Accelerating CBPP research towards a better vaccine through the application of genome transplantation

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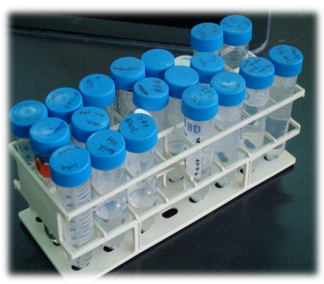
ABSTRACT

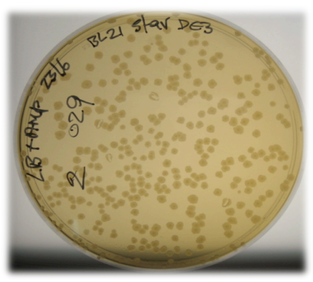
Contagious bovine pleuropneumonia (CBPP) caused by *Mycoplasma mycoides* subsp. *mycoides* (*Mmm*) is one of the most important livestock diseases in sub-Saharan Africa. CBPP impacts health and poverty of livestock-dependent people through decreased animal productivity, reduced food supply, and the cost of control measures. Additionally, CBPP is a barrier to trade in many African countries and this reduces the value of livestock and the income of many value chain stakeholders.

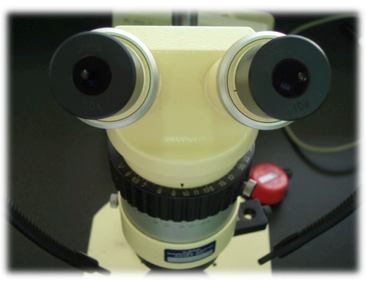
Presently control of CBPP relies mainly on a live vaccine of limited efficacy and duration of immunity. Vaccines with better efficacy are necessary for control and eradication programmes within all African regions.

The identification of Mycoplasma target molecules for vaccines has been hampered by the non-existence of genetic tools to manipulate the pathogen's genome in a systematic way. Recently, techniques for the targeted mutagenesis of the closely related Mycoplasma species *Mycoplasma mycoides* subsp. *capri* (*Mmc*) have been developed as part of synthetic biology efforts. Yeast is used as a host cell for the whole Mycoplasma genome, opening up to the fast and efficient genome manipulation toolbox for yeast. Back-transplantation of the genome to Mycoplasma cells will enable the subsequent testing of targeted genes for their role in host pathogen interactions using *in vitro* and *in vivo* systems.

Targeted mutagenesis will accelerate the knowledge gain with respect to pathogenicity and host specificity and therefore vaccine development.
We are in the process of transferring the genome transplantation technology to ILRI in Africa and adapting the method to field strains of *Mycoplasma mycoides* subsp. *mycoides.* We have also started the procedure of targeted mutagenesis of putative virulence genes in the already established *Mycoplasma mycoides* subsp. *capri* model





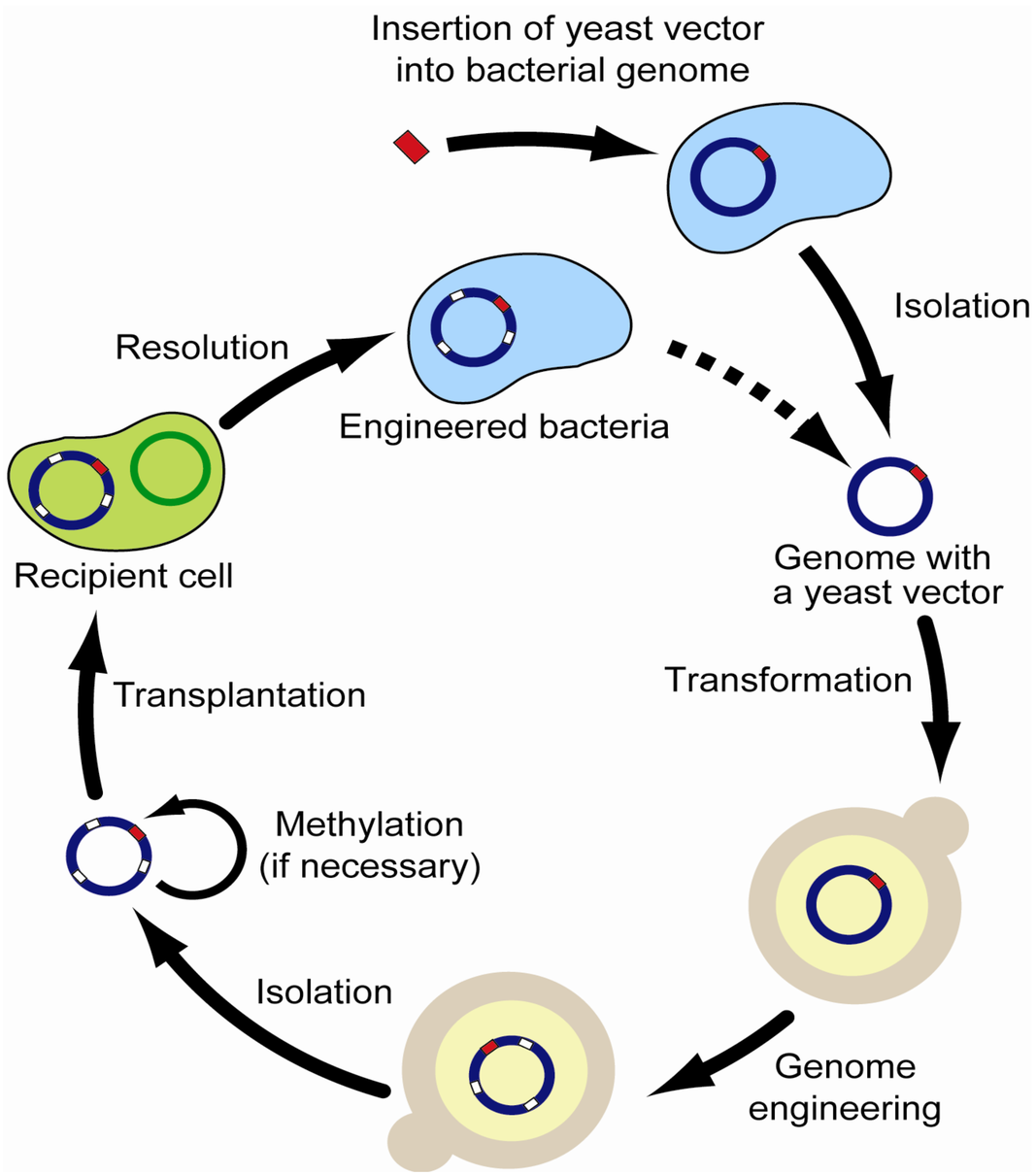
Long-term Aims (>6yrs):
Recombinant vaccine

Short-term Aims:
Identify genes involved in virulence

Why genome transplantation?
Offers a rapid and site directed way of altering the genome:

Once the genome is in yeast we can easily target any gene of interest, using the yeast genetic manipulation toolbox.

For example TREC (tandem repeat coupled with endonuclease cleavage): Offering seamless deletions.



Where are we now?

All steps in the genome transplantation cycle have been performed by us using *Mycoplasma mycoides* subsp. *capri*

The insertion of a yeast vector into the genome of *Mycoplasma mycoides* subsp. *mycoides* is ongoing.

First knock-outs of putative virulence genes (n=2) have been done, now awaiting back-transplantation to Mycoplasma cells followed by *in vitro* testing.

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