

Characterising the (epi+) Genome of *Legionella longbeachae* Serogroup 1 Clinical Isolates

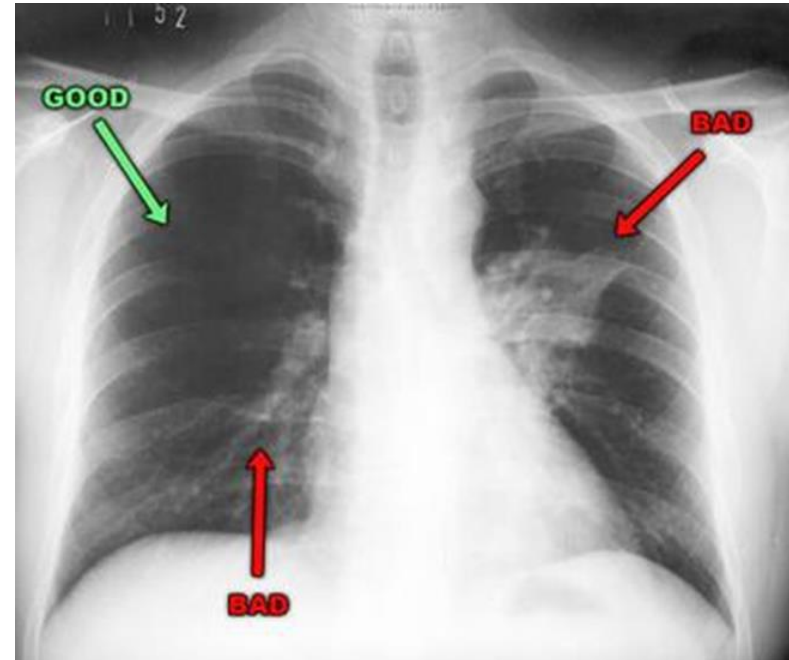
Sandy Slow

The Infection Group, Department of Pathology & Biomedical Science

University of Otago, Christchurch

Legionnaires' Disease

- Pneumonia caused by *Legionella* bacteria
- Can be severe and life threatening
- No human-to-human transmission
- Requires specific antibiotic treatment
- Notifiable disease
- *L. longbeachae* predominant cause in NZ-soil/composted plant material





Potting mix nearly kills Wellington man

RHIANNON MCCONNELL
Last updated 18:52, February 17 2016



Legionnaires' puts 10 Aucklanders in hospital

AMY MAAS

How to protect yourself against Legionnaires' disease

DR CATHY STEPHENSON
Last updated 11:27, November 21 2017



Legionnaires' disease puts him in intensive care

Tom Kitchin • 19:08, Oct 05 2018



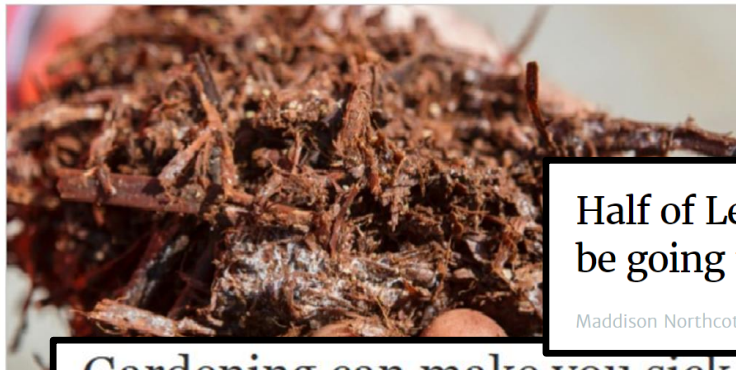
Spring garden chores linked to spike in Legionnaires' disease

KATIE NEWTON
Last updated 17:43, October 9 2018



Health boards, including Hutt Valley DHB, seeing more cases of legionellosis

BLAKE CRAYTON-BROWN
Last updated 08:14, January 22 2016



Half of Legionnaires' disease sufferers may be going undiagnosed

Maddison Northcott • 15:39, Nov 17 2017



Gardening can make you sick

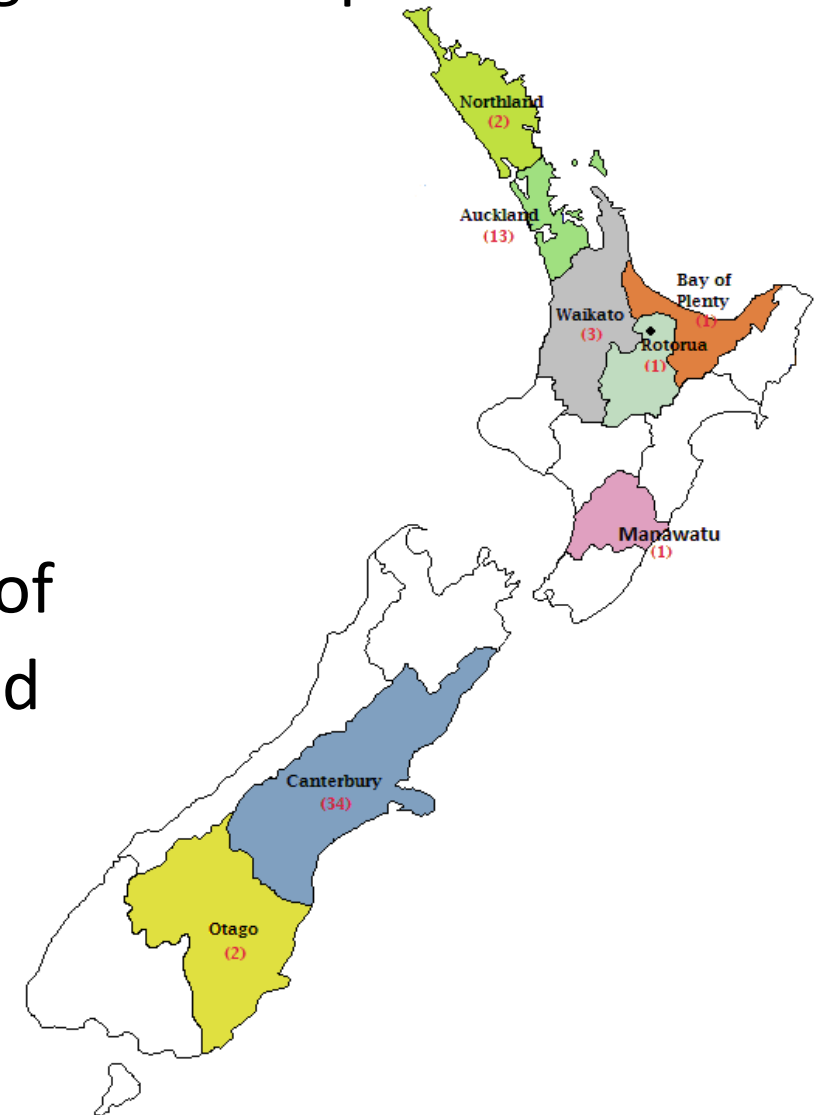


L. Longbeachae genome

- Until 2017 isolate NSW 150 only complete genome sequence
 - 4.1 Mb chromosome
 - 71 kb plasmid

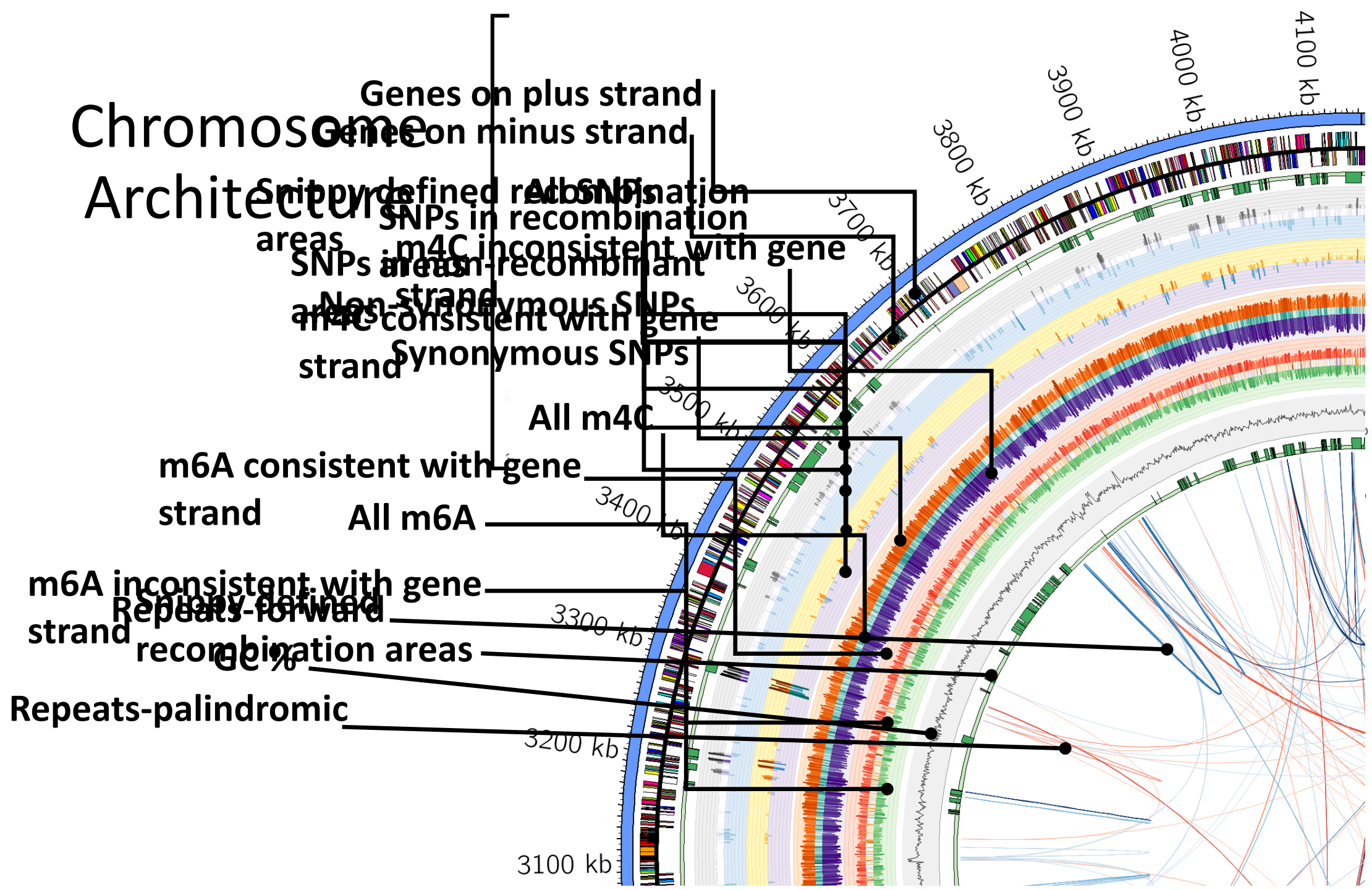
Objective

- Assess the geo-temporal genetic diversity of *L. longbeachae* clinical isolates from around NZ over a 22 year period (1993-2015)

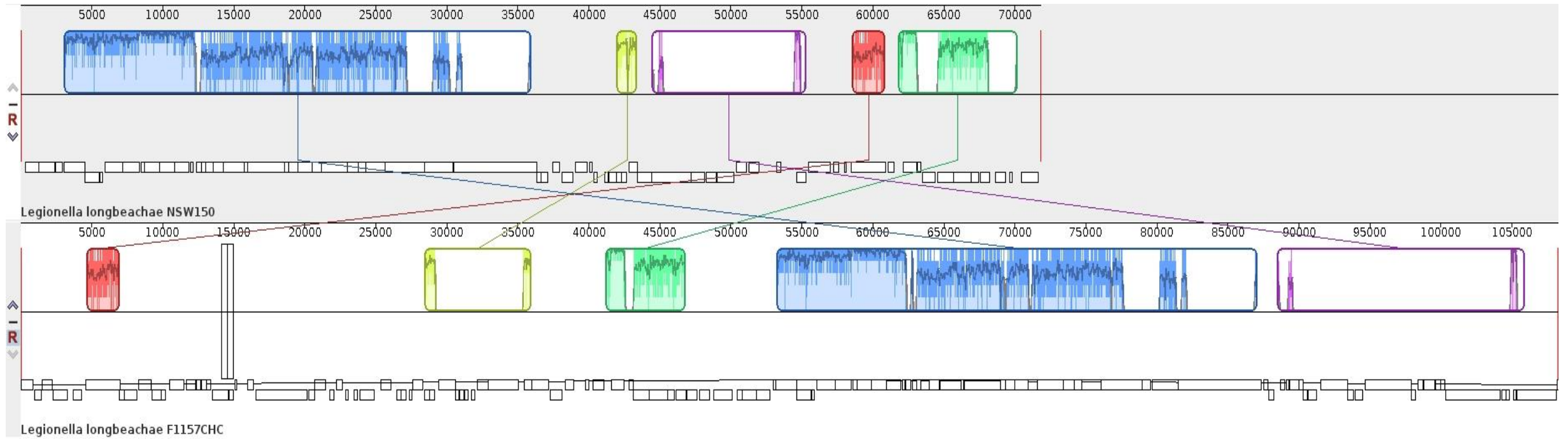


Sequencing and NZ Ref Genome

- Isolates sequenced on Illumina MiSeq (short-read-250 bp PE)
- One isolate (F1157CHC-2014, Canterbury Isolate) selected for further sequencing to get NZ Ref genome-used PacBio RSII Platform (long-read, 15,000 kb library cut-off)
- PacBio also provides epigenome data
- Isolate had chromosome of ≈ 4.1 Mb and a plasmid of $\approx \underline{108}$ kb
- 3,577 coding sequences in chromosome and 114 in plasmid



Plasmid

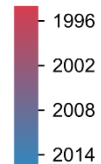


Geo-temporal analyses

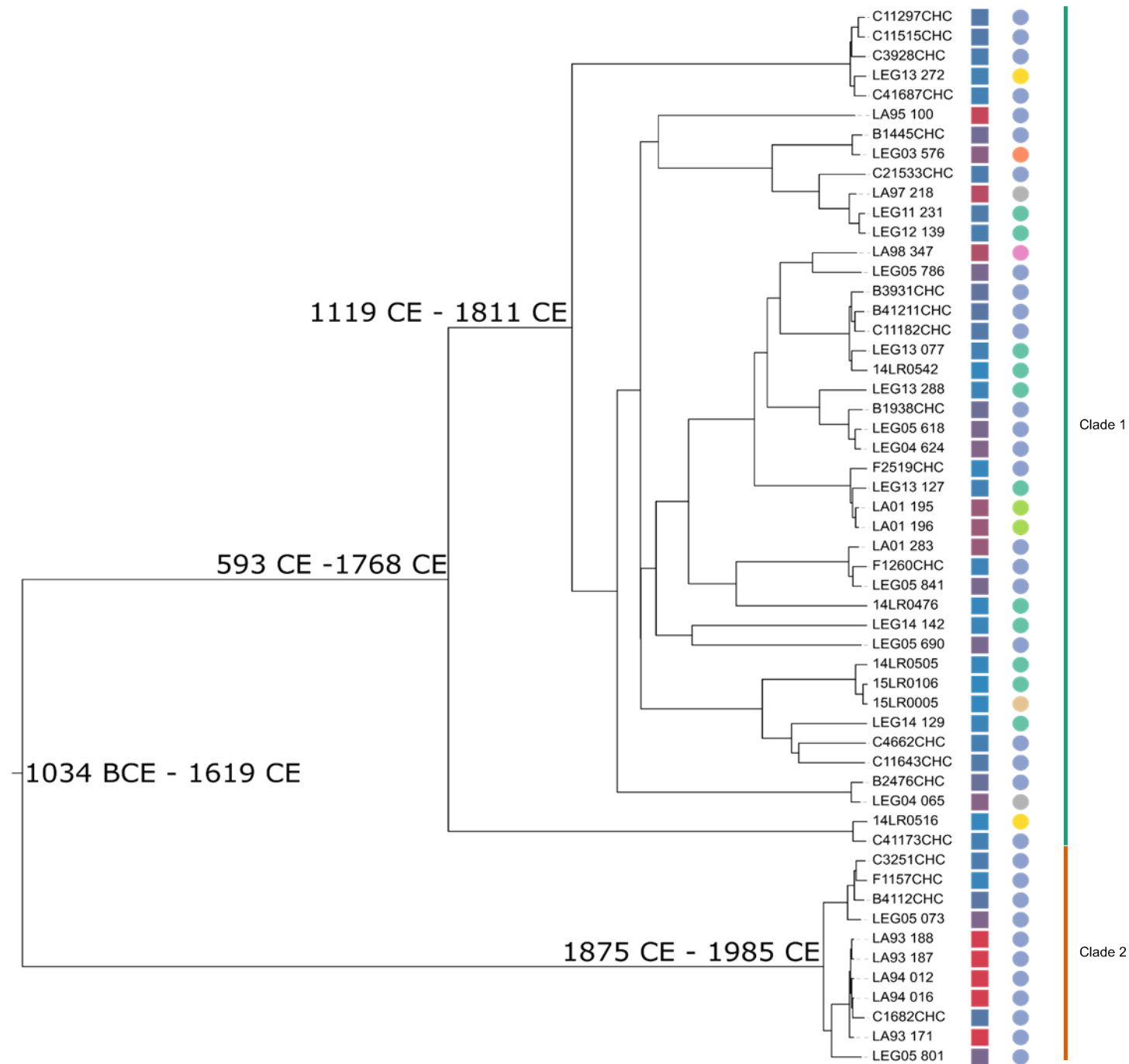
- The 54 sg1 isolates analysed shared 5,383 core SNPs. Only 2,338 were non-recombinant.
- Phylogenetic modelling of non-recombinant SNPs estimated that isolates mutated at a rate of $3.45 \times 10^{-8} - 1.71 \times 10^{-7}$ substitutions/site/year.
- Isolates had a shared common ancestor between 1034 BCE and 1619 CE.
- One large clade and one small clade were observed. The largest clade consisted of isolates from multiple regions, while the second clade contained isolates only from Canterbury.

100

Date of collection



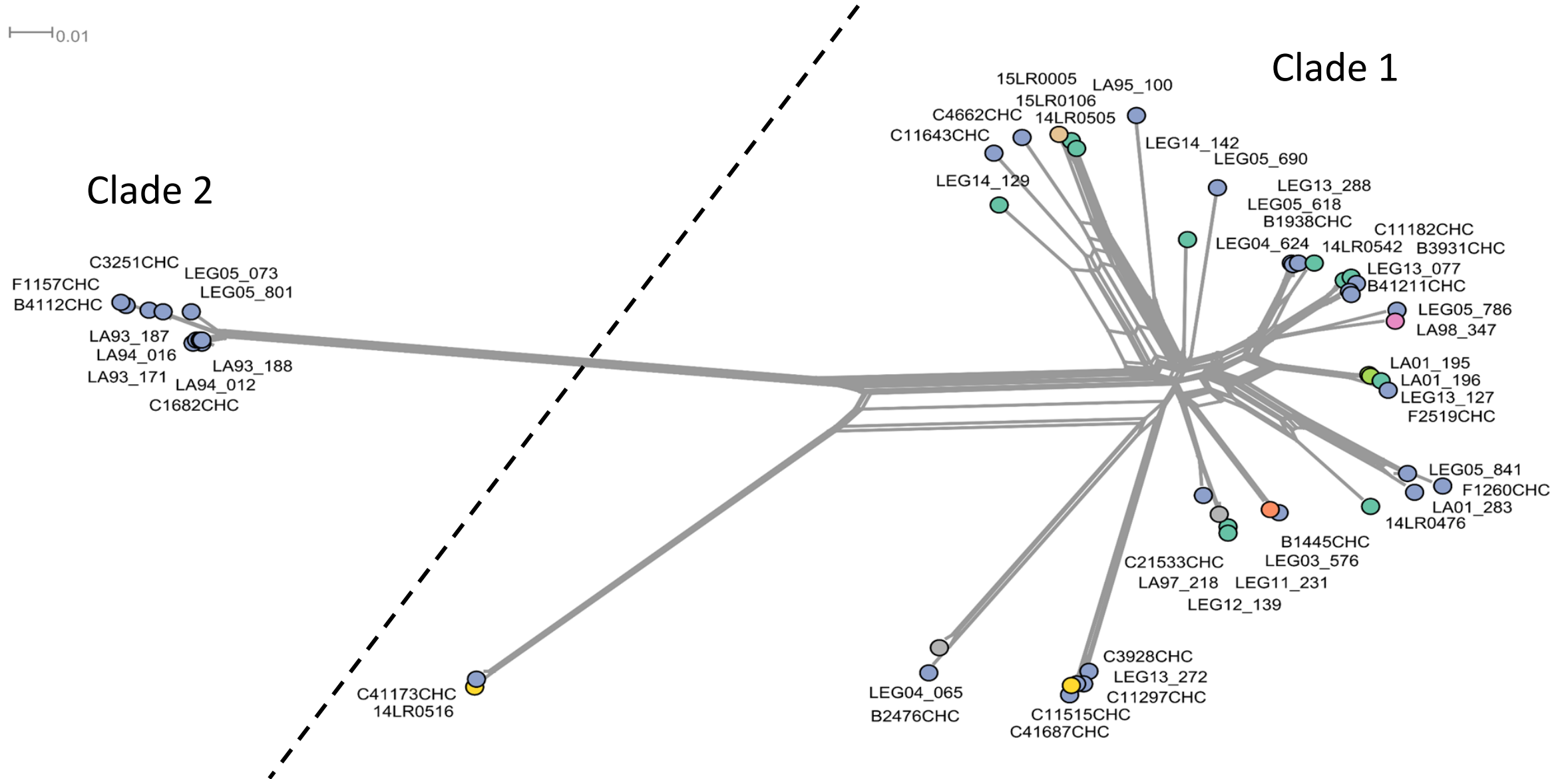
Sources

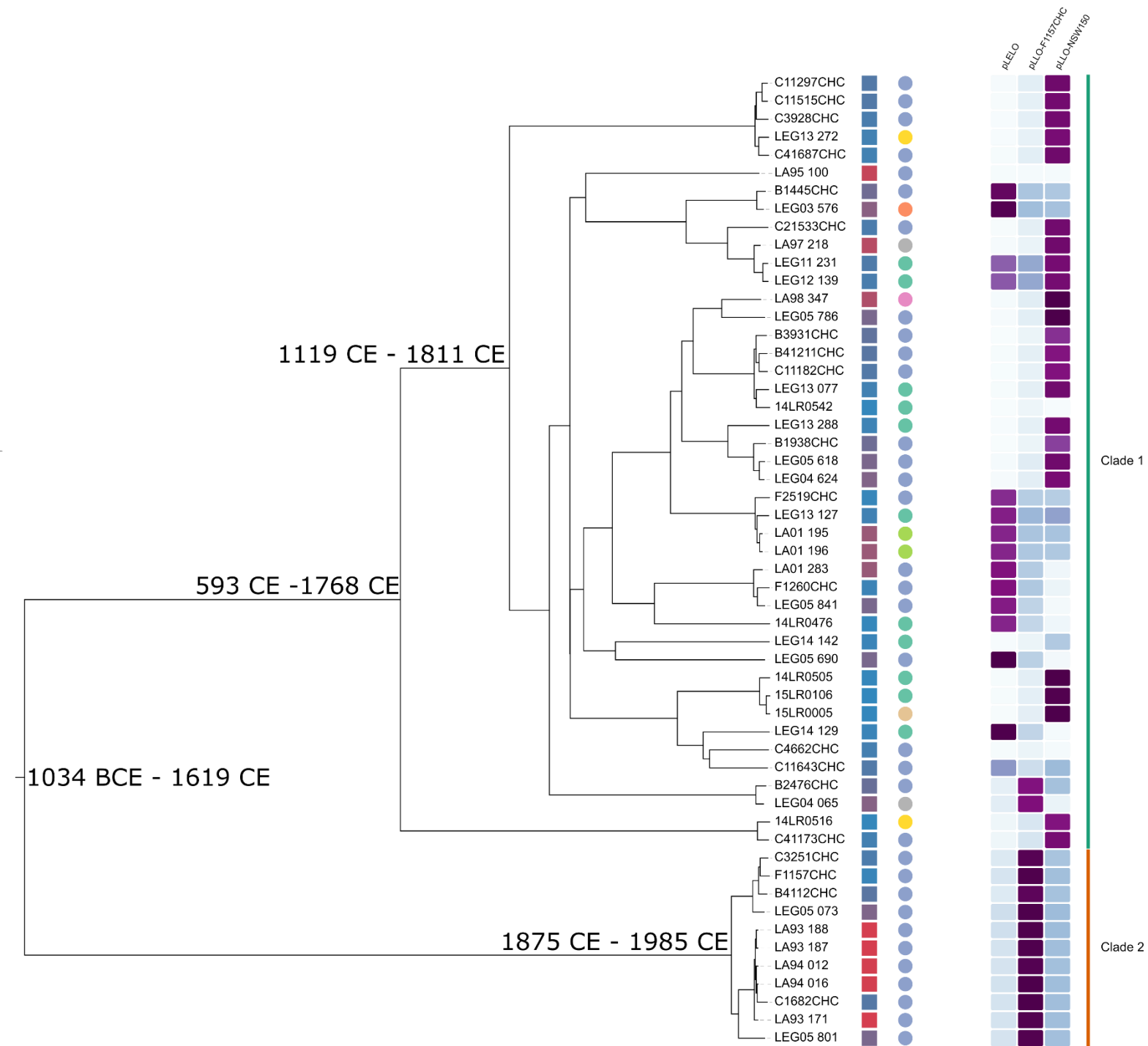
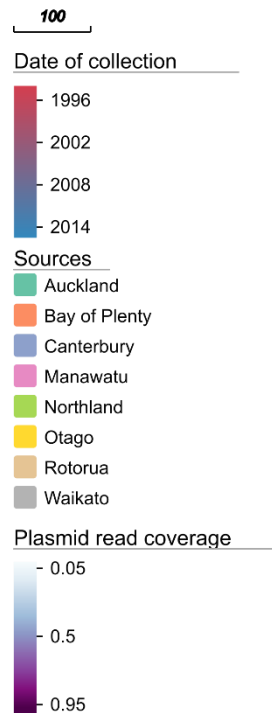


0.01

Clade 2

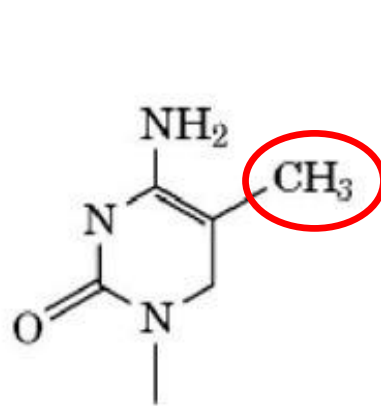
Clade 1



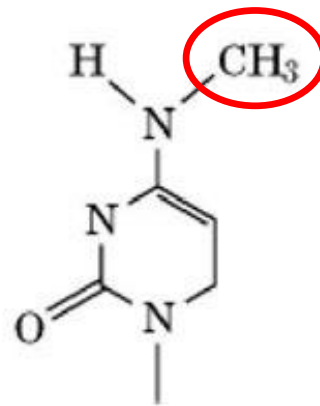


Epigenetic Modification

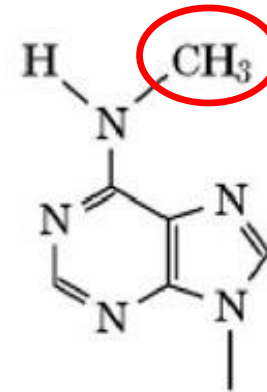
- Epigenetic = “on top of”
- Methylation 6mA, 4mC and 5mC
- Restriction modification system-protects against viral DNA insertion



5-methylcytosine
(5mC)



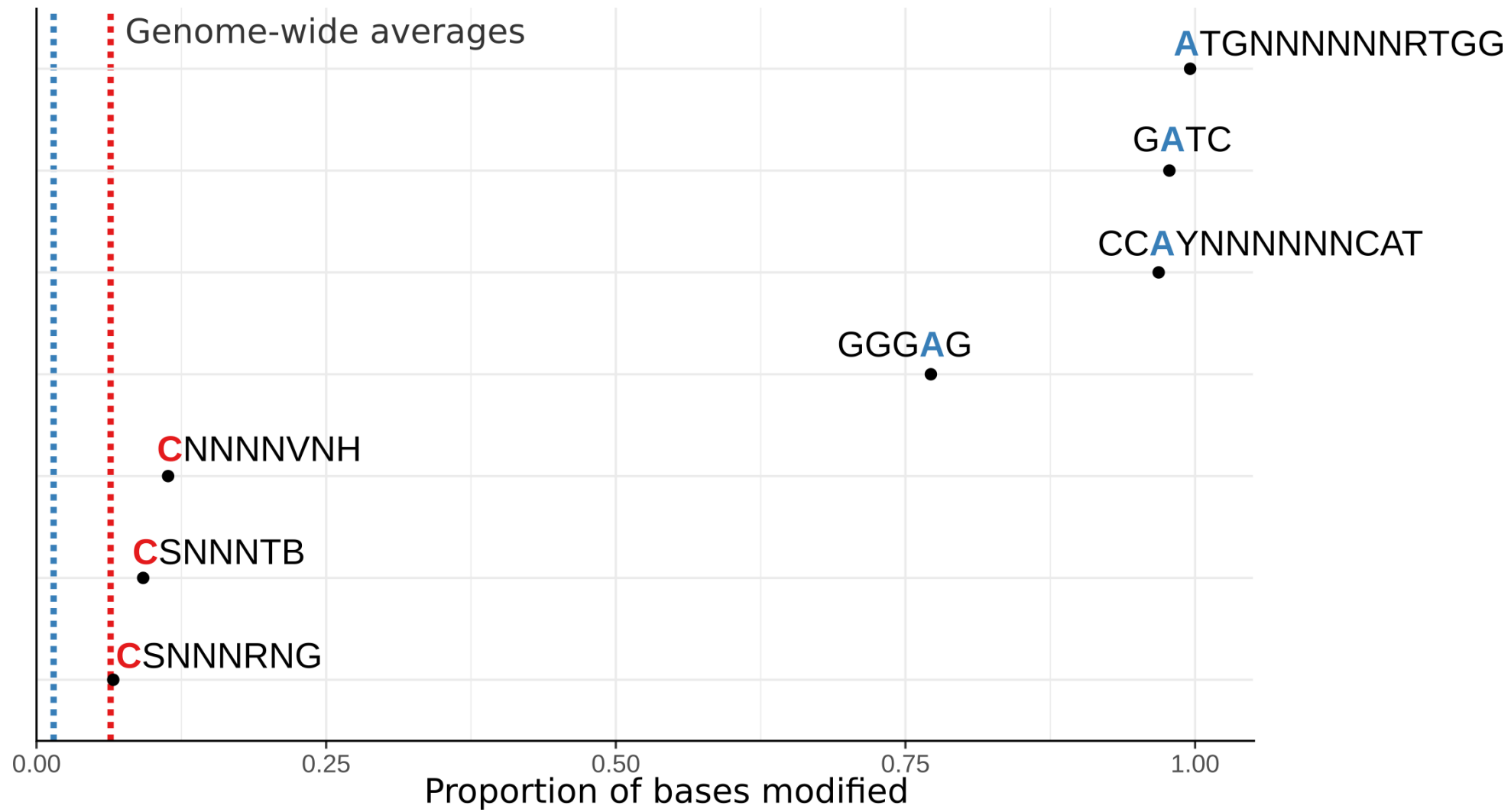
N4-methylcytosine
(N4mC)

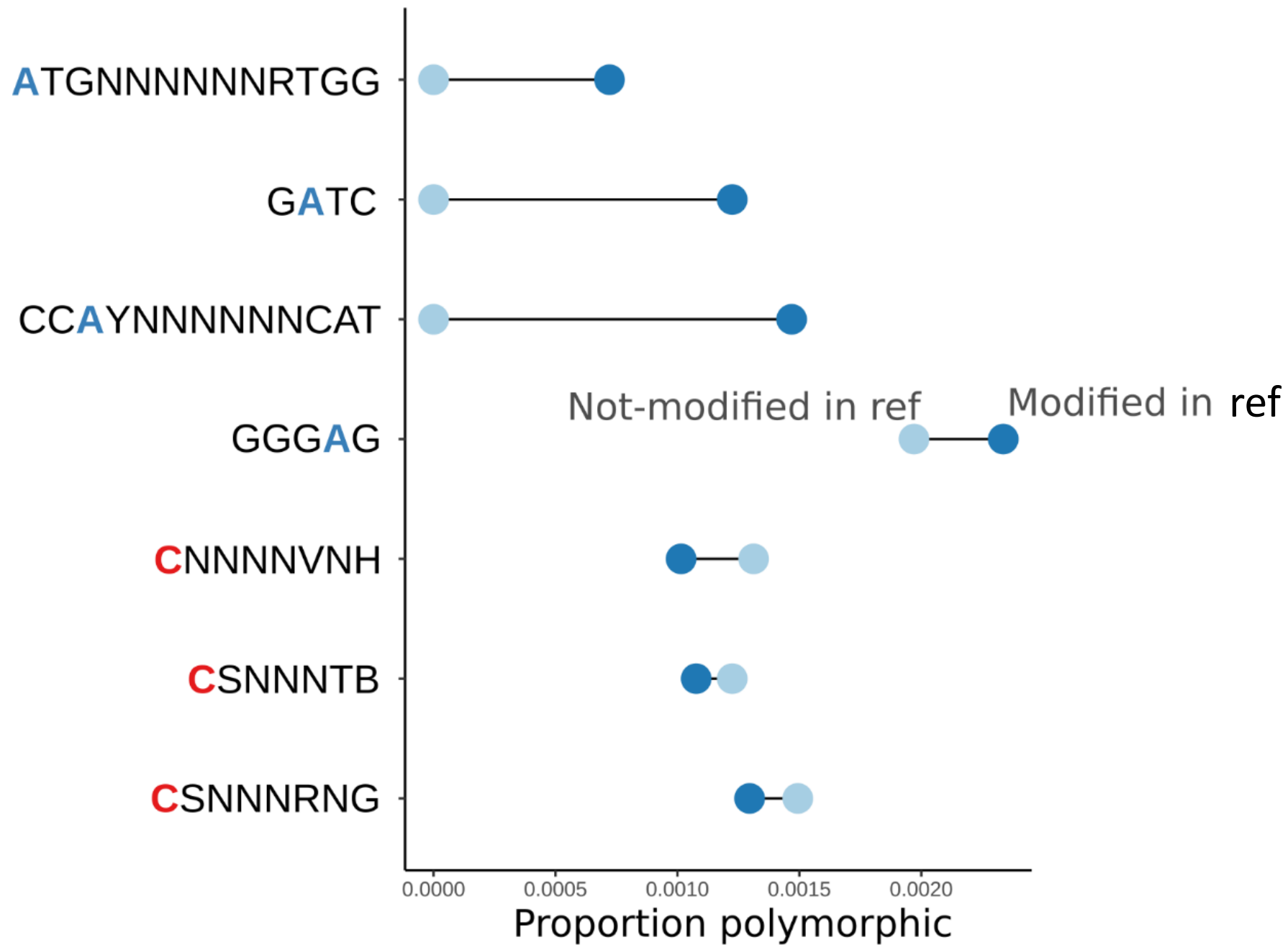


N6-methyladenine
(N6mA)

Least common
modification

Most common
modification





Summary

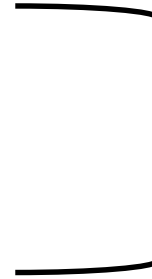
- Most variability in the *L. longbeachae* genome is from recombination and there have been large-scale rearrangements.
- Two highly related clades -occur throughout NZ and persist over time.
- Most genetically distinct clade consisted of isolates only from Canterbury BUT oversampling from this region.
- Mosaic plasmids-intra and inter-species exchange
- Epigenic modification- highly modified genome, m6A most common, m4C modified bases conserved

Acknowledgements

Patrick Biggs

David Winter

Samuel Bloomfield



Massey University

Trevor Anderson

Canterbury Health Laboratories

David Murdoch

David Harte- *Legionella* Reference Laboratory and CHL for access to the isolates

Funding: University of Otago-UORG; Millennium Science-SMRT Grant

NZGL: Illumina Sequencing

Doherty Institute, Melbourne: PacBio Sequencing