

Video Article

Erratum: Creation of a Dense Transposon Insertion Library Using Bacterial Conjugation in Enterobacterial Strains Such As *Escherichia Coli* or *Shigella flexneri*

URL: https://www.jove.com/video/6113

DOI: doi:10.3791/6113

Keywords:

Date Published: 5/14/2018

Citation: Erratum: Creation of a Dense Transposon Insertion Library Using Bacterial Conjugation in Enterobacterial Strains Such As Escherichia Coli or Shigella flexneri. J. Vis. Exp. (), e6113, doi:10.3791/6113 (2018).

Abstract

An erratum was issued for: Creation of a Dense Transposon Insertion Library Using Bacterial Conjugation in Enterobacterial Strains Such As *Escherichia Coli* or *Shigella flexneri*. The Discussion and References sections have been corrected.

The second paragraph in the Discussion section was updated from:

A significant benefit of this method is that, in theory, the user has wide latitude in the choice of enterobacterial recipient strains. This paper, as well as others¹¹, use *E. coli* as a recipient strain, however the pJA1 plasmid has been used successfully with other enterobacterial recipient species such as *Shigella flexneri*⁶ and *Salmonella enterica* serovar Typhimurium strain SL1344¹⁰. Theoretically, the γ origin of replication ($ori_{R6K\gamma}$) in pJA1 allows this plasmid to be maintained in a broad host range¹⁹, allowing that the recipient strain is pir+. Recently, new methods have been described that allow for construction of the pir+ in a range of enterobacterial strains²⁰, giving additional flexibility. Additionally, the 300 base pair mob region from the RP4 plasmid in pJA1 allows conjugative transfer of this plasmid to a wide range of gram negative bacterial strains¹⁹. Simply put, this method could theoretically be used with a variety of recipient strains, as long as several conditions are met: the strain is pir+, and is marked with an antibiotic resistance other than kanamycin and other than the donor strain.

to

A significant benefit of this method is that, in theory, the user has wide latitude in the choice of enterobacterial recipient strains. This paper, as well as others¹¹, use *E. coli* as a recipient strain, however the pJA1 plasmid has been used successfully with other enterobacterial recipient species such as *Shigella flexneri*⁶ and *Salmonella enterica* serovar Typhimurium strain SL1344¹⁰. Theoretically, the γ origin of replication ($\sigma i_{R6K\gamma}$) in pJA1 allows this plasmid to be maintained in a broad host range¹⁹, allowing that the recipient strain is ρir . The 300 base pair mob region from the RP4 plasmid in pJA1 allows conjugative transfer of this plasmid to a wide range of gram negative bacterial strains¹⁹. Simply put, this method could theoretically be used with a variety of recipient strains, as long as several conditions are met: the strain is ρir , and is marked with an antibiotic resistance other than kanamycin and other than the donor strain.

The References section was updated from:

- 18. Blattner, F. R., Plunkett, G., et al. The Complete Genome Sequence of Escherichia coli K-12. Science. 277 (5331), 1453-1462 (1997).
- 19. Alexeyev, M. F., & Shokolenko, I. N. Mini-Tn10 transposon derivatives for insertion mutagenesis and gene delivery into the chromosome of gram-negative bacteria. *Gene.* **160** (1), 59-62 (1995).
- 20. Kvitko, B. H., Bruckbauer, S., et al. A simple method for construction of pir+ Enterobacterial hosts for maintenance of R6K replicon plasmids. *BMC Res Notes.* **5** (1), 157 (2012).
- Barrick, J. E., Yu, D. S., et al. Genome evolution and adaptation in a long-term experiment with Escherichia coli. Nature. 461 (7268), 1243-1247 (2009).
- 22. Jacobs, M. A., Alwood, A., et al. Comprehensive transposon mutant library of Pseudomonas aeruginosa. PNAS. 100 (24), 14339-14344 (2003).
- 23. van Opijnen, T., & Camilli, A. Transposon insertion sequencing: a new tool for systems-level analysis of microorganisms. *Nature Rev Microbiol.* **11** (7), 435-442 (2013).

to:

- 18. Blattner, F. R., Plunkett, G., et al. The Complete Genome Sequence of Escherichia coli K-12. Science. 277 (5331), 1453-1462 (1997).
- 19. Alexeyev, M. F., & Shokolenko, I. N. Mini-Tn10 transposon derivatives for insertion mutagenesis and gene delivery into the chromosome of gram-negative bacteria. *Gene.* **160** (1), 59-62 (1995).
- Barrick, J. E., Yu, D. S., et al. Genome evolution and adaptation in a long-term experiment with Escherichia coli. Nature. 461 (7268), 1243-1247 (2009).
- 21. Jacobs, M. A., Alwood, A., et al. Comprehensive transposon mutant library of Pseudomonas aeruginosa. PNAS. 100 (24), 14339-14344 (2003).
- 22. van Opijnen, T., & Camilli, A. Transposon insertion sequencing: a new tool for systems-level analysis of microorganisms. *Nature Rev Microbiol.* **11** (7), 435-442 (2013).



Protocol

An erratum was issued for: Creation of a Dense Transposon Insertion Library Using Bacterial Conjugation in Enterobacterial Strains Such As *Escherichia Coli* or *Shigella flexneri*. The Discussion and References sections have been corrected.

The second paragraph in the Discussion section was updated from:

A significant benefit of this method is that, in theory, the user has wide latitude in the choice of enterobacterial recipient strains. This paper, as well as others 11 , use *E. coli* as a recipient strain, however the pJA1 plasmid has been used successfully with other enterobacterial recipient species such as *Shigella flexneri* and *Salmonella enterica* serovar Typhimurium strain SL1344 10 . Theoretically, the γ origin of replication ($ori_{R6K\gamma}$) in pJA1 allows this plasmid to be maintained in a broad host range 19 , allowing that the recipient strain is pir+. Recently, new methods have been described that allow for construction of the pir+ in a range of enterobacterial strains 20 , giving additional flexibility. Additionally, the 300 base pair mob region from the RP4 plasmid in pJA1 allows conjugative transfer of this plasmid to a wide range of gram negative bacterial strains 19 . Simply put, this method could theoretically be used with a variety of recipient strains, as long as several conditions are met: the strain is pir+, and is marked with an antibiotic resistance other than kanamycin and other than the donor strain.

to:

A significant benefit of this method is that, in theory, the user has wide latitude in the choice of enterobacterial recipient strains. This paper, as well as others¹¹, use *E. coli* as a recipient strain, however the pJA1 plasmid has been used successfully with other enterobacterial recipient species such as *Shigella flexneri*⁶ and *Salmonella enterica* serovar Typhimurium strain SL1344¹⁰. Theoretically, the γ origin of replication ($\sigma r_{R6K\gamma}$) in pJA1 allows this plasmid to be maintained in a broad host range¹⁹, allowing that the recipient strain is ρr . The 300 base pair mob region from the RP4 plasmid in pJA1 allows conjugative transfer of this plasmid to a wide range of gram negative bacterial strains¹⁹. Simply put, this method could theoretically be used with a variety of recipient strains, as long as several conditions are met: the strain is ρr , and is marked with an antibiotic resistance other than kanamycin and other than the donor strain.

The References section was updated from:

- 18. Blattner, F. R., Plunkett, G., et al. The Complete Genome Sequence of Escherichia coli K-12. Science. 277 (5331), 1453-1462 (1997).
- 19. Alexeyev, M. F., & Shokolenko, I. N. Mini-Tn10 transposon derivatives for insertion mutagenesis and gene delivery into the chromosome of gram-negative bacteria. *Gene.* **160** (1), 59-62 (1995).
- 20. Kvitko, B. H., Bruckbauer, S., et al. A simple method for construction of pir+ Enterobacterial hosts for maintenance of R6K replicon plasmids. *BMC Res Notes.* **5** (1), 157 (2012).
- Barrick, J. E., Yu, D. S., et al. Genome evolution and adaptation in a long-term experiment with Escherichia coli. Nature. 461 (7268), 1243-1247 (2009).
- Jacobs, M. A., Alwood, A., et al. Comprehensive transposon mutant library of Pseudomonas aeruginosa. PNAS. 100 (24), 14339-14344 (2003).
- 23. van Opijnen, T., & Camilli, A. Transposon insertion sequencing: a new tool for systems-level analysis of microorganisms. *Nature Rev Microbiol.* **11** (7), 435-442 (2013).

to:

- 18. Blattner, F. R., Plunkett, G., et al. The Complete Genome Sequence of Escherichia coli K-12. Science. 277 (5331), 1453-1462 (1997).
- 19. Alexeyev, M. F., & Shokolenko, I. N. Mini-Tn10 transposon derivatives for insertion mutagenesis and gene delivery into the chromosome of gram-negative bacteria. *Gene.* **160** (1), 59-62 (1995).
- Barrick, J. E., Yu, D. S., et al. Genome evolution and adaptation in a long-term experiment with Escherichia coli. Nature. 461 (7268), 1243-1247 (2009).
- 21. Jacobs, M. A., Alwood, A., et al. Comprehensive transposon mutant library of Pseudomonas aeruginosa. PNAS. 100 (24), 14339-14344 (2003).
- 22. van Opijnen, T., & Camilli, A. Transposon insertion sequencing: a new tool for systems-level analysis of microorganisms. *Nature Rev Microbiol.* **11** (7), 435-442 (2013).

Disclosures

No conflicts of interest declared.