Rotavirus VP6 extracellular secretion by *Kluyveromyces lactis*

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Background
Rotavirus is the leading cause of diarrhoea in children under the age of five. Due to the high cost and possible risk of a global shortage of the current licensed live-attenuated vaccines, production of local, non-live vaccines is necessary. Rotavirus VP6 protein is highly immunogenic and an attractive vaccine candidate as anti-VP6 antibodies inhibit viral replication. It also has the ability to self-assemble in tubular structures which have shown to confer protection in mice. Yeast, as a recombinant protein production platform, is cost-effective and scalable. Previously, VP6 has been expressed intracellularly in various yeast strains. Extracellular production of VP6 by yeast would be desirable for scalable, low-cost production.

Methods
The wide-range yeast expression vector, pKM177, previously used for intracellular expression contained the VP6 open reading frame optimised for expression in *Pichia pastoris* and *Hansenula polymorpha* driven by a TEF promoter from *Yarrowia lipolytica*. Here the promoter was exchanged for the *LAC4* promoter from *Kluveromyces lactis* for improved expression. A native signal sequence (*K. lactis* αMF) was also introduced to facilitate secretion of VP6. These changes in the expression vector were introduced using the NEBuilder HiFi DNA Assembly cloning kit. Following transformation of *K. lactis* using electroporation, best expressing colonies were identified during a deep-well plate screen. Expression was monitored via ELISA. N-terminal processing was evaluated by mass spectrometry analysis.

Results
Sanger sequencing confirmed the successful introduction of a signal peptide sequence as well as the exchange of the TEF promoter. Hundred colonies were obtained following transformation of *K. lactis*. Secretion of VP6 was detected following deep-well culture screening. Results investigating N-terminal processing of the signal sequence will be presented.

Conclusion
Secretion of rotavirus VP6 by yeast was obtained for the first time. Extracellular production of VP6 will considerably reduce downstream processing and subsequently, lower production costs.