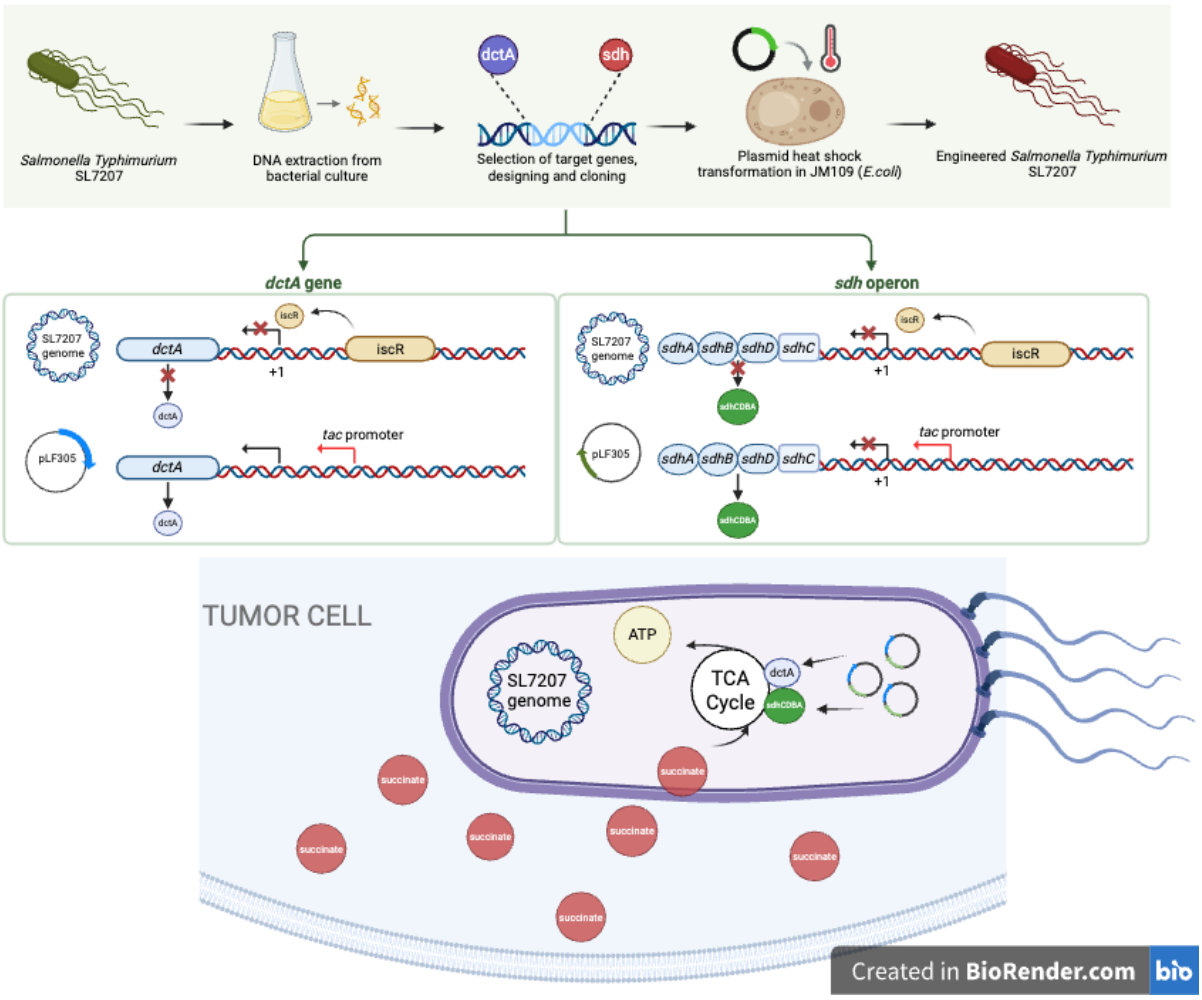
CHAU (CHLOE) TRAN / SWANSEA UNIVERSITY

Engineering *Salmonella* to explore a metabolic vulnerability in cancer

MSc Biomedical Science · Supervisor: Professor Paul Dyson

THE RESEARCH QUESTION

Could engineering bacterial succinate transport and metabolism provide a route to consuming excess succinate in the tumour microenvironment?



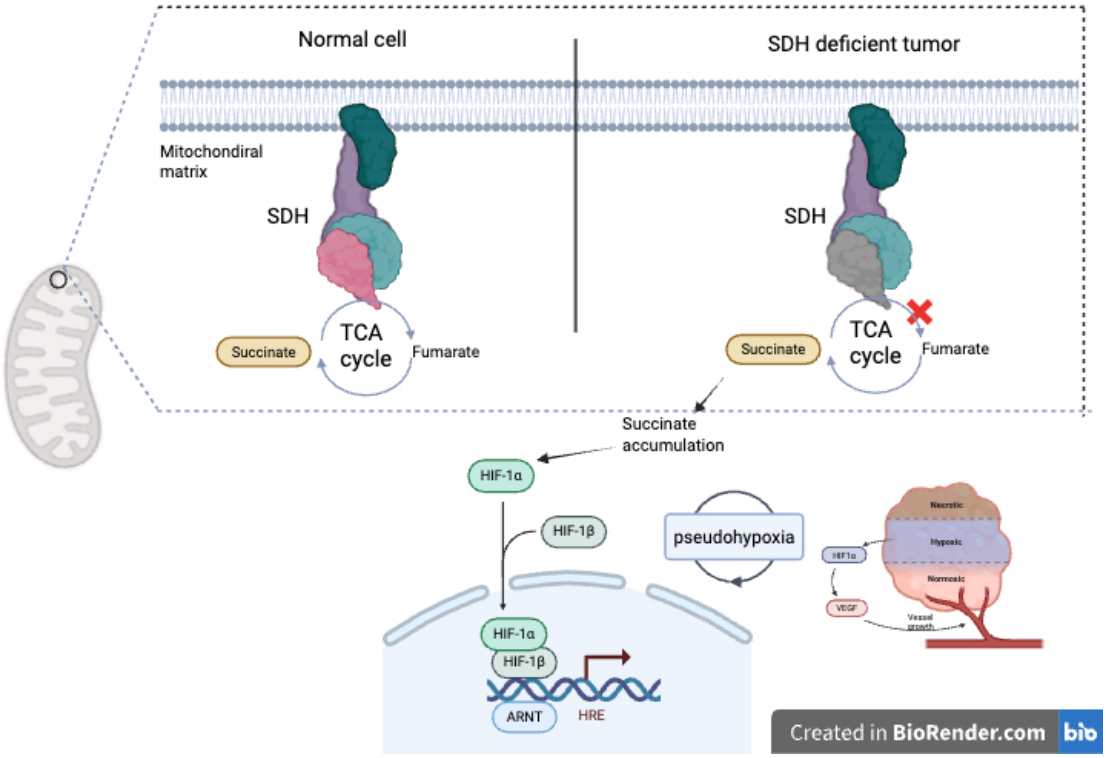
Graphical abstract. Original dissertation illustration showing the proposed engineering strategy and its intended metabolic application. The complete scheme is a research concept, not evidence that every illustrated step was achieved. Original BioRender credit retained.

PROJECT OVERVIEW

The project targeted two complementary functions: *dctA* for succinate transport and *sdhCDAB* for succinate oxidation. A sequence-verified *dctA* construct was obtained; attempts to clone the larger *sdh* operon revealed technical barriers that required further investigation.

Why target succinate?

Connecting a cancer-metabolism question to a molecular engineering project.



Dissertation Figure 1. Succinate metabolism in normal and SDH-deficient tumour cells. The illustration explains the biological rationale; it is not a result from a tumour experiment in this project.

In SDH-deficient cancers, impaired conversion of succinate to fumarate can lead to succinate accumulation. The dissertation explores how this can influence oxygen sensing and tumour-supporting signalling, and considers whether engineered bacteria could eventually act as a metabolic sink.

TWO TARGETS, TWO DIFFERENT FUNCTIONS

Transport — *dctA*. The 1,287 bp gene encodes an aerobic dicarboxylate transporter. Cloning it into pLF305 was intended to support succinate uptake.

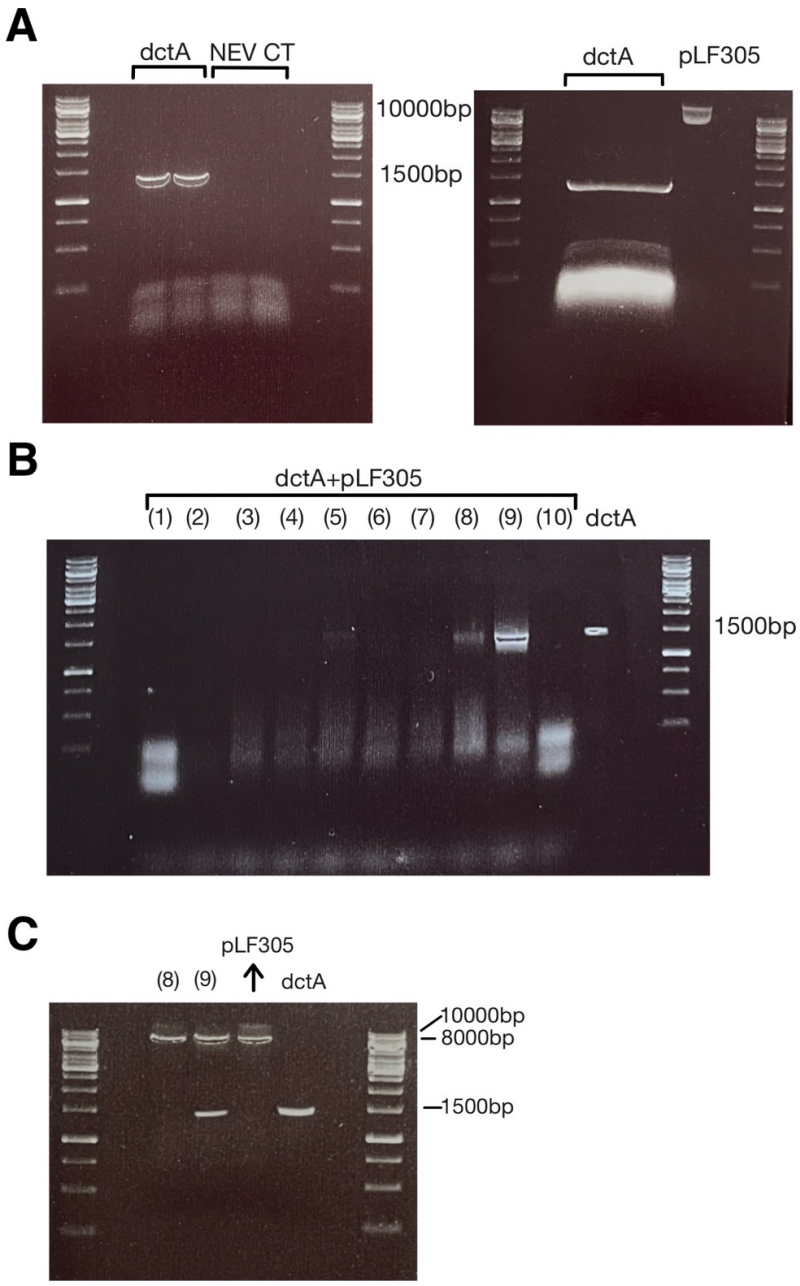
Oxidation — *sdhCDAB*. The approximately 3.5 kb operon encodes succinate dehydrogenase. Its larger size made amplification and stable cloning a separate experimental challenge.

MY PRACTICAL CONTRIBUTION

Genomic DNA preparation and primer design; PCR optimisation; restriction–ligation cloning; bacterial transformation and plasmid preparation; colony PCR, restriction analysis and sequencing-based verification. The work combined construct development with investigation of unexpected results.

Building the dctA construct

Complementary assays supported progression from an amplicon to a verified plasmid.

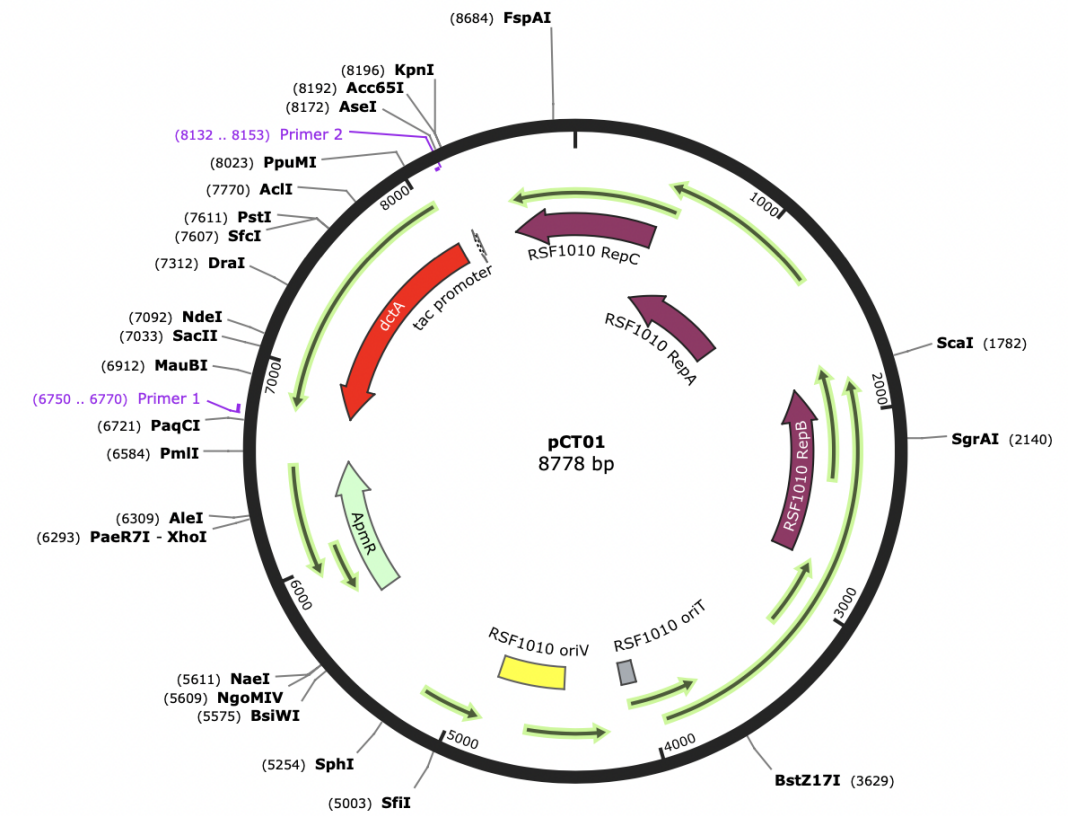


Original dissertation Figure 3A–C. Gel panels and lane annotations reproduced unchanged at a larger scale.

A — Amplification: a product at the expected *dctA* size supported cloning.
B — Screening: colony PCR identified candidate transformants.
C — Restriction analysis: diagnostic digestion examined the insert and vector. Sequencing provided the final check of identity and orientation.

From sequence to function

The plasmid map and the biological question address different levels of validation.



Original dissertation Figure 3D. Map labelled pCT01; the written results call the construct pCT1. Sequencing confirmed *dctA* identity and orientation, with no mutations or frameshifts reported.

BIOLOGICAL FUNCTION

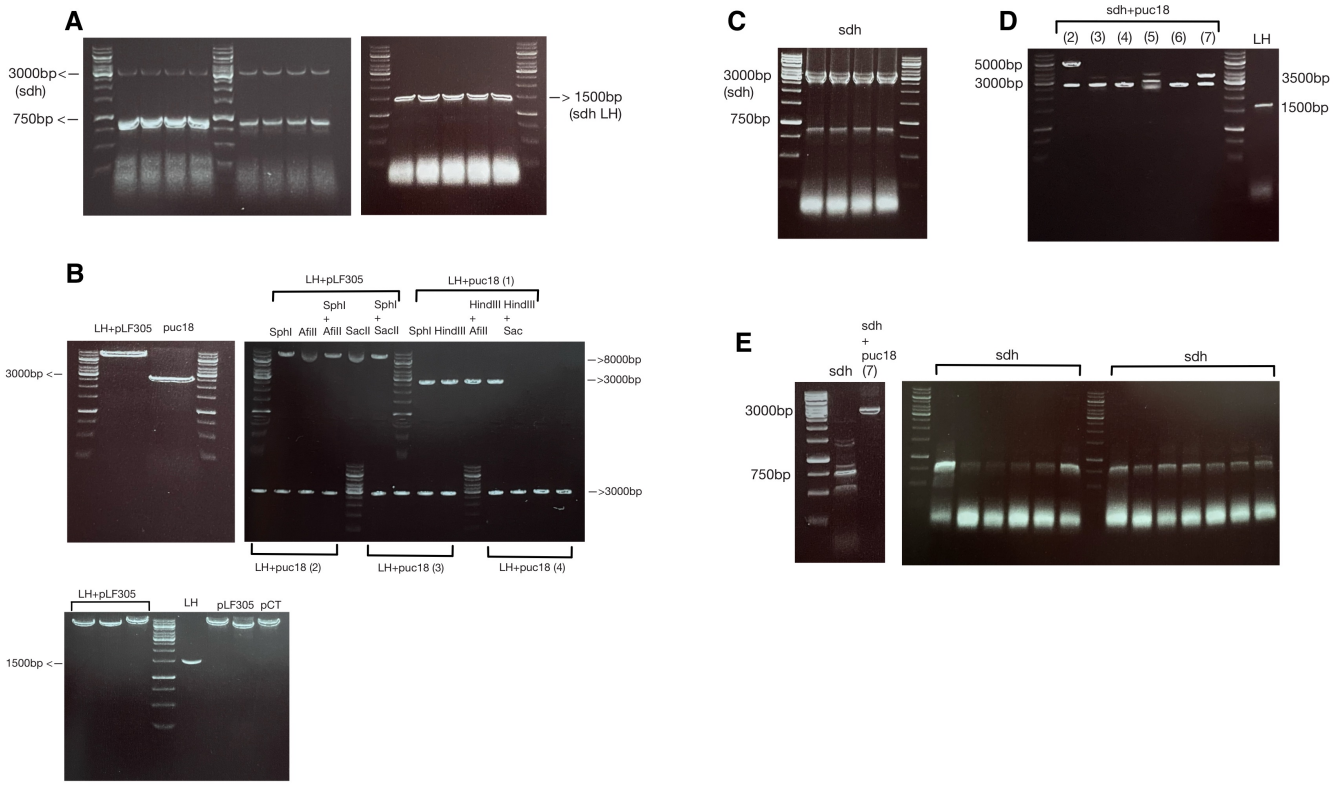
Updated growth result supplied by Chloe — wording pending confirmation.

The new account describes growth of the pCT1-bearing recombinant in minimal medium with succinate as the sole carbon source, compared with no growth of the parental strain under the same conditions. This differs from the supplied dissertation's statement that functional assays were not performed. The experiment's status and strain identifier are being confirmed before this section is finalised.

Growth on succinate would support utilisation under the tested conditions. It would not by itself establish extracellular succinate depletion in a tumour model or an anticancer effect.

The sdh cloning challenge

Successful amplification did not establish a stable, correct recombinant construct.



Original dissertation Figure 4A–E. Left: amplification and screening of the LH fragment. Right: full-operon amplification, candidate screening and follow-up PCR. Original panels retained.

WHAT THE CHECKS SHOWED

PCR optimisation produced the full-length operon, and one candidate initially showed a promising banding pattern. Follow-up PCR did not confirm the intended insert. Sequencing of selected plasmids instead identified approximately 5.5 kb fragments of unrelated *E. coli* genomic DNA, rather than the intended 3.5 kb operon.

INTERPRETATION & NEXT STEPS

Incomplete digestion and DNA rearrangement were proposed explanations, not established causes. The dissertation proposes alternative assembly approaches and hosts suited to unstable inserts. The key lesson was the value of checking construct identity beyond an initial gel band.

Sources: Chau Tran, MSc dissertation, *Developing tumor-targeting Salmonella strain optimised for utilisation of succinate*, Swansea University; original graphical abstract and Figures 1, 3 and 4. Biological-function update supplied by the author; clarification pending.