

Supplementary file of the manuscript:
**Detection of *Rickettsia* spp. in questing ticks (Acari: Ixodidae)
from forest fragments adjacent to agricultural and livestock
farms in Casanare, Colombia**

Next files correspond only to the most representative samples (positive DNA samples only) gels made for the different genes amplified (16S mRNA for tick species identification and *gltA*, *htrA* and *ompA* for *Rickettsia* detection and identification).

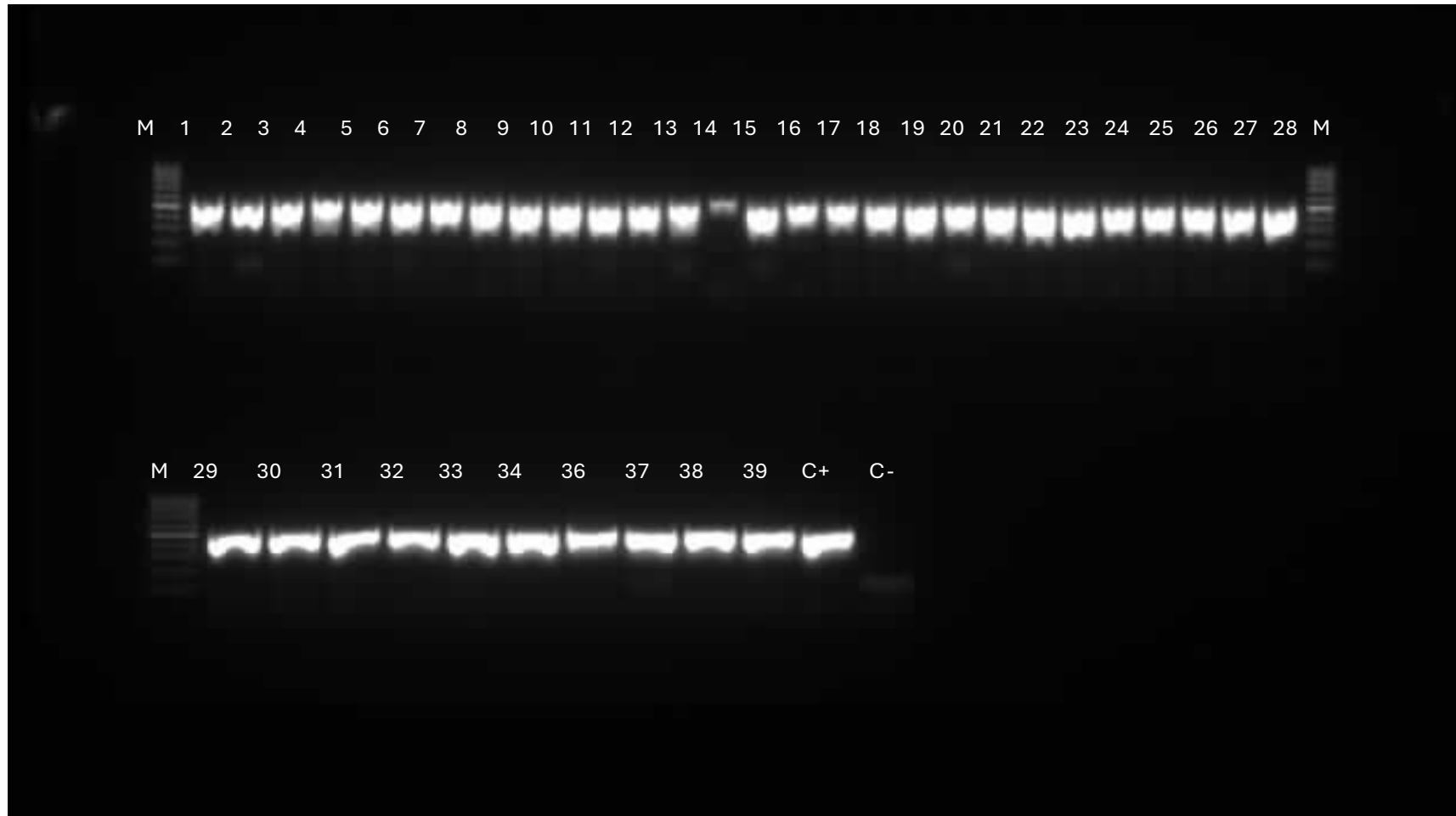
The total number of gels in this study was over 90 gels.

I hope this completes the information required by the reviewers. In any case, I remain attentive to any other request.

16S mitochondrial *RNA* gene amplification for tick species identification

- The gel in the next slide shows the nymph DNA samples that were amplified for the 16S *mRNA* gene.
- As mentioned in the manuscript, only samples from *Rickettsia* sp. positive adult ticks or larvae were amplified for the 16S mRNA gene. Gels are not shown.

16S *mRNA* amplification. Nymphs DNA.



gltA gene amplification

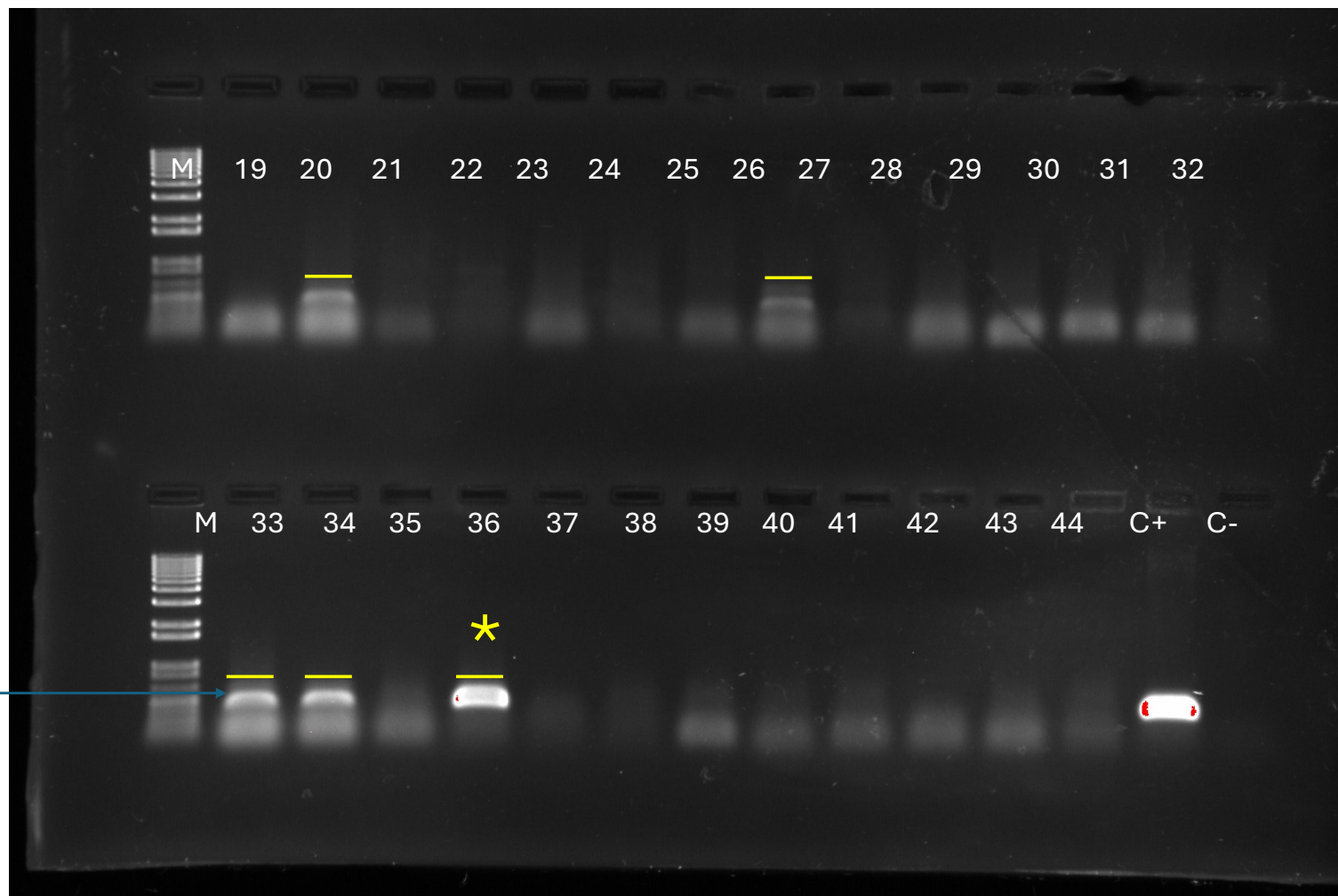
- The following slides are related to the amplification of the *gltA* gene (400bp or 800bp fragment size) in DNA samples from larval, nymph and adult ticks. Only a few are shown. As positive results were obtained, they were sent for sequencing.
- *800bp size fragments were used for sequencing

Adults plate1
(*A. cajennense*)
Gel 3. **gltA** gene

gltA

Result:
1 positive sample
#36 (*)

400bp

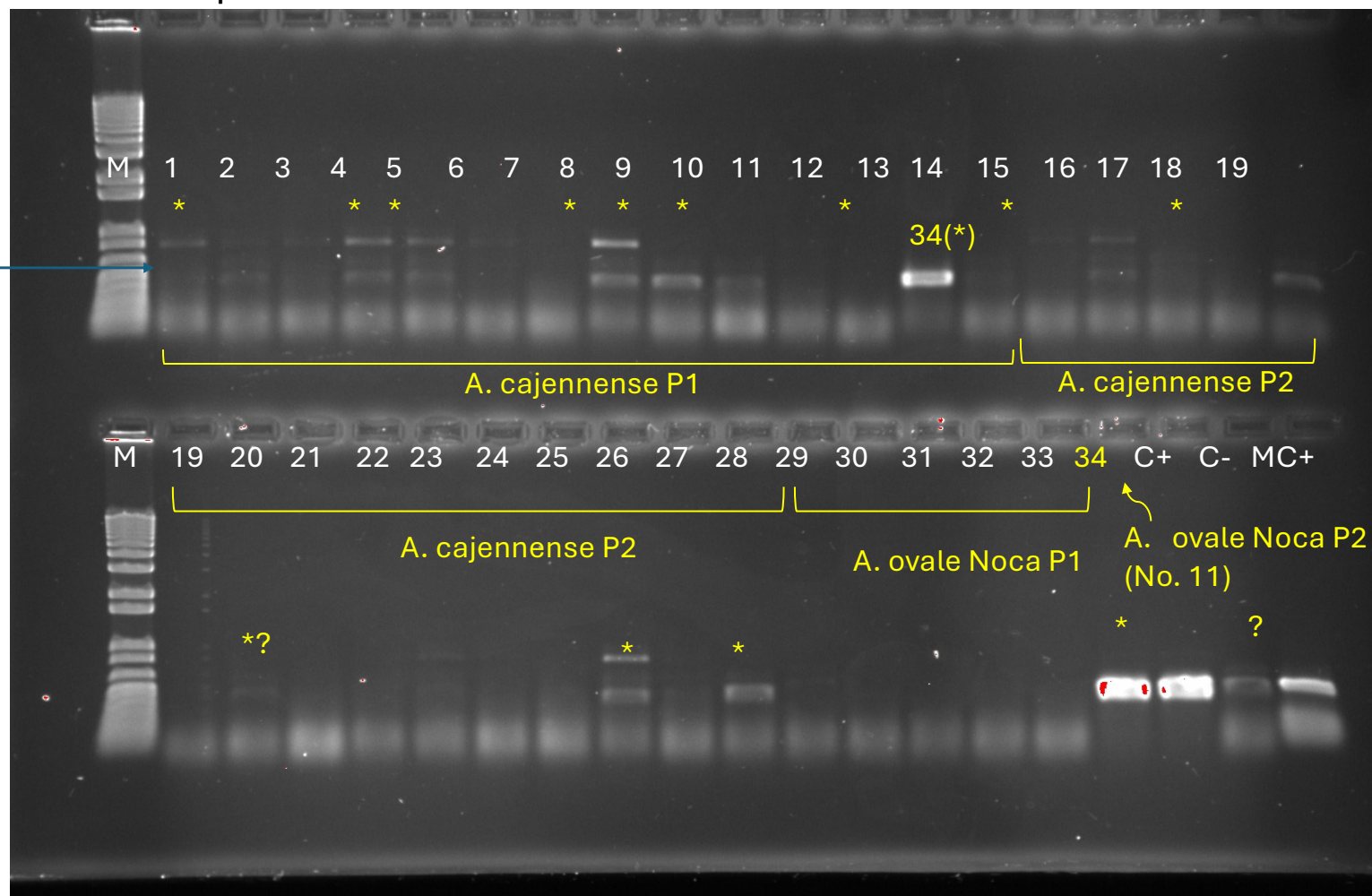


gltA 400. Adult ticks Yopal and Nocaima Diluted DNA 1:10

400bp

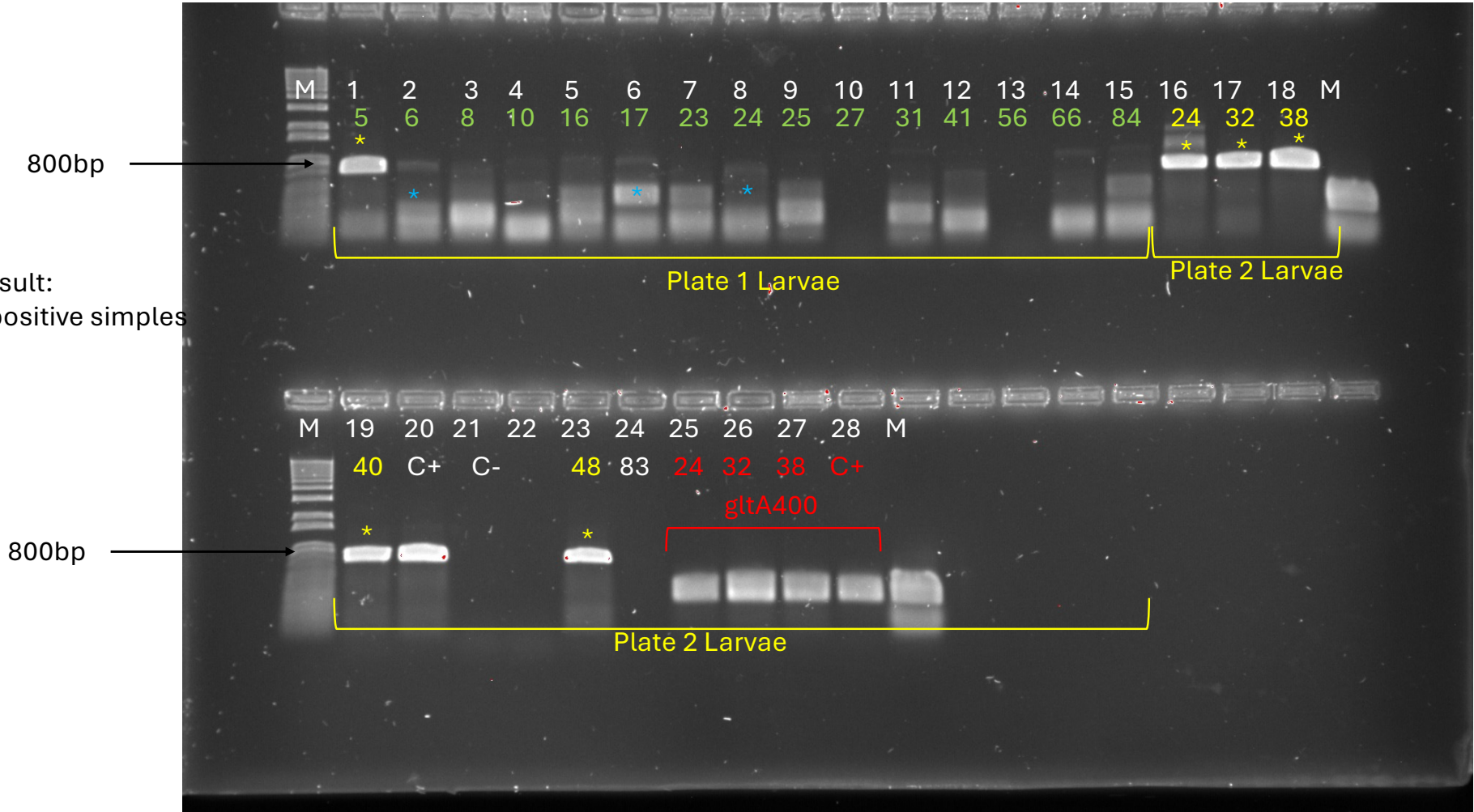
Result:
1 positive sample

37(*)



gltA (800bp). Larvae. Plate 1

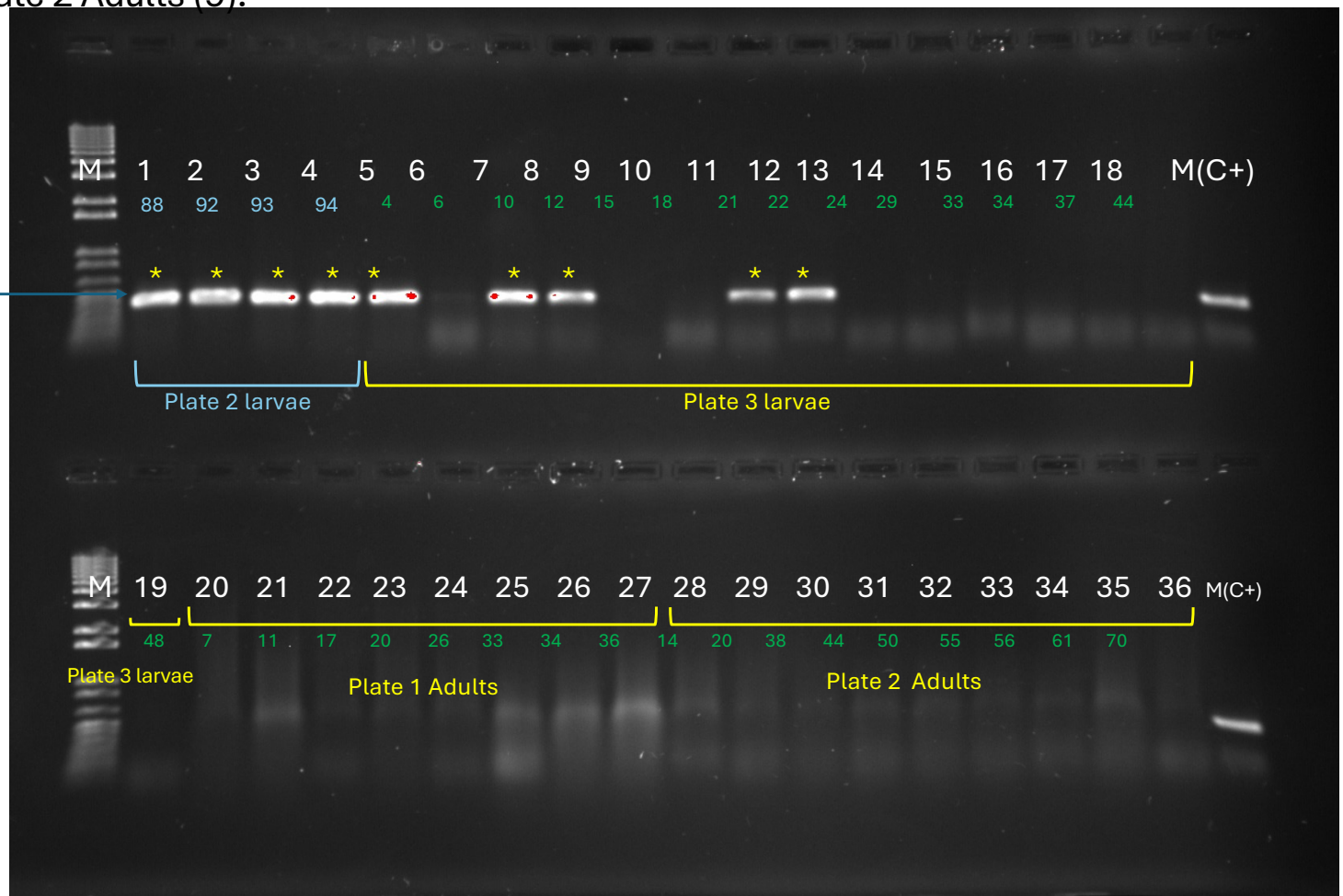
Result:
6 positive samples
(*)



gltA Confirmative. Gel 1. Samples: Positive gltA 400bp) from plate **2 Larvae** (N:4), Plate 3 larvae (N:15), Plate 1 Adults (N:8) y plate 2 Adults (9).

400bp

Result:
9 positive sample
37(*)



gltA 800. **Larvae** - Plate 1, 2 y 3 & Nymphs plates 1, 2 y 3 . Gel 1

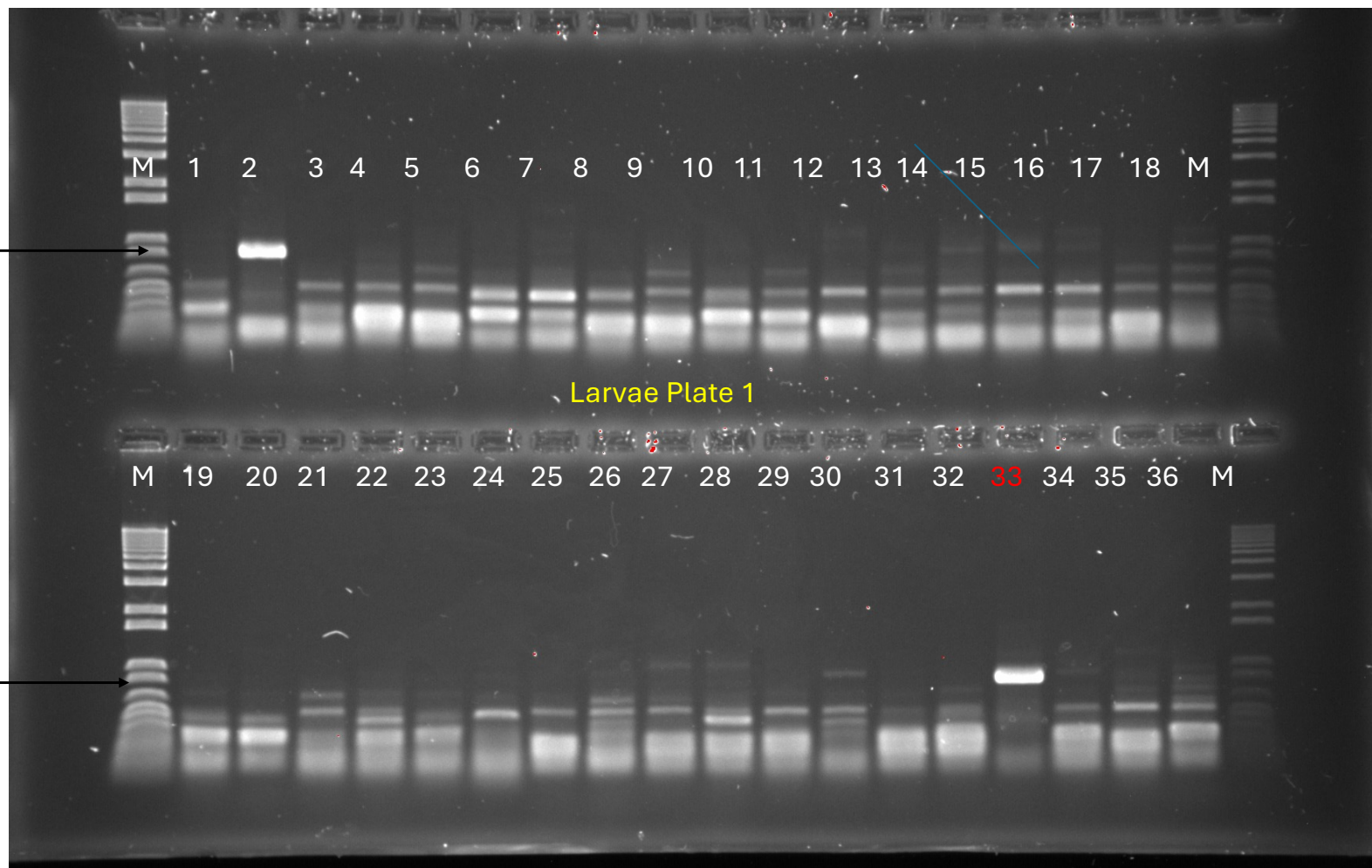
Larvae

gltA

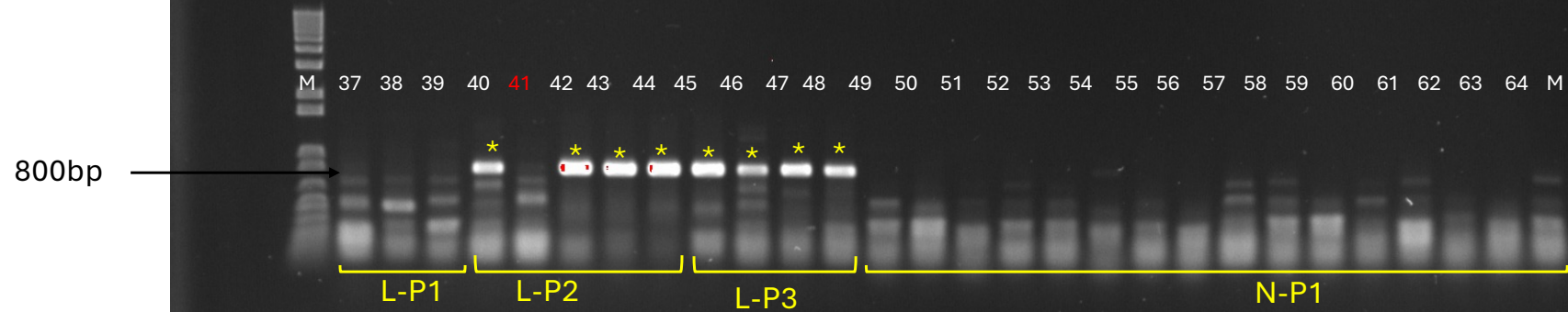
800bp

Result:
2 positive sample
37(*)

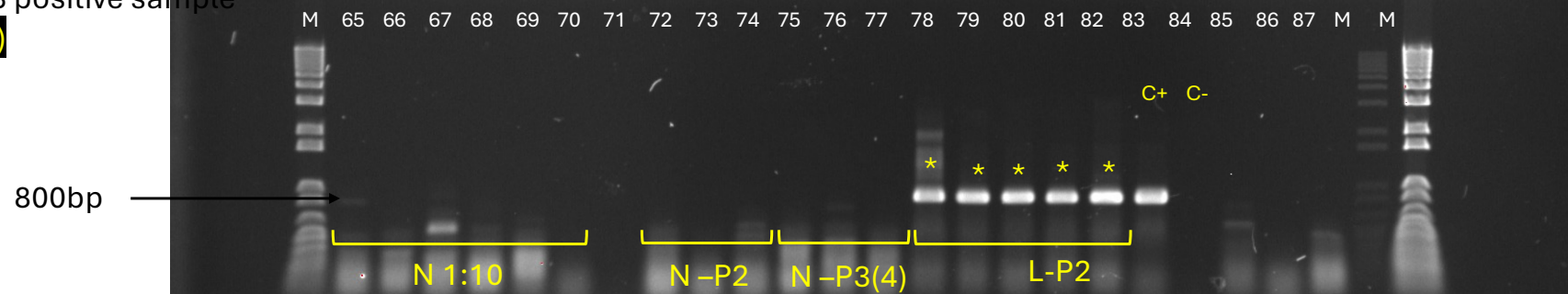
800bp



gltA 800bp. Larvae Plate 1, 2 y 3 & Nymphs Plates 1, 2 y 3. Gel 2



Result:
13 positive sample
(*)



htrA gene amplification

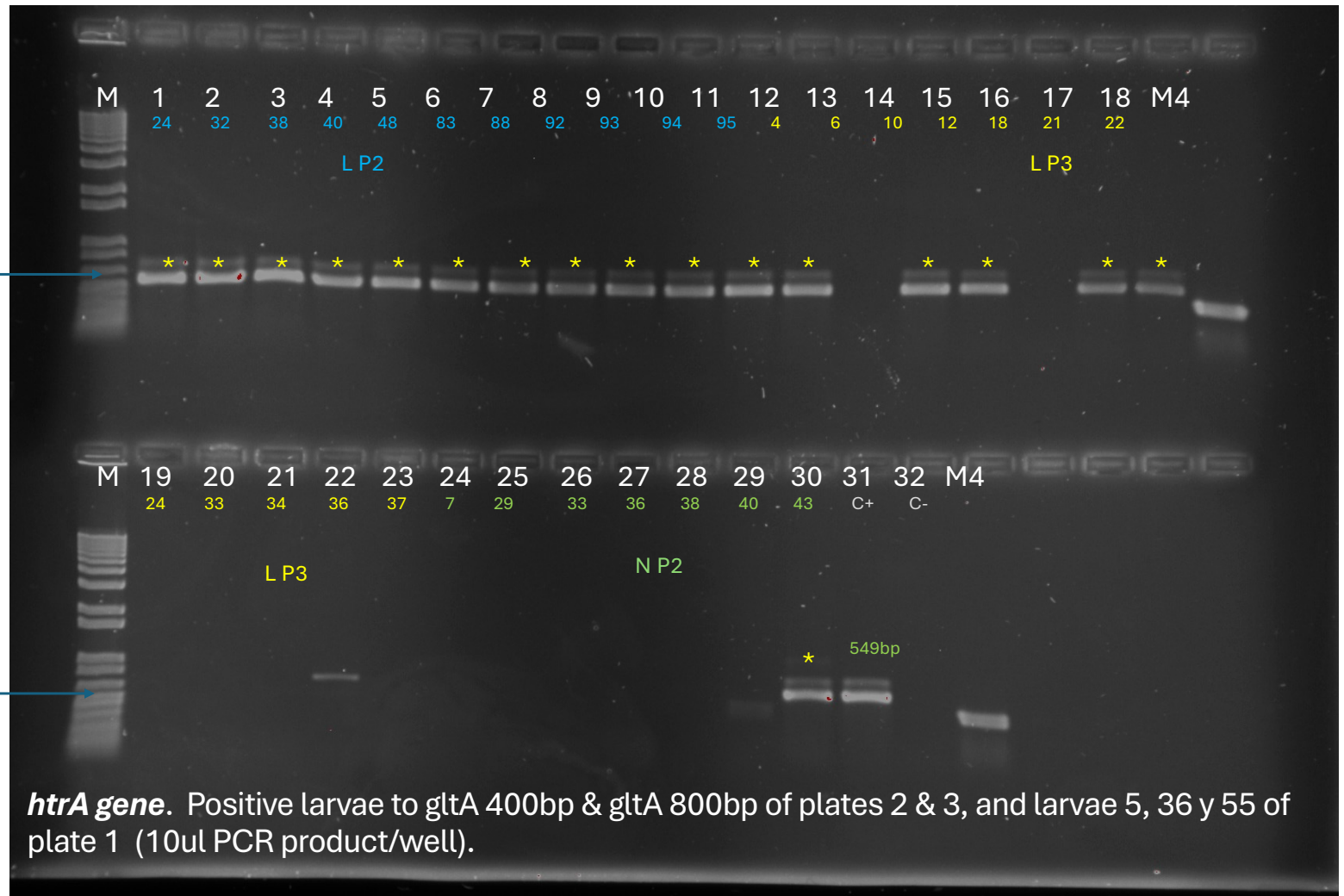
The following slide shows the amplification of *htrA* gene fragments from larval DNA samples that were positive for the *gltA* gene.

htrA gene.
Larvae DNA

549bp

Result:
16 positive samples
from larvae
1 positive sample
from Nymph
(*)

549bp



ompA amplification

- The following slide shows the amplification of *ompA* gene fragments from some larval DNA samples that were positive for the *gltA* gene.

ompA PCR. Larvae Plate 1 (N:49). 7 September 2021

