

Expression of Rabies Antibodies in Transgenic Maize

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INTRODUCTION

- Rabies, an important disease in Asia and Africa, is an acute viral disease of the central nervous system that affects humans and other mammals (fig 1 and 2). Upon bites or contact with rabid animals patients are immediately immunized with antibodies followed by human diploid cell vaccine (HDCV). Lack of post-exposure treatment usually results in 100% fatality.
- Monoclonal antibodies are inherently stable human mucosal surface defence proteins with high specificity, low toxicity, and appropriate for chronic conditions as injectable, topical and orally applied. Plants have proven to be efficient production system for therapeutic proteins with advantages of scalability and product safety compared to other production systems.
- The monoclonal antibody (MAb) E559 is part of a cocktail of antibodies that are used as post exposure prophylaxis against rabies.
- Rabies antibody production in plants to be used as model for other high-value antibody targets

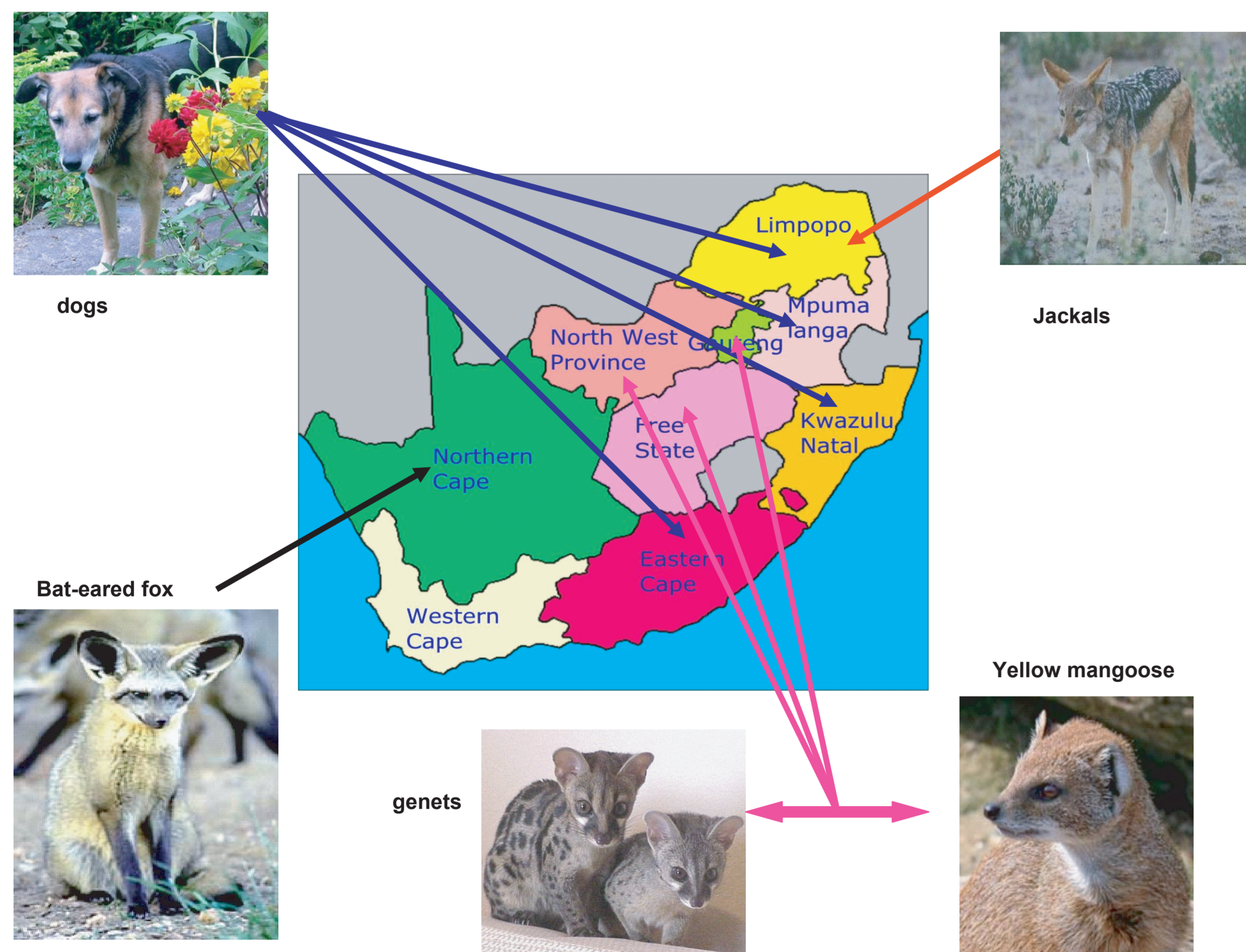


Figure 1: Rabies transmission in South Africa.

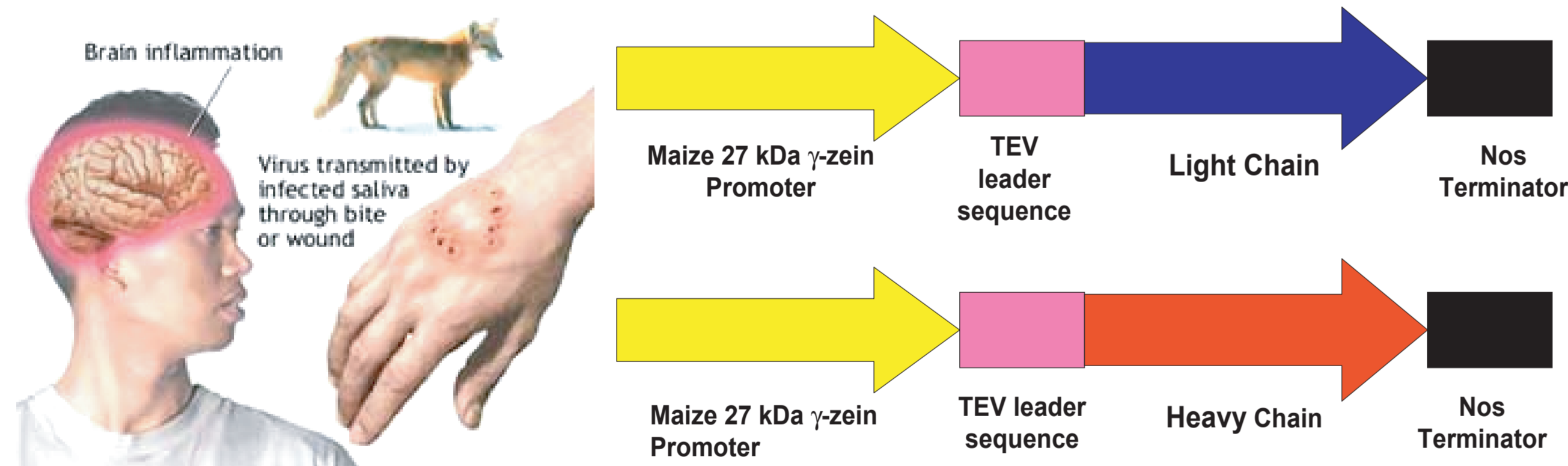


Figure 2: Process of infection.

Figure 3: Minimal cassettes used for transformation.

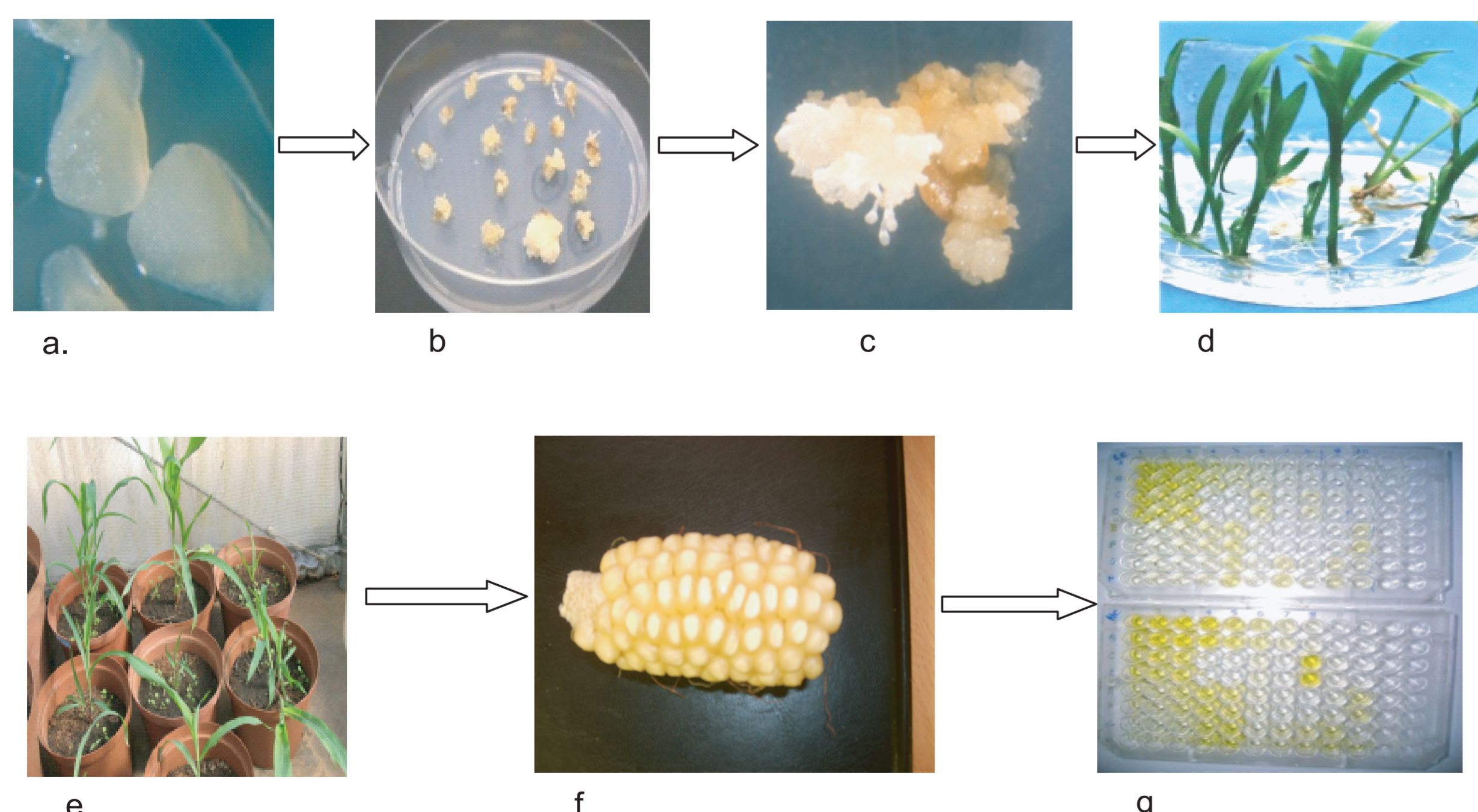


Figure 4: Tissue culture procedure leading to production of transgenic maize. The pictures showing the process of tissue culture leading to production of transgenic maize. (a) immature embryos ready for bombardment, (b and c) callus on selection media (d) plantlets growing on maturation media containing mannose (e) plants growing in the green house (soil), (f) fully grown cob ready for protein extraction used in the ELISA.

REFERENCES

- Breitschneider, R., Becker, D, and Lorz, H. (1997) Efficient transformation of scutellar tissue of mature maize embryos. Theor Genet 94:94 737-748
- Chakauya, E., Lotter, T., Dube, N., Ramesaar, K. and Chikwamba, R. Novel platforms for Essential Biomolecules: Expression of rabies antibodies in tobacco. CSIR Report. Pretoria, SA.

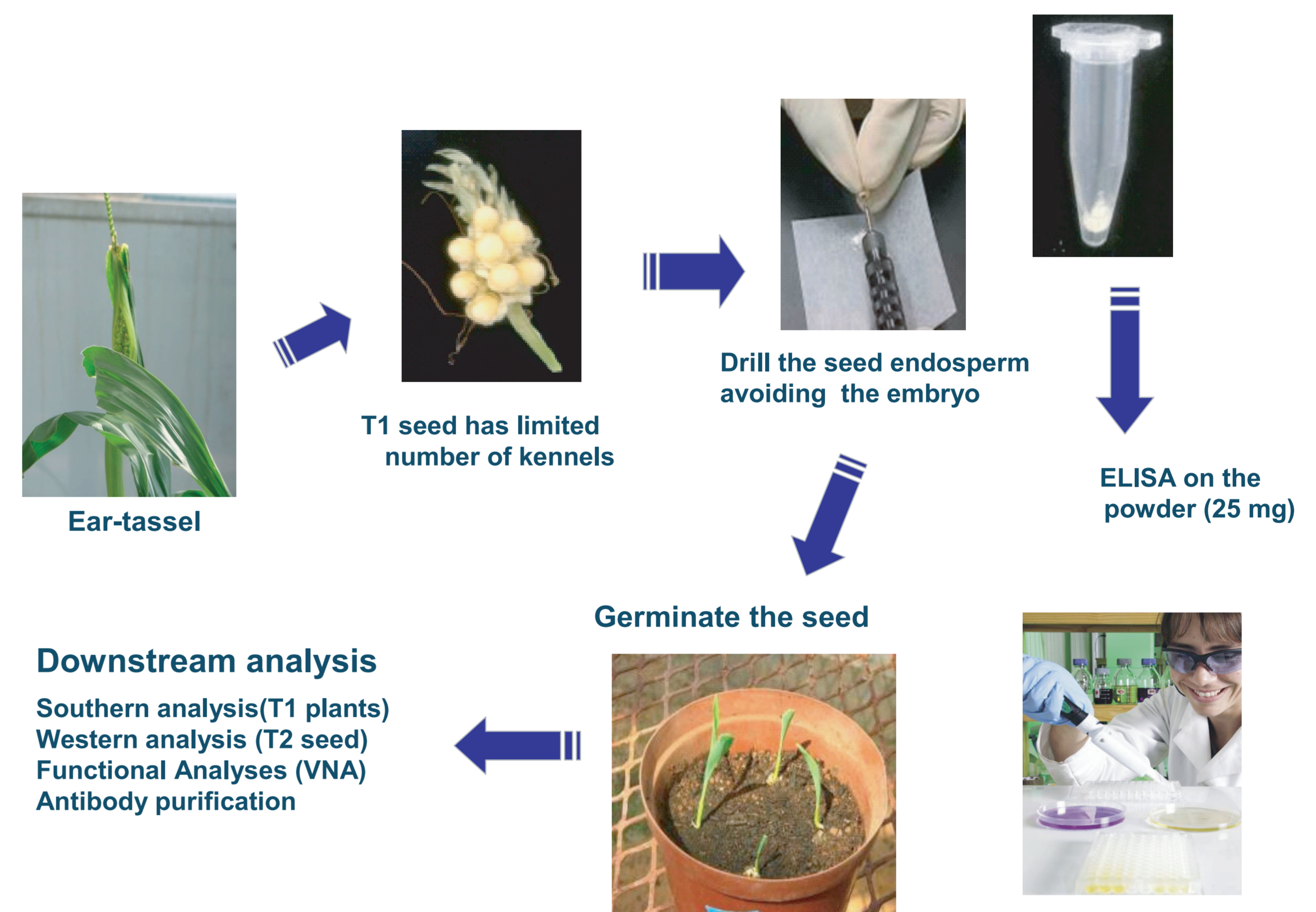


Figure 5: Procedure for screening maize seeds. The seed is drilled, protein extracted and analysed by sandwich ELISA.

SUMMARY OF RABIES ELISA DATA

- bialaphos (bar)- 25 putative transgenics in the greenhouse
- Phosphomannose isomerase (PMI)- 24 events screened by ELISA
 - 9 LC independent events
 - 7 HC independent events
 - 7 seeds from 4 different events have HC/LC

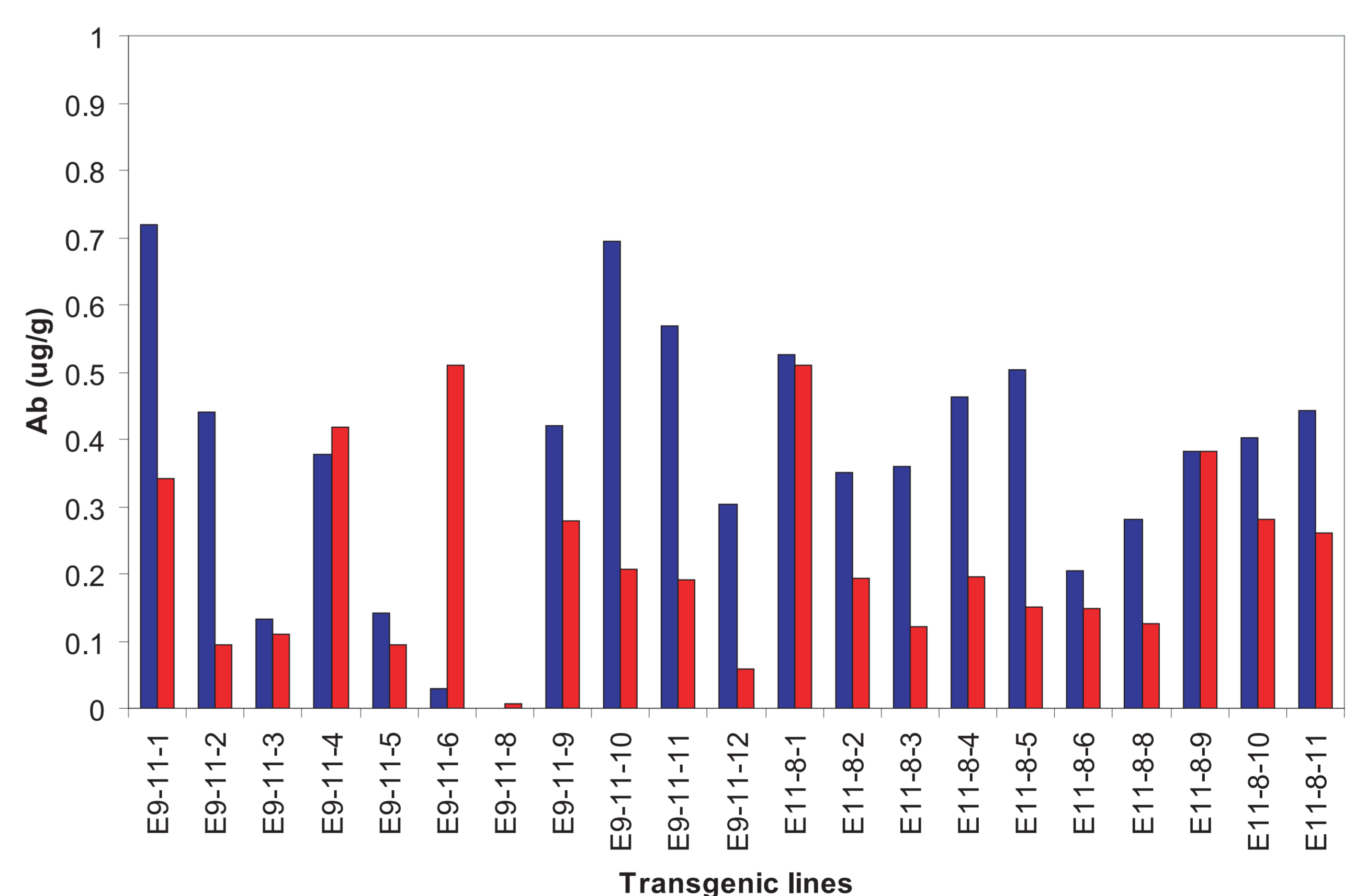
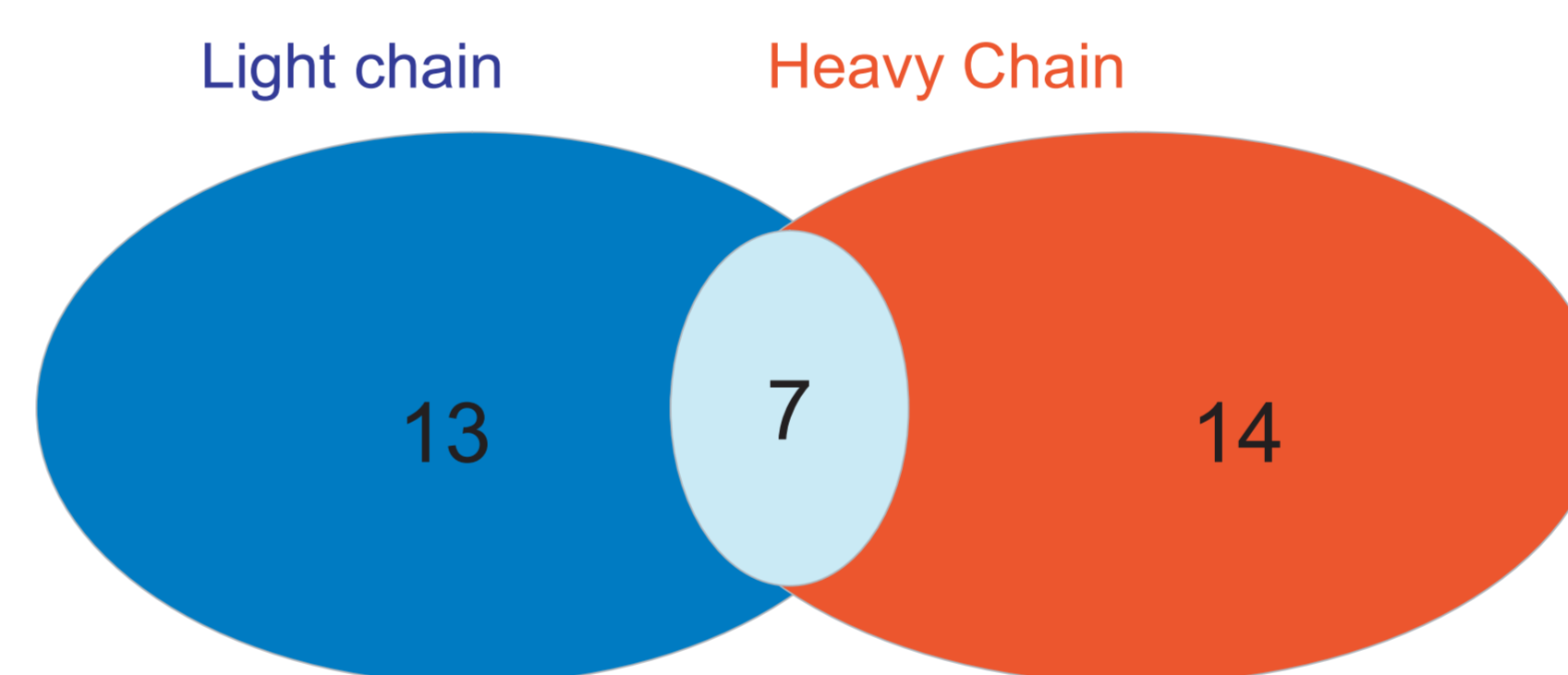


Figure 6: Analysis of maize transgenic seeds (T2) using ELISA using the antibodies sheep anti-human IgG, Sheep anti human IgG Gamma chain-HRP and Sheep anti human Kappa-HRP conjugate.

DISCUSSION AND CONCLUSION

- Seeds from the maize were drilled and analysed using the ELISA technique. The expression levels for the transgenic lines is shown on fig 6. with the highest expression of 0.7 $\mu\text{g.g}^{-1}$. The seeds expressing both heavy and light chains were planted for the next generation.
- Plant-made pharmaceuticals is arguably the fastest growing area of biotechnology, where the potential of addressing some of the troublesome public health issues in the developing world cannot be emphasised. In the current study we showed the production of anti rabies mAb in transgenic maize.
- We also believe that plant production systems, be they transgenic, transient or cell culture are a genuine and scalable system for producing affordable anti-rabies mAb for prophylaxis therapy in the developing world.