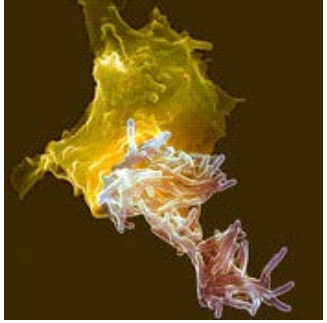
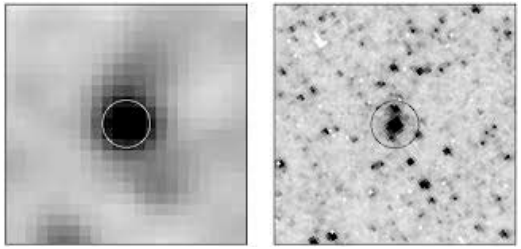
Whole Genome Sequencing for sourcing Bovine Tuberculosis herd breakdowns in New Zealand

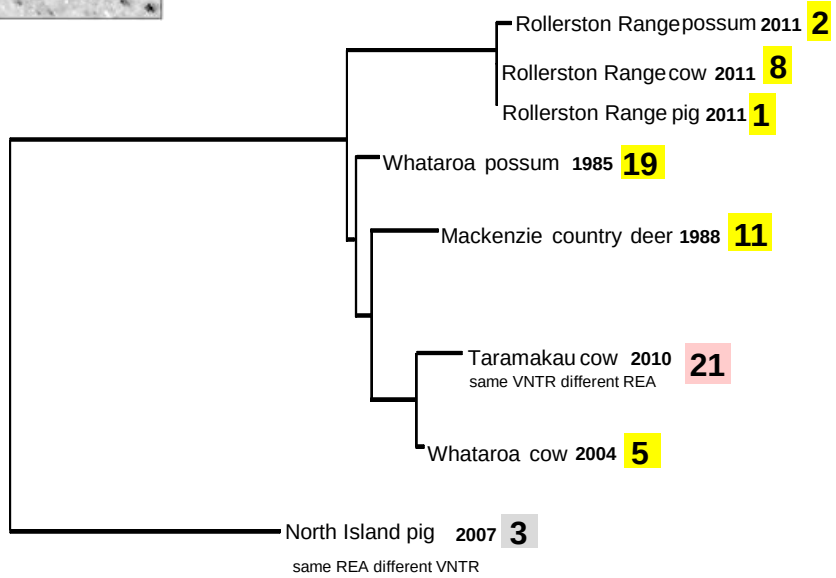


Marian Price-Carter

Geoffrey de Lisle and Desmond Collins



Strain #	Source	Year	Host	REA # and Type		VNTR type
2	Mt. Algidus	2011	possum	19	A1 A4/0 A4	2 3 4 3 5 3 9 4(15)5(10)
8	Mt. Algidus	2011	cow	19	A1 A4/0 A4	2 3 4 3 5 3 9 4(15)5(10)
1	Mt. Oakden	2011	pig	19	A1 A4/0 A4	2 3 4 3 5 3 9 4(15)5(10)
19	Whataroa	1985	possum	19	A1 A4/0 A4	2 3 4 3 5 3 9 4(15)5(10)
11	Mackenzie C.	1988	deer	19	A1 A4/0 A4	2 3 4 3 5 3 9 4(15)5(10)
21	Taramakau	2010	cow	299	A17 A4/0 A4	2 3 4 3 5 3 9 4(15)5(10)
5	Whataroa	2004	cow	19	A1 A4/0 A4	2 3 4 3 5 3 9 4(15)5(10)
3	Otaki	2007	pig	19	A1 A4/0 A4	2 5 4 3 5 3 9 4(15)5 6



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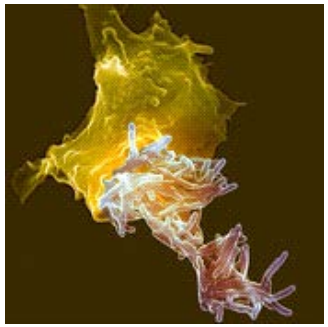
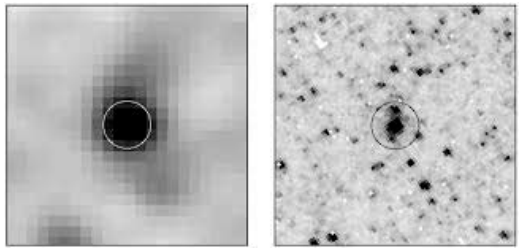
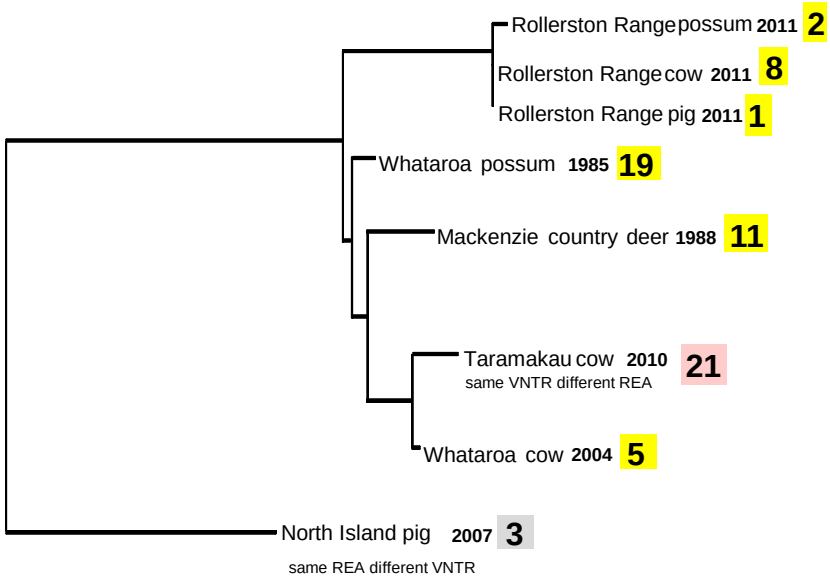
Whole Genome Sequencing for sourcing Bovine Tuberculosis herd breakdowns in New Zealand



Marian Price-Carter
Geoffrey de Lisle and Desmond Collins

- Introduction –
- Current typing system
- Results of pilot WGS study
- Next steps





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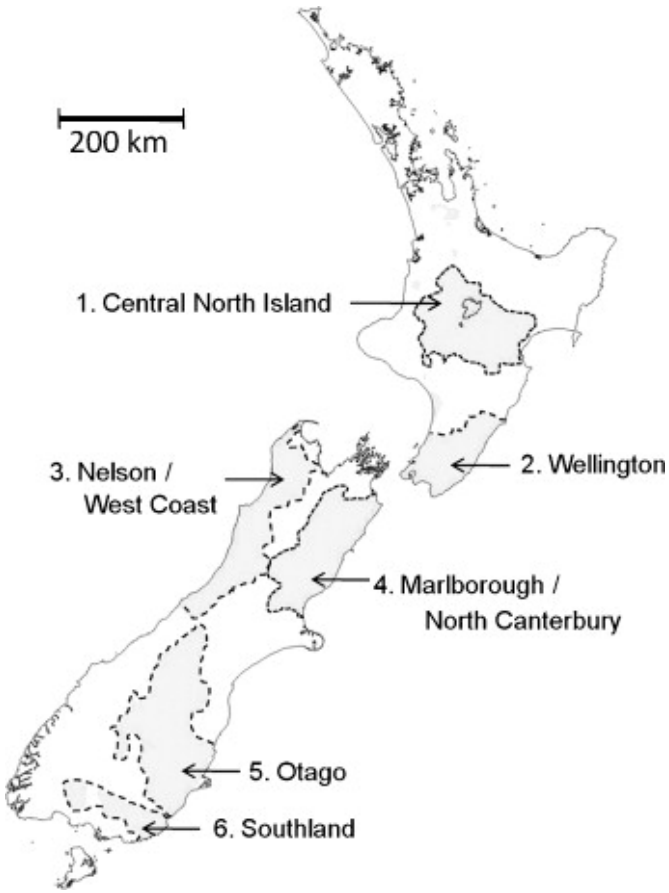
TBfree NEW ZEALAND
THE PROGRAMME OF THE ANIMAL HEALTH BOARD

OSPRI NEW ZEALAND

- TB control assists in maintaining the production and reputation of dairy, beef and deer exports, worth around $14 billion a year to NZ

- Eradication efforts are hampered by wildlife reservoirs mainly the Australian brush tail possum



- There are six regions in NZ where wildlife carry bovine TB

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Geoff de Lisle

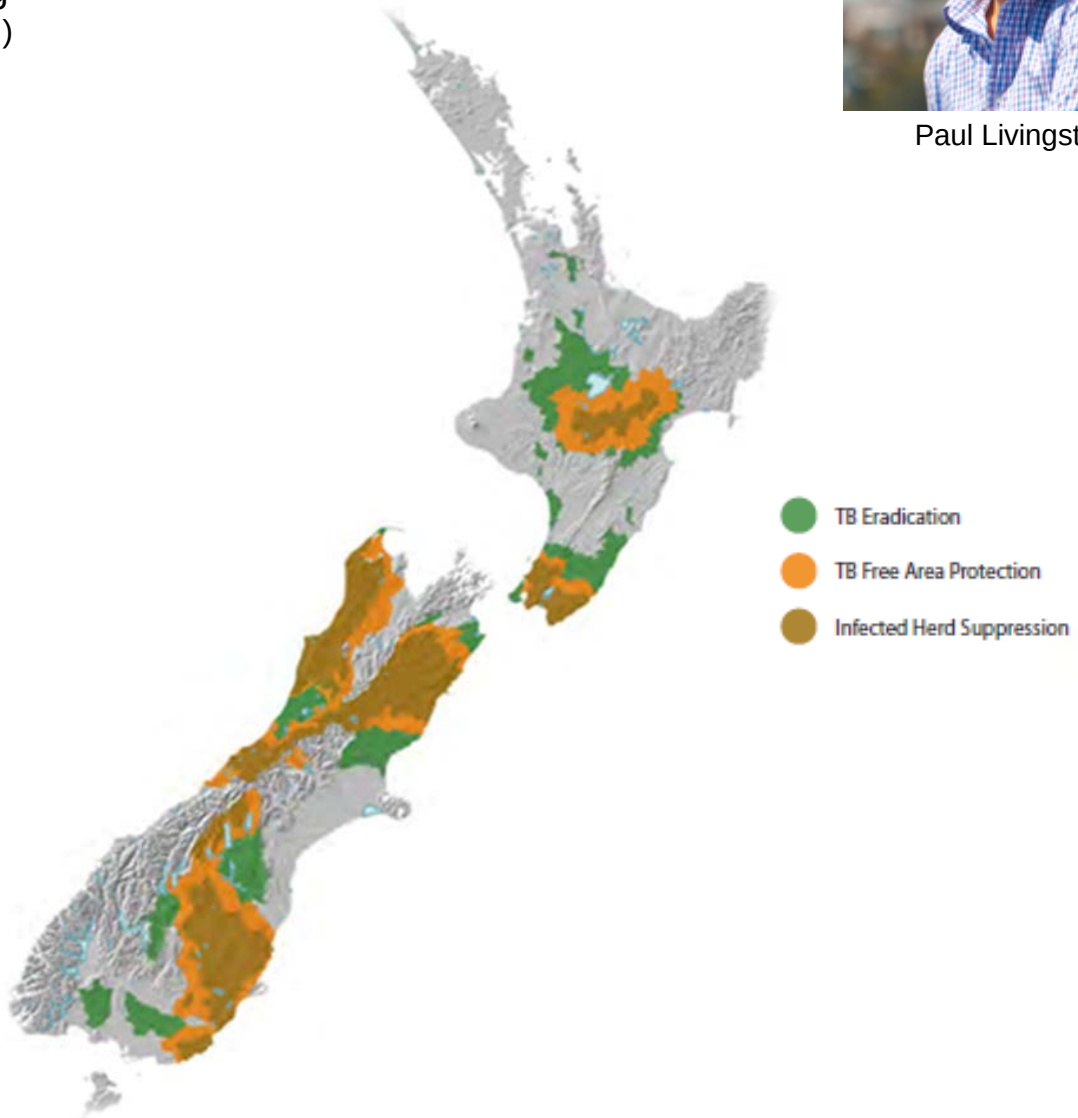
Herd Testing
(Bovigam ELISA)

M. bovis
culture

Strain typing
method development

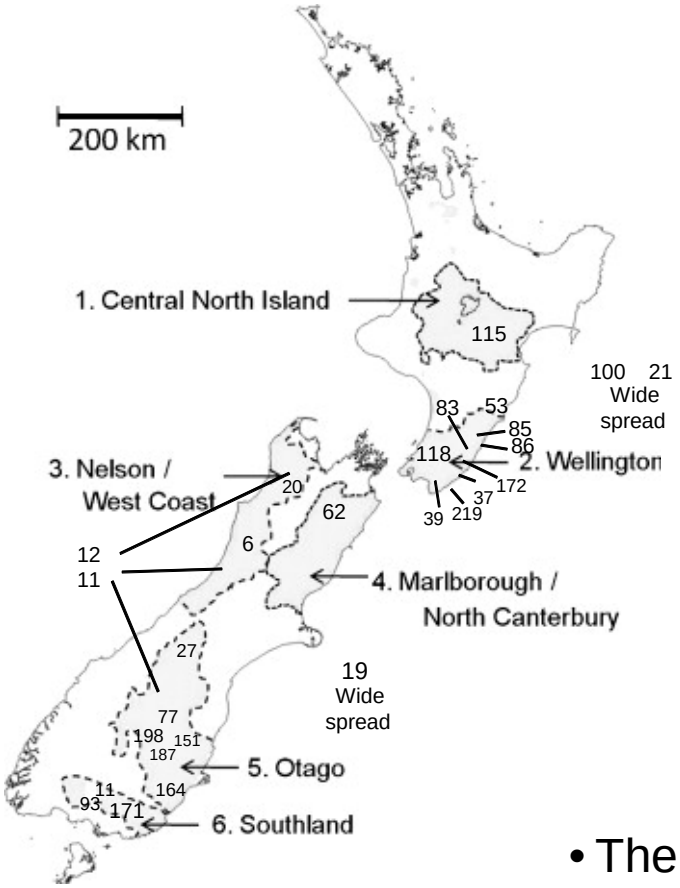
Des Collins

Paul Livingstone



NZ *M. bovis* Types

Distinguishing frequent *M. bovis* isolates aids in control of bovine TB



- DNA typing of *Mycobacterium bovis* has been used for over 30 years as a tool to study the epidemiology of bovine tuberculosis in New Zealand and as an aid for optimising wildlife control operations

- There are over 300 different types- 8 major families with variants and minor subgroups

- Many types are isolated only from certain regions of the country

- The same types are usually found in farmed animals and wildlife in the same area.

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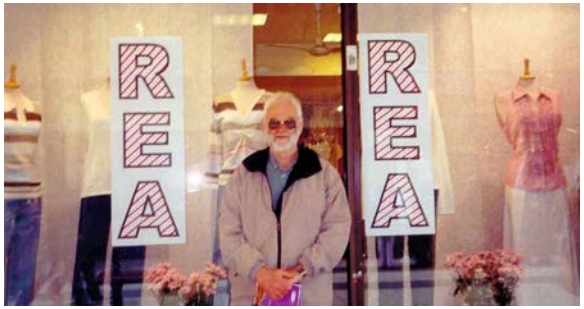
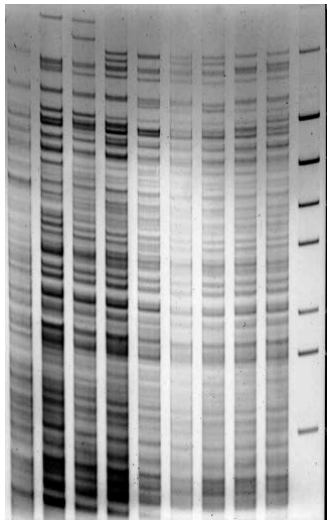
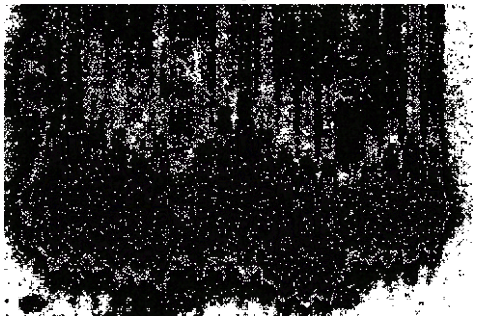
Evolution of NZ *M. bovis* Typing Methods

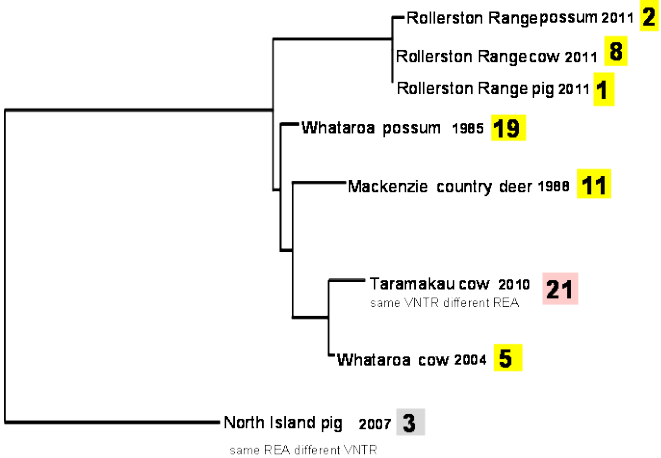




VNTR/DR



Whole Genome Sequencing



1985 1995 2005 2015 2020

Time

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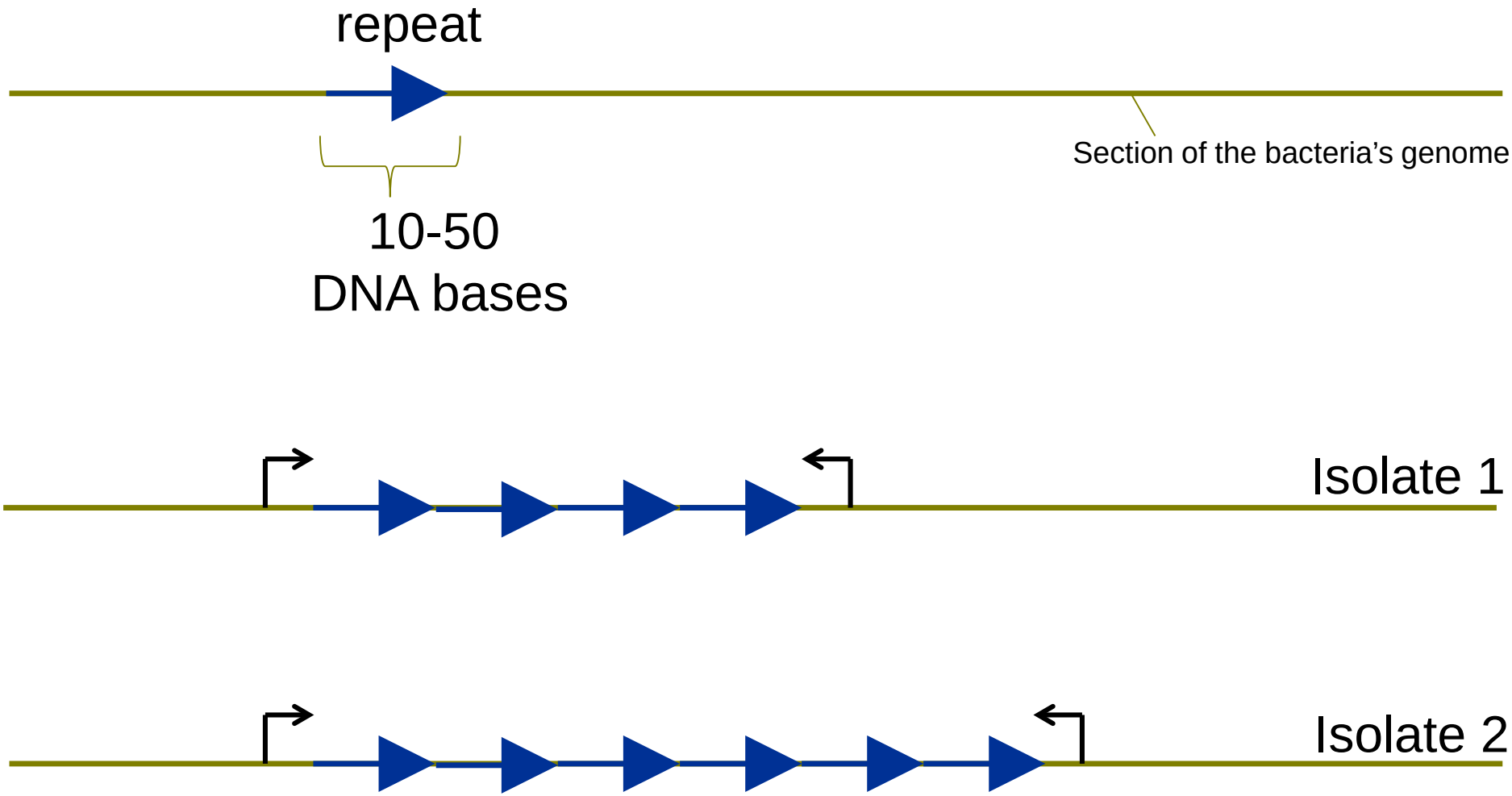
Variable Number Tandem Repeat Assay (VNTR)



PCR amplified and analysed by electrophoresis

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VNTR /DR TYPING OF NZ BOVINE TB ISOLATES

Comparison of 45 variable number tandem repeat (VNTR) and two direct repeat (DR) assays to restriction endonuclease analysis for typing isolates of *Mycobacterium bovis*

Veterinary Microbiology 150(2011) 107-114

Marian Price-Carter, Serena Rooker [1], Desmond M. Collins *

AgResearch, National Centre for Biosecurity and Infectious Disease, Wallaceville, P.O. Box 40063, Upper Hutt, New Zealand

VNTR region

TCGGCTAGCCGAGCAGGGCGTCGACGAATGCGGCGGGTTCGAAAGGCGCCAGGTCATCGGGGCCT
TCACCAAGCCCGACCAGCTTCACCGGCACCCCAAGTTCCTGTTGAACGCGGAACACAATGCCGCC
CTTGGCCGTTCCGTCCAGTTTGGTGAGCACCGCGCCGCTGATGTCGACGACCTCGGCGAACACTC
TGGCCTGCGCCAACCCGTTCTGTCCGATCGTGGCATCGAGCACCAGCAACACCTCGTCAACGGAC
GCTCGCCGAGTCACCACGCGCTTGACCTTGTCCAGCTCGTCCATCAGGCCAACCTTGGTGTGCAG
CCGCCCGGCTGTATCGATGAGCACGACGTCTGCGCCGGCGGCGATGCCCTTGTCGACGGCGTCGA
ACGCCACCGATGCCGGGTCGGCGCCTTCGGGCCCGCGAACCACCGCTGCGCCAACCCGCGCCGCC
CAGGTCTGTAGCTGATCGGCGGCGGCCGCACGGAAGGTGTCAGC

CCGTGGCGATCGCAAGCGCGGCGCAGCCGCGCGCAGCGGGTCGCCACCATCAGACC
CCGTGGCGATCGCAAGCGCGGCGCAGCCGCGCGCAGCGGGTCGCCACCATCAGACC
CCGTGGCGATCGCAAGCGCGGCGCAGCCGCGCGCAGCGGGTCGCCACCATCAAACC
CCGTGGCGATCGCAAGCGCGGCGCAGCCGCGCGCAGCGGGTCGCCACCATCAAACC
CCGTGGCGATCGCAAGCGCGGCGCAGCCGCGCGCAGCGGGTCGCCACCATCAAACC
CCGTGGCGATCGCAAGCGCGGCGCAGCCGCGCGCAGCGGGTCGCCACC

TCGGCTAGCCGAGCAGGGCGTCGACGAATGCGGCGGGTTCGAAAGGCGCCAGGTCATCGGGGCCT
TCACCAAGCCCGACCAGCTTCACCGGCACCCCAAGTTCCTGTTGAACGCGGAACACAATGCGCCC
TTGGGAGCTTCACCGGCACCCCAAGTTCCTGTTGAACGCGGAACACAATGCCGCCCTTGGAGGCT
TGACAGCTAGTCGAACTTCTCCTTGTCATGGGAACTCGAGGGCGGTGCCTACAGCGGAAGGATGC
TGGTGAGTTCAGCGCTCCTGCTGTTGATGACGGCGGGTCTAGTTCA

DR region (exploited in spoligotyping)

AGAGGGAATGGCAATGATGGTCGACGAA
GTTTCCGTCCCCTCTCGGGGTTTTGGGTCTGACGAC
TCAAAAACGGGGACGGCATGCTGCATGCCCTAACGTCGT
GTTTCCGTCCCCTCTCGGGGTTTTGGGTCTGACGAC
CAGCGCAGACGGCAGCCCCGAGTACTCGCTCTCCTCAG
GTTTCCGTCCCCTCTCGGGGTTTTGGGTCTGACGAC
CAGCGCAGACGGCAGCCCCGAGTACTCGCTCTCCTCAG
GTTTCCGTCCCCTCTCGGGGTTTTGGGTCTGACGAC
CTAAGCCCGCTAATCCCGCACAAGTGGTCAGAAAA
GTTTCCGTCCCCTCTCGGGGTTTTGGGTCTGACGAC
CTGATGATTGGTCGGCGTATGACGTGCTACTGAGGTGTT
GTTTCCGTCCCCTCTCGGGGTTTTGGGTCTGACGAC
ACCCCTGAGCCACGGCATGTGCACGGCTCCGTGTTCAA
GTTTCCGTCCCCTCTCGGGGTTTTGGGTCTGACGAC
TCGATGAATGATGCGCGCCAGCGGTAACGCGTGGGATGT
GTTTCCGTCCCCTCTCGGGGTTTTGGGTCTGACGAC
TCTGAAAATTCGTGGTCAACGAATTGTCGTCGAAAT
GTTTCCGTCCCCTCTCGGGGTTTTGGGTCTGACGAC
ACGGGCCGTGGGGCACTTACGGCGACGGCGCACAT
GTTTCCGTCCCCTCTCGGGGTTTTGGGTCTGACGAC
CCCCTAGCCACGCCTGCCGTGCCATGCCGCGCCGCGA
GTTTCCGTCCCCTCTCGGGGTTTTGGGTCTGACGAC
TTGGTCAAAAGCTGTCGCCCAAGCATGAGGCAAAA

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VNTR TEST REPORT

Batch Number **Report ID:** **Date Result sent:**

Sample number	Case No.	Animal ID	Host	Location	Area	DDCM	MIRU40	EtrD	EtrC	EtrE	NZ2	QUB19	QUB11a	QUB26	DR2	DR1	QUB3232
1	W12/0495	DM1006731	Bovine	Taumaranui	Taumaranui	J. Sinclair	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
2	W12/0534	DM1006757	Bovine	Middlemarch	Otago	B. Paterson	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
3	W12/0535	DM1006787	Bovine	Taipo	West Coast	M. Neil	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
4	W12/0536	DM1006772	Bovine	Karamea	West Coast	M. Neil	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
5	W12/0564	DM1006810	Bovine	Karamea	West Coast	M. Neil	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
6	W12/0574	DM1006828	Bovine	Taramakau	West Coast	M. Neil	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
7	W12/0576	DM1006832	Bovine	Otematata	Otago	B. Paterson	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Analysed by: **Authorised by:**

- This VNTR/DR method is now accredited and is currently used routinely for distinguishing NZ isolates
- Isolate type information is stored in a NZ *M. bovis* VNTR database (>800 entries) and compared to information in the NZ *M. bovis* REA database (>3,800 entries)

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- VNTR typing is often sufficient for sourcing herd breakdowns

- Because a particular DNA type is often found across a wide region or in some instances multiple regions, it can sometimes be difficult to determine the source of infection

- On these occasions a more discriminating typing method could improve the efficiency of control measures.

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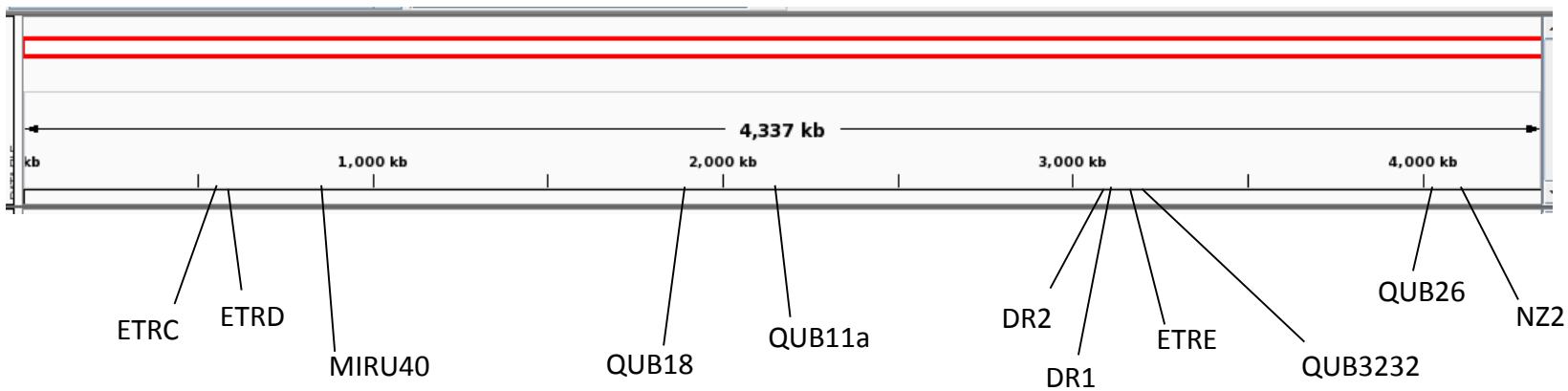
- The 11 repeated sequences used for distinguishing NZ *M. bovis* isolates are a tiny fraction of the bacteria's genome (< 0.2%)

Whole Genome Sequencing (WGS)



- More resolution can be achieved by comparing differences throughout the genome

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Mycobacterial relationships are well suited for analysis by WGS

- They evolve slowly
- They evolve by mutation- almost no horizontal gene transfer
- Most species do not harbour plasmids

WGS studies compare SNP content of isolates

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Data processing

- Python scripts for

  Data trimming – Dynamictrim software

  Mapping - Burrows Wheeler Aligner (BWA) software

  aligned to **Genebank *M. bovis* reference AF2122/97**

  Identification of SNPs and other variants-

  SAM Tools and Variant Call Format software

- Filtering erroneous data

  Iterative evaluation of filter parameters

Scripts developed by Rudiger Brauning

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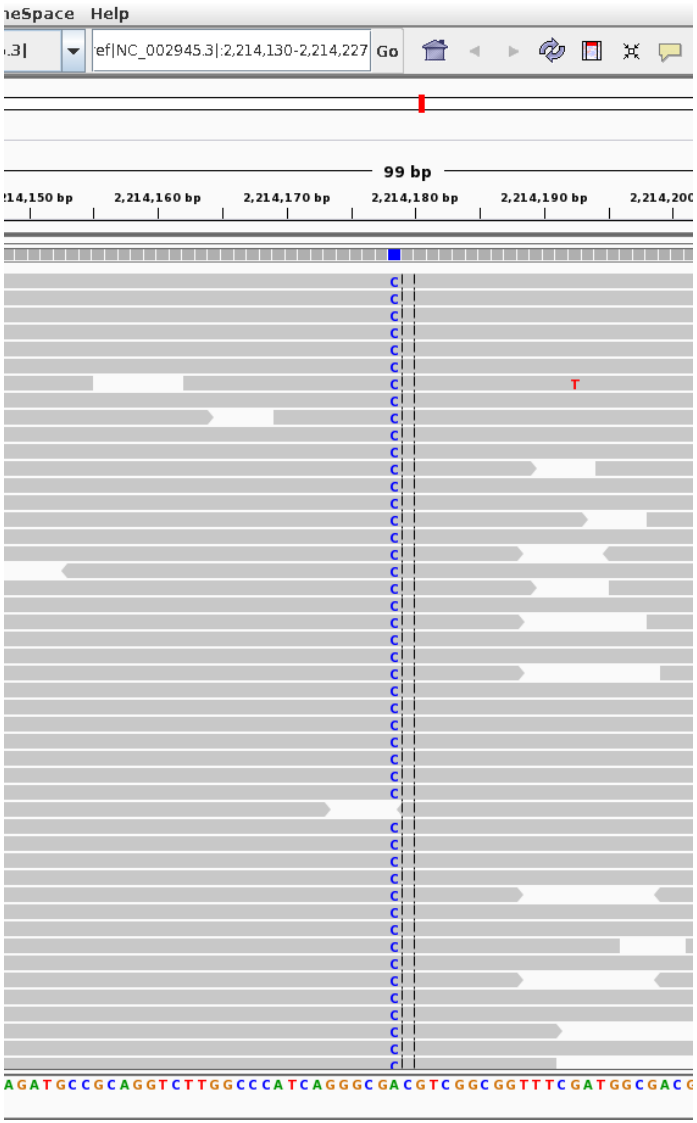
SNP Filtering

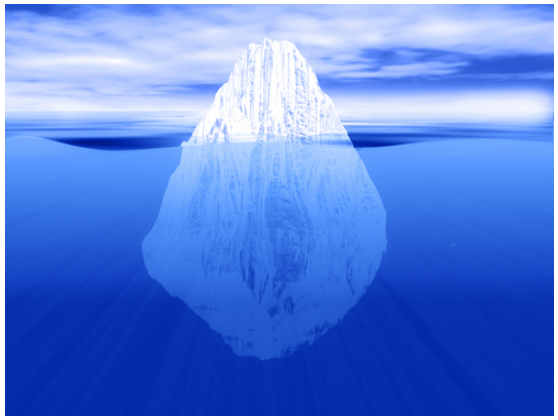


- Each detected SNP must be carefully characterised
- Erroneous SNPs disregarded

  Is it in a region where there is good quality data?

  Do all the reads have the base change?

  Are there lots of other SNPs nearby?



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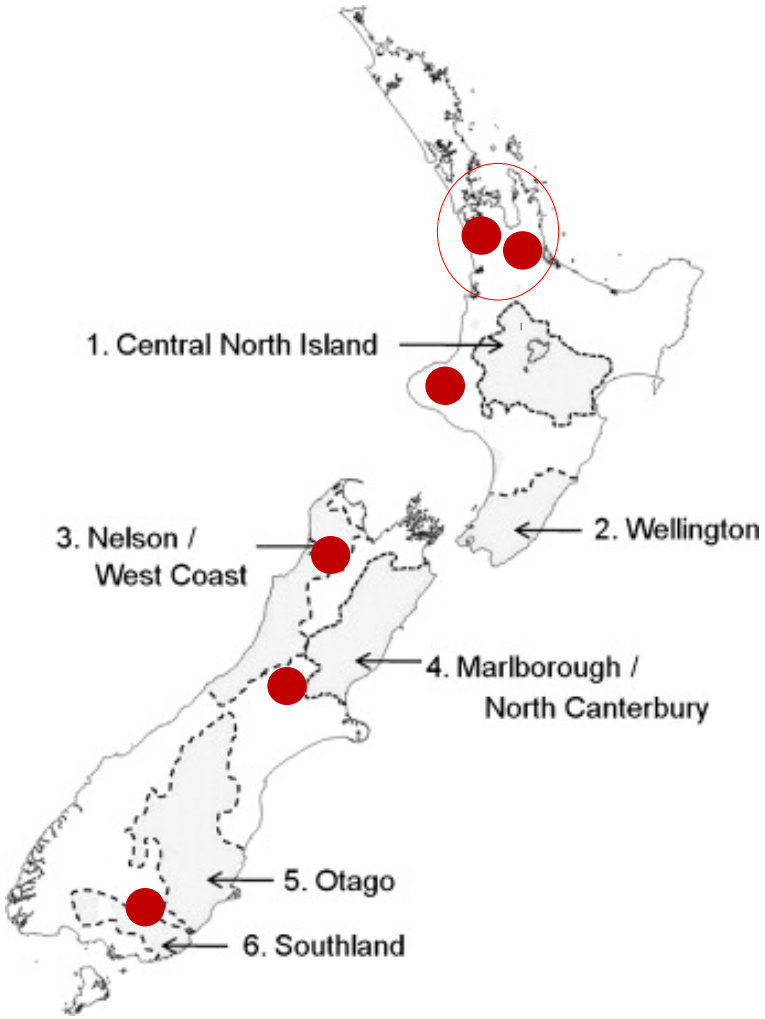
Out Breaks Investigated by WGS



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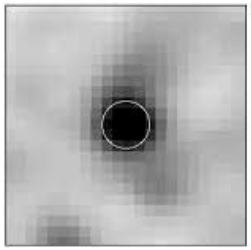
Waikato Outbreak Investigation

Very similar VNTR and REA type

Strain #	Source	Year	Host	REA # and Type		VNTR type
12	Patetonga	2011	cow	161	B2 A5/3 C1	2 4 4 3 5 4(10)3(12)5 7
16	Patetonga	2007	cow	161	B2 A5/3 C1	2 4 4 3 5 4(10)3(12)5 7
20	Patetonga	2004	cow	151	B2 A5 C1	2 4 4 3 5 4(10)3(12)5 7
15	Patetonga	1997	ferret	151	B2 A5 C1	2 4 2 3 5 4(10)4(12)5 9
13	Hatepe	2011	possum	115	B1 A5 C1	2 4 4 3 5 4(10)3(12)5 7
14	Te Aroha	1994	cow	115	B1 A5 C1	2 4 4 3 5 4(10)3(12)5 7



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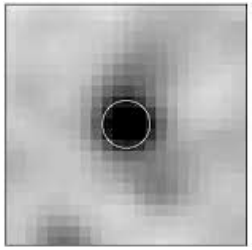
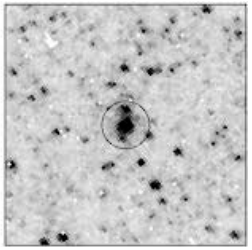
WGS = and adds resolution to VNTR REA

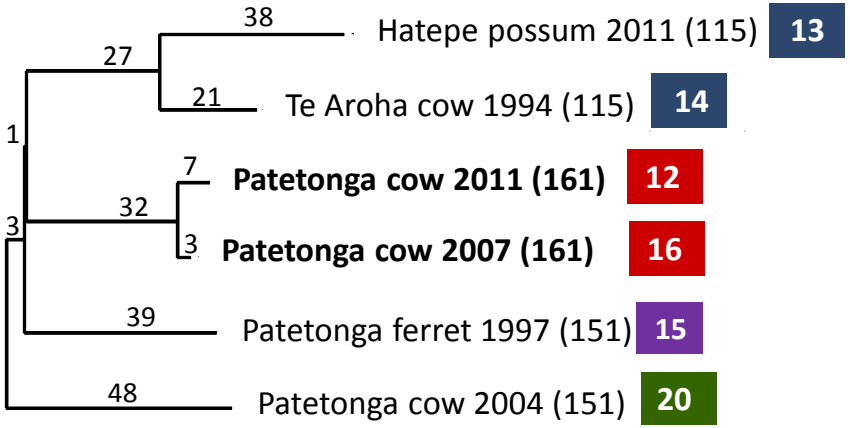
Waikato Outbreak Investigation

Very similar VNTR and REA type

Strain #	Source	Year	Host	REA # and Type	VNTR type
12	Patetonga	2011	cow	161 B2 A5/3 C1	2 4 4 3 5 4(10)3(12)5 7
16	Patetonga	2007	cow	161 B2 A5/3 C1	2 4 4 3 5 4(10)3(12)5 7
20	Patetonga	2004	cow	151 B2 A5 C1	2 4 4 3 5 4(10)3(12)5 7
15	Patetonga	1997	ferret	151 B2 A5 C1	2 4 2 3 5 4(10)4(12)5 9
13	Hatepe	2011	possum	115 B1 A5 C1	2 4 4 3 5 4(10)3(12)5 7
14	Te Aroha	1994	cow	115 B1 A5 C1	2 4 4 3 5 4(10)3(12)5 7







- WGS adds resolution

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Patetonga Farm

Waiuku Outbreak

Te Aroha

Hatepe

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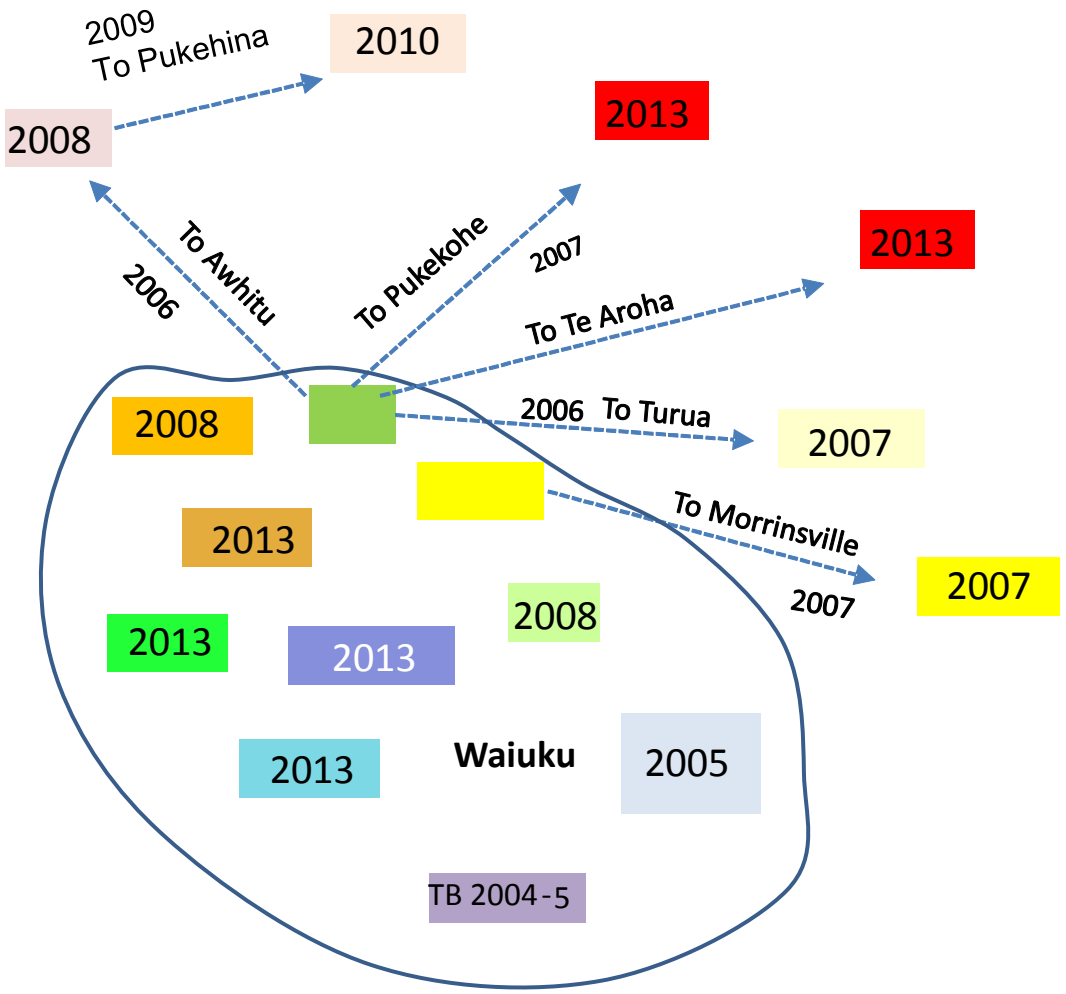
Waiuku Outbreak



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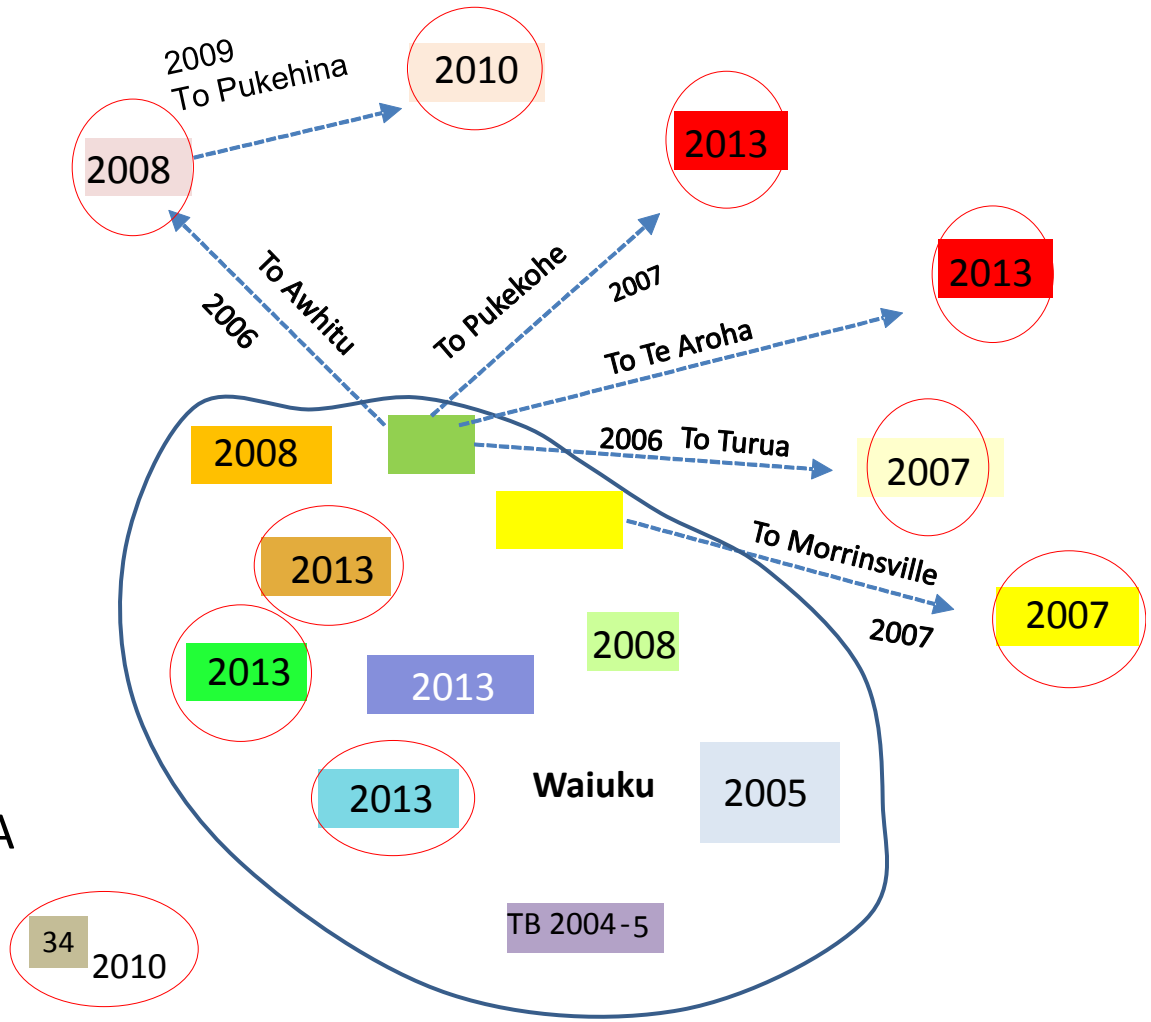
Waiuku Outbreak

Isolates for WGS analysis

- Sourced from Waiuku stock
- Sourced from stock moved from farms in Waiuku
- Sourced from elsewhere in the Central North Island
- All identical by VNTR and REA



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Aria

These North Island isolates are very similar when compared with VNTR

Sample ID	Year	Host	Location	REA TYPE	M40	ETRD	ETRC	ETRE	NZ2	Q18	Q11a	Q26	DR2	DR1	Q3232
54	2007	cow	Turua	115	2	4	4	3	5	4	10	3	12	5	7
24	2008	cow	Waiuku	115	2	4	4	3	5	4	10	3	12	5	7
33	2010	cow	Awhitu	115	2	4	4	3	5	4	10	3	12	5	7
40	2007	cow	Morrinsville	115	2	4	4	3	5	4	10	3	12	5	7
46	2013	cow	Waiuku		2	4	4	3	5	4	10	3	12	5	7
8	2013	cow	Waiuku		2	4	4	3	5	4	10	3	12	5	7
48	2013	cow	Waiuku		2	4	4	3	5	4	10	3	12	5	7
35	2010	cow	Pukehina	115	2	4	4	3	5	4	10	3	12	5	7
18	2013	cow	Te Aroha		2	4	4	3	5	4	10	3	12	5	7
1	2013	cow	Te Aroha		2	4	4	3	5	4	10	3	12	5	7
43	2013	cow	Te Aroha		2	4	4	3	5	4	10	3	12	5	7
25	2013	cow	Pukekohe		2	4	4	3	5	4	10	3	12	5	7
34	2010		Aria	115	2	4	4	3	5	4	10	3	12	5	7
13	2011	possum	Hatepe	115	2	4	4	3	5	4	10	3	12	5	7
14	1994	cow	Te Aroha	115	2	4	4	3	5	4	10	3	12	5	7
12	2011	cow	Patetonga	161	2	4	4	3	5	4	10	3	12	5	7
16	2007	cow	Patetonga	161	2	4	4	3	5	4	10	3	12	5	7
20	2004	cow	Patetonga	151	2	4	4	3	5	4	10	3	12	5	7
15	1997	ferret	Patetonga	151	2	4	2	3	5	4	10	4	12	5	9

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The genomes of these isolates are >95 % identical

Sample ID	chrom1-683059	chrom1-740995	chrom1-831079	chrom1-903385	chrom1-973194	chrom1-988040	chrom1-2475044	chrom1-740910	chrom1-221839	chrom1-221927	chrom1-2702921	chrom1-714709	chrom1-3715671
UK reference	C	C	G	C	T	A	T	C	T	G	T	G	G
54	C	C	G	C	T	A	T	C	T	G	T	G	G
24	C	C	G	C	T	A	T	C	T	G	T	G	G
33	C	C	G	C	T	A	T	C	T	G	T	G	G
40	C	C	G	C	T	A	T	C	T	G	T	G	G
46	C	C	G	C	T	A	T	C	T	G	T	G	G
8	C	C	G	C	T	A	T	C	T	G	T	G	G
48	C	C	G	C	T	A	T	C	T	G	T	G	G
35	C	C	G	C	T	A	T	C	T	G	T	G	G
18	C	C	G	C	T	A	T	C	T	G	T	G	G
1	C	C	G	C	T	A	T	C	T	G	T	G	G
43	C	C	G	C	T	A	T	C	T	G	T	G	G
25	C	C	G	C	T	A	T	C	T	G	T	G	G
34	C	C	G	C	T	A	T	C	T	G	T	G	G
13	C	C	G	C	T	A	T	C	T	G	T	G	G
14	C	C	G	C	T	A	T	C	T	G	T	G	G
12	C	C	G	C	T	A	T	C	T	G	T	G	G
16	C	C	G	C	T	A	T	C	T	G	T	G	G
20	C	C	G	C	T	A	T	C	T	G	T	G	G
15	C	C	G	C	T	A	T	C	T	G	T	G	G

Single Nucleotide Polymorphisms (SNPs) detected by WGS

Sample ID	chrom1-683059	chrom1-740995	chrom1-831079	chrom1-903385	chrom1-973194	chrom1-988040	chrom1-2475044	chrom1-740910	chrom1-221839	chrom1-221927	chrom1-2702921	chrom1-714709	chrom1-3715671	chrom1-1202528	chrom1-1230453	chrom1-1239379	chrom1-1337278	chrom1-1526730	chrom1-1676888	chrom1-1944285	chrom1-1944286	chrom1-2140627	chrom1-2544626
UK reference	C	C	G	C	T	A	T	C	T	G	T	G	G	C	C	G	G	C	A	C	G	C	G
54	C	C	G	C	T	A	T	C	T	G	T	G	G	T	T	A	A	T	G	T	T	G	A
24	C	C	G	C	T	A	T	C	T	G	T	G	G	T	T	A	A	T	G	T	T	G	A
33	C	C	G	C	T	A	T	C	T	G	T	G	G	T	T	A	A	T	G	T	T	G	A
40	C	C	G	C	T	A	T	C	T	G	T	G	G	T	T	A	A	T	G	T	T	G	A
46	C	C	G	C	T	A	T	C	T	G	T	G	G	T	T	A	A	T	G	T	T	G	A
8	C	C	G	C	T	A	T	C	T	G	T	G	G	T	T	A	A	T	G	T	T	G	A
48	C	C	G	C	T	A	T	C	T	G	T	G	G	T	T	A	A	T	G	T	T	G	A
35	C	C	G	C	T	A	T	C	T	G	T	G	G	T	T	A	A	T	G	T	T	G	A
18	C	C	G	C	T	A	T	C	T	G	T	G	G	T	T	A	A	T	G	T	T	G	A
1	C	C	G	C	T	A	T	C	T	G	T	G	G	T	T	A	A	T	G	T	T	G	A
43	C	C	G	C	T	A	T	C	T	G	T	G	G	T	T	A	A	T	G	T	T	G	A
25	C	C	G	C	T	A	T	C	T	G	T	G	G	T	T	A	A	T	G	T	T	G	A
34	C	C	G	C	T	A	T	C	T	G	T	G	G	T	T	A	A	T	G	T	T	G	A
13	C	C	G	C	T	A	T	C	T	G	T	G	G	T	T	A	A	T	G	T	T	G	A
14	C	C	G	C	T	A	T	C	T	G	T	G	G	T	T	A	A	T	G	T	T	G	A
12	C	C	G	C	T	A	T	C	T	G	T	G	G	T	T	A	A	T	G	T	T	G	A
16	C	C	G	C	T	A	T	C	T	G	T	G	G	T	T	A	A	T	G	T	T	G	A
20	C	C	G	C	T	A	T	C	T	G	T	G	G	T	T	A	A	T	G	T	T	G	A
15	C	C	G	C	T	A	T	C	T	G	T	G	G	T	T	A	A	T	G	T	T	G	A

Single Nucleotide Polymorphisms (SNPs) detected by WGS

	chrom1-3336322	chrom1-3336343	chrom1-3336347	chrom1-3397871	chrom1-3524771	chrom1-3739257	chrom1-3950678	chrom1-4064911	chrom1-40811	chrom1-4162759	chrom1-439435	chrom1-4507	chrom1-530751	chrom1-801214	chrom1-883831	chrom1-514887	chrom1-2344880	chrom1-728179	chrom1-3243762	chrom1-2370286	chrom1-1317973	chrom1-1482939	chrom1-243654	chrom1-2535413	chrom1-3220499	chrom1-567195	chrom1-1134975	chrom1-1339804	chrom1-1654609	chrom1-1903214	chrom1-3860411
UK reference	A	A	G	G	T	T	G	C	C	T	A	T	G	G	C	T	T	T	G	C	A	C	G	G	C	A	C	G	G	T	C
54	G	C	C	C	A	C	A	T	A	C	G	C	A	C	T	T	T	T	G	C	A	C	G	G	C	A	C	G	G	T	C
24	G	C	C	C	A	C	A	T	A	C	G	C	A	C	T	T	T	T	G	C	A	C	G	G	C	A	C	G	G	T	C
18	G	C	C	C	A	C	A	T	A	C	G	C	A	C	T	T	T	T	G	C	A	C	G	G	C	A	C	G	G	T	C
33	G	C	C	C	A	C	A	T	A	C	G	C	A	C	T	C	T	T	G	C	A	C	G	G	C	A	C	G	G	T	C
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46	G	C	C	C	A	C	A	T	A	C	G	C	A	C	T	T	T	T	T	C	A	C	G	G	C	A	C	G	G	T	C
8	G	C	C	C	A	C	A	T	A	C	G	C	A	C	T	T	T	T	T	C	A	C	G	G	C	A	C	G	G	T	C
48	G	C	C	C	A	C	A	T	A	C	G	C	A	C	T	T	T	T	T	C	A	C	G	G	C	A	C	G	G	T	C
35	G	C	C	C	A	C	A	T	A	C	G	C	A	C	T	T	T	T	G	G	A	C	G	G	C	A	C	G	G	T	C
1	G	C	C	C	A	C	A	T	A	C	G	C	A	C	T	T	T	T	G	C	G	C	G	G	C	A	C	G	G	T	C
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25	G	C	C	C	A	C	A	T	A	C	G	C	A	C	T	T	T	T	G	C	A	C	G	G	C	A	T	A	A	C	A
34	A	A	G	G	T	T	G	C	C	T	A	T	G	G	C	T	T	T	G	C	A	C	G	G	C	A	C	G	G	T	C
13	A	A	G	G	T	T	G	C	C	T	A	T	G	G	C	T	T	T	G	C	A	C	G	G	C	A	C	G	G	T	C
14	A	A	G	G	T	T	G	C	C	T	A	T	G	G	C	T	T	T	G	C	A	C	G	G	C	A	C	G	G	T	C
12	A	A	G	G	T	T	G	C	C	T	A	T	G	G	C	T	T	T	G	C	A	C	G	G	C	A	C	G	G	T	C
16	A	A	G	G	T	T	G	C	C	T	A	T	G	G	C	T	T	T	G	C	A	C	G	G	C	A	C	G	G	T	C
20	A	A	G	G	T	T	G	C	C	T	A	T	G	G	C	T	T	T	G	C	A	C	G	G	C	A	C	G	G	T	C
15	A	A	G	G	T	T	G	C	C	T	A	T	G	G	C	T	T	T	G	C	A	C	G	G	C	A	C	G	G	T	C

Waiuku isolates

other CNI

Patetonga isolates

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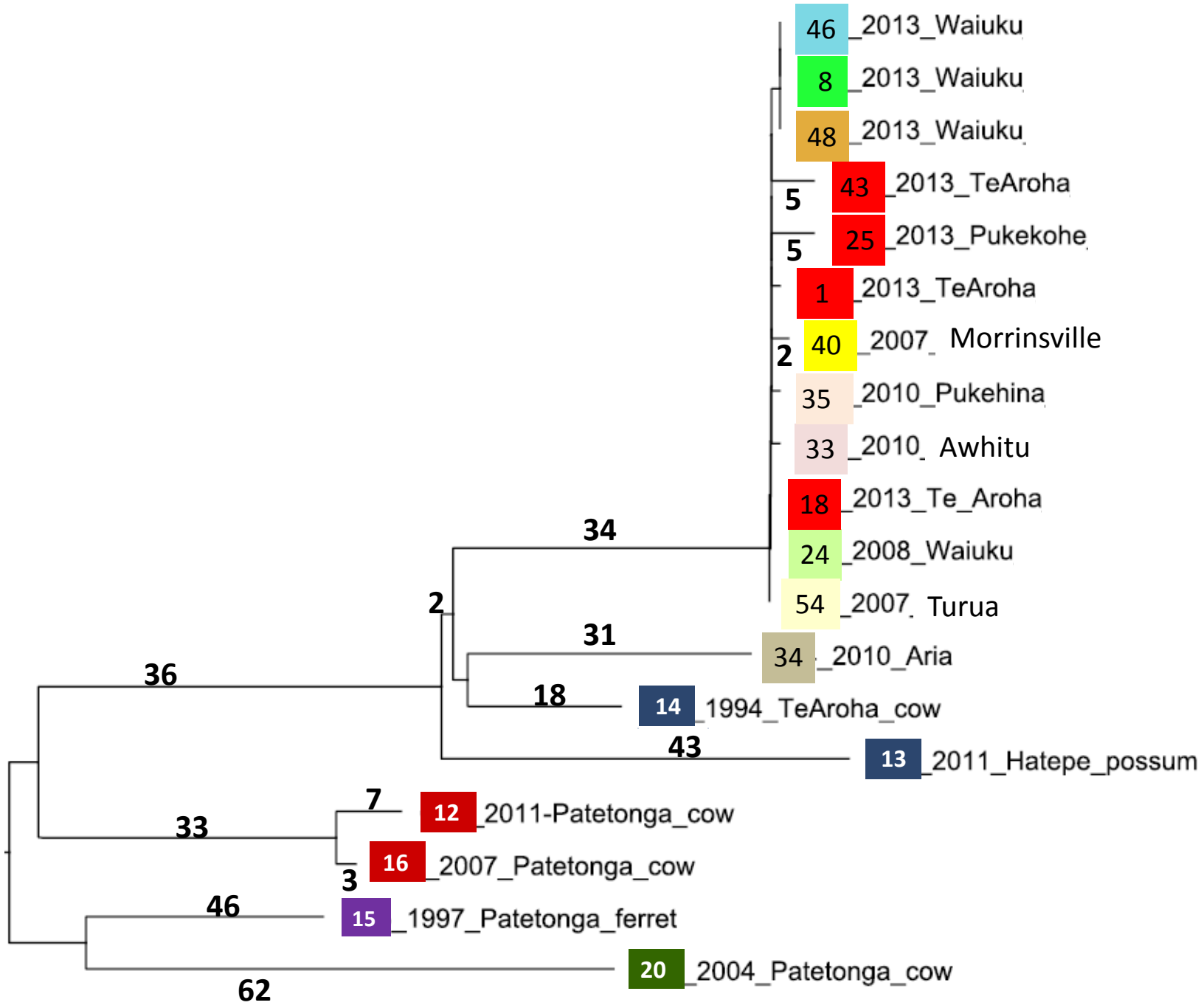
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35 _2010_Pukehina
33 _2010_ Awhitu
18 _2013_Te_Aroha
24 _2008_Waiuku
54 _2007_ Turua
34 _2010_Aria
14 _1994_TeAroha_cow
13 _2011_Hatepe_possum
12 _2011-Patetonga_cow
16 _2007_Patetonga_cow
15 _1997_Patetonga_ferret
20 _2004_Patetonga_cow
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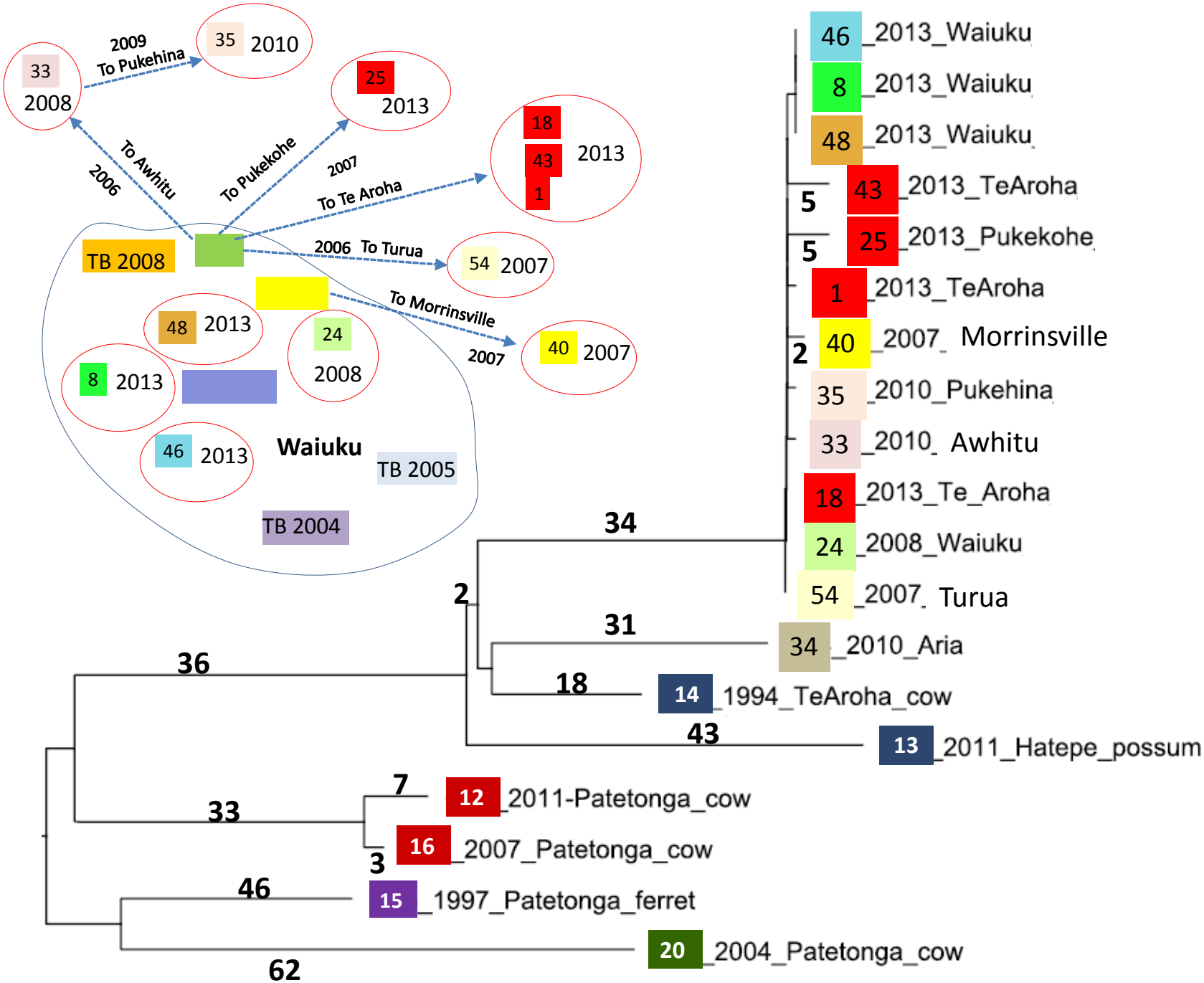
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To Te Aroha
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To Awhitu
TB 2008
2006 To Turua
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To Morrinsville
2007
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Waiuku
TB 2005
TB 2004
46 _2013_Waiuku
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13 _2011_Hatepe_possum
12 _2011-Patetonga_cow
16 _2007_Patetonga_cow
15 _1997_Patetonga_ferret
20 _2004_Patetonga_cow
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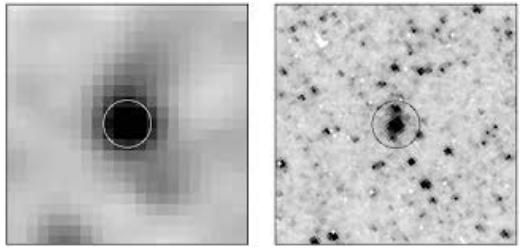
Conclusions



- WGS data has already added clarity to unresolved situations involving NZ *M. bovis* infections

- The more precise sourcing of *M. bovis* outbreaks will enable more directed and efficient control

- Much needs to be worked out before it can used as a routine typing method

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Best results with this technique will require effective collaboration between lab scientists and epidemiologists

Development of NZ *M. bovis* WGS typing system
Paul Livingstone and DCMs at TB Free NZ

Mutation rate
Molecular Clock estimates for CNI and Marlborough / NC bovine TB cycles
Josephine Bryant, Simon Harris, James Wood & Julian Parkhill

Molecular Clock estimates for West Coast bovine TB cycles
Rowland Kao and Thom Doherty

Variation in a population
Dan Thompkins, Carlos Rouco, Bryce Buddle and Nigel French
shedders Vs spreaders project

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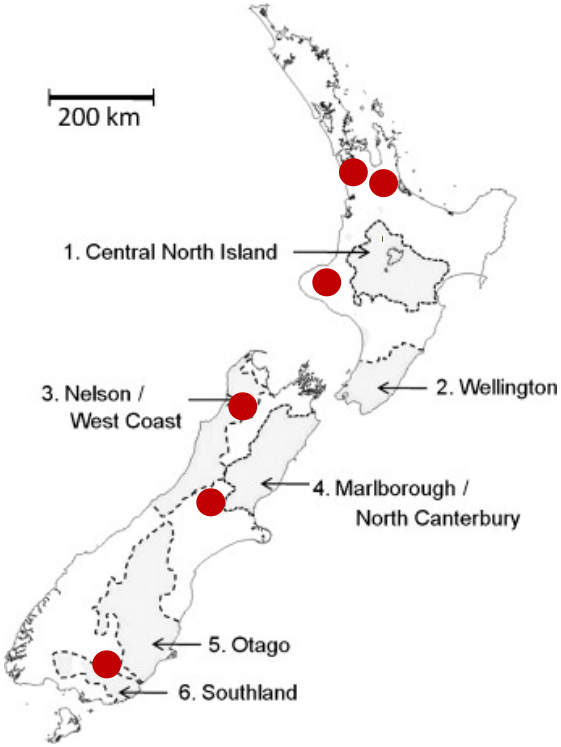
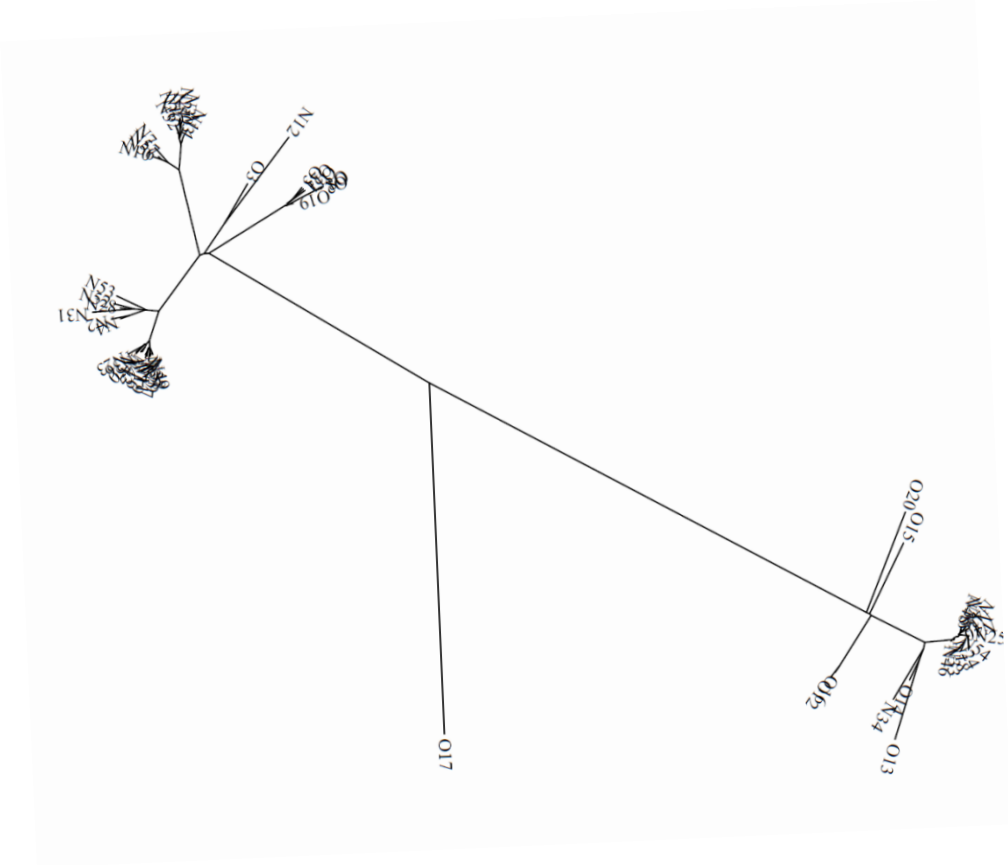
Current Studies:

- Relatedness of different families of *M. bovis*
  - Define SNP lineages of important families
  - Determine significance of changes in REA/VNTR
  - Deletions
  - Optimal filtering

(so far 60 genomes from recent breakdowns NZGL)





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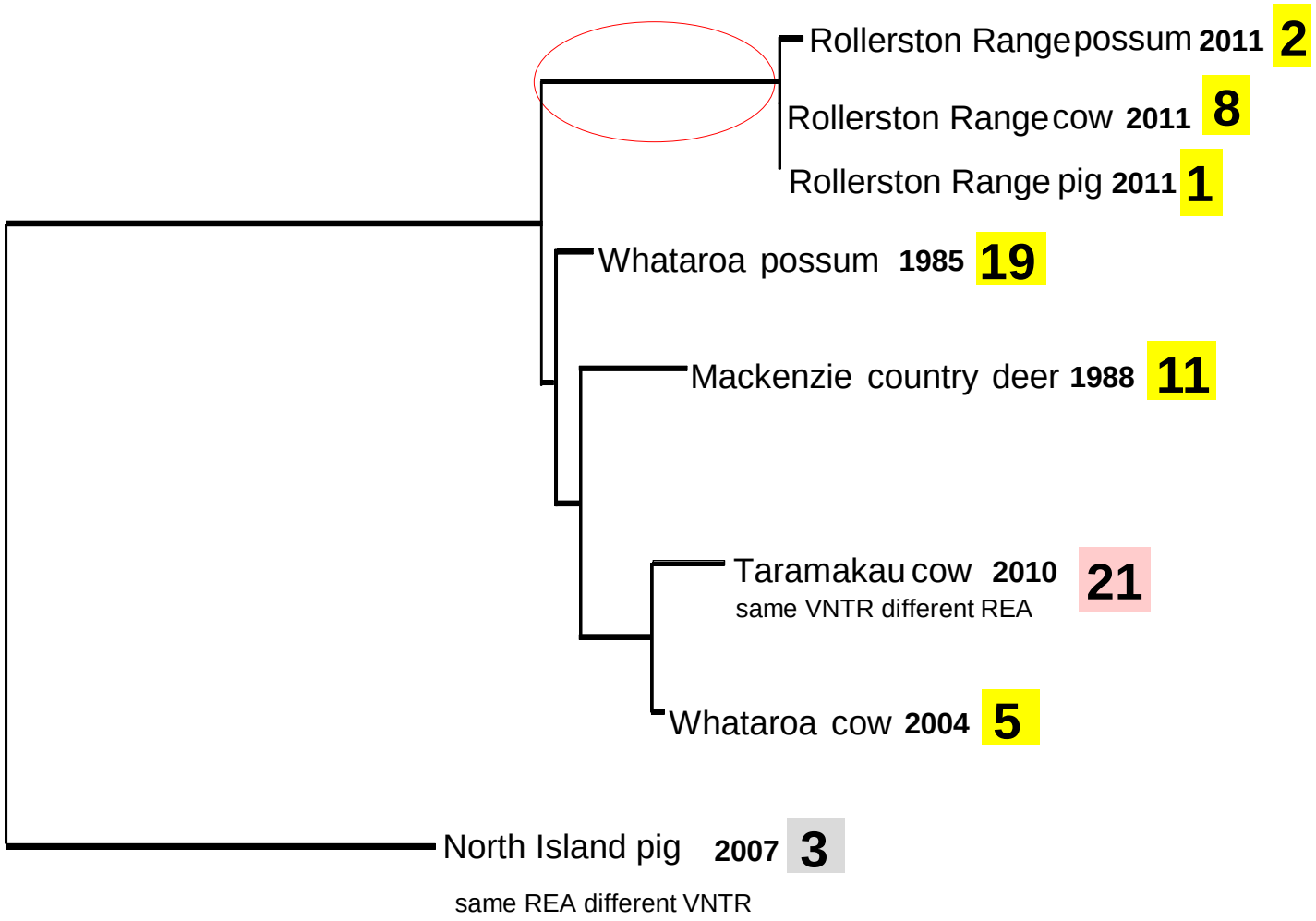
Molecular clock- How LOOOONG????



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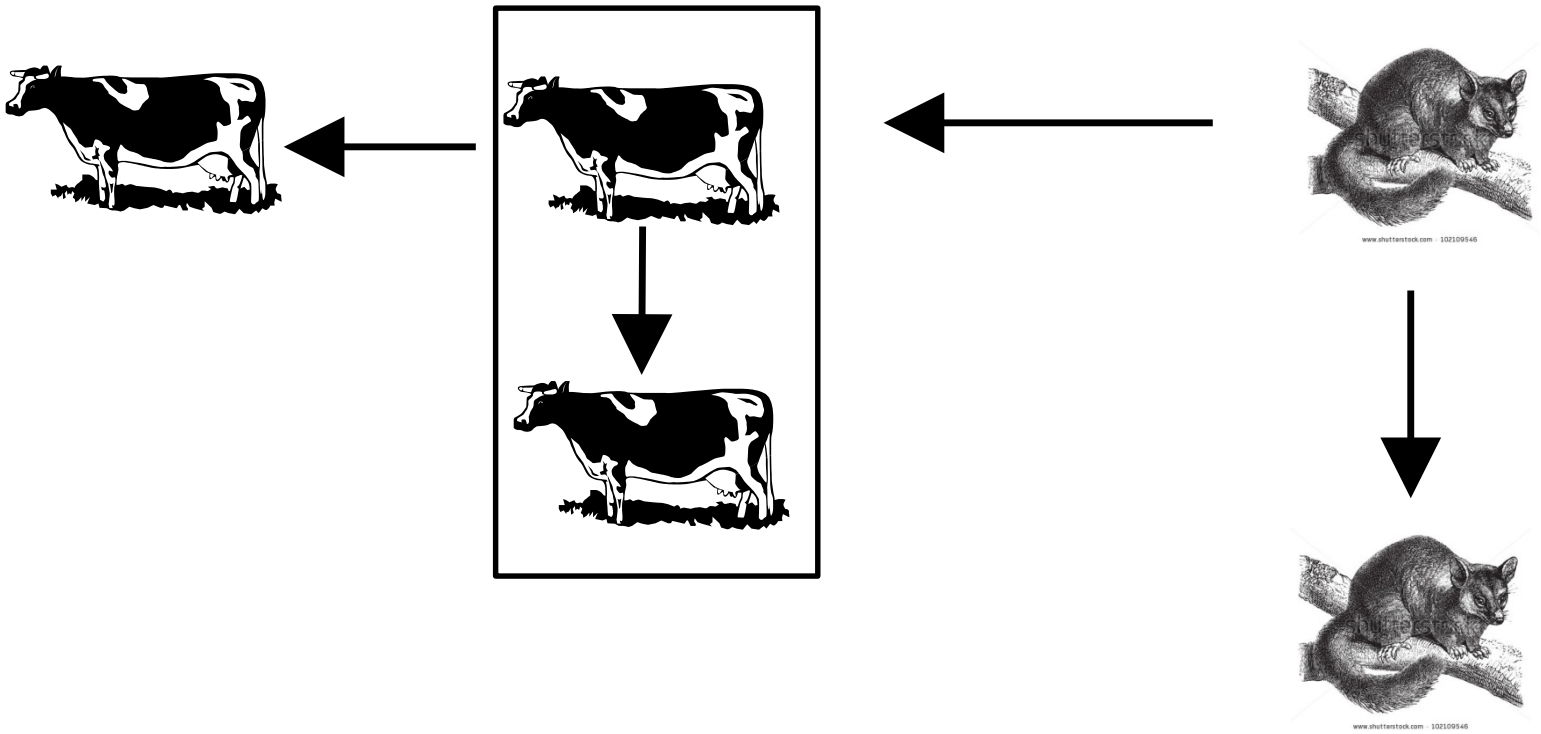
Molecular clock- How LOOOONG????

Vector risk / Vector free?

Latent Vs active?

Doubling time?



Time to most recent common ancestor – mutation rate

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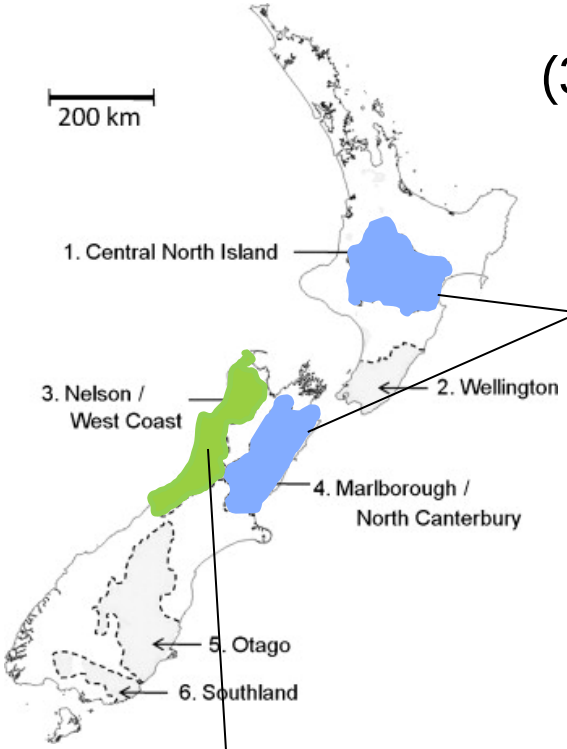
Current Studies:

- Estimation of NZ bovine TB cycle molecular clocks

mutation rate of different types

mutation rate in different regions
(312 genomes Welcome Trust Sanger and Glasgow)



Welcome Trust- Sanger-
Josephine Bryant, Simon Harris, Julian Parkhill & James Wood
(218 genomes)

Welcome Trust- Glasgow-
Rowland Kao & Thom Doherty (94 genomes)

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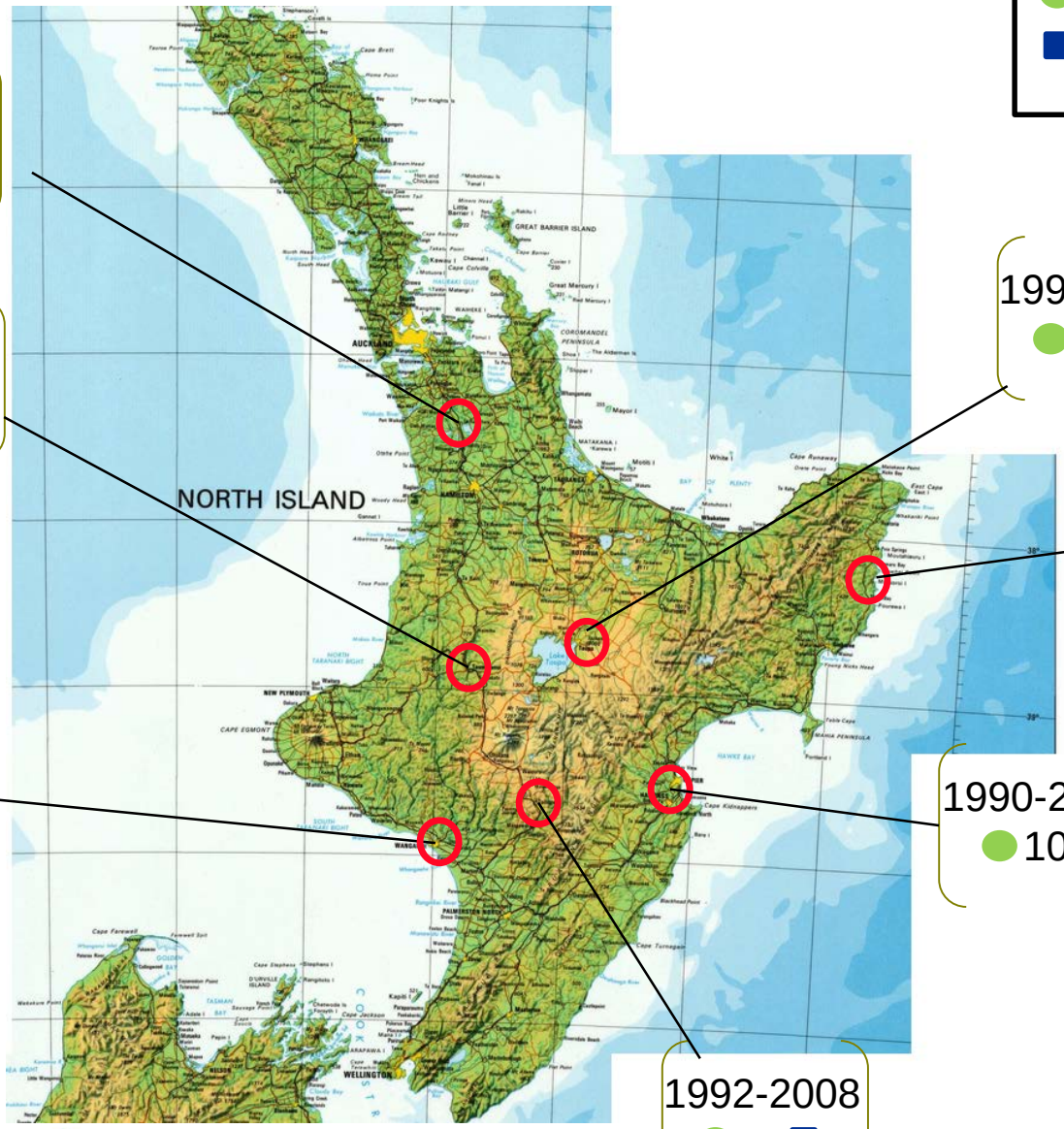
Current Studies mutation rate estimations:

Livestock
Wildlife



1996-2007
4

1983-1999
4 6

1994-2001
5 4

1994-1996
3

1988-2002
6 1

1990-2008
10 2

1992-2008
8 1

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Acknowledgement

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Des Collins
Geoff de Lisle

Isolate Culture
Gary Yates
Maree Joyce

Bioinformatics
Rudiger Brauning

IDRec Organisers

You

TB Free NZ (formerly the AHB)

Funding
Advice on *M. bovis* epidemiology
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Brent Patterson (Case manager)
Jane Sinclair (ADM)
Mark Neil (ADM)
Gillian Atkinson(ADM)

New Zealand Genomics Ltd.

Genome Sequencing
Jenny Schackelford
Patrick Biggs
Lorraine Berry

Data filtering and Phylogeny

University of Glasgow

Rowland Kao
Roman Biek
Hannah Trewby

USDA

SueLee Robbe-Austerman
Todd Stuber

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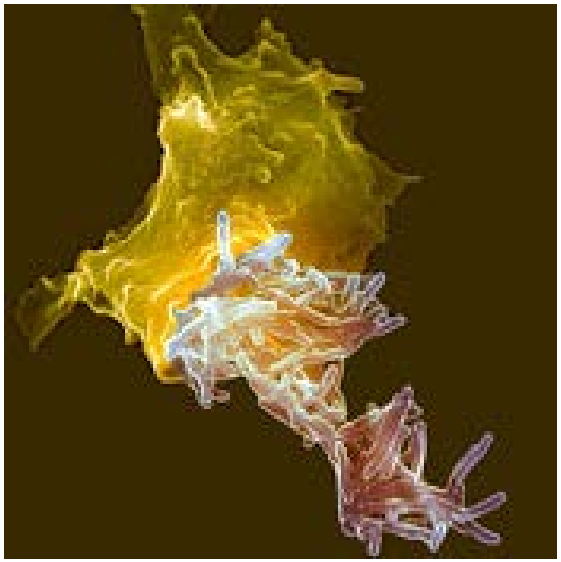

Questions?

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