

https://dspace.org/

Mutation rates in mycobacterial hosts with altered Dna metabolic activity

Barichievy, Samantha

2006-02-08
http://hdl.handle.net/10539/163

Downloaded from a DSpace repository. https://dspace.org/

Appendix A : Media and some general solutions

A1 Media

All final volumes were made up to one litre with deionised sterile water. All media was sterilised by autoclaving for 10 minutes at 121^{o}C.

A1.1 Luria Bertani broth (LB)
10 g tryptone powder, 5 g yeast extract, 10 g sodium chloride

A1.2 Luria Bertani agar (LA)
10 g tryptone powder, 5 g yeast extract, 10 g sodium chloride, 15 g agar powder

A1.3 Middlebrook 7H9 broth (MADC-Tw)
4.7 g Middlebrook 7H9 media and 2 mL glycerol. To this, ADC (50 g/L albumin, 20 g/L dextrose and 0.04 g/L catalase as supplied by the manufacturer) and 5.0 mL of filter sterilised 20% Tween 80 were added aseptically after autoclaving

A1.4 Middlebrook 7H10 agar plates
19 g Middlebrook 7H10 media and 5 mL glycerol. To this, OADC (0.5 g/L oleic acid, 50 g/L albumin, 20 g/L dextrose and 0.04 g/L catalase as supplied by the manufacturer) was added aseptically after autoclaving

A1.5 2TY broth
16 g tryptone powder, 10 g yeast extract, 5 g sodium chloride

A2 General Solutions

All final volumes were one litre unless otherwise specified. All solutions were filter sterilised using a 0.22 µM filter

A2.1 Antibiotic stocks

A2.1.1 Ampicillin: 100 mg/mL made up in 50% ethanol and stored at -20^{o}C
A2.1.2 Gentamicin: 10 mg/mL made up in 50% ethanol and stored at -20^{o}C
A2.1.3 Hygromycin B: 50 mg/mL supplied in phosphate buffered saline and stored at 4^{o}C
A2.1.4 Kanamycin: 50 mg/mL made up in sterile water and stored at 4^{o}C
A2.1.5 Rifampicin: 200 mg/mL made up in DMF and stored in dark glass at 4^{o}C

A2.2 L-Histidine

100 mg/mL made up in sterile water and stored at 4^{o}C

A2.3 Solution I (plasmid isolation)

0.5 M glucose, 50 mM Tris-HCl pH 8.0, 10mM EDTA

A2.4 Solution II (plasmid isolation)

0.2 M NaOH, 1% SDS

A2.5 Solution III (plasmid isolation)

3 M potassium acetate, pH 5.5

A2.6 Cetyltrimethylammoniumbromide (CTAB) solution

10% cetyltrimethylammoniumbromide prepared in 0.7 M NaCl

A2.7 TE buffer

10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 7.6

A2.8 Loading dye

Recipe for 15 mL stock solution: 4.5mL glycerol, 10.5mL deionised sterile distilled water, 0.04g bromophenol blue

Appendix B : Plasmid maps

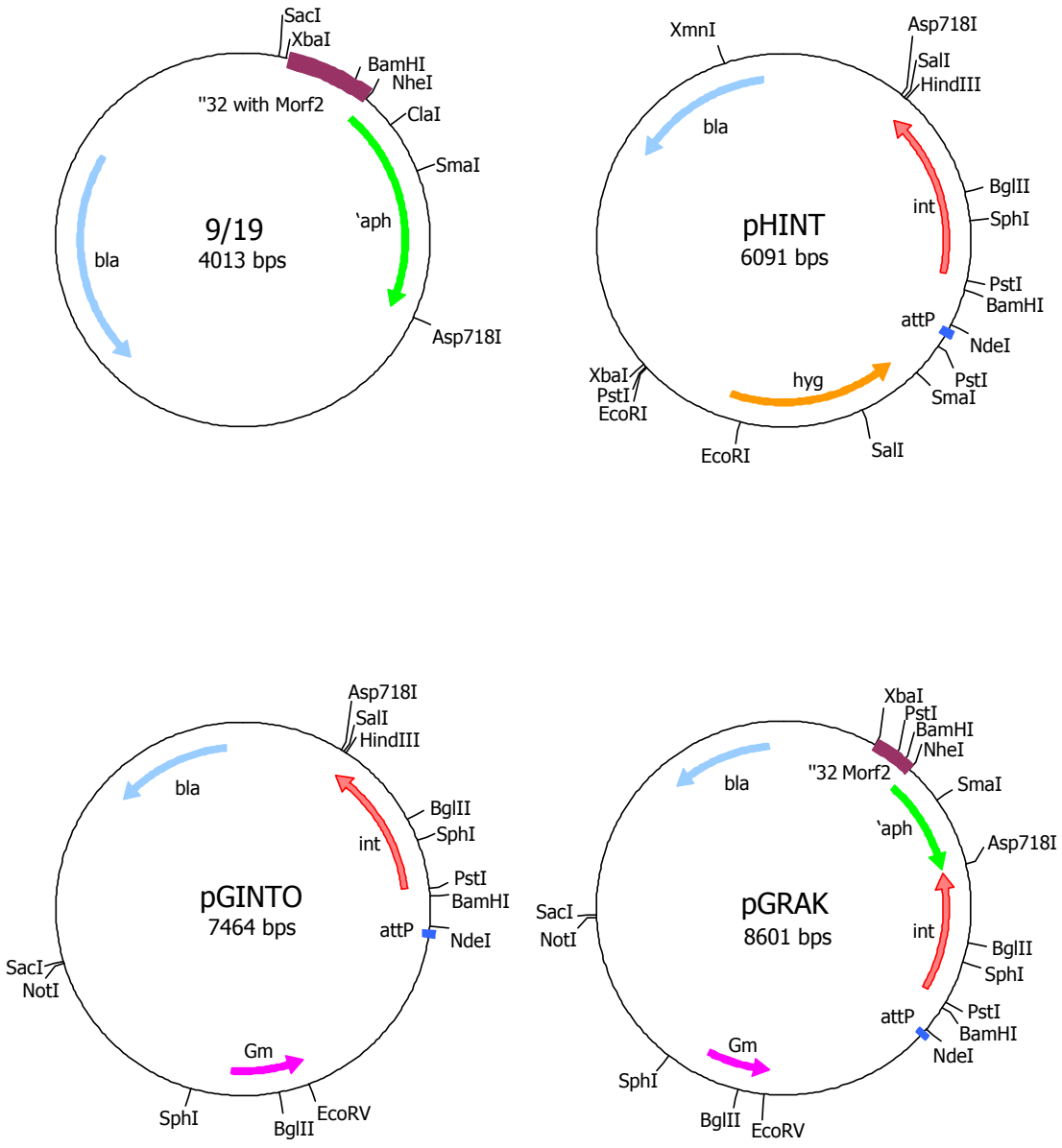


Figure B1: Plasmids p9/19, pHINT, pGINTO and pGRAK.

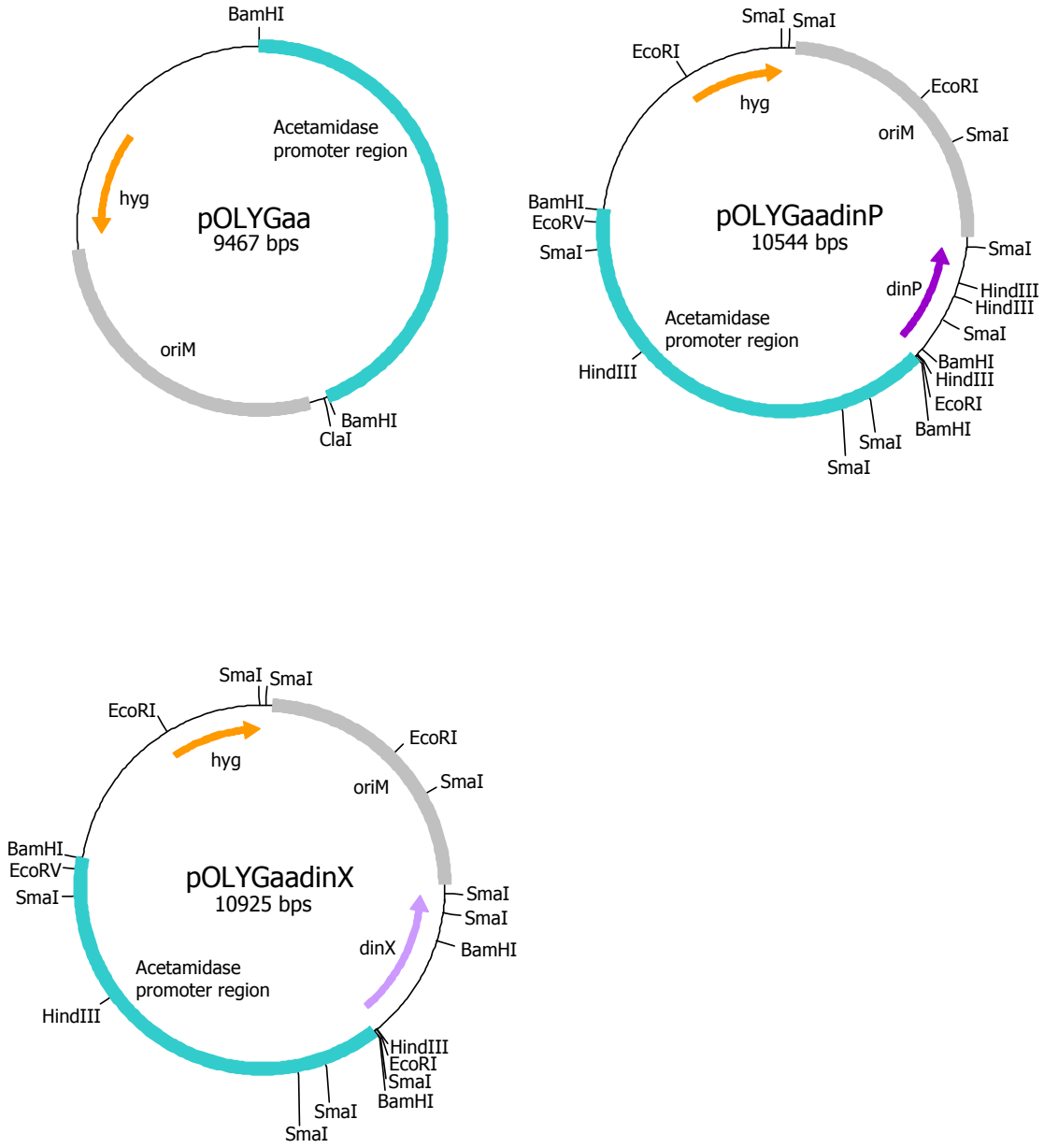
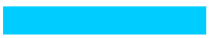
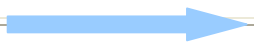
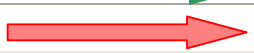
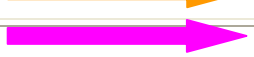


Figure B2: Plasmids pOLYGaa, pOLYGaadinP and pOLYGaadinX.

Table B1: Symbol definitions of the plasmid maps in Appendix B

Symbol	Definition
	*attP* site on plasmid that integrates with *attB* site on bacterial chromosome
	"32 region flanked by *Xba*I and *Nhe*I containing Morf2 construct
	*Bam*HI cassette containing acetamidase promoter
	Origin of replication (OriM) of plasmid
	Repeat 1 (R1) of Morf2 construct
	Distal repeat 3 (dR3) of Morf2 construct
	Proximal repeat 2 (pR2) of Morf2 construct
	3' PCR region used to generate ΔrecA and ΔL knockout (KO) plasmids
	5' PCR region used to generate ΔrecA and ΔL KO plasmids
	*bla* gene encoding β-lactamase for Ampicillin resistance
	'aph cassette containing Km resistance gene without its own ATG codon
	complete aph cassette containing Km resistance gene
	*int* gene encoding factors used to integrate with bacterial chromosome
	*hyg* cassette encoding Hygromycin resistance factors
	*Gm* cassette encoding Gentamicin resistance factors
	dinP gene under control of the acetamidase promoter
	dinX gene under control of the acetamidase promoter

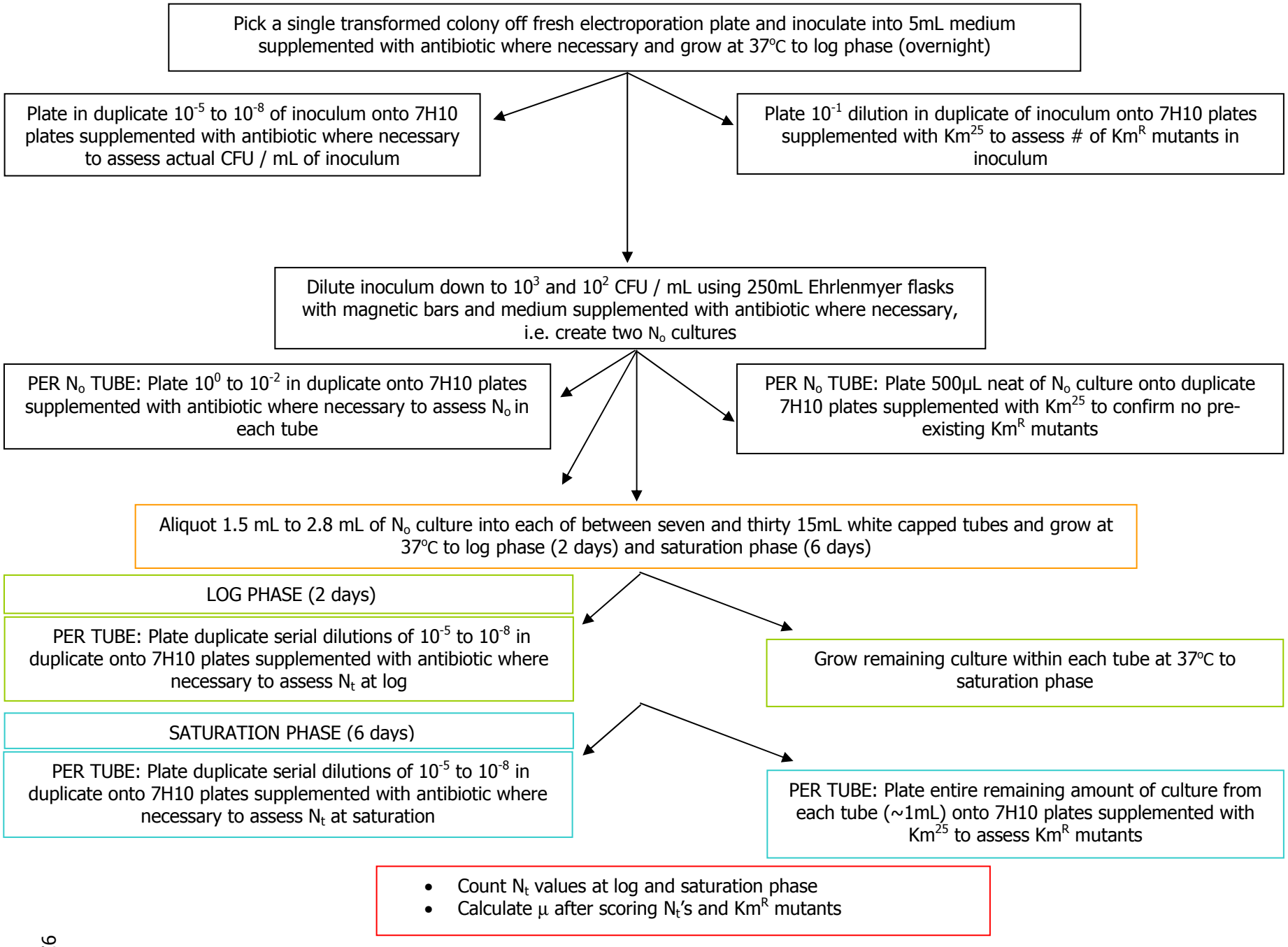
Appendix C : Specific experimental requirements for Fluctuation Tests used in this study

The principles of the Luria Delbrück Fluctuation Test remained the same for each experiment, but the individual experimental requirements differed and are set out Table C1. The flow diagram on page 2 of Appendix C illustrates the general protocol used for all Fluctuation Tests in this study. Changes to the protocol used with the mc^2 *his*G380E strain are detailed in Section 2.11.2.

Table C1: Specific experimental parameters used for each Fluctuation Test in this study

Strain	**Medium**	**Additional constituents**	**# N_t tubes assessed**
mc^2 155 (pGRAK)	LB	Gm^2	10
mc^2 155 (pGRAK)	2TY	Gm^2	10
mc^2 155 (pGRAK)	MADC-Tw 80	Gm^2	15
mc^2 155 ΔrecA (pGRAK)	MADC-Tw 80	Gm^2	7
mc^2 155 (pGRAK / pOLYGaa)	LB	Gm^2 and Hyg^{50}	30
mc^2 155 (pGRAK / pOLYGaa)	MADC-Tw 80	Gm^2 and Hyg^{50}	15
mc^2 155 (pGRAK / pOLYGaadinP)	MADC-Tw 80	Gm^2 and Hyg^{50}	15
mc^2 155 (pGRAK / pOLYGaadinX)	MADC-Tw 80	Gm^2 and Hyg^{50}	15
mc^2 155 ΔY (pGRAK)	MADC-Tw 80	Gm^2 and Hyg^{50}	14
mc^2 155 ΔL (pGRAK)	MADC-Tw 80	Gm^2 and Hyg^{50}	14
mc^2 155 (Rif resistance)	MADC-Tw 80	Rif^{150}	30
mc^2 155 *his*G380E	MADC-Tw 80	His^{100}	7

General Fluctuation Test protocol using *Mycobacterium smegmatis* mc^2 155



Appendix D : Techniques to limit clumping of mycobacterial cultures

To reduce the extent of clumping, culture tubes were placed in a beaker of water and secured with paper towel thereby creating a 'water jacket' in which the cultures were incubated (Figure D1A). This probably ensured even heat distribution to all culture tubes. Cultures that had clumped were mixed by aspiration with a 1 mL syringe needle. Furthermore, when assessing N_t values, all serial dilutions were made in 0.5% Tween 80. Combined, these three techniques proved effective in minimising the effects of clumping on N_t value determination (Figure D1B).

A



B

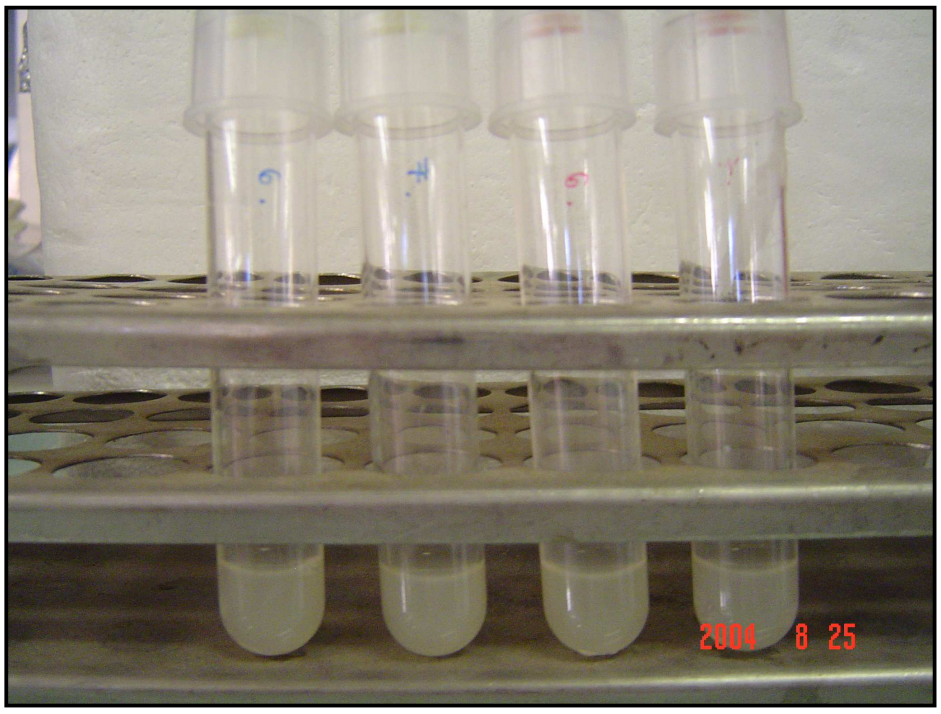


Figure D1: Clumping of mycobacterial cultures. (A) Culture tubes used in Fluctuation Tests were placed in a beaker partly filled with water and secured with paper towel. Cultures grown in this manner for 2 or 6 days (B) did not clump, therefore N_t values assessed from these tubes were reproducible.

Appendix E : Additional sequencing data from Morf2 region

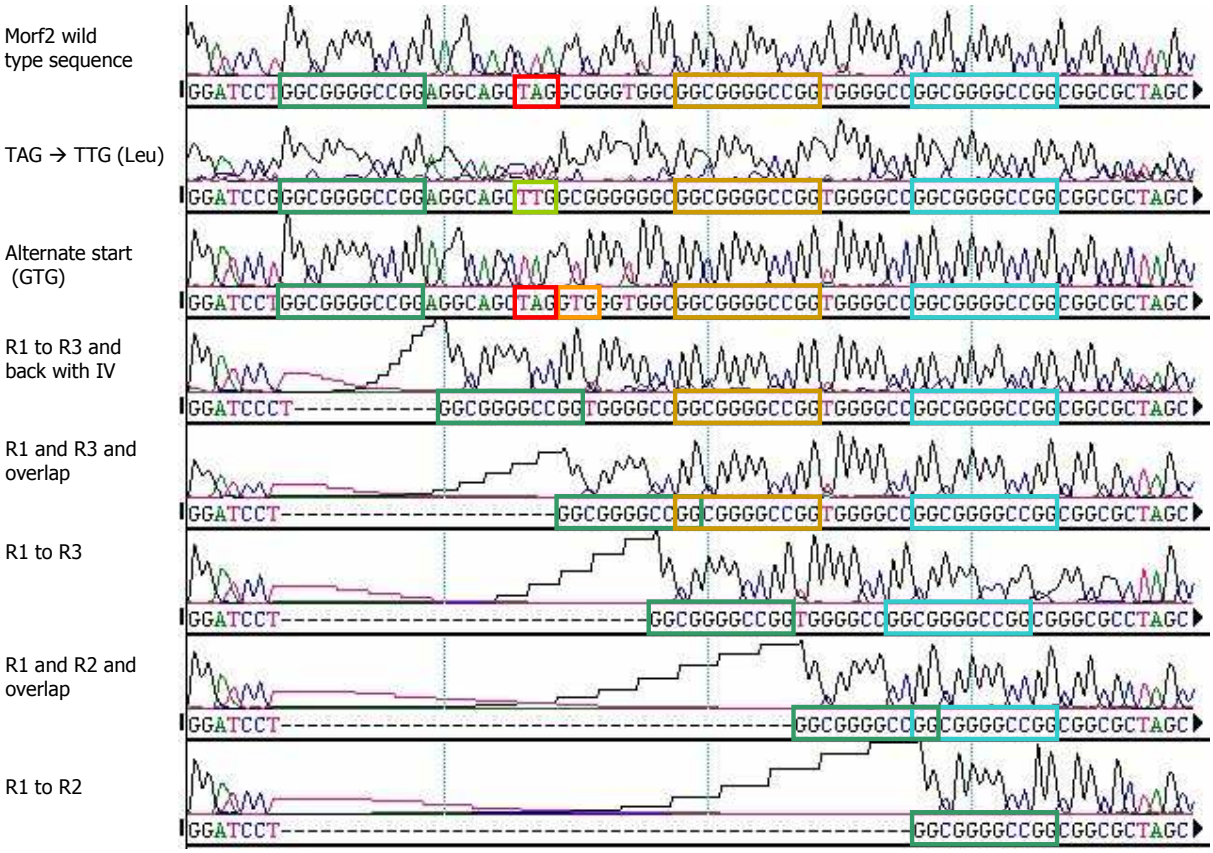


Figure E1: Sequence data of Morf2 region indicating a spectrum of mutations resulting in Km^R. G↓GATCC at the 5' end of the sequences indicates the *Bam*HI site at the start of the Morf2 region. G↓CTAGC at the 3' end of the sequence indicates the *Nhe*I site at the start of the 'aph cassette. R1 is highlighted by green blocks. The TAG stop codon is highlighted by red blocks. R2 and R3 are highlighted by blue and yellow blocks respectively. The point mutation within TAG to form a codon encoding Leucine is highlighted by the bright green block. The point mutation to form an alternative in frame start codon (GTG) is highlighted by the orange block. IV = intervening sequence.

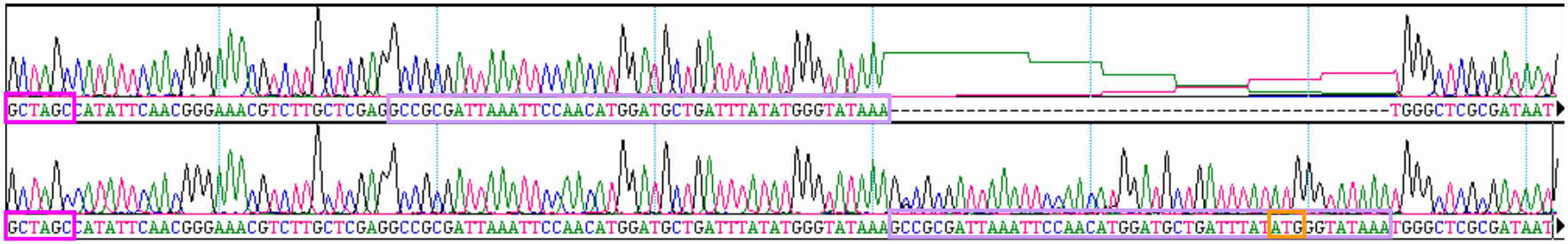


Figure E2: Sequence data indicating a Km^R clone with duplication in the 'aph cassette. The upper sequence indicates the wild type 'aph cassette from the *NheI* site at the 3' end of the Morf2 sequence (indicated by pink block at the 5' end of the sequence). The lower sequence indicates the Km^R mutant with the duplicated portion of the 'aph cassette (indicated by the purple blocks) that in turn created a new in frame ATG start codon (indicated by the orange block).

References

Adwan, K., N. Abu-Hasan*, et al.* (1998). "Analysis of Neomycin, Kanamycin, Tobramycin and Amikacin resistance mechanisms in Gentamicin-resistant isolates of Enterobacteriaceae." J Med Microbiol 47(11): 1019-1021.

Allred, D. R., T. C. McGuire*, et al.* (1990). "Molecular basis for surface antigen size polymorphisms and conservation of a neutralisation-sensitive epitope in *Anaplasma marginale*." Proceedings of the National Academy of Science. USA 87: 3220-3224.

Alm, R. A., L. S. Ling*, et al.* (1999). "Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*." Nature 397: 176-180.

Arain, T. M., A. E. Resconi*, et al.* (1996). "Reporter gene technology to assess activity of antimycobacterial agents in macrophages." Antimicrobial Agents and Chemotherapy 40(6): 1542-1544.

Armstrong, J. A. and P. D. Hart (1971). "Response of cultured macrophages to *Mycobacterium tuberculosis* with observation on fusion of lysosomes within phagosomes." Journal of Experimental Medicine 134: 713-740.

Banu, S., N. Honoré*, et al.* (2002). "Are the PE-PGRS proteins of *Mycobacterium tuberculosis* variable surface antigens?" Molecular Microbiology 44(1): 9-19.

Billington, O. J., T. D. McHugh*, et al.* (1999). "Physiological cost of Rifampin resistance induced *in vitro* in *Mycobacterium tuberculosis*." Antimicrobial Agents and Chemotherapy 43(8): 1866–1869.

Black, W. J., R. S. Schwalbe*, et al.* (1984). "Characterisation of *Neiserria gonorrhoeae* protein II phase variation by use of monoclonal antibodies." Infection and Immunity 45: 453-457.

Blower, S. M. and T. Chou (2004). "Modeling the emergence of the 'hot zones': tuberculosis and the amplification dynamics of drug resistance." Nature Medicine 10(10): 1111-1116.

Boshoff, H. I. M., M. B. Reed*, et al.* (2003). "DnaE2 polymerase contributes to *in vivo* survival and the emergence of drug resistance in *Mycobacterium tuberculosis*." Cell 113: 183-193.

Brandt, L., J. F. Cunha*, et al.* (2002). "Failure of the *Mycobacterium bovis* BCG vaccine: some species of environmental mycobacteria block multiplication of BCG and induction of protective immunity to Tuberculosis." Infection and Immunity 70(2): 672-678.

Brennan, M. J. and G. Delogu (2002). "The PE multigene family: a 'molecular mantra' for mycobacteria." TRENDS in Microbiology 10(5): 246-249.

Brennan, M. J., G. Delogu*, et al.* (2001). "Evidence that mycobacterial PE-PGRS proteins are cell surface constituents that influence interactions with other cells." Infection and Immunity 69(12): 7326-7333.

Brennan, P. J. and H. Nikaido (1995). "The envelope of mycobacteria." Annual Review of Biochemistry 64: 29-63.

Brosch, R., S. V. Gordon*, et al.* (2000). Genomics, biology and evolution of the *Mycobacterium tuberculosis* complex. Molecular Genetics of Mycobacteria. W. R. Jacobs Jr. Washington, D.C., ASM Press: 19-36.

Bruand, C., V. Bidnenko*, et al.* (2001). "Replication mutations differentially enhance RecA dependant and RecA independant recombination between tandem repeats in *Bacillus subtilis*." Molecular Microbiology 39(5): 1248-1258.

Caminero, J. A., M. J. Pena*, et al.* (2001). "Epidemiological evidence of the spread of a *Mycobacterium tuberculosis* strain of the Beijing genotype on Gran Canaria Island." Am J Respir Crit Care Med 164: 1165-1170.

Caws, M. and F. A. Drobniewski (2001). "Molecular techniques in the diagnosis of *Mycobacterium tuberculosis* and the detection of drug resistance." Annals of the New York Academy of Sciences 953: 138-145.

Cohen, T. and M. Murray (2004). "Modelling epidemics of multi-drug-resistant *Mycobacterium tuberculosis* of heterogeneous fitness." Nature Medicine 10(10): 1117-1121.

Colditz, G. A., T. F. Brewer*, et al.* (1994). "Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature." JAMA 271: 698-702.

Cole, S. (1998). "Comparative mycobacterial genomics." Current Opinion in Microbiology 1: 567-571.

Cole, S. (1999). "Learning from the genome sequence of *Mycobacterium tuberculosis* H37Rv." FEBS Letters 452: 7-10.

Cole, S., R. Brosch*, et al.* (1998). "Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence." Nature 393: 537-544.

Corbett, E. L., C. J. Watt*, et al.* (2003). "The growing burden of tuberculosis." Archives of International Medicine 163: 1009-1021.

Cosma, C. L., O. Humbert*, et al.* (2004). "Superinfecting mycobacteria home to established tuberculous granulomas." Nature Immunology 5(8): 828-835.

David, H. L. (1970). "Probability distribution of drug-resistant mutants in unselected populations of *Mycobacterium tuberculosis*." Applied Microbiology 20(5): 810-814.

de Visser, J. A. G. M., C. W. Zeyl*, et al.* (1999). "Diminishing returns from mutation supply rate in asexual populations." Science 283: 404-406.

Delogu, G. and M. J. Brennan (2001). "Comparative immune response to PE and PE-PGRS antigens of *Mycobacterium tuberculosis*." Infection and Immunity 69(9): 5606-5611.

Draper, P. (1998). "The outer parts of the mycobacterial envelope as permeability barriers." Frontiers in Bioscience 3: 1253-1261.

Duigou, S., S. D. Ehrlich*, et al.* (2004). "Distinctive genetic features exhibited by the Y-family DNA polymerases in *Bacillus subtilis*." Molecular Microbiology 54(2): 439-451.

Durbach, S. I., B. Springer*, et al.* (2003). "DNA alkylation damage as a sensor of nitrosative stress in *Mycobacterium tuberculosis*." Infection and Immunity 71(2): 997-1000.

Dye, C., S. Scheele*, et al.* (1999). "Global burden of tuberculosis - estimated incidence, prevalence, and mortality by country." Journal of American Medical Associations 282: 677-686.

Echols, H. and M. F. Goodman (1991). "Fidelity mechanisms in DNA replication." Annual Review of Biochemistry 60: 477-511.

Espitia, C., J. P. Laclette*, et al.* (1999). "The PE-PGRS glycine-rich proteins of *Mycobacterium tuberculosis*: a new family of fibronectin-binding proteins?" Microbiology 145: 3487-3495.

Fine, P. E. M. (1990). "Modern vaccines. Mycobacterial diseases." Lancet 35: 1016-1020.

Fleischmann, R. D., D. Alland*, et al.* (2002). "Whole-genome comparison of *Mycobacterium tuberculosis* clinical and laboratory strains." Journal of Bacteriology 184(19): 5479–5490.

Fonesca, D. P. A. J., B. Benaissa-Trouw*, et al.* (2001). "Induction of cell-mediated immunity against *Mycobacterium tuberculosis* using DNA vaccines encoding cytotoxic and helper T-cell epitopes of the 38-kilodalton protein." Infection and Immunity 69(8): 4839-4845.

Friedberg, E. C. (2003). "DNA damage and repair." Nature 421: 436-440.

Friedberg, E. C. and P. L. Fischhaber (2003). "TB or Not TB: How *Mycobacterium tuberculosis* may evade drug treatment." Cell 113: 139-145.

Friedberg, E. C., R. Wagner*, et al.* (2002). "Specialized DNA polymerases, cellular survival, and the genesis of mutations." Science 296: 1627-1630.

Gandy, M. and A. Zumla (2003). Return of the white plague: global poverty and the 'new' Tuberculosis. London, England, Nature Publishing Group: 320.

Glickman, M. S. and W. R. Jacobs Jr (2001). "Microbial Pathogenesis of *Mycobacterium tuberculosis*: Dawn of a Discipline." Cell 104: 477-485.

Goodman, M. F. (2002). "DNA polymerases in prokaryotes and eukaryotes." Annual Review of Biochemistry 71: 17-50.

Gordon, S. V., K. Eiglmeier*, et al.* (2001). "Genomics of *Mycobacterium bovis*." Tuberculosis 81(1/2): 157-163.

Guleria, I., R. Teitelbaum*, et al.* (1996). "Auxotrophic vaccines for tuberculosis." Nature Medicine 2(3): 334-337.

Hacker, J., O. G. Blum*, et al.* (1997). "Pathogenicity islands of virulent bacteria: structure, function and impact." Molecular Microbiology 23: 1089-1097.

Hanahan, D. (1983). "Studies in the transformation of *Eschericia coli* with plasmids." Journal of Molecular Biology 166: 557-580.

Harkin, T. J. and H. W. Harris (1995). Treatment of multi-drug resistant tuberculosis. Tuberculosis. S. Garay. Boston, Massachusetts, Little, Brown and Company: 843-850.

Hayashi, C. Y. and R. V. Lewis (2000). "Molecular Architecture and evolution of a modular spider silk protein gene." Science 287: 1477-1479.

Hinshelwood, S. and N. G. Stoker (1992). "Cloning of the mycobacterial histidine synthesis genes by complementation of a *Mycobacterium smegmatis* auxotroph." Molecular Microbiology 6(19): 2887-2895.

Hirsh, A. E., A. G. Tsolaki*, et al.* (2004). "Stable association between strains of *Mycobacterium tuberculosis* and their human host populations." Proceedings of the National Academy of Science. USA 101(14): 4871-4876.

Hollingshead, S. K., V. A. Fischetti*, et al.* (1986). "Complete nucleotide sequence of type 6 M protein of the Group A Streptococcus." The Journal of Biological Chemistry 261(4): 1677-1686.

Hollingshead, S. K., V. A. Fischetti*, et al.* (1987). "Size variation in group A streptococcal M protein is generated by homologous recombination between intragenic repeats." Molecular and General Genetics 207: 196-203.

Jacobs Jr, W. R., R. Barletta*, et al.* (1991). "Genetic systems for mycobacteria." Methods in Enzymology 204: 537-555.

Jones, M. E., S. M. Thomas*, et al.* (1994). "Luria-Delbrück fluctuation experiments: design and analysis." Genetics 136: 1209-1216.

Jonsson, A., J. Pfeifer*, et al.* (1992). "*Neisseria gonorrhoeae* PilC expression provides a selective mechanism for structural diversity of pili." Proceedings of the National Academy of Science. USA 89: 3204-3208.

Josenhans, C., K. A. Eaton*, et al.* (2000). "Switching of flagellar motility in *Helicobacter pylori* by reversible length variation of a short homopolymeric sequence repeat in *fliP*, a gene encoding a basal body protein." Infection and Immunity 68(8): 4598-4603.

Jungblut, P. R., U. E. Schaible*, et al.* (1999). "Comparative proteome analysis of *Mycobacterium tuberculosis* and *Mycobacterium bovis* BCG strains: towards functional genomics of microbial pathogens." Molecular microbiology 33(6): 1103-1117.

Kana, B. and V. Mizrahi (2004). "Molecular genetics of *Mycobacterium tuberculosis* in relation to the discovery of novel drugs and vaccines." Tuberculosis 84: 63-75.

Kanduma, E., M. T. D*, et al.* (2003). "Molecular methods for *Mycobacterium tuberculosis* strain typing: a users guide." Journal of Applied Microbiology 94: 781-791.

Kendal, W. S. and P. Frost (1988). "Pitfalls and practice of Luria-Delbrück Fluctuation Analysis: A review." Cancer Research 48: 1060-1065.

Kostrzynska, M., J. D. Betts*, et al.* (1991). "Identification, characterisation, and spatial localisation of two flagellin species in *Helicobacter pylori* flagella." Journal of Bacteriology 173: 937-946.

Kremer, K., D. van Soolingen*, et al.* (1999). "Comparison of methods based on different molecular epidemiological markers for typing of *Mycobacterium tuberculosis* complex strains: inter-laboratory study of discriminatory power and reproducibility." Journal of Clinical Microbiology 37: 2607-2618.

Kuzminov, A. (1999). "Recombinational repair of DNA damage in *Eschericia coli* and bacteriophage lambda." Microbiology and Molecular Biology Reviews 63(4): 751-813.

Labes, M., A. Puhler*, et al.* (1990). "A new family of RSF1010-derived expression and lac-fusion broad host range vectors for Gram-negative bacteria." Gene 89: 37-46.

Ladefoged, S. A., S. Birkelund*, et al.* (1995). "A 135-kilodalton surface antigen of *Mycoplasma hominis* PG21 contains multiple directly repeated sequences." Infection and Immunity 63(1): 212-223.

Lea, D. E. and C. A. Coulsen (1949). "The distribution of the numbers of mutants in bacterial populations." Journal of Genetics 49: 264-286.

Lee, M. H., L. Pascopella*, et al.* (1991). "Site-specific integration of mycobacteriophage L5: integration-proficient vectors for *Mycobacterium smegmatis*, *Mycobacteruim tuberculosis* and bacille Calmette-Guerin." Proceedings of the National Academy of Science, USA 88: 3111-3115.

Levitskaya, J., M. Coram*, et al.* (1995). "Inhibition of antigen processing by the internal repeat region of the Epstein-Barr virus nuclear antigen-1." Nature 375: 685-688.

Levitskaya, J., A. Sharipo*, et al.* (1997). "Inhibition of ubiquitin/proteosome-dependent protein degredation by the Gly-Ala repeat domain of the Epstein-Barr virus nuclear antigen 1." Proceedings of the National Academy of Science. USA 94: 12616-12621.

Lovett, S. T. (2004). "Encoded errors: mutations and rearrangements mediated by misalignment at repetitive DNA sequences." Molecular Microbiology 52(5): 1243-1253.

Luria, S. E. and M. Delbrück (1943). "Mutations of bacteria from virus sensitivity to virus resistance." Genetics 28: 491–511.

MacMicking, J. D., G. A. Taylor*, et al.* (2003). "Immune control of tuberculosis by IFN-gamma–inducible LRG-47." Science 302: 654-659.

Mahairas, G., P. J. Sabo*, et al.* (1996). "Molecular analysis of genetic differences between *Mycobacterium bovis* BCG and virulent *M. bovis*." Journal of Bacteriology 178(5): 1274-1282.

Makino, S., J. P. M. van Putten*, et al.* (1991). "Phase variation of the opacity outer membrane protein controls invasion by *Neisseria gonorrhoeae* into human epithelial cells." The EMBO journal 10(6): 1307-1315.

Mariam, D. H., Y. Mengistu*, et al.* (2004). "Effect of *rpoB* mutations conferring Rifampicin resistance on fitness of *Mycobacterium tuberculosis*." Antimicrobial Agents and Chemotherapy 48(4): 1289-1294.

Martinez, J. L. and F. Baquero (2000). "Mutation frequencies and antibiotic resistance." Antimicrobial Agents and Chemotherapy 44(7): 1771–1777.

Mayer, L. W. (1982). "Rates of *in vitro* changes of gonococcal colony opacity phenotypes." Infection and Immunology 37: 481-485.

McKenzie, G. and S. Rosenberg (2001). "Adaptive mutations, mutator DNA polymerases and genetic change strategies of pathogens." Current Opinions in Microbiology 4: 586-594.

Meier, J. T., M. I. Simon*, et al.* (1985). "Antigenic variation is associated with DNA rearrangements in a relapsing fever *Borellia*." Cell 41: 403-409.

Metzgar, D. and C. Wills (2000). "Evidence for the Adaptive Evolution of Mutation Rates." Cell 101: 581–584.

Meyer, T. F. and J. P. M. van Putten (1989). "Genetic mechanisms and biological implications of phase variation in pathogenic *Neisseriae*." Clinical Microbiology Reviews 2(supplement): S139-S145.

Miller, B. H., R. A. Fratti*, et al.* (2004). "Mycobacteria inhibit nitric oxide synthase recruitment to phagosomes during macrophage infection." Infection and Immunity 72(5): 2872-2878.

Mitchison, D. A. (2004). "Tuberculosis hits back." Nature 427: 295.

Morlock, G. P., B. P. Plikaytis*, et al.* (2000). "Characterisation of spontaneous, *in vitro* selected, Rifampicin resistant mutants of *Mycobacterium tuberculosis* strain H37Rv." Antimicrobial Agents and Chemotherapy 44(12): 3298-3301.

Murphy, G. L., T. D. Connell*, et al.* (1989). "Phase variation of gonococcal protein II; regulation of gene expression by slipped-strand mispairing of a repetitive DNA sequence." Cell 56: 539-547.

Nathan, C. and M. U. Shiloh (2000). "Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens." Proceedings of the National Academy of Science. USA 97: 8841-8848.

Navin, T. R., S. J. McNabb*, et al.* (2002). "The continued threat of tuberculosis." Emerging Infectious Diseases 8: 1187.

O'Gaora, P., S. Barnini*, et al.* (1997). "Mycobacteria as immunogens: development of expression vectors for use in multiple mycobacterial species." Med. Principles Prac. 6: 91-96.

Pena, C. A. E., M. H. Lee*, et al.* (1997). "Characterisation of the mycobacteriophage attachment site, *attP*." Journal of Molecular Biology 266: 76-92.

Poulet, S. and S. T. Cole (1995). "Characterisation of the highly abundant polymorphic GC-rich-repetitive sequence (PGRS) present in *Mycobacterium tuberculosis*." Archives of Microbiology 163: 87-95.

Poulet, S. and S. T. Cole (1995). "Repeated DNA sequences in mycobacteria." Archives of Microbiology 163: 79-86.

Pozos, T. C. and L. Ramakrishnan (2004). "New models for the study of mycobacterium–host interactions." Current Opinion in Immunology 16: 499-505.

Pym, A. S., P. Brodin*, et al.* (2002). "Loss of RD1 contributed to the attenuation of the live tuberculosis vaccines *Mycobacterium bovis* BCG and *Mycobacterium microti*." Molecular Microbiology 46: 709-717.

Rad, M. E., P. Bifani*, et al.* (2003). "Mutations in putative mutator genes of *Mycobacterium tuberculosis* strains of the W-Beijing family." Emerging Infectious Diseases 9(7): 838-845.

Radman, M. (1999). "Enzymes of evolutionary change." Nature 401: 866-869.

Ramakrishnan, L., N. A. Federspiel*, et al.* (2000). "Granuloma-Specific Expression of Mycobacterium Virulence Proteins from the Glycine-Rich PE-PGRS Family." Science 288: 1436-1439.

Ramaswamy, S. and J. M. Musser (1998). "Molecular genetic basis of antimicrobial agent resistance in *Mycobacterium tuberculosis*: 1998 update." Tuberc. Lung Dis. 79: 3-29.

Ratliff, T. L., J. A. McGarr*, et al.* (1988). "Attachment of mycobacteria to fibronectin-coated surfaces." Journal of General Microbiology 134: 1307-1313.

Rattray, A. J. and J. N. Strathern (2003). "Error-prone DNA polymerases: when making a mistake is the only way to get ahead." Annual Review of Genetics 37: 31-66.

Reed, S. G., M. R. Alderson*, et al.* (2003). "Prospects for a better vaccine against tuberculosis." Tuberculosis: 1-7.

Reuven, N. B., G. Arad*, et al.* (1999). "The mutagenesis protein UmuC is a DNA polymerase activated by UmuD', RecA and SSB and is specialised for translesion replication." Journal of Biological Chemistry 274: 1763-1766.

Richardson, M., G. D. van der Spuy*, et al.* (2004). "Stability of polymorphic GC-rich repeat sequence-containing regions of *Mycobacterium tuberculosis*." Journal of Clinical Microbiology 42(3): 1302-1304.

Robbins, R. J. (2001). "Mutations of bacteria from virus sensitivity to virus resistance: an introduction." Genetics 28: i - xi.

Robertson, B. D. and T. F. Meyer (1992). "Genetic variation in pathogenic bacteria." TIG 8(12): 422-427.

Rosche, W. A. and P. L. Foster (2000). "Determining mutation rates in bacterial populations." Methods 20: 4-17.

Rosenberg, S. M. (2001). "Evolving responsively: adaptive mutation." Nature 2: 504-511.

Ross, B. C., K. Raios*, et al.* (1992). "Molecular cloning of a highly repeated DNA element from *Mycobacterium tuberculosis* and its use as an epidemiological tool." Journal of Clinical Microbiology 30: 942-946.

Sambrook, J., E. F. Fritsch*, et al.* (1989). Molecular Cloning: a laboratory manual. Cold Spring Harbour, New York, Cold Spring Harbour Laboratory.

Sassetti, C. M., D. H. Boyd*, et al.* (2003). "Genes required for mycobacterial growth defined by high density mutagenesis." Molecular Microbiology 48(1): 77–84.

Sassetti, C. M. and E. J. Rubin (2003). "Genetic requirements for mycobacterial survival during infection." Proceedings of the National Academy of Science. USA 100(22): 12989-12994.

Saunders, B. M. and A. M. Cooper (2000). "Restraining mycobacteria: role of granulomas in mycobacterial infections." Immunology and Cell Biology 78: 334-341.

Schaaper, R. M. (1988). "Mechanisms of mutagenesis in the *Eschericia coli* mutator mutD5: role of DNA mismatch repair." Proceedings of the National Academy of Science, USA 85: 8126-8130.

Schaaper, R. M., B. N. Danforth*, et al.* (1986). "Mechanisms of spontaneous mutagenesis: an analysis of the spectrum of spontaneous mutation in the *Eschericia coli lacI* gene." Journal of Molecular Biology 189: 273-284.

Schlesinger, L. S. (1993). "Macrophage phagocytosis of virulent but not attenuated strains of *Mycobacterium tuberculosis* is mediated by mannose receptors in addition to complement receptors." Journal of Immunology 150: 2920-2930.

Schlesinger, L. S., C. G. Bellinger-Kawahara*, et al.* (1990). "Phagocytosis of *Mycobacterium tuberculosis* is mediated by human monocyte complement receptors and complement component C3." Journal of Immunology 144: 2771-2780.

Schnappinger, D., S. Ehrt*, et al.* (2003). "Transcriptional adaptation of *Mycobacterium tuberculosis* within macrophages: insights into the phagosomal environment." The Journal of Experimental Medicine 198(5): 693-704.

Shen, X., J. M. Sayer*, et al.* (2002). "Efficiency and accuracy of SOS-induced DNA polymerases replicating benzo[a]pyrene-7,8-diol 9,10-epoxide A and G adducts." Journal of Biological Chemistry 277: 5265-5274.

Singh, K. K., X. Zhang*, et al.* (2001). "Antigens of *Mycobacterium tuberculosis* expressed during preclinical tuberculosis: serological immunodominance of proteins with repetitive amino acid sequences." Infection and Immunity 69(6): 4185-4191.

Snapper, S. B., R. E. Melton, *et al* . (1990). "Isolation and characterisation of efficient plasmid transformation mutants of *Mycobacterium smegmatis*." Molecular Microbiology 4: 1911-1919.

Snewin, V., G. Stewart*, et al.* (2001). Genetic strategies for vaccine development. Molecular Genetics of Mycobacteria. W. R. Jacobs Jr. Washington DC, ASM Press: 279-296.

Stern, A., M. Brown*, et al.* (1986). "Opacity genes in *Neisseria gonorrhoeae*: control of phase and antigenic variation." Cell 47: 61-71.

Streicher, E. M., R. M. Warren*, et al.* (2004). "Genotypic and phenotypic characterization of drug-resistant *Mycobacterium tuberculosis* isolates from rural districts of the western cape province of South Africa." Journal of Clinical Microbiology 42(4): 891–894.

Sturgill-Kosycki, S., P. H. Schlesinger*, et al.* (1994). "Lack of acidification in mycobacterium phagosomes produced by the exclusion of the vesicular proton-ATPase." Science 263: 678-681.

Tang, M., P. Pham*, et al.* (2000). "Roles of *Eschericia coli* DNA polymerases IV and V in lesion-targeted and untargeted SOS mutagenesis." Nature 404: 1014-1018.

Tippin, B., P. Pham*, et al.* (2004). "Error-prone replication for better or worse." TRENDS in Microbiology 12(6): 288-295.

Toossi, Z. and J. Ellner (1998). "Host response to *Mycobacterium tuberculosis*." Frontiers in Bioscience 3: e133-e140.

Traub, W. H. and P. I. Fukushima (1979). "Non-specific aminoglycoside cross-resistance of *Serratia marcescens*." Chemotherapy 25(4): 204-213.

Trias, J. and R. Benz (1994). "Permeability of the cell wall of *Mycobacterium smegmatis*." Molecular micobiology 14(2): 283-290.

Turner, O. C., R. J. Basaraba*, et al.* (2003). "Immunopathogenesis of pulmonary granulomas in the guinea pig after infection with *Mycobacterium tuberculosis*." Infection and Immunity 71(2): 864-871.

van Helden, P. D. (2003). "The economic divide and tuberculosis." EMBO reports 4(special issue): S24-S28.

van Putten, J. P. M. (1993). "Phase variation of lipopolysaccharide directs interconversion of invasive and immuno-resistant phenotypes of *Neisseria gonorrhoeae*." The EMBO journal 12(11): 4043-4051.

Victor, T. C., P. E. W. de Haas*, et al.* (2004). "Molecular characteristics and global spread of *Mycobacterium tuberculosis* with a Western Cape F11 genotype." Journal of Clinical Microbiology 42(2): 769-772.

Voskuil, M. I., D. Schnappinger*, et al.* (2004). "Regulation of the *Mycobacterium tuberculosis* PE/PPE genes." Tuberculosis 84: 256-262.

Weber, M. (1996). "Evolutionary plasticity in prokaryotes: A panglossian view." Biol. Phil. 11: 67-88.

Werngren, J. and S. E. Hoffner (2003). "Drug-susceptible *Mycobacterium tuberculosis* Beijing genotype does not develop mutation-conferred resistance to Rifampicin at an elevated rate." Journal of Clinical Microbiology 41(4): 1520-1524.

Willems, R., A. Paul*, et al.* (1990). "Fimbrial phase variation in *Bordetella pertussis*: a novel mechanism for transcriptional regulation." The EMBO journal 9(9): 2803-2809.

Yang, W. (2003). "Damage repair DNA polymerases Y." Current Opinion in Structural Biology 13: 23-30.

Ye, Z. H., Y. R. Song*, et al.* (1991). "Comparative localisation of three classes of cell wall proteins." Plant Journal 1(175-183).

Young, D. B. and K. Duncan (1995). "Prospects for new interventions in the treatment and prevention of mycobacterial disease." Annual Review of Microbiology: 641-673.

Zhang, Q. and K. S. Wise (1996). "Molecular basis of size and antigenic variation of a *Mycoplasma hominis* adhesin encoded by divergent *vaa* genes." Infection and Immunity 64(7): 2737-2744.

Zhang, Y. and A. Telenti (2000). Genetics of drug resistance in *Mycobacterium tuberculosis*. Molecular Genetics of Mycobacteria. W. R. Jacobs Jr. Washington, D.C., ASM Press: 235-254.