

# High throughput techniques as a strategy to overcome crystal twinning

Stephen Carr, Simon Phillips and Chris Thomas

## Background

Staphylococcal plasmids of the pT181 family use a “rolling circle” mode of replication. This requires a plasmid-encoded initiator protein, Rep. These proteins share over 80% sequence identity, yet, *in vivo*, the Rep proteins are specific for their cognate plasmid: thus RepD protein initiates replication for pC221, RepC for pT181 and RepN for pCW7.

Previously crystallisation trials have been performed with RepD protein and chimeric constructs in which the C-terminal domain of RepD has been exchanged with that of either RepC or RepN. The latter constructs produced diffraction quality crystals, but structure solution has been hampered by the presence of near-perfect merohedral twinning in crystals of both proteins.

## Recent Findings

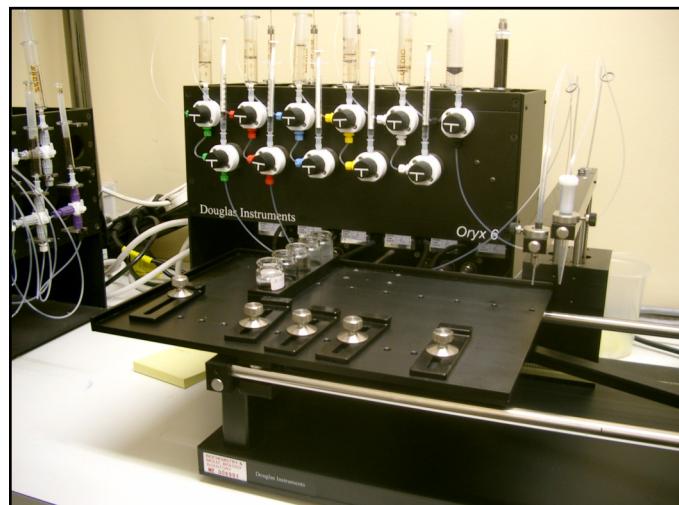
In an attempt to produce non-twinned crystals suitable for diffraction studies, the choice of potential target proteins has been expanded to encompass the entire protein family. These can be considered point mutations on a larger scale as multiple residues will be altered between constructs.

Rep proteins C, D, E, I, J and N have all been cloned into plasmid pET11a. Truncated variants of the above proteins, which lack a highly variable region of 30 amino acids at the extreme N-terminus, have also been produced. The truncated constructs remove what is likely to be a highly flexible region of the proteins which could hamper crystallisation. These have been shown to be active in the case of RepD.

The novel Rep targets have all been over-expressed and can be readily purified in milligram quantities. These targets are currently undergoing automated crystallisation trials using a robot produced by Douglas Instruments.

## Acknowledgements

We thank Val sergeant for technical support. This work is funded by the Wellcome Trust.



**Fig 1.** Crystallisation robot (Douglas Instruments)