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Calcium chloride-mediated transformation of different Chlamydia species

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Abstract

Summary

The genetic toolbox for the obligate intracellular bacterial species *Chlamydia trachomatis* and *C. muridarum* has rapidly expanded in recent years (Sixt and Valdivia, 2016; O'Neill *et al.*, 2020; Fields *et al.*, 2022), and has now extended to *C. caviae, C. felis, C. pecorum, C. pneumoniae, C. psittaci* and *C. suis* (Shima *et al.*, 2018, 2020; Filcek *et al.*, 2019; Marti *et al.*, 2023; Faessler *et al.*, In Preparation). This document comprises details for the design, construction, and transformation of various vectors into different *Chlamydia* species and was adapted from the original publication on calcium chloride-mediated transformation (Wang *et al.*, 2011).

References:

Bauler, L. D., and Hackstadt, T. (2014). Expression and Targeting of secreted Proteins from *Chlamydia trachomatis*. *Journal of Bacteriology,* 196, 1325–1334. doi:10.1128/JB.01290-13

Faessler, N., Biggel, M., Jelocnik, M., Borel, N., and Marti, H. (In Preparation). Development of shuttle vector-based transformation systems for *Chlamydia pecorum* and *Chlamydia caviae*.

Fields, K. A., Bodero, M. D., Scanlon, K. R., Jewett, T. J., and Wolf, K. (2022). A Minimal Replicon Enables Efficacious, Species-Specific Gene Deletion in Chlamydia and Extension of Gene Knockout Studies to the Animal Model of Infection Using *Chlamydia muridarum*. *Infection and Immunity* 90, e00453-22. doi: 10.1128/iai.00453-22

Filcek, K., Vielfort, K., Muraleedharan, S., Henriksson, J., Valdivia, R. H., Bavoil, P. M., *et al.* (2019). Insertional mutagenesis in the zoonotic pathogen *Chlamydia caviae*. *PLoS One* 14, e0224324. doi: 10.1371/journal.pone.0224324

Marti, H., Biggel, M., Shima, K., Onorini, D., Rupp, J., Charette, S. J., *et al.* (2023). *Chlamydia suis* displays high transformation capacity with complete cloning vector integration into the chromosomal rrn-nqrF plasticity zone. *Microbiol Spectr* 11, e02378-23. doi: 10.1128/spectrum.02378-23

O'Neill, C. E., Clarke, I. N., and Fisher, D. J. (2020). "Chlamydia Genetics," in *Chlamydia Biology: From Genome to Disease*, eds. M. Tan, J. H. Hegeman, and C. Sütterlin (Norfolk: Caister Academic Press), 241–262. doi: 10.21775/9781912530281.11

Shima, K., Wanker, M., Skilton, R. J., Cutcliffe, L.T., Schnee, C., Kohl, T. A., *et al.* (2018). The Genetic Transformation of *Chlamydia pneumoniae*. *mSphere* 3, e00412-18. doi: 10.1128/mSphere.00412-18

Shima, K., Weber, M. M., Schnee, C., Sachse, K., Käding, N., Klinger, M., *et al.* (2020). Development of a Plasmid Shuttle Vector System for Genetic Manipulation of *Chlamydia psittaci*. *mSphere* 5, e00787-20. doi: 10.1128/mSphere.00787-20

Sixt, B. S., and Valdivia, R. H. (2016). Molecular Genetic Analysis of *Chlamydia* Species. *Annual Review of Microbiology* 70, 179–198. doi: 10.1146/annurev-micro-102215-095539

Tam, J. E., Davis, C. H., Thresher, R. J., and Wyrick, P. B. (1992). Location of the origin of replication for the 7.5-kb *Chlamydia trachomatis* plasmid. *Plasmid* 27, 231–236. doi: 10.1016/0147-619x(92)90025-6

Wang, Y., Kahane, S., Cutcliffe, L. T., Skilton, R.J., Lambden, P. R., and Clarke, I. N. (2011). Development of a Transformation System for *Chlamydia trachomatis*: Restoration of Glycogen Biosynthesis by Acquisition of a Plasmid Shuttle Vector. *PLoS Pathogens* 7, e1002258. doi: 10.1371/journal.ppat.1002258

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Protocol references

Bauler, L. D., and Hackstadt, T. (2014). Expression and Targeting of secreted Proteins from *Chlamydia trachomatis*. *Journal of Bacteriology,* 196, 1325–1334. doi:10.1128/JB.01290-13

Faessler, N., Biggel, M., Jelocnik, M., Borel, N., and Marti, H. (In Preparation). Development of shuttle vector-based transformation systems for *Chlamydia pecorum* and *Chlamydia caviae*.

Fields, K. A., Bodero, M. D., Scanlon, K. R., Jewett, T. J., and Wolf, K. (2022). A Minimal Replicon Enables Efficacious, Species-Specific Gene Deletion in Chlamydia and Extension of Gene Knockout Studies to the Animal Model of Infection Using *Chlamydia muridarum*. *Infection and Immunity* 90, e00453-22. doi: 10.1128/iai.00453-22

Filcek, K., Vielfort, K., Muraleedharan, S., Henriksson, J., Valdivia, R. H., Bavoil, P. M., *et al.* (2019). Insertional mutagenesis in the zoonotic pathogen *Chlamydia caviae*. *PLoS One* 14, e0224324. doi: 10.1371/journal.pone.0224324

Marti, H., Biggel, M., Shima, K., Onorini, D., Rupp, J., Charette, S. J., *et al.* (2023). *Chlamydia suis* displays high transformation capacity with complete cloning vector integration into the chromosomal rrn-nqrF plasticity zone. *Microbiol Spectr* 11, e02378-23. doi: 10.1128/spectrum.02378-23

O'Neill, C. E., Clarke, I. N., and Fisher, D. J. (2020). "Chlamydia Genetics," in *Chlamydia Biology: From Genome to Disease*, eds. M. Tan, J. H. Hegeman, and C. Sütterlin (Norfolk: Caister Academic Press), 241–262. doi: 10.21775/9781912530281.11

Shima, K., Wanker, M., Skilton, R. J., Cutcliffe, L.T., Schnee, C., Kohl, T. A., *et al.* (2018). The Genetic Transformation of *Chlamydia pneumoniae*. *mSphere* 3, e00412-18. doi: 10.1128/mSphere.00412-18

Shima, K., Weber, M. M., Schnee, C., Sachse, K., Käding, N., Klinger, M., *et al.* (2020). Development of a Plasmid Shuttle Vector System for Genetic Manipulation of *Chlamydia psittaci*. *mSphere* 5, e00787-20. doi: 10.1128/mSphere.00787-20

Sixt, B. S., and Valdivia, R. H. (2016). Molecular Genetic Analysis of *Chlamydia* Species. *Annual Review of Microbiology* 70, 179–198. doi: 10.1146/annurev-micro-102215-095539

Tam, J. E., Davis, C. H., Thresher, R. J., and Wyrick, P. B. (1992). Location of the origin of replication for the 7.5-kb *Chlamydia trachomatis* plasmid. *Plasmid* 27, 231–236. doi: 10.1016/0147-619x(92)90025-6

Wang, Y., Kahane, S., Cutcliffe, L. T., Skilton, R.J., Lambden, P. R., and Clarke, I. N. (2011). Development of a Transformation System for *Chlamydia trachomatis*: Restoration of Glycogen Biosynthesis by Acquisition of a Plasmid Shuttle Vector. *PLoS Pathogens* 7, e1002258. doi: 10.1371/journal.ppat.1002258