



## Antibiotic-free Plasmid Maintenance System: ORT<sup>®</sup>

Cobra Biologics, with locations in Matfors and Södertälje, Sweden and Keele, UK, provides a full range of services from gene cloning to cGMP manufacturing for pre-clinical through to Phase III clinical trials and commercial supply. In support of customer programmes, the company has developed a range of expression technologies. ORT<sup>®</sup> is the only technology able to replicate any plasmid without antibiotics, antibiotic resistance or other selectable marker genes in *Escherichia coli*.

### Features and Benefits

- ORT<sup>®</sup> provides antibiotic-free selection and maintenance of high copy-number plasmids
- ORT<sup>®</sup> plasmids are highly stable
- No antibiotic contamination of plasmid DNA
- Minimized plasmid vectors allow more therapeutic gene per milligram of DNA
- No metabolic burden due to selectable marker gene expression
- ORT<sup>®</sup> vaccines used in numerous clinical trials

### Applications

- DNA vaccines: Smaller plasmids for improved antigen gene delivery
- Gene therapy: Plasmid vector with an improved safety profile for delivery of therapeutic genes
- Recombinant protein production: Antibiotic-free fermentation with potential for higher product yields through enhanced stability

### Background

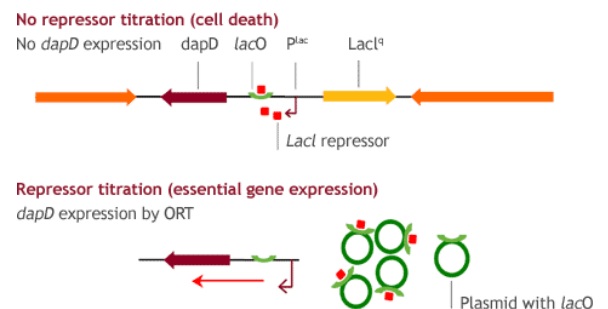
The isolation of bacterial transformants and subsequent stable plasmid maintenance has traditionally been accomplished using plasmid-borne selectable marker genes. The marker genes typically encode resistance to an antibiotic, or alternatively encode an essential gene that complements a host cell deficiency (auxotrophy). The disadvantages of these systems are:

- Imposition of a metabolic burden on the host from selectable marker gene expression
- Potential reduction in recombinant protein yield
- Additional cost and downstream processing requirements to remove traces of antibiotics

### Technical Design

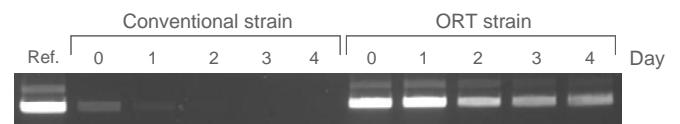
The ORT<sup>®</sup> technology employs plasmid-mediated repressor titration to activate a host selectable marker, removing the requirement for a plasmid-borne marker gene. Current ORT<sup>®</sup> strains are engineered to contain an

essential gene, *dapD*, under transcriptional control of the *lac* operator/promoter (*lacO/P*), although any essential gene can be used. In the absence of an inducer such as IPTG, this strain cannot grow due to the repression of *dapD* by the LacI repressor protein binding to *lacO/P*. Transformation with a high copy number plasmid containing the *lac* operator (*lacO*) effectively induces *dapD* expression by titrating LacI from the operator. Regulation of the *dapD* ensures the growth of bacteria and maintenance of recombinant plasmids containing *lacO* and an origin of replication.



### Superior Plasmid Maintenance

Stable plasmid maintenance is essential for generating high yields of plasmid DNA and recombinant protein. A typical DNA vaccine vector was replicated in a conventional and an ORT-VAC strain of *E. coli* and sampled at daily intervals.



The ORT<sup>®</sup> strain enabled significantly better plasmid maintenance over multiple generations than the conventional strain from which it was derived.

### References

- Durany *et al.* 2005, *Biotechnol. Bioeng.* 91: 460-467.  
 Cranenburgh *et al.* 2004, *JMMB* 7: 197-203.  
 Cranenburgh *et al.* 2001, *Nucleric Acids Res.* 29: e26.