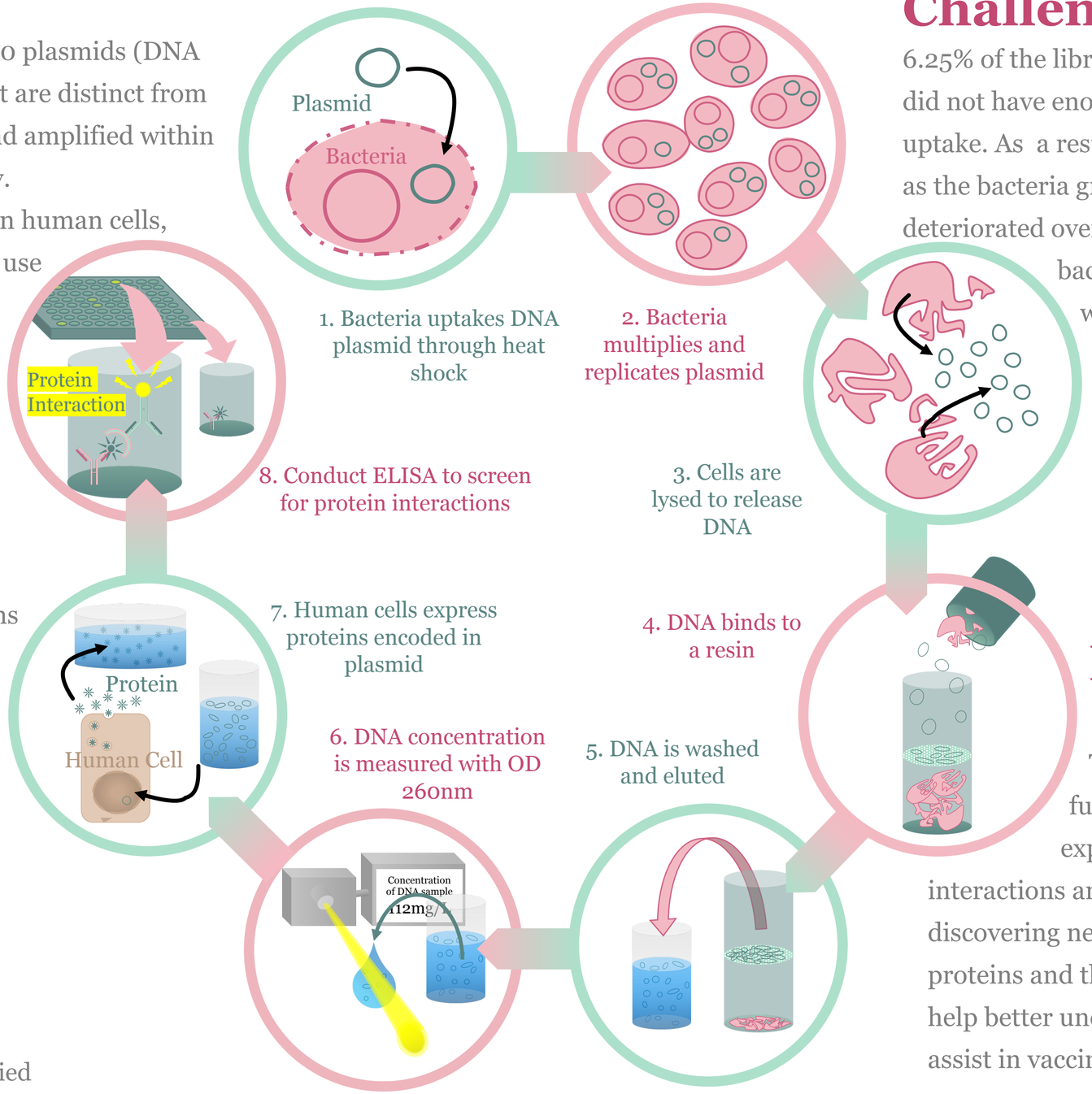
Restoration of a cDNA library for ELISA

Samara Maxwell, Tom Bird, Liam Turk, Davide Comoletti
School of Biological Science

The Task

Over the duration of this project, 150 plasmids (DNA molecules found inside bacteria that are distinct from its chromosome) were replicated and amplified within bacteria, restoring a plasmid library.
The plasmids were then expressed in human cells, producing the encoded proteins for use in an ELISA.



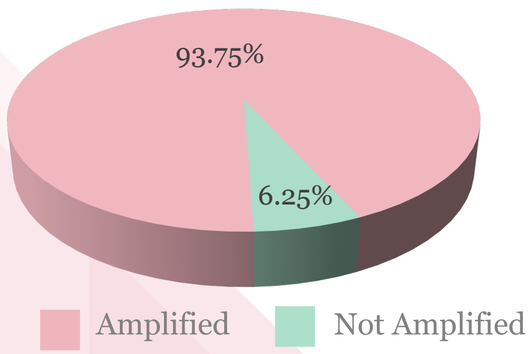
Challenges Faced

6.25% of the library was not amplified. Some plasmids did not have enough DNA remaining for bacteria to uptake. As a result, there was no plasmid amplification as the bacteria grew. Secondly, some of the plasmids had deteriorated over time while in storage. This meant the bacteria could not replicate the plasmid when replicating its own genome, resulting in no plasmid amplification.

The Outcome

93.75% of the PD-Fc library was replenished, with final concentrations for DNA ranging from 305 – 3,067ng/ul with an average of 1,566ng/ul.

Total Proportion of Library Amplified



Future Endeavours

The amplified DNA is to be used in future ELISAs and crystallisation experiments to discover novel protein interactions and structures. Of particular interest is discovering new interactions between human proteins and the COVID-19 spike receptor. This will help better understand infection mechanisms and assist in vaccine production.

References

Machery-Nagel (2017). *NucleoBond Xtra Midi Plasmid DNA Purification*.
Ozgul, Sinem, et al. 'An ELISA-Based Screening Platform for Ligand–Receptor Discovery'. *Methods in Enzymology*, vol. 615, Elsevier, 2019, pp. 453–75. *DOI.org (Crossref)*, https://doi.org/10.1016/bs.mie.2018.10.001

VICTORIA UNIVERSITY OF WELLINGTON TE HERENGA WAKA