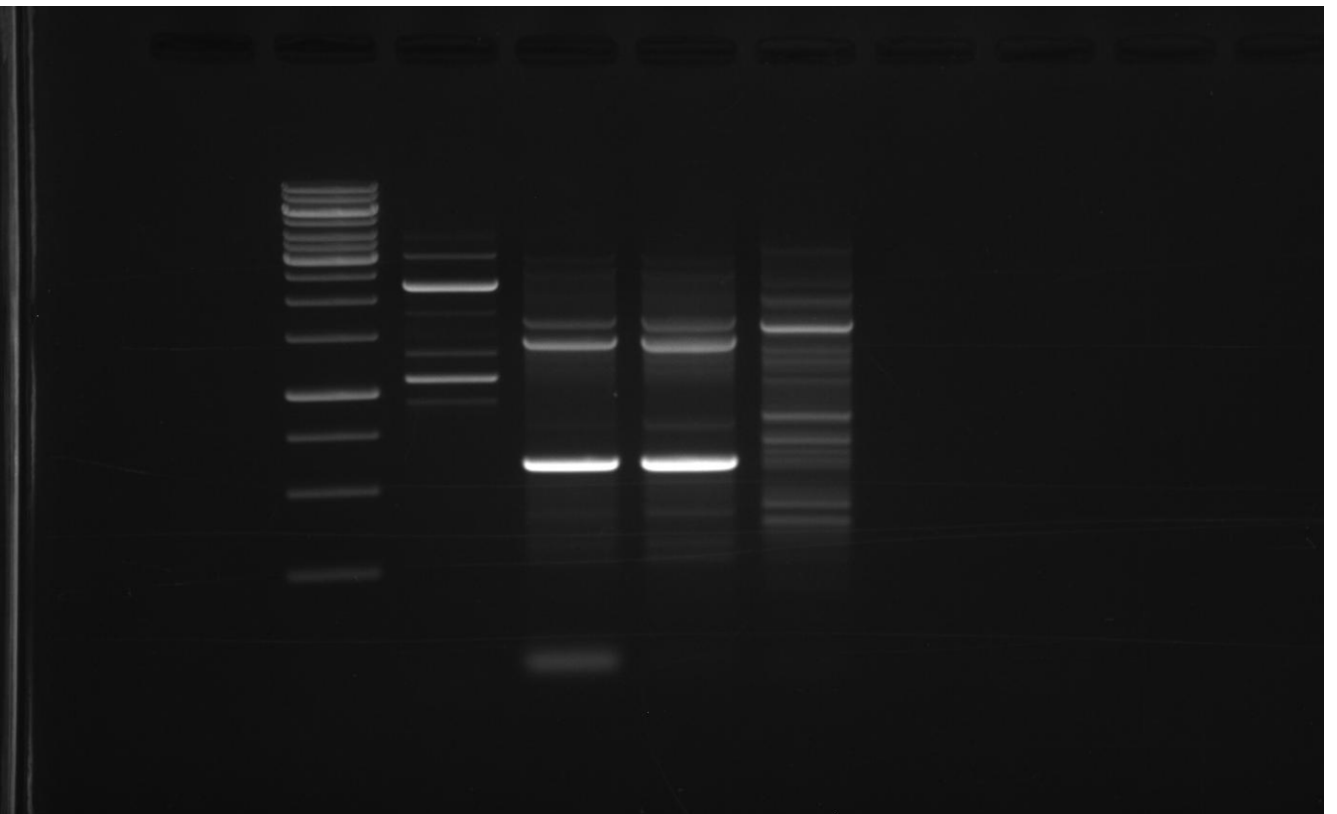
d)



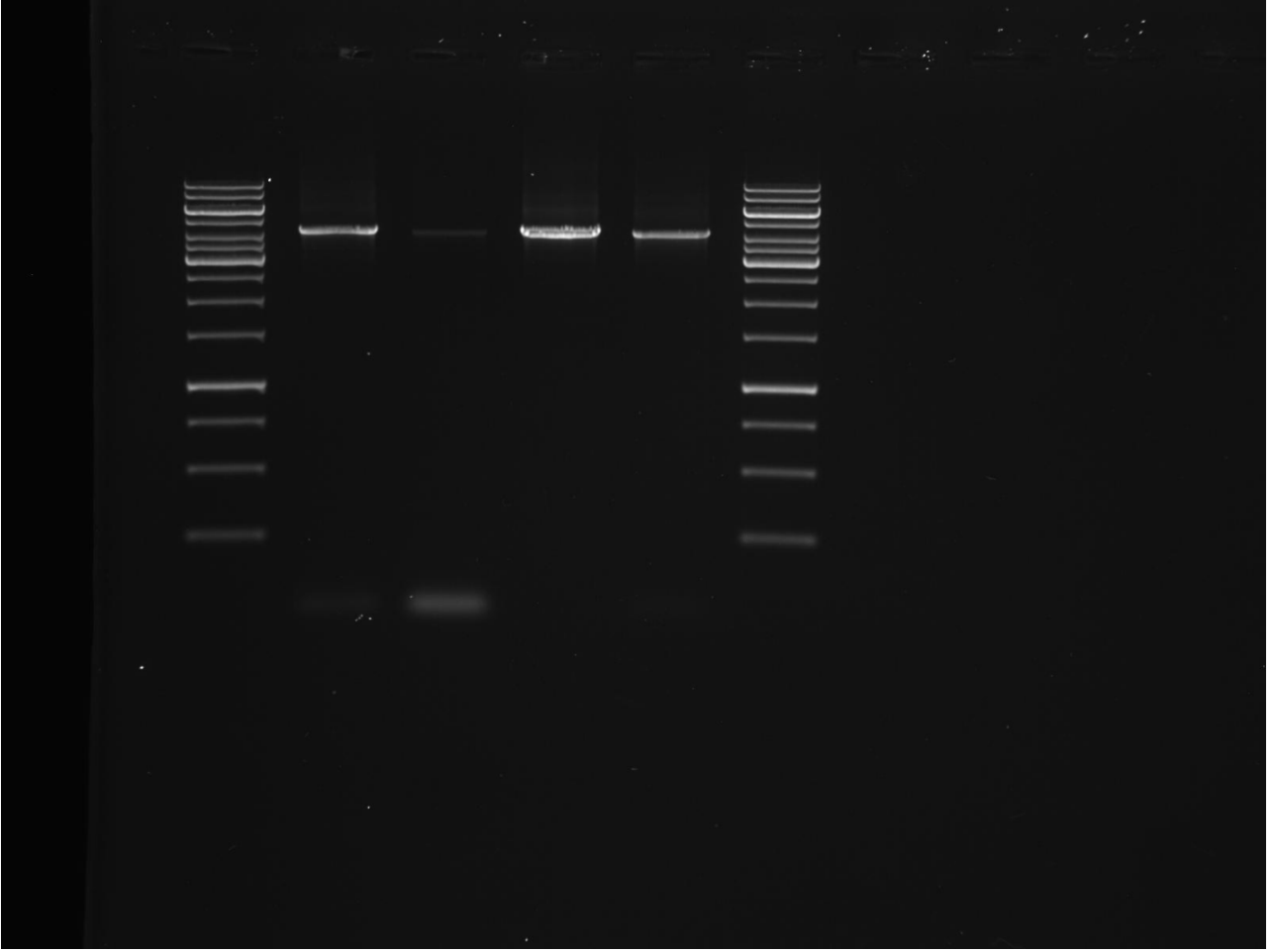
e)



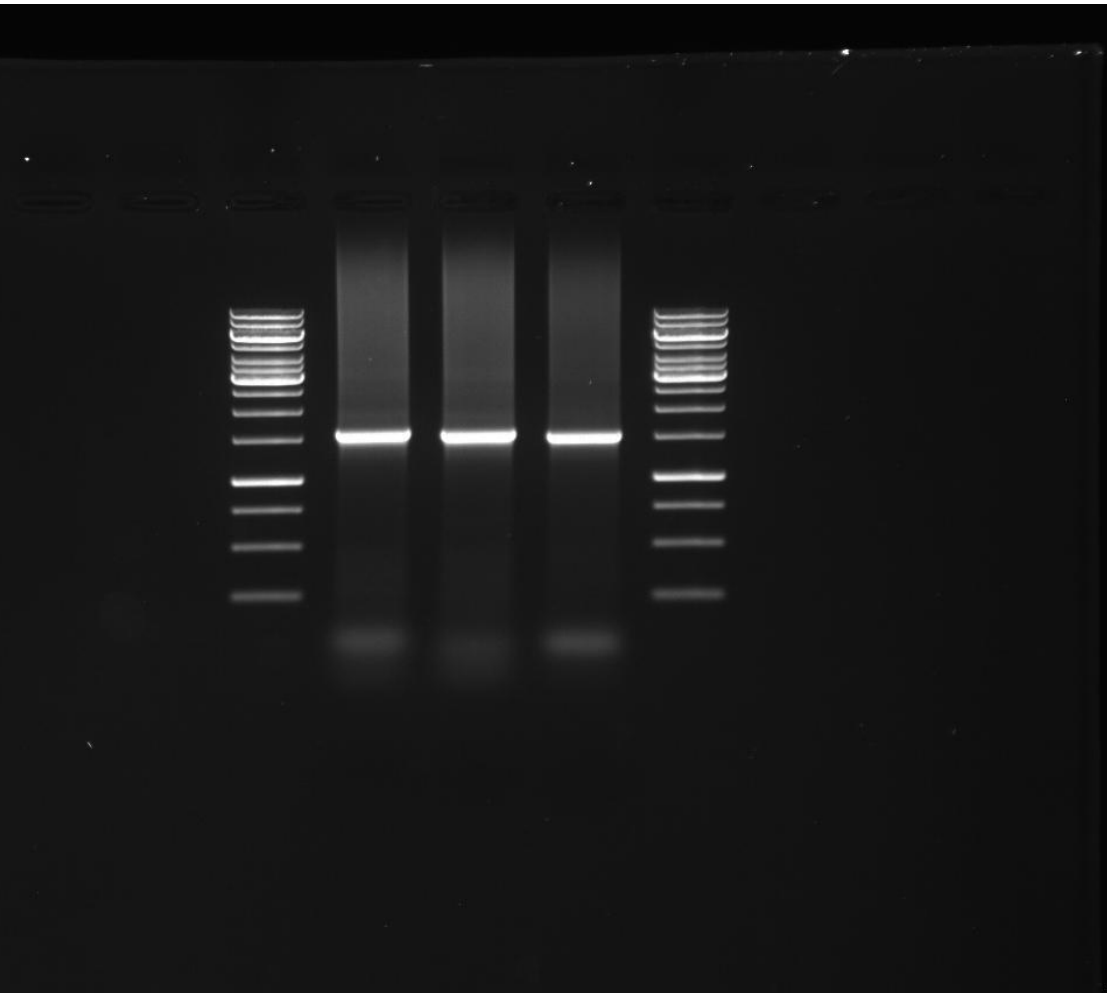
f)



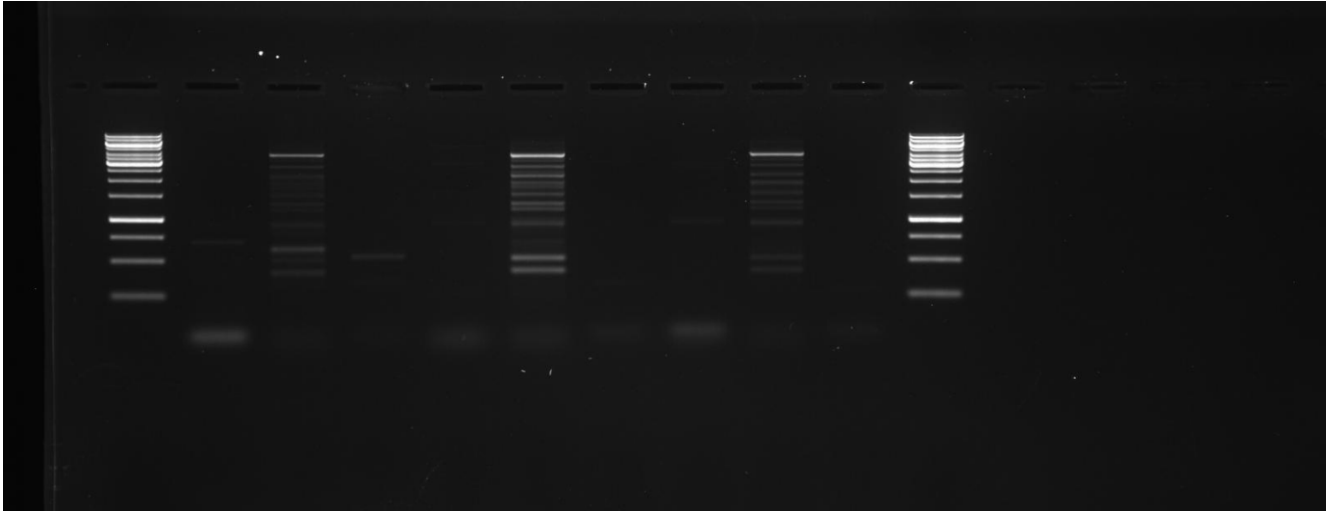
g)



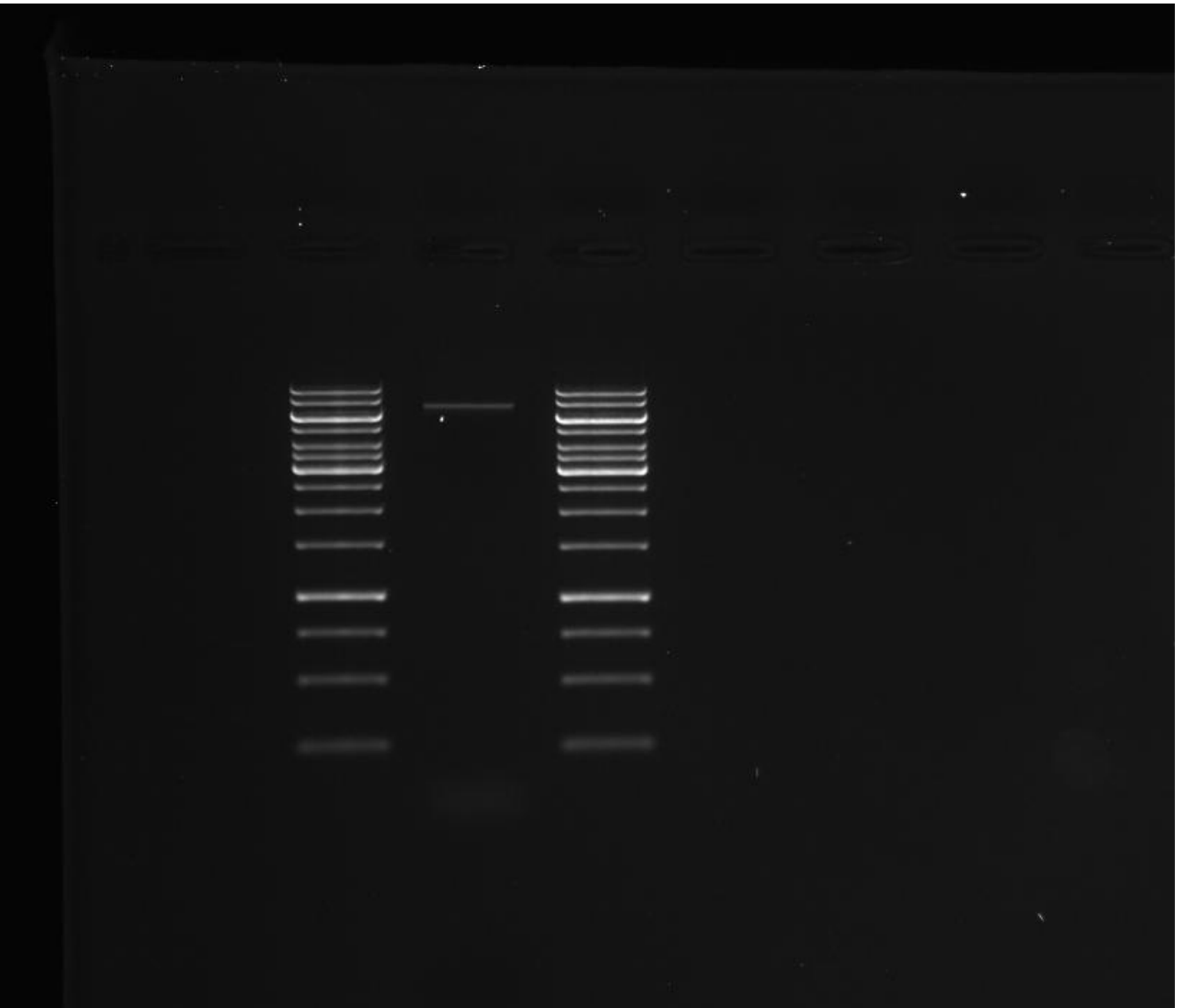
h)



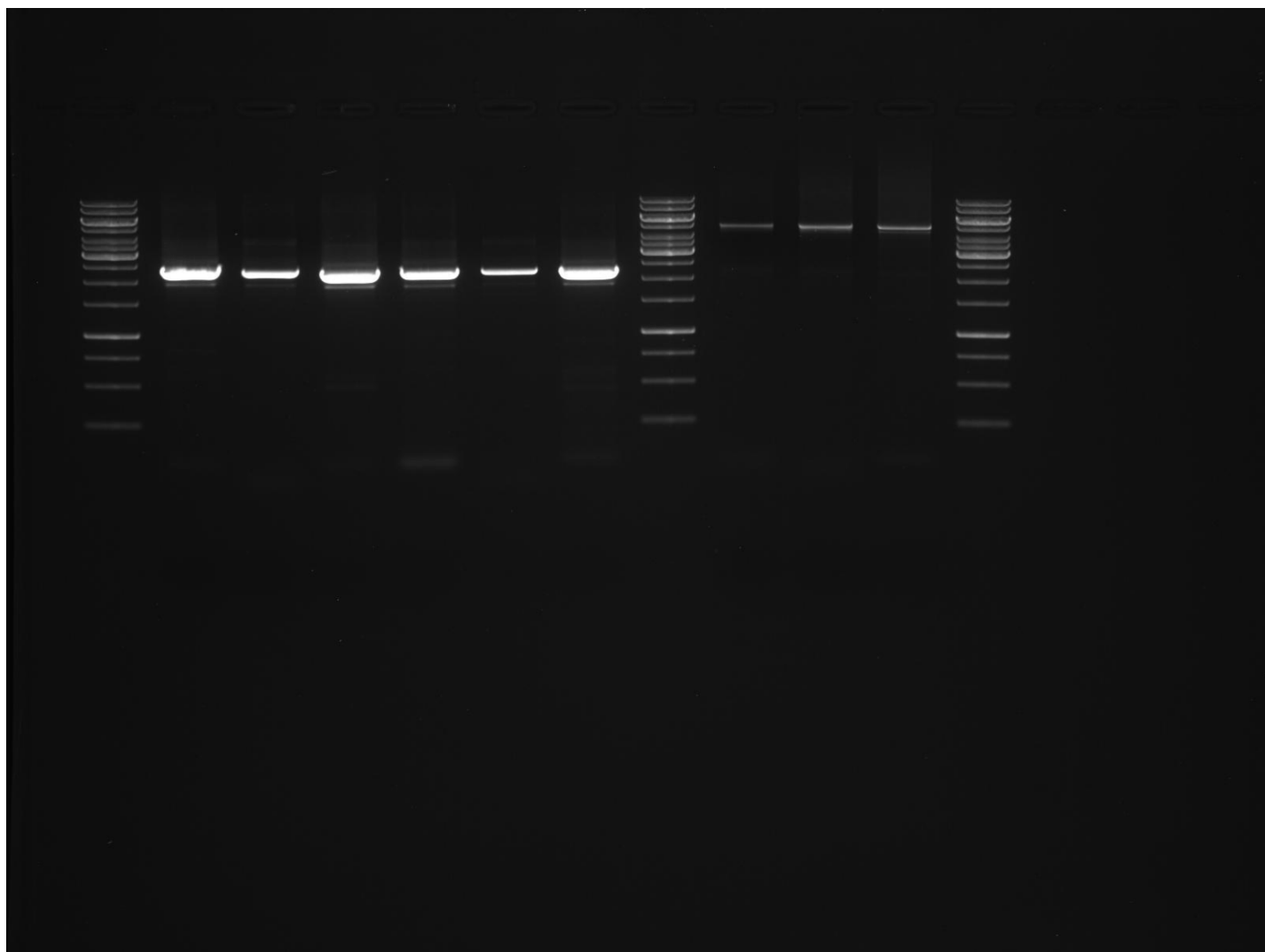
i)



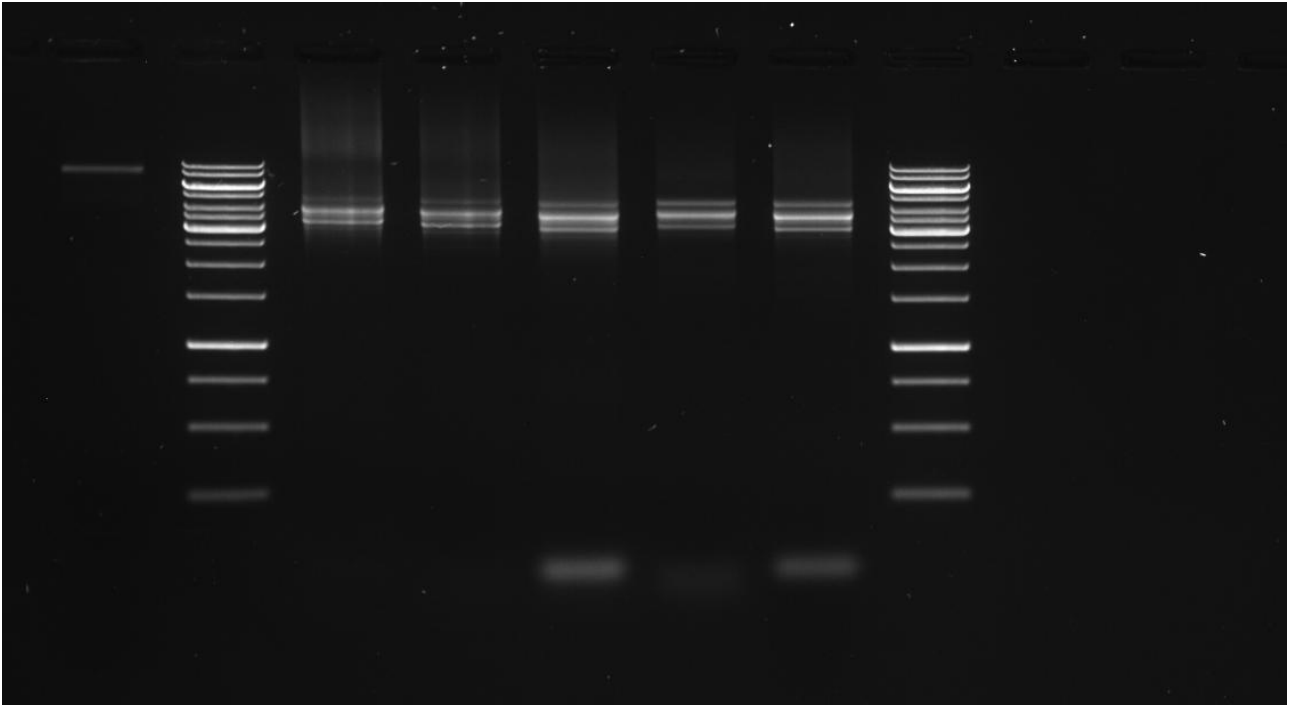
j)



k}



l)



m)

