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**Effects of aerobic and anaerobic
environments on bacterial mutation rates
and mutation spectra assessed by whole
genome analyses**

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Abstract

For organisms that are exposed to different environments, the rates and types of spontaneous mutations that arise in each environment can vary, and potentially impact the direction of evolution as a whole. Oxidative stress is a major cause of mutation, but the effect of oxygen availability on the mutation rates and spectra of organisms grown in aerobic as compared to anaerobic environments is not well understood at the whole genome level. To investigate the mutation rates and spectra of a facultative anaerobic bacterium grown under strictly aerobic or anaerobic conditions, 24 mutation accumulation lineages, derived from *Escherichia coli* REL4536, were established and propagated through 180 and 144 single-colony population bottlenecks, respectively. Spontaneous mutation rates of 2.50×10^{-10} and 4.14×10^{-10} mutations per nucleotide per generation were obtained for aerobically and anaerobically grown cells, respectively. Mutations in the aerobic environment were significantly biased towards G \rightarrow T mutations and IS186 transposition, while C \rightarrow A, T \rightarrow G, A \rightarrow C mutations, gross chromosomal rearrangements (GCRs) and IS150 transposition were significantly more prevalent under anaerobic conditions. Transcriptional profiling, *via* RNAseq, of REL4536 grown under aerobic and anaerobic environments revealed that repair genes, especially those involved in the repair of GCRs, were generally up-regulated in the anaerobic environment, consistent with findings that mutation rates, especially for GCRs, are higher in the anaerobic environment.

GCRs have long been thought to play an important role in the evolutionary process, though their contributions to the process have not been specifically defined. SbcCD, an exonuclease, is involved in the repair of DNA secondary structures, and is thought to help prevent the occurrence of GCRs. Transcriptome analyses showed that in *E. coli*, *sbcC* was up-regulated during growth in an anaerobic environment, as compared to an aerobic environment. To investigate the impact of GCRs on adaptive evolution, an *E. coli* REL4536 strain with disrupted *sbcC* was constructed and evolved under anaerobic conditions for 1,000 generations in glucose-limited media in 14 parallel populations. Mutations that arose during evolution were determined by whole genome re-sequencing of selected clones, and evolved *sbcC* mutant strains displayed more GCRs and enhanced population-level fitness on average. Together, these results suggest that GCRs may play an important role in the rate of adaptation.

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Table of Contents

Abstract	i
Acknowledgements	iii
Table of Contents	v
List of tables	xiii
List of figures	xvii
Non-standard abbreviations	xxi
Chapter One: Introduction	1
1.1 Mutations and evolution.....	1
1.2 Sources of mutations	2
1.2.1 Reactive oxygen species (ROS)	3
1.3 Classes of mutations.....	3
1.3.1 Base pair substitutions (BPSs)	4
1.3.2 Indels	5
1.3.3 Gross chromosomal rearrangements (GCRs).....	6
1.3.3.1 Formation of GCRs	7
1.3.3.1.1 Mobile genetic elements (MGEs).....	9
1.3.3.1.1.1 Transposable elements (TEs)	9
1.3.3.1.1.1.1 IS elements	10
1.3.3.1.1.1.2 MITEs and transposons.....	11
1.4 DNA replication and repair systems	11
1.4.1 DNA replication	12
1.4.1.1 Translesion synthesis (TLS)	13
1.4.2 Base excision repair (BER)	13
1.4.3 Nucleotide excision repair (NER)	15
1.4.4 Mismatch repair (MMR)	15
1.4.5 SOS response.....	17
1.4.6 Stringent response	17
1.4.7 Recombinational repair	18
1.4.7.1 Homologous recombination	18

1.4.7.1.1 The RecBCD pathway	19
1.4.7.1.2 The RecFOR pathway.....	19
1.4.8 Expression of repair pathways in aerobic and anaerobic environments	19
1.4.8.1.1 Transcriptome analysis	19
1.5 Mutation detection	21
1.5.1 Genome-wide mutation detection	21
1.5.1.1 Genome-wide mutation detection by genome sequencing.....	22
1.6 Mutation rates.....	23
1.6.1 Mutation rate measurement.....	25
1.6.1.1 Relative rates <i>via</i> fluctuation assays.....	25
1.6.1.2 Inference of rates <i>via</i> phylogenetic analyses	26
1.6.1.3 Direct detection of rates <i>via</i> mutation accumulation (MA) assays.....	27
1.7 The role of GCRs in adaptive evolution	28
1.7.1 Experimental evolution	29
1.7.1.1 Long-term evolution experiment (LTEE).....	30
1.7.2 The <i>sbcC</i> gene	30
1.8 Project aims.....	31

Chapter Two: Materials and Methods..... 33

2.1 Materials.....	33
2.1.1 Bacterial strains	33
2.1.2 Plasmids and phage	33
2.1.3 Antibiotics	34
2.1.4 Oligonucleotides.....	34
2.1.5 Laboratory chemicals and enzymes	39
2.1.6 Buffers and solutions.....	39
2.1.6.1 Tris-acetate-EDTA (TAE) buffer	39
2.1.6.2 EDTA/SDS solution	39
2.1.6.3 Lysis buffer.....	40
2.1.6.4 Resazurin solution	40
2.1.6.5 Reducing agent	40
2.1.6.6 Diethylpyrocarbonate (DEPC) H ₂ O	40
2.1.6.7 Glycerol saline.....	40
2.1.6.8 Glucose	40
2.1.6.9 Magnesium sulphate	40
2.1.6.10 Thiamine.....	41

2.1.7 Media.....	41
2.1.7.1 Luria-Bertani (LB) medium.....	41
2.1.7.1.1 Aerobic preparation	41
2.1.7.1.2 Anaerobic preparation.....	41
2.1.7.2 Davis Minimal (DM) medium	42
2.1.7.2.1 DM25 Aerobic preparation	42
2.1.7.2.2 DM25 Anaerobic preparation	42
2.1.7.3 DM solid medium	43
2.1.7.3.1 Aerobic preparation	43
2.1.7.3.2 Anaerobic preparation.....	43
2.1.7.4 Minimal glucose (MG) solid medium	43
2.1.7.5 Minimal arabinose (MA) solid medium	43
2.2 Methods.....	44
2.2.1 Aerobic cultivation of <i>E. coli</i>	44
2.2.2 Anaerobic cultivation of <i>E. coli</i>	44
2.2.3 Culture resuscitation from frozen stocks.....	44
2.2.4 Bacterial growth courses	44
2.2.5 Gram staining	45
2.2.6 Agarose gel electrophoresis.....	45
2.2.7 Polymerase chain reaction (PCR).....	46
2.2.7.1 PCR purifications	47
2.2.8 Quantitative PCR (qPCR)	47
2.2.9 Generation of <i>sbcC</i> deletion mutant.....	48
2.2.9.1 Preparation of electrocompetent cells.....	48
2.2.9.2 Transformation of electrocompetent cells	48
2.2.9.3 Lambda Red (λ -Red) recombination	48
2.2.9.3.1 First homologous recombination step.....	49
2.2.9.3.1.1 Electrocompetent <i>E. coli</i> REL4536.....	49
2.2.9.3.1.2 Transformation with pWRG100.....	49
2.2.9.3.2 Second homologous recombination step	49
2.2.9.3.2.1 Transformation of <i>E. coli</i> REL4536 Δ <i>sbcC</i> :: <i>cat</i> with pWRG99.....	49
2.2.9.3.2.2 Scarless deletion of <i>sbcC</i>	50
2.2.10 Nucleic acid extraction.....	50
2.2.10.1 DNA extractions	50

2.2.10.1.1 Cell lysis	50
2.2.10.1.2 Extractions with Phenol: Chloroform: Isoamyl alcohol	51
2.2.10.2 RNA extractions	51
2.2.10.2.1 RNA handling practises	51
2.2.10.2.2 Growth conditions.....	52
2.2.10.2.3 RNA extraction	52
2.2.10.2.4 DNase treatment	53
2.2.10.2.5 RNA purifications.....	53
2.2.10.2.6 RNA quality analysis	53
2.2.10.3 cDNA synthesis	53
2.2.10.4 Nucleic acid quantity and quality analysis	53
2.2.11 Sanger sequencing.....	54
2.2.12 Fluctuation assays.....	54
2.2.13 Fitness assays	55
2.2.13.1 Generation of neutrally marked strain	55
2.2.13.2 Fitness assays with rifampicin resistant marker	55
2.2.13.2.1 Fitness calculation.....	56
2.2.14 MA assays	57
2.2.14.1 Establishment of lineages	57
2.2.14.2 Maintenance of lineages	57
2.2.14.2.1 Single-colony bottleneck procedure	57
2.2.14.2.1.1 Estimation of number of generations	58
2.2.14.2.2 Storage	58
2.2.14.2.3 Contamination test	58
2.2.14.3 Mutation rate calculation	59
2.2.15 <i>sb</i> c <i>C</i> adaptive lineages	59
2.2.15.1 Establishment of lineages	59
2.2.15.2 Maintenance of lineages	60
2.2.15.2.1 Daily lineage sub-culture	60
2.2.15.2.2 Storage	60
2.2.15.2.3 Contamination test	61
2.2.16 <i>In silico</i> analysis	61
2.2.16.1 Bioinformatic resources and software	61
2.2.16.2 R simulator modelling	64
2.2.16.3 DNA high throughput sequencing analysis	64

2.2.16.3.1	Illumina sequencing	64
2.2.16.3.2	Mutation detection	64
2.2.16.3.2.1	Reference-based mutation detection	65
2.2.16.3.2.2	<i>De novo</i> assembly based mutation detection.....	66
2.2.16.3.2.2.1	Genome alignment	66
2.2.16.3.3	Cumulative GC-skew analysis	67
2.2.16.3.4	Codon usage analysis	67
2.2.16.4	RNA high throughput sequencing analysis	67
2.2.16.4.1	RNAseq data analysis	67
2.2.16.4.2	Illumina sequencing.....	67
2.2.16.4.3	Removal of rRNA and tRNA reads	68
2.2.16.4.4	Sequence read alignment	68
2.2.16.4.4.1	Differential gene expression.....	69
2.2.16.4.4.2	Gene ontology (GO) analysis.....	69
2.2.17	Statistical analysis	69
Chapter Three: Mutation rate in aerobic and anaerobic environments		71
3.1	Introduction	71
3.2	Results and discussion.....	71
3.2.1	Fluctuation assays.....	71
3.2.2	MA simulations <i>in silico</i>	74
3.2.3	MA lineages	75
3.2.3.1	Next-generation sequence analysis.....	75
3.2.3.2	Mutation identification	76
3.2.3.2.1	BPS and indel detection	76
3.2.3.2.2	GCR detection.....	79
3.2.3.2.2.1	Challenges with GCR detection	79
3.2.3.2.2.2	Large-scale GCRs	80
3.2.3.3	Summary of number of mutations found in lineages	86
3.2.3.4	Estimation of mutation rates	87
3.2.3.4.1	Mutation accumulation assays versus fluctuation assays	89
3.2.3.4.2	Mutation accumulation assays versus <i>in silico</i> mutations	90
3.3	Summary	92
Chapter Four: Mutation spectra in aerobic and anaerobic environments.....		93
4.1	Introduction	93

4.2 Results and discussion.....	94
4.2.1 Spectra of mutations in aerobic and anaerobically grown <i>E. coli</i>	94
4.2.1.1 BPSs.....	96
4.2.1.1.1 Nucleotide mutation rates	97
4.2.1.1.2 Transition bias.....	100
4.2.1.1.3 Synonymous vs non-synonymous BPSs.....	102
4.2.1.2 Indels	105
4.2.1.3 GCRs	109
4.2.1.3.1 Different GCR types	109
4.2.1.3.1.1 IS element transposition.....	111
4.2.1.3.2 Large-scale GCRs	114
4.2.1.3.2.1 Cumulative GC-skew	114
4.2.2 Genome-wide distribution of mutations.....	119
4.2.2.1 Genome organisation.....	119
4.2.2.1.1 Distribution of mutations within MDs.....	120
4.2.2.1.2 First replichore versus second replichore	123
4.2.2.2 Mutation hotspots	126
4.2.3 Activities of genes involved in maintaining genome integrity.....	130
4.2.3.1 Differential gene expression.....	130
4.2.3.1.1 Expression of DNA repair and replication genes	136
4.2.3.1.1.1 DNA replication	137
4.2.3.1.1.2 Recombinational repair	138
4.2.3.1.1.2.1 IS element genes involved in transposition.....	138
4.2.3.1.1.3 The NER pathway	139
4.2.3.1.1.4 The MMR pathway	140
4.2.3.1.1.5 The BER pathway	140
4.2.3.1.1.6 SOS response	142
4.2.3.1.1.7 The stringent response.....	142
4.2.3.1.2 Genes involved in acid response.....	142
4.3 Summary	143

Chapter Five: The contribution of gross chromosomal rearrangements to adaptive evolution..... 145

5.1 Introduction.....	145
5.2 Results and discussion.....	146
5.2.1 Experimental expression of <i>sbcC</i>	146

5.2.1.1 Gene expression of <i>sbcC</i> as determined using RNAseq.....	146
5.2.1.2 Validation using reverse transcription quantitative PCR (RT-qPCR).....	147
5.2.1.2.1 Determination of reference genes	148
5.2.1.2.2 <i>sbcC</i> gene expression verification by RT-qPCR	151
5.2.1.3 Comparison of gene expression data	152
5.2.2 Contribution of <i>sbcC</i> to genome fidelity and fitness.....	154
5.2.2.1 Generation of <i>sbcC</i> mutant	154
5.2.2.2 Growth dynamics of <i>sbcC</i> mutant under anaerobic environment	155
5.2.2.3 Establishment of long-term lineages in anaerobic environment.....	155
5.2.2.4 Mutation analysis.....	156
5.2.2.4.1 Rates of GCRs per generation per genome.....	158
5.2.2.4.2 Mutations of interest	160
5.2.2.4.2.1 REL4536 Δ <i>sbcC</i> :: <i>cat</i> -1K-1 and REL4536 Δ <i>sbcC</i> :: <i>cat</i> -1K-8 ..	160
5.2.2.4.2.2 The <i>adhE</i> gene.....	161
5.2.2.4.2.3 IS insertions.....	162
5.2.2.5 Fitness assessment of evolved populations.....	163
5.2.2.5.1 Assessment of neutrally marked strains.....	164
5.2.2.5.2 Population fitness.....	164
5.2.2.6 Polymorphic evolved lineages.....	168
5.2.2.6.1 Genetic basis of polymorphism	169
5.3 Summary	169
Chapter Six: General discussion.....	171
6.1 Background	171
6.2 General discussion of findings and future directions	171
6.3 Conclusions	175
References	177
Appendices	199
Appendix A	199
Appendix B	207
Appendix C	211

List of tables

Table 2.1. <i>E. coli</i> strains used in this study.....	33
Table 2.2. Plasmids used in this study.	33
Table 2.3. Antibiotics used in this study.....	34
Table 2.4. Oligonucleotides used in this study.	35
Table 2.5. DM medium components.....	42
Table 2.6. Molecular weight-size markers used in this study.....	45
Table 2.7. Reaction components for a standard PCR reaction.....	46
Table 2.8. Reaction components for a standard qPCR reaction.....	47
Table 2.9. Bioinformatic resources and software used.	62
Table 3.1. <i>E. coli</i> mutation rates calculated from fluctuation assays.....	73
Table 3.2. Genome re-sequencing reference-based mapping and <i>de novo</i> assembly statistics for aerobic MA clones.....	77
Table 3.3. Genome re-sequencing reference-based mapping and <i>de novo</i> assembly statistics for anaerobic MA clones.	78
Table 3.4. Details of IS elements present in <i>E. coli</i> REL4536.	80
Table 3.5. Large-scale rearrangement break points identified <i>in silico</i> and verified by PCR.....	85
Table 3.6. Frequency of mutations found in each sequenced lineage.....	86
Table 3.7. Genome-wide spontaneous mutation rates for <i>E. coli</i> grown aerobically and anaerobically.	87
Table 4.1. Slippage events detected in aerobic and anaerobic MA lineages.	108
Table 4.2. Relative distribution of mutations within the macrodomains of <i>E. coli</i>	121
Table 4.3. Mutations identified in multiple MA lineages.....	129
Table 4.4. Summary statistics for RNAseq data obtained from Bowtie2 and EDGE-Pro output.....	131

Table 4.5. List of the 20 most up-regulated genes in aerobically grown REL4536.	134
Table 4.6. List of the 20 most up-regulated genes in anaerobically grown REL4536..	135
Table 5.1. Summary of RT-qPCR amplification of reference genes for cDNA derived from aerobic and anaerobically grown REL4536.	150
Table 5.2. Summary of RT-qPCR amplification of <i>sbcC</i> for cDNA derived from aerobic and anaerobically grown REL4536.	151
Table 5.3. Quantification of <i>sbcC</i> expression using <i>rpoD</i> and <i>proC</i> expression values.	152
Table 5.4. Genome re-sequencing reference-based mapping and <i>de novo</i> assembly statistics for anaerobic REL4536 Δ <i>sbcC</i> :: <i>cat</i> -1K and REL4536 AN-1K clones.	157
Table 5.5. Frequency of mutations found in the genome of each lineage clone.	158
Table 5.6. Details of <i>adhE</i> mutations detected in this study.	161
Table 5.7. Correlations between relative fitness and number of accumulated GCRs; and population morphology of clones under aerobic conditions.	168
Table A.1. Examples of oxidative lesions and the mutations they produce.	199
Table A.2. Expression values of all genes in REL4536 known to be involved in DNA repair and replication.	200
Table A.3. List of all mutations detected in aerobic and anaerobic MA clone genomes.	211
Table A.4. Mutation rates calculated from individual lineages.	264
Table A.5. Codon usage of <i>E. coli</i> REL4536 genome.	265
Table A.6. Genes induced in response to acidic stress.	270
Table A.7. List of all mutations found in REL4536 Δ <i>sbcC</i> :: <i>cat</i> -1K and REL4536 AN-1K clone genomes.	271
Table A.8. Genes deleted in the 33 kb deletion event observed in REL4536	

<i>ΔsbcC::cat-1K-1</i> and REL4536 <i>ΔsbcC::cat-1K-8</i> clones.	282
Table A.9. Colony morphotypes of REL4536 <i>ΔsbcC::cat-1K</i> populations and clones after 1,000 generations.	283

List of figures

Figure 1.1. Transition versus transversion mutations.	5
Figure 1.2. Examples of GCRs.	6
Figure 1.3. Homologous recombination of repeated sequences resulting in the formation of GCRs.	8
Figure 1.4. Proposed model of the GO system.	14
Figure 1.5. Mutation rates per nucleotide versus genome size for different organisms	25
Figure 1.6. Mutation accumulation (MA) studies of bacteria.	27
Figure 2.1. Bioinformatics pipeline developed to identify mutations within the genome sequences analysed in this study.	65
Figure 2.2. Overview of the workflow for RNAseq <i>in silico</i> analysis.	67
Figure 3.1. MA simulations <i>in silico</i>	75
Figure 3.2. Synteny plots for aerobic lineages.	83
Figure 3.3. Synteny plots for anaerobic lineages.	84
Figure 3.4. Comparison of MA mutations and <i>in silico</i> theoretical mutations.	91
Figure 4.1. Mutation rates for different types of mutations observed in the MA study.	95
Figure 4.2. Mutation rates of single base substitutions in aerobically and anaerobically grown <i>E. coli</i>	100
Figure 4.3. Mutation rates of transitions and transversions in aerobically and anaerobically grown <i>E. coli</i>	102
Figure 4.4. Rates of coding, non-coding, synonymous and non-synonymous mutations in aerobically and anaerobically grown <i>E. coli</i>	104
Figure 4.5. Mutation rates of indels in aerobically and anaerobically grown <i>E. coli</i>	107

Figure 4.6. Mutation rates of different classes of GCRs in aerobically and anaerobically grown <i>E. coli</i> .	110
Figure 4.7. Transposition rates of different IS elements in REL4536 when grown aerobically and anaerobically.	113
Figure 4.8. Gene diagrams of the seven large-scale GCRs that occurred in AE-180-04, AE-180-06, AE-180-24, AN-144-12 and AN-144-46.	115
Figure 4.9. The cumulative GC-skew of <i>E. coli</i> REL4536, AE-180-04, AE-180-06, AE-180-24, AN-144-12 and AN-144-46 as a function of position in genome.	117
Figure 4.10. Circular plots displaying the GC-skews of: a) <i>E. coli</i> REL4536, b) AE-180-04 and c) AE-180-24 genomes.	118
Figure 4.11. Genomic distribution of mutations mapped onto <i>E. coli</i> REL4536.	122
Figure 4.12. Mutation rates of different mutation types in the two replichores in aerobically and anaerobically grown <i>E. coli</i> .	124
Figure 4.13. Genome organisation of <i>E. coli</i> .	126
Figure 4.14. Significantly up-regulated genes, as a proportion of genes in the pathway, known to be involved in DNA repair and replication, classified by pathway.	136
Figure 5.1. Principal component analysis (PCA) of the DESeq2-normalised RPKM values of the three aerobic and two anaerobic samples.	147
Figure 5.2. Comparison of relative log ₂ fold changes observed for six genes quantified by both RNAseq and RT-qPCR.	153
Figure 5.3. Growth dynamics of <i>E. coli</i> REL4536 and <i>E. coli</i> REL4536 $\Delta sbcC::cat$ under anaerobic growth conditions.	156
Figure 5.4. Mutation rates for different types of mutations observed amongst the REL4536 $\Delta sbcC::cat$ -1K and REL4536 AN-1K clones.	159

Figure 5.5. Relative fitness of anaerobic populations to an ancestral strain after 1,000 generations of evolution.	166
Figure A.1. Mutation rates of different BPSs in the two replichores in aerobically and anaerobically grown <i>E. coli</i>	267
Figure A.2. Mutation rates of different BPSs in the two replichores in aerobically and anaerobically grown <i>E. coli</i> per day of growth.	268
Figure A.3. Functional category enrichment analysis of differentially expressed genes.....	269

Non-standard abbreviations

λ -Red	Lambda Red
A	Adenine (nucleotide base)
AE	Aerobic
AHT	Anhydrotetracycline
Amp ^R	Ampicillin resistant strain
AN	Anaerobic
Anc	Ancestor
Ara ⁻	Strain that cannot utilise arabinose
Ara ⁺	Strain that can utilise arabinose
ATP	Adenosine triphosphate
BER	Base excision repair
BLAST	Basic Local Alignment Sequence Tools
bp	Base pairs
BPS	Base pair substitution
C	Cytosine (nucleotide base)
cDNA	Complementary DNA
CFU	Colony forming units
CL	Confidence limits
Cm ^R	Chloramphenicol resistant strain
CQ	Quantification cycle
DEPC H ₂ O	Diethylpyrocarbonate treated water
dH ₂ O	Distilled water
DM	Davis minimal
DNA	Deoxyribonucleic acid
DSB	Double-strand break
dsDNA	Double-stranded DNA
E	Amplification efficiency for qPCR reactions
G	Guanine (nucleotide base)
GCR	Gross chromosomal rearrangement
IR	Inverted repeat
IS	Insertion elements
kb	Kilobase
LB	Luria-Bertani
LTEE	Long-term experimental evolution
MA	Mutation accumulation
Mb	Megabase
MB	Mega bytes

MD	Macrodomain
MGE	Mobile genetic element
MMR	Mismatch repair
mRNA	Messenger RNA
MSS-MLE	Ma-Sandri-Sarkar Maximum Likelihood Estimator method
Nal ^R	Nalidixic acid resistant strain
ncRNA	Non-coding RNA
NER	Nucleotide excision repair
NS	Non-structured regions
OD	Optical density
oriC	Origin of replication
PCA	Principal component analysis
PCR	Polymerase chain reaction
pol	Polymerase
ppGpp	Guanosine tetra-phosphates
pppGpp	Guanosine penta-phosphates
qPCR	Quantitative polymerase chain reaction
R ²	Correlation coefficient
Rif ^r	Rifampicin resistant reference strain
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RPKM	Reads per kilobase of gene per million reads mapped
RPM	Rotations per minute
rRNA	Ribosomal RNA
RT	Room temperature
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
SCM	Small colony morphotype
SEM	Standard error of the mean
SSB	Single-strand break
ssDNA	Single-stranded DNA
T	Thymine (nucleotide base)
TCM	Typical colony morphotype
TE	Transposable element
tRNA	Transfer RNA
Ts/Tv	Transition to transversion ratio
v/v	Volume per volume
v/v/v	Volume per volume per volume
w/v	Weight per volume