CLAIMS:

1. A method for identifying an essential gene which serves as an antibiotic target in a bacterium, the method comprising the steps of:

(a) generating a pool of mutant bacteria by transposon mutagenesis with an activating transposon (Tn_A), wherein the Tn_A comprises a promoter such that transposon insertion into bacterial DNA increases the transcription of a gene at or near the insertion site;

(b) growing bacteria from the mutant pool in the presence of different amounts of said antibiotic to produce two or more test cultures; and

(c) comparing the distribution of Tn_A insertions between test cultures to identify a putative essential gene serving as a target of said antibiotic in said bacterium.

2. The method of claim 1 wherein in step (a) a combination of two or more different transposons is used to generate the pool of mutant bacteria.

3. The method of claim 1 or claim 2 wherein the pool of mutant bacteria comprises at least 0.5 x 10^5 mutants, for example at least 1 x 10^5 mutants.

~~3~~4. The method of claim 1 or claim 2 wherein the pool of mutant bacteria comprises at least 5x10^5 mutants.

~~4~~5. The method of claim 1 or claim 2 wherein the pool of mutant bacteria comprises at least 1x10^6 mutants.

~~5~~6. The method of claim 1 or claim 2 wherein the pool of mutant bacteria comprises 0.5 x10^6 to 2 x10^6 mutants.

~~6~~7. The method of claim ~~5~~ 6 wherein the pool of mutant bacteria comprises about 1 x10^6 mutants.

~~7~~8. The method of any one of the preceding claims wherein the transposon mutagenesis step (a) yields an insertion rate of at least one transposon per 50 base pairs of bacterial DNA.

~~8~~9. The method of any one of the preceding claims wherein the transposon mutagenesis step (a) yields an insertion rate of at least one transposon per 30 base pairs of bacterial DNA.

~~9~~10. The method of any one of the preceding claims wherein the transposon mutagenesis step (a) yields an insertion rate of at least one transposon per 25 base pairs of bacterial DNA.

~~10~~11. The method of any one of the preceding claims wherein the transposon mutagenesis step (a) yields an insertion rate of at least one transposon per 15 base pairs of bacterial DNA.

~~11~~12. The method of any one of the preceding claims wherein the transposon mutagenesis step (a) yields an insertion rate of at least one transposon per 10 base pairs of bacterial DNA.

~~12~~13. The method of any one of the preceding claims wherein the bacterial DNA of step (a) is chromosomal (genomic) DNA.

~~13~~14. The method of any one of claims 1 to ~~11~~ 12 wherein the bacterial DNA of step (a) is plasmid DNA or a mixture of chromosomal (genomic) and plasmid DNA.

~~14~~15. The method of any one of the preceding claims wherein the transposon mutagenesis of step (a) occurs *in vivo*.

~~15~~16. The method of any one of claims 1 to ~~13~~ 14 wherein the transposon mutagenesis of step (a) occurs *in vitro.*

~~16~~17. The method of any one of the preceding claims wherein the bacterium is a Gram-positive bacterium.

~~17~~18. The method of claim ~~16~~ 17 wherein the bacterium is selected from *Enterococcus faecalis*, *Enterococcus faecium* and *Neisseria gonorrhoeae.*

~~18~~19. The method of any one of claims 1 to ~~15~~ 16 wherein the bacterium is a Gram-negative bacterium.

~~19~~20. The method of claim ~~18~~ 19 wherein the bacterium is selected from: *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Escherichia coli*, *E. coli* ST131 strains, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Enterobacter aerogenes* and *Neisseria gonorrhoeae.*

~~20~~21. The method of any one of the preceding claims wherein bacteria are grown from the mutant pool in step (b) by inoculating growth medium with 10^7 to 10^9, for example about 10^8, cfu from the mutant pool.

~~21~~22. The method of any one of the preceding claims wherein bacteria are grown from the mutant pool in step (b) in the presence of antibiotic at a concentration of about 0.5, about 1 and about 2 x MIC to produce at least three test cultures.

~~22~~23. The method of any one of the preceding claims wherein the distribution of Tn_A insertions between test cultures is compared by sequencing DNA adjacent or near the insertion site of the Tn_A.

~~23~~24. The method of claim ~~22~~ 23 wherein the sequencing of DNA adjacent or near the insertion site of the Tn_A comprises selective amplification of transposon-bacterial DNA junctions.

~~24~~25. The method of claim ~~22~~ 23 or claim ~~23~~ 24 wherein the sequencing comprises sequencing-by-synthesis (SBS) biochemistry.

~~25~~26. The method of any one of claims ~~22~~ 23 to ~~24~~ 25 wherein about 25, 50, 75, 100 or greater than 100 base pairs of DNA adjacent or near the Tn_A insertion site are sequenced.

~~26~~27. The method of any one of claims ~~22~~ 23 to ~~25~~ 26 wherein the sequenced DNA is 5' and/or 3' to the Tn_A insertion site.

~~27~~28. The method of any one of the preceding claims further comprising the step of assigning an essential function to said putative essential gene by sequence comparison with one or more essential gene(s) of said bacterium.

~~28~~29. The method of claim ~~27~~ 28 wherein said essential gene(s) are identified by transposon directed insertion site sequencing using a transposon which inactivates on insertion.

~~29~~30. The method of any one of the preceding claims further comprising the step of assigning an essential function to said putative essential gene by determining that it is not an antibiotic resistance gene.

~~30~~31. The method of claim ~~29~~ 30 wherein the determining step comprises identifying an antibiotic resistance gene by transposon directed insertion site sequencing using a transposon which inactivates on insertion.

~~31~~32. The method of any one of the preceding claims further comprising the steps of: generating an antibiotic resistant mutant of said bacterium by a method comprising the step of selecting for growth in the presence of said antibiotic to produce an antibiotic resistant mutant clone (Ab^R mutant); transforming the Ab^R mutant with: (i) one or more essential genes of said bacterium; and (ii) a transposon which insertionally inactivates bacterial DNA, to produce a pool of transposon mutants which are merodiploid for said one or more essential genes; growing bacteria from the merodiploid pool in the presence of different amounts of said antibiotic to produce two or more test cultures; and comparing the distribution of transposon insertions between test cultures to identify a putative essential gene serving as a target of said antibiotic in said bacterium.

~~32~~33. A method of identifying an antibiotic comprising identifying an essential gene which serves as a target of said antibiotic according to a method as defined in any one of the preceding claims.

~~33~~34. A process for producing an antibiotic comprising the method as defined in claim ~~32~~33.

~~34~~35. The process of claim ~~33~~ 34 further comprising synthesising said antibiotic.

~~35~~36. The process of claim ~~34~~ 35 further comprising mixing the synthesised antibiotic with a pharmaceutically acceptable excipient to produce a pharmaceutical composition.

SEQUENCE LISTING

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CLAIMS:

1. A method for identifying an essential gene which serves as an antibiotic target in a bacterium, the method comprising the steps of:

(a) generating a pool of mutant bacteria by transposon mutagenesis with an activating transposon (Tn_A), wherein the Tn_A comprises a promoter such that transposon insertion into bacterial DNA increases the transcription of a gene at or near the insertion site;

(b) growing bacteria from the mutant pool in the presence of different amounts of said antibiotic to produce two or more test cultures; and

(c) comparing the distribution of Tn_A insertions between test cultures to identify a putative essential gene serving as a target of said antibiotic in said bacterium.

2. The method of claim 1 wherein in step (a) a combination of two or more different transposons is used to generate the pool of mutant bacteria.

3. The method of claim 1 or claim 2 wherein the pool of mutant bacteria comprises at least 0.5×10^5 mutants, for example at least 1×10^5 mutants.

4. The method of claim 1 or claim 2 wherein the pool of mutant bacteria comprises at least 5×10^5 mutants.

5. The method of claim 1 or claim 2 wherein the pool of mutant bacteria comprises at least 1×10^6 mutants.

6. The method of claim 1 or claim 2 wherein the pool of mutant bacteria comprises 0.5×10^6 to 2×10^6 mutants.

7. The method of claim 6 wherein the pool of mutant bacteria comprises about 1×10^6 mutants.

8. The method of any one of the preceding claims wherein the transposon mutagenesis step (a) yields an insertion rate of at least one transposon per 50 base pairs of bacterial DNA.

9. The method of any one of the preceding claims wherein the transposon mutagenesis step (a) yields an insertion rate of at least one transposon per 30 base pairs of bacterial DNA.

10. The method of any one of the preceding claims wherein the transposon mutagenesis step (a) yields an insertion rate of at least one transposon per 25 base pairs of bacterial DNA.

11. The method of any one of the preceding claims wherein the transposon mutagenesis step (a) yields an insertion rate of at least one transposon per 15 base pairs of bacterial DNA.

12. The method of any one of the preceding claims wherein the transposon mutagenesis step (a) yields an insertion rate of at least one transposon per 10 base pairs of bacterial DNA.

13. The method of any one of the preceding claims wherein the bacterial DNA of step (a) is chromosomal (genomic) DNA.

14. The method of any one of claims 1 to 12 wherein the bacterial DNA of step (a) is plasmid DNA or a mixture of chromosomal (genomic) and plasmid DNA.

15. The method of any one of the preceding claims wherein the transposon mutagenesis of step (a) occurs *in vivo*.

16. The method of any one of claims 1 to 14 wherein the transposon mutagenesis of step (a) occurs *in vitro.*

17. The method of any one of the preceding claims wherein the bacterium is a Gram-positive bacterium.

18. The method of claim 17 wherein the bacterium is selected from *Enterococcus faecalis*, *Enterococcus faecium* and *Neisseria gonorrhoeae.*

19. The method of any one of claims 1 to 16 wherein the bacterium is a Gram-negative bacterium.

20. The method of claim 19 wherein the bacterium is selected from: *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Escherichia coli*, *E. coli* ST131 strains, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Enterobacter aerogenes* and *Neisseria gonorrhoeae.*

21. The method of any one of the preceding claims wherein bacteria are grown from the mutant pool in step (b) by inoculating growth medium with 10^7 to 10^9, for example about 10^8, cfu from the mutant pool.

22. The method of any one of the preceding claims wherein bacteria are grown from the mutant pool in step (b) in the presence of antibiotic at a concentration of about 0.5, about 1 and about 2 x MIC to produce at least three test cultures.

23. The method of any one of the preceding claims wherein the distribution of Tn_A insertions between test cultures is compared by sequencing DNA adjacent or near the insertion site of the Tn_A.

24. The method of claim 23 wherein the sequencing of DNA adjacent or near the insertion site of the Tn_A comprises selective amplification of transposon-bacterial DNA junctions.

25. The method of claim 23 or claim 24 wherein the sequencing comprises sequencing-by-synthesis (SBS) biochemistry.

26. The method of any one of claims 23 to 25 wherein about 25, 50, 75, 100 or greater than 100 base pairs of DNA adjacent or near the Tn_A insertion site are sequenced.

27. The method of any one of claims 23 to 26 wherein the sequenced DNA is 5’ and/or 3’ to the Tn_A insertion site.

28. The method of any one of the preceding claims further comprising the step of assigning an essential function to said putative essential gene by sequence comparison with one or more essential gene(s) of said bacterium.

29. The method of claim 28 wherein said essential gene(s) are identified by transposon directed insertion site sequencing using a transposon which inactivates on insertion.

30. The method of any one of the preceding claims further comprising the step of assigning an essential function to said putative essential gene by determining that it is not an antibiotic resistance gene.

31. The method of claim 30 wherein the determining step comprises identifying an antibiotic resistance gene by transposon directed insertion site sequencing using a transposon which inactivates on insertion.

32. The method of any one of the preceding claims further comprising the steps of: generating an antibiotic resistant mutant of said bacterium by a method comprising the step of selecting for growth in the presence of said antibiotic to produce an antibiotic resistant mutant clone (Ab^R mutant); transforming the Ab^R mutant with: (i) one or more essential genes of said bacterium; and (ii) a transposon which insertionally inactivates bacterial DNA, to produce a pool of transposon mutants which are merodiploid for said one or more essential genes; growing bacteria from the merodiploid pool in the presence of different amounts of said antibiotic to produce two or more test cultures; and comparing the distribution of transposon insertions between test cultures to identify a putative essential gene serving as a target of said antibiotic in said bacterium.

33. A method of identifying an antibiotic comprising identifying an essential gene which serves as a target of said antibiotic according to a method as defined in any one of the preceding claims.

34. A process for producing an antibiotic comprising the method as defined in claim 33.

35. The process of claim 34 further comprising synthesising said antibiotic.

36. The process of claim 35 further comprising mixing the synthesised antibiotic with a pharmaceutically acceptable excipient to produce a pharmaceutical composition.

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