Landcare Research
Manaaki Whenua

ministry of science + innovation
TE PŪNAHA HIHONGA WHAIAHEA

Applying pathogen discovery techniques to wildlife disease issues in New Zealand

Dan Tompkins (LCR)

Dan White (LCR)

Richard Hall (ESR)

Richard Jakob-Hoff (NZCCM)

Kate McInnes (DOC)

ESR

NZCCM
NEW ZEALAND CENTRE FOR CONSERVATION MEDICINE
TE RŌPU TAIAO RONGOA O AOTEAROA

Department of Conservation
Te Papa Atawhai

Acknowledgements

Jing Wang (ESR)

Phil Lyver (LCR)

Keven Drew (LCR)

David Pulford (MPI)

Ron Moorhouse (DOC)

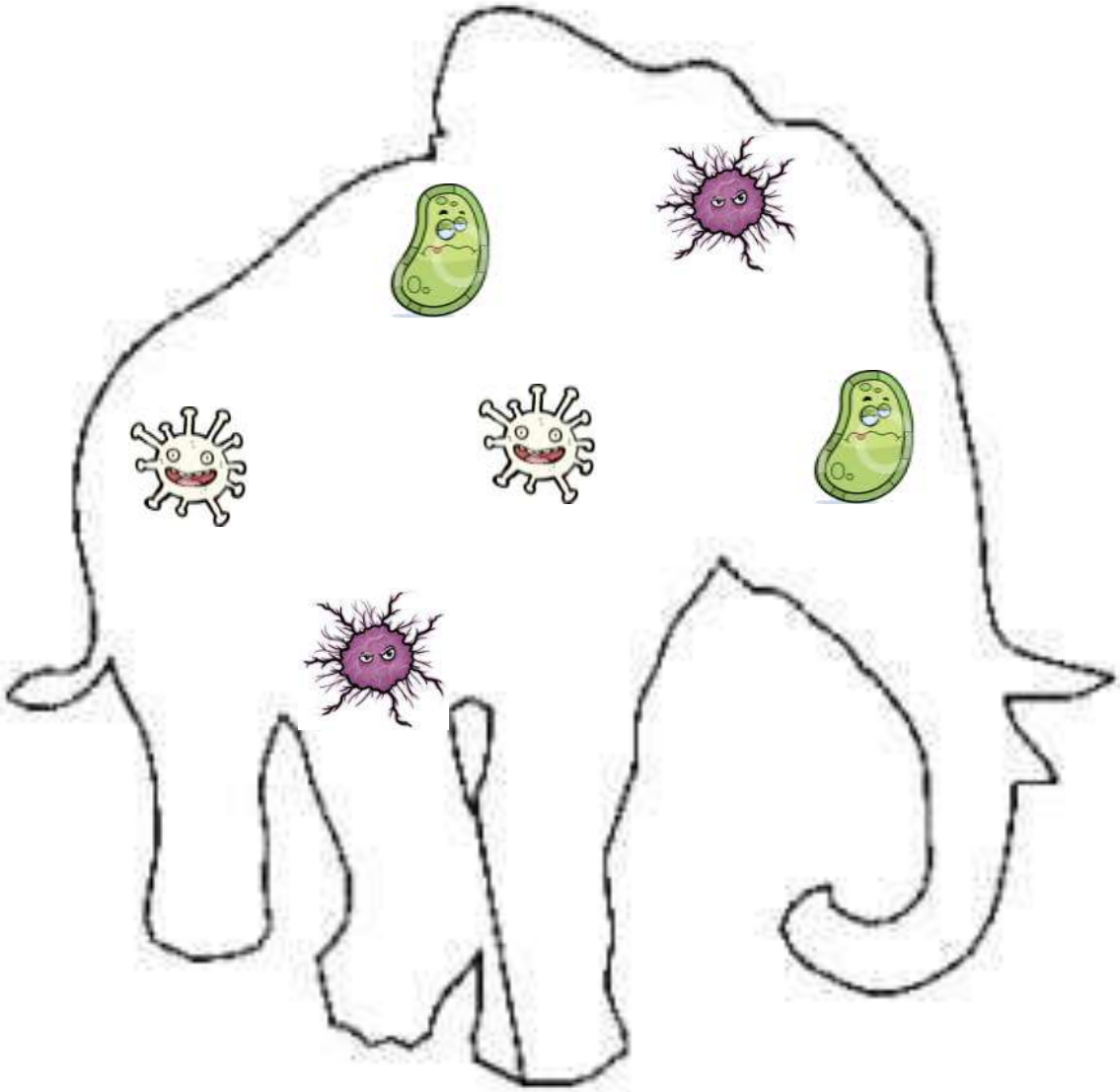
Catriona MacLeod (LCR)

Bethany Jackson (NZCCM)

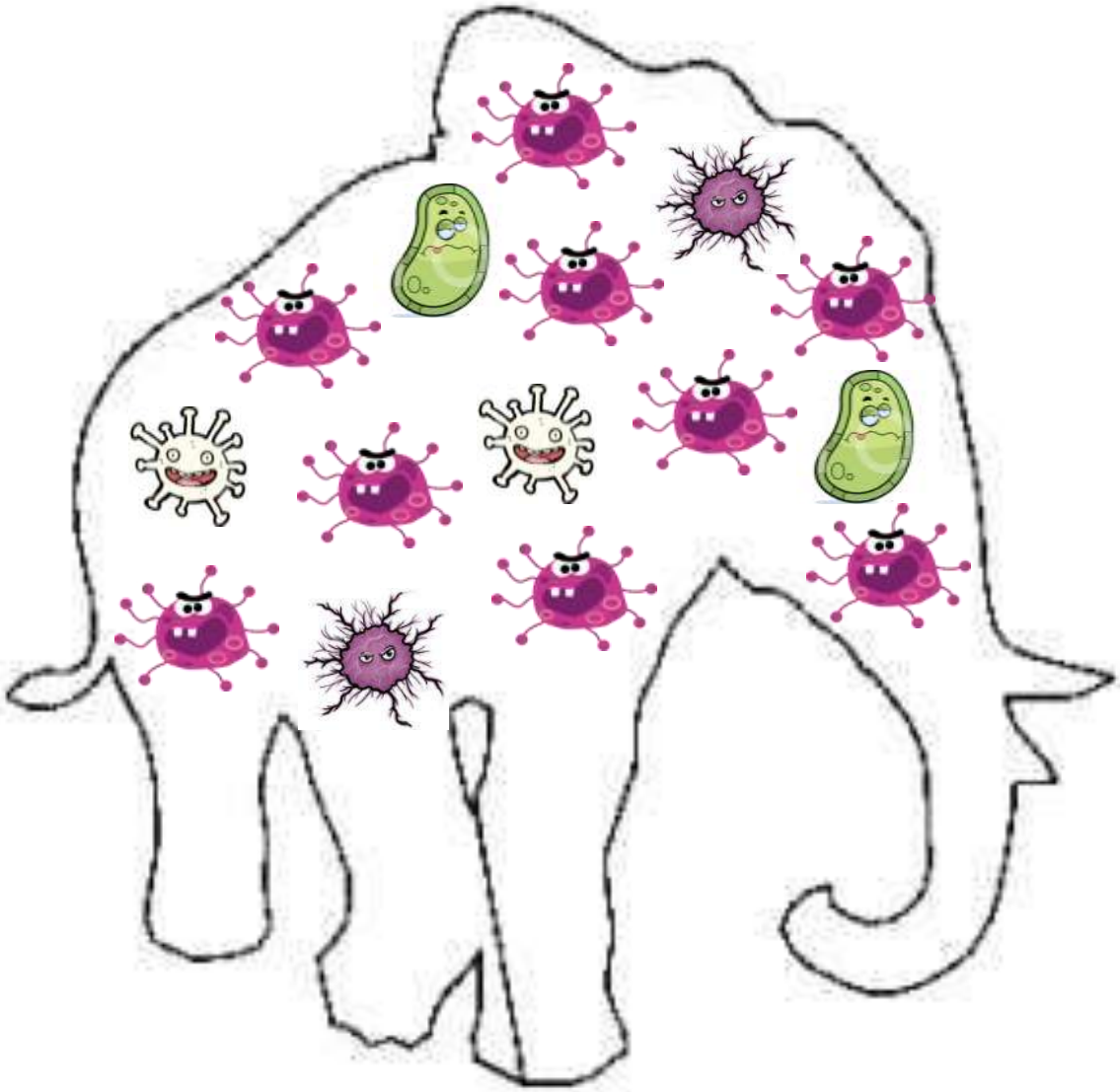
Wray Grimaldi (University of Otago)

Amy Whitehead (University of Melbourne)

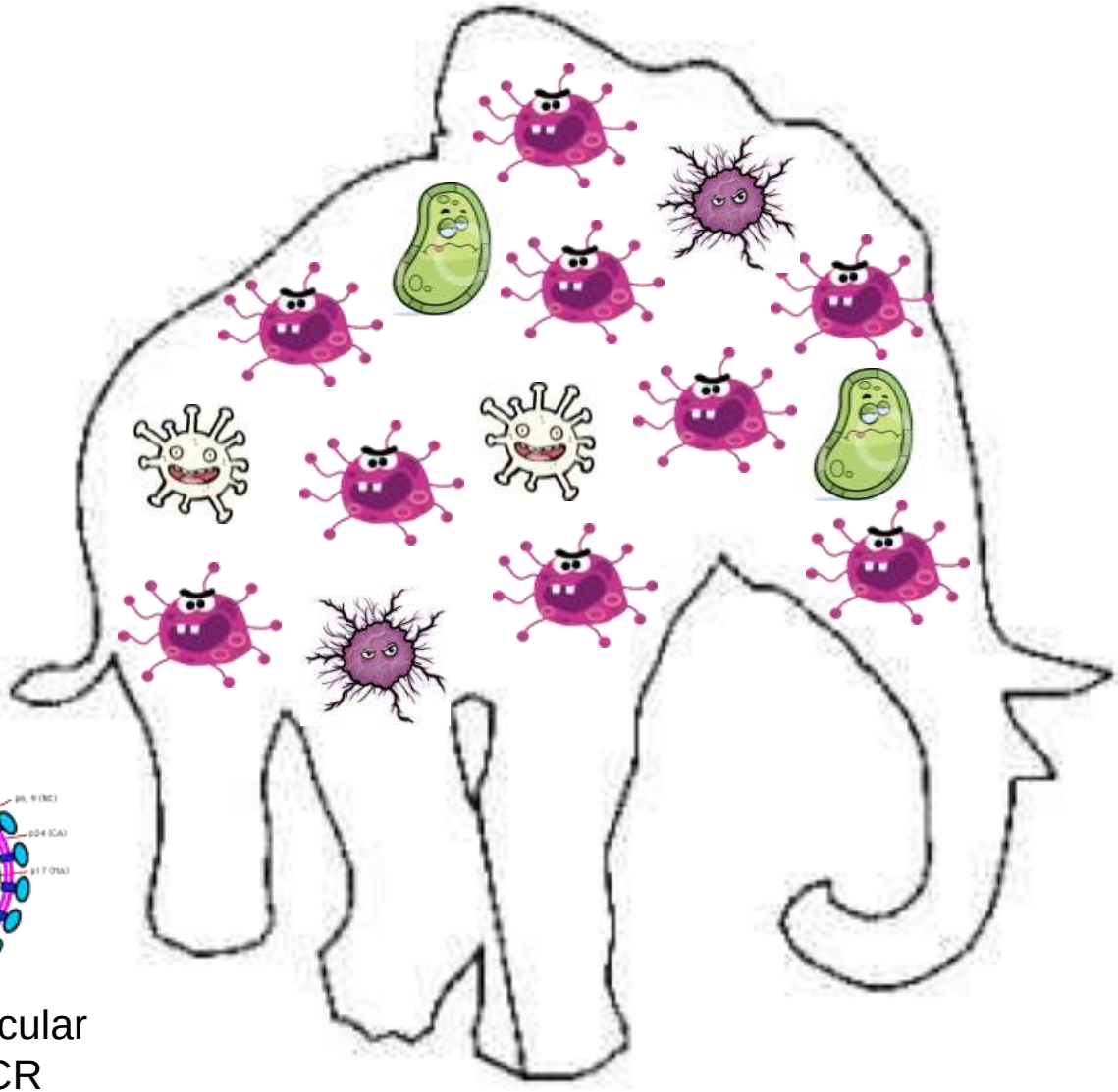
Pathogen discovery

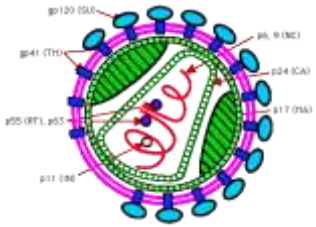


Pathogen discovery



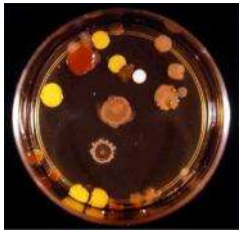
Pathogen discovery



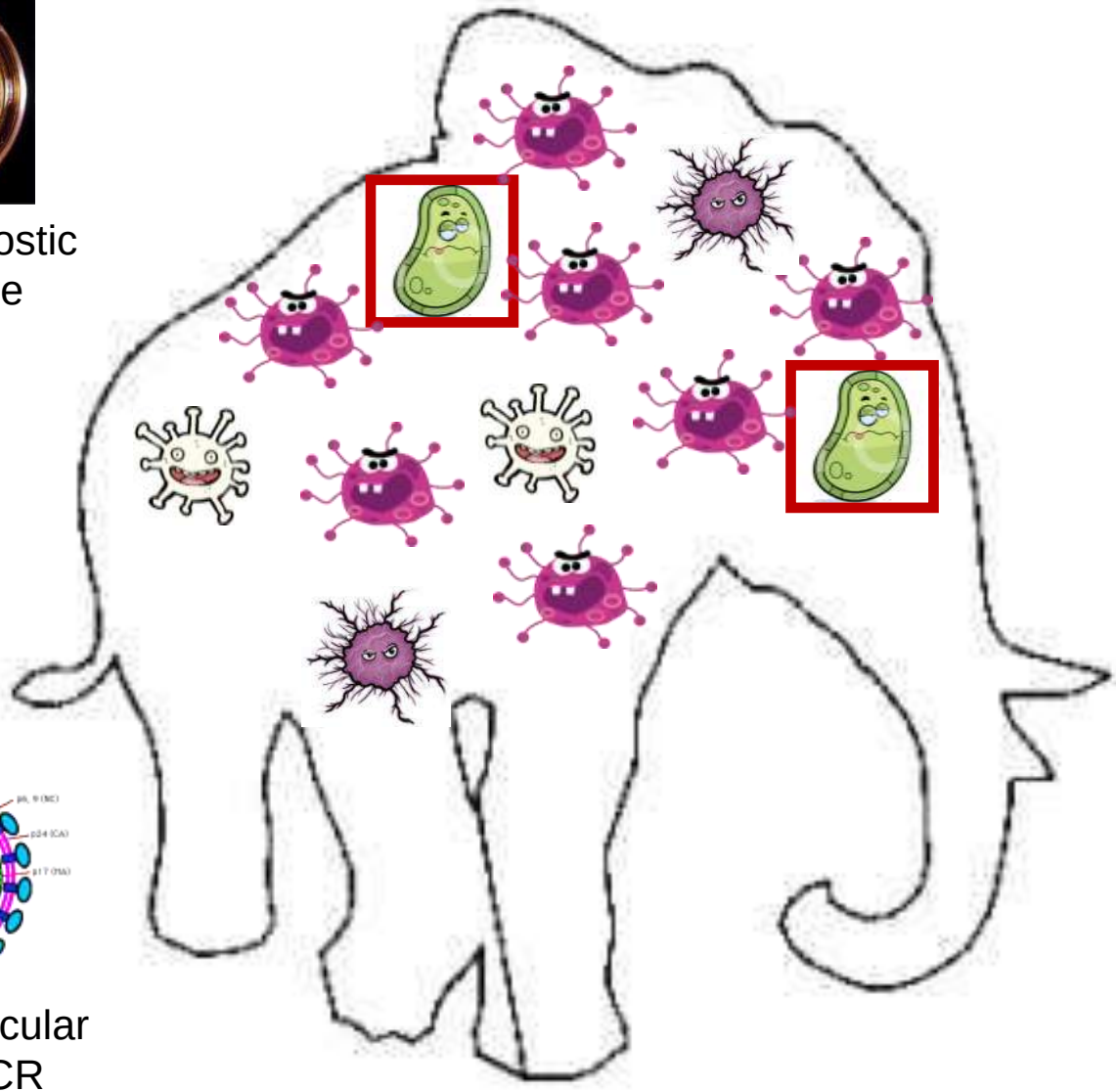


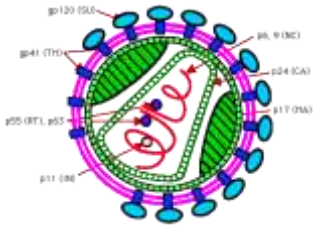
Specific molecular test e.g. PCR

Pathogen discovery



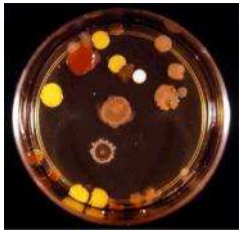
Group diagnostic
e.g. culture



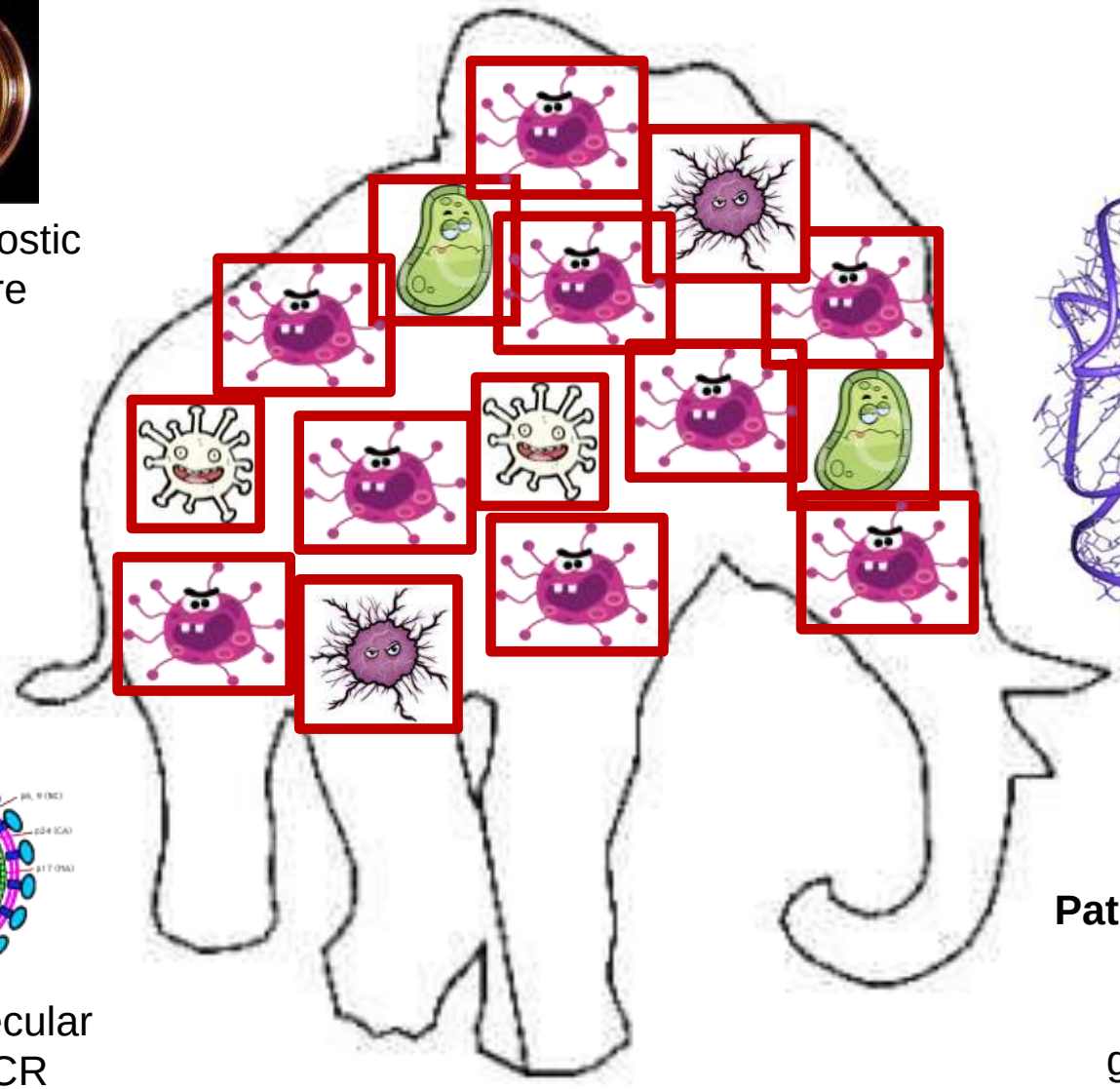


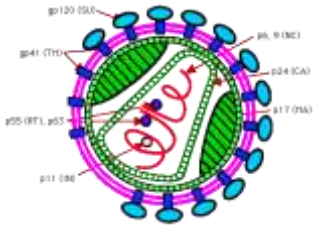
Specific molecular
test e.g. PCR

Pathogen discovery



Group diagnostic
e.g. culture

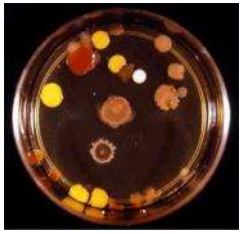




Specific molecular
test e.g. PCR

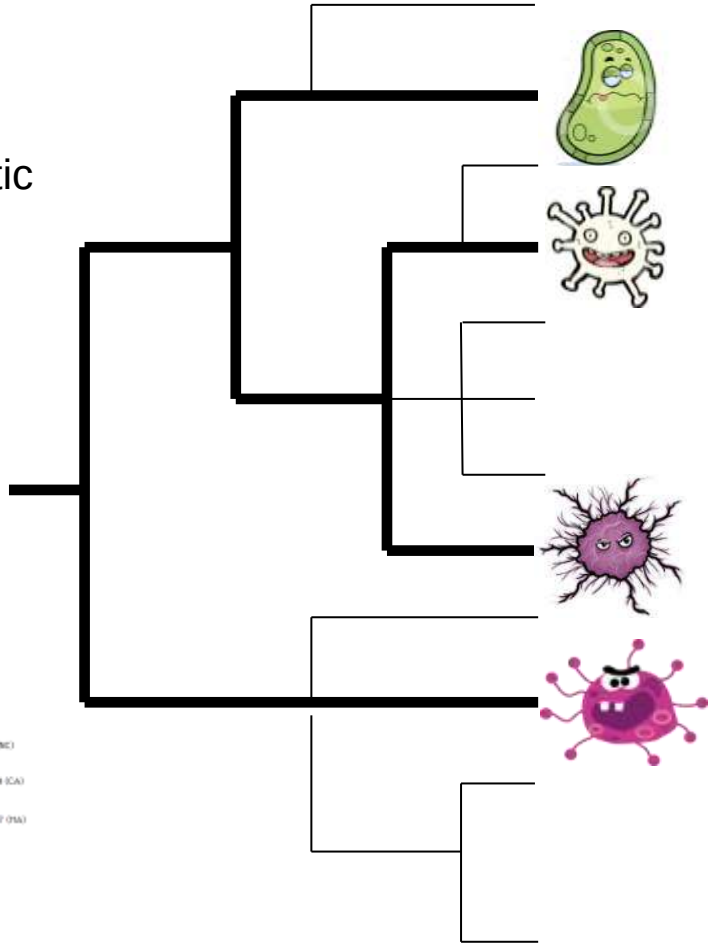
Pathogen Discovery
Procedures to
detect all
genetic material

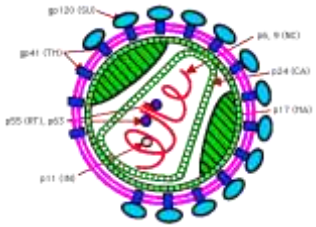
Pathogen discovery



Group diagnostic
e.g. culture

NCBI GENBANK

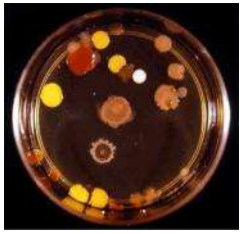




Specific molecular
test e.g. PCR

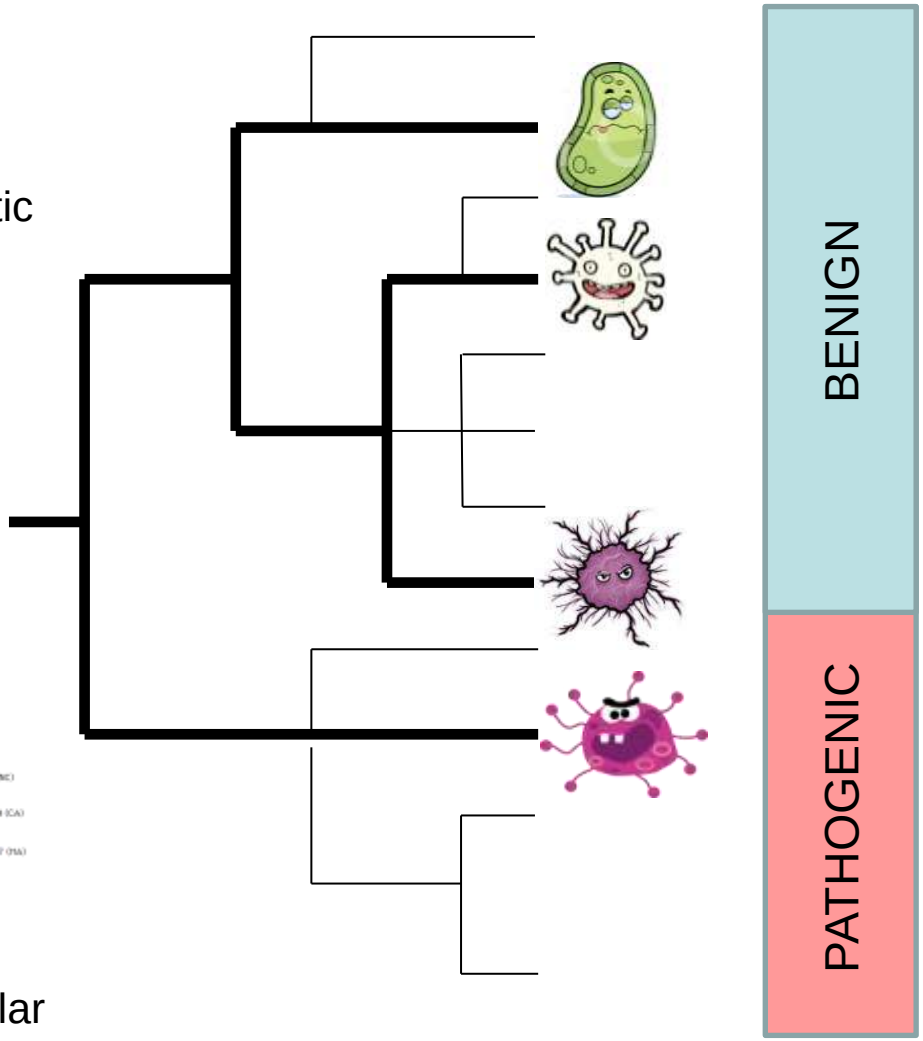
Pathogen Discovery
Procedures to
detect all
genetic material

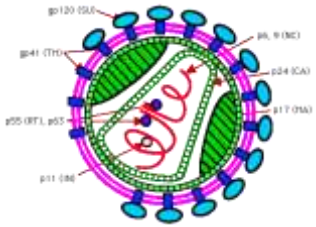
Pathogen discovery



Group diagnostic
e.g. culture

NCBI GENBANK





Specific molecular
test e.g. PCR

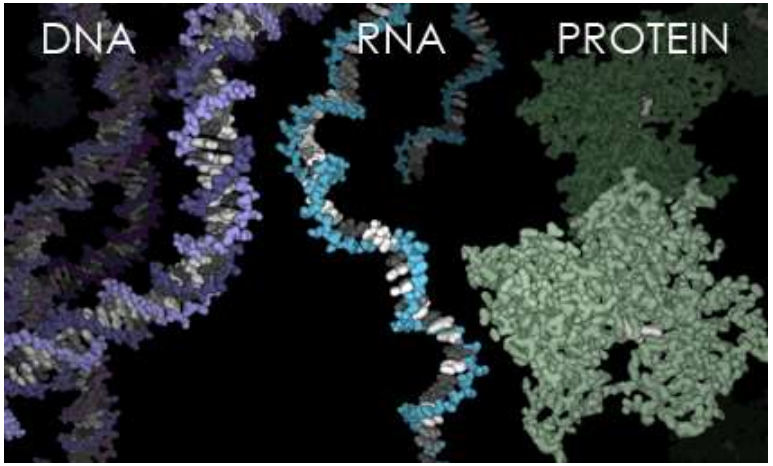
Pathogen Discovery
Procedures to
detect all
genetic material

What does it involve?

DNAse

EXTRACTION

MULTIPLE DISPLACEMENT AMPLIFICATION

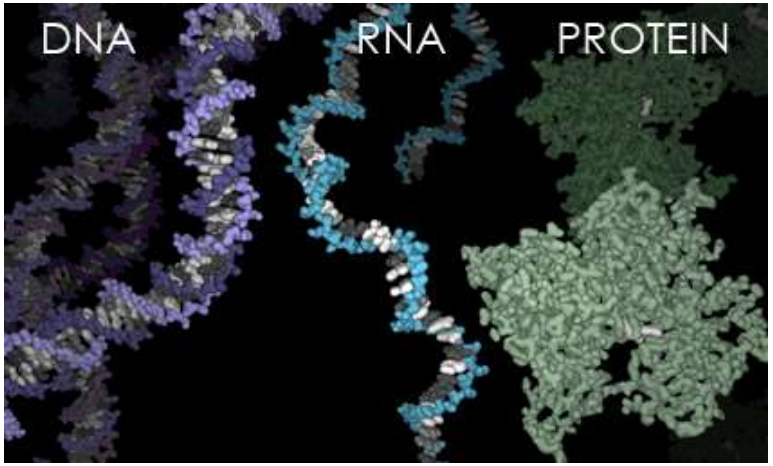


What does it involve?

DNAse

EXTRACTION

MULTIPLE
DISPLACEMENT
AMPLIFICATION



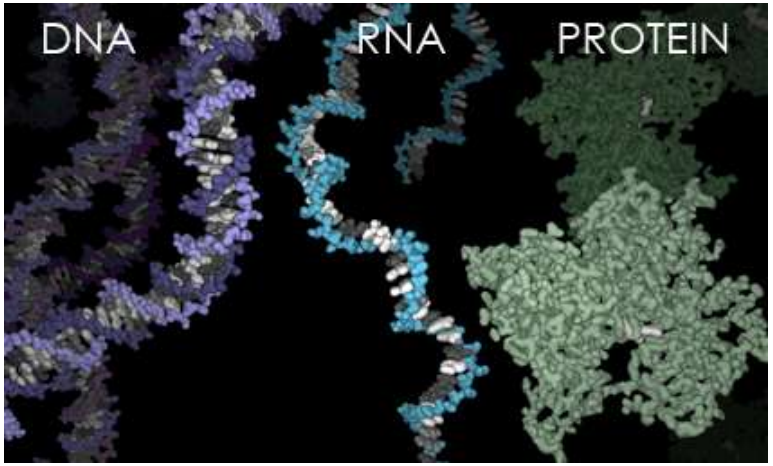
SEQUENCING

What does it involve?

DNAse

EXTRACTION

MULTIPLE
DISPLACEMENT
AMPLIFICATION



SEQUENCING

ASSEMBLY

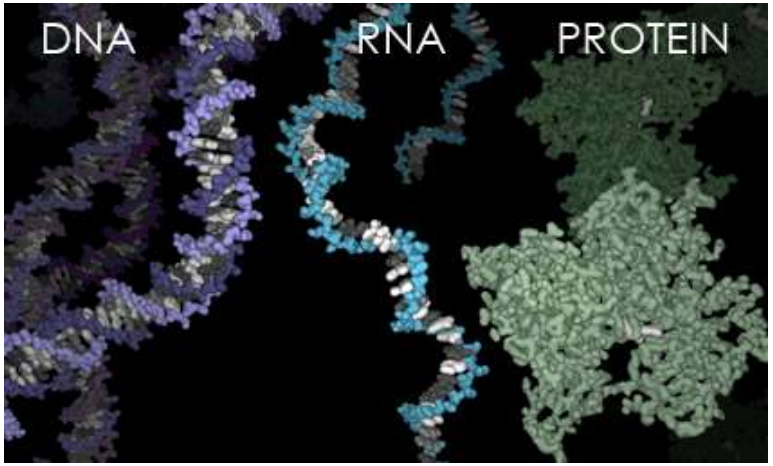
'CONTIGS'

What does it involve?

DNAse

EXTRACTION

MULTIPLE DISPLACEMENT AMPLIFICATION

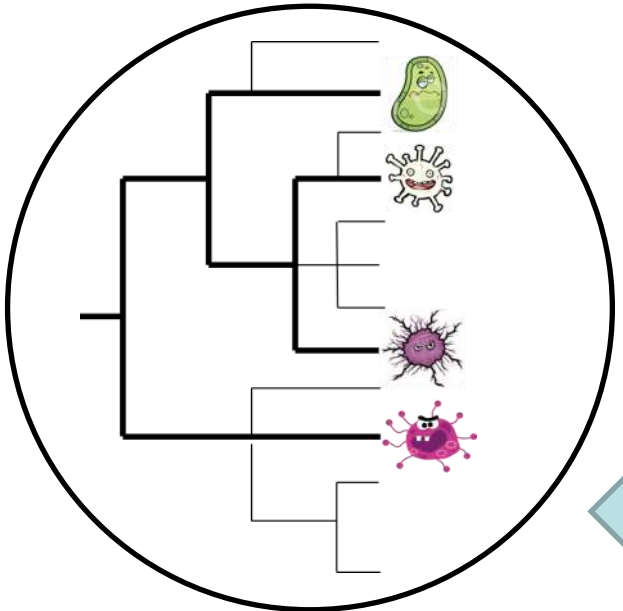


SEQUENCING

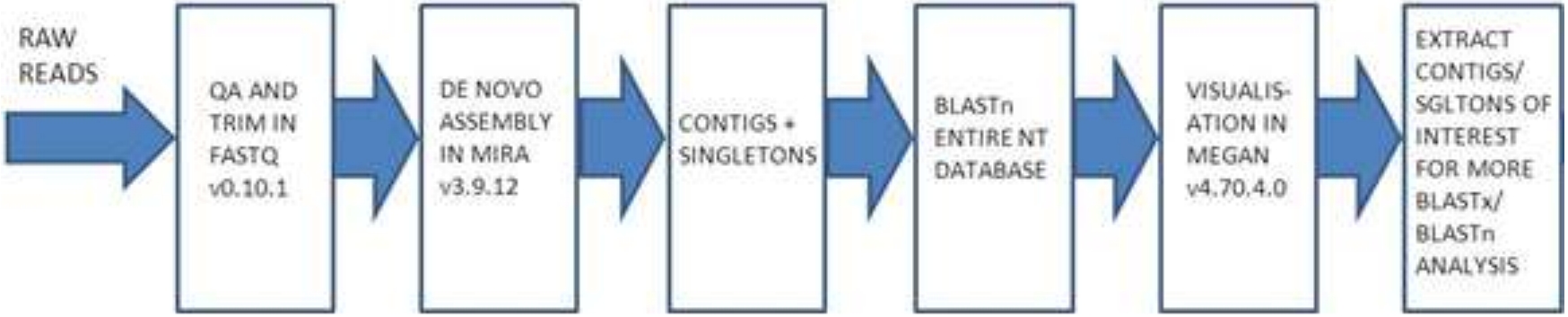
ASSEMBLY

'CONTIGS'

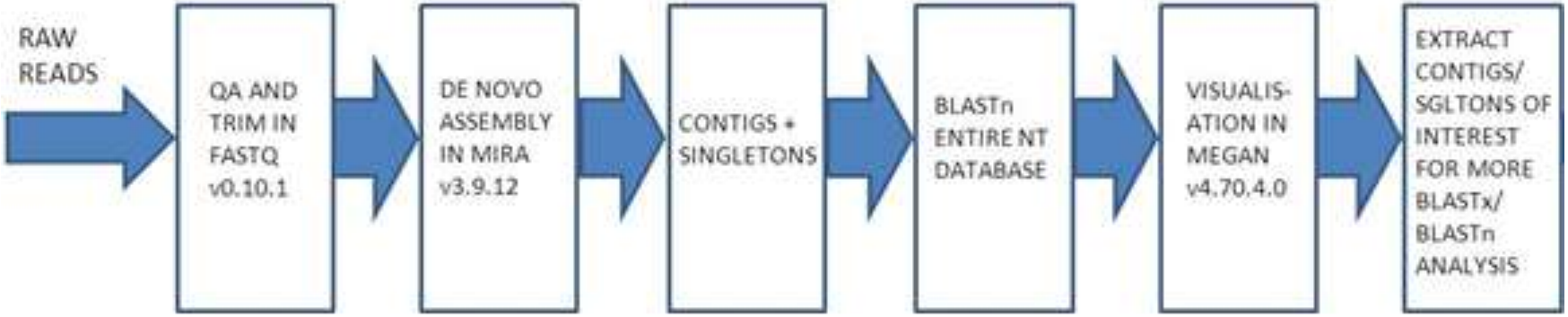
GENBANK



Bioinformatics work flow

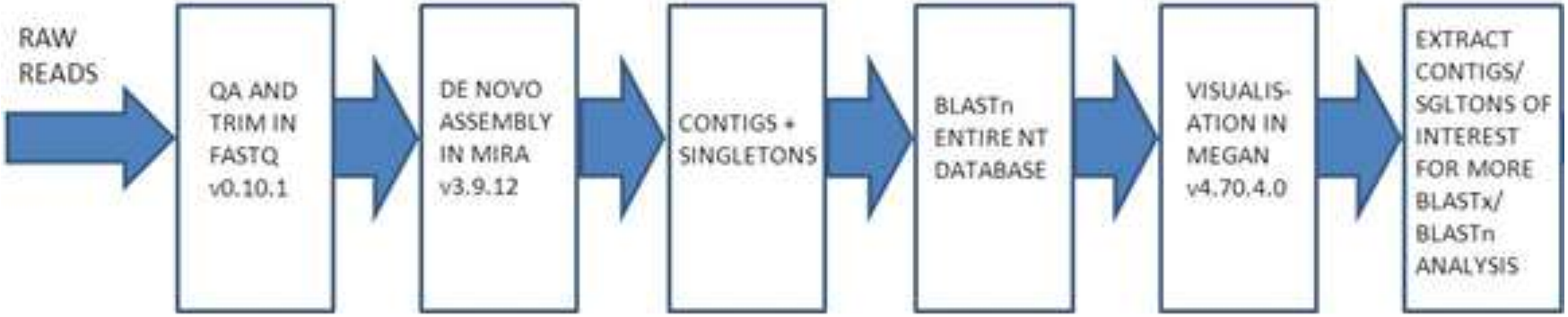


Bioinformatics work flow



Cannot prove or disprove the presence or role of causative agents

Bioinformatics work flow



Cannot prove or disprove the presence or role of causative agents

Offers guidance on where to focus traditional veterinary approaches; useful when causative agents are cryptic or unknown to science

Exudative cloacitis in kakapo

Inflammation of the cloaca observed in nine individuals since 2002

Can become lesioned with associated bacterial growth.

Can cause infertility and potentially death. Population currently 124 birds

Exudative cloacitis in kakapo

Need to identify cause to guide both treatment and preventive management.

Bacteria present bacteria (principally *Escherichia coli* and Enterococcus spp.) are common intestinal inhabitants.

Underlying viral cause?

Exudative cloacitis in kakapo

Preliminary investigation of faecal material from one diseased individual, compared to a pooled sample of material from eight non-diseased individuals

Exudative cloacitis in kakapo

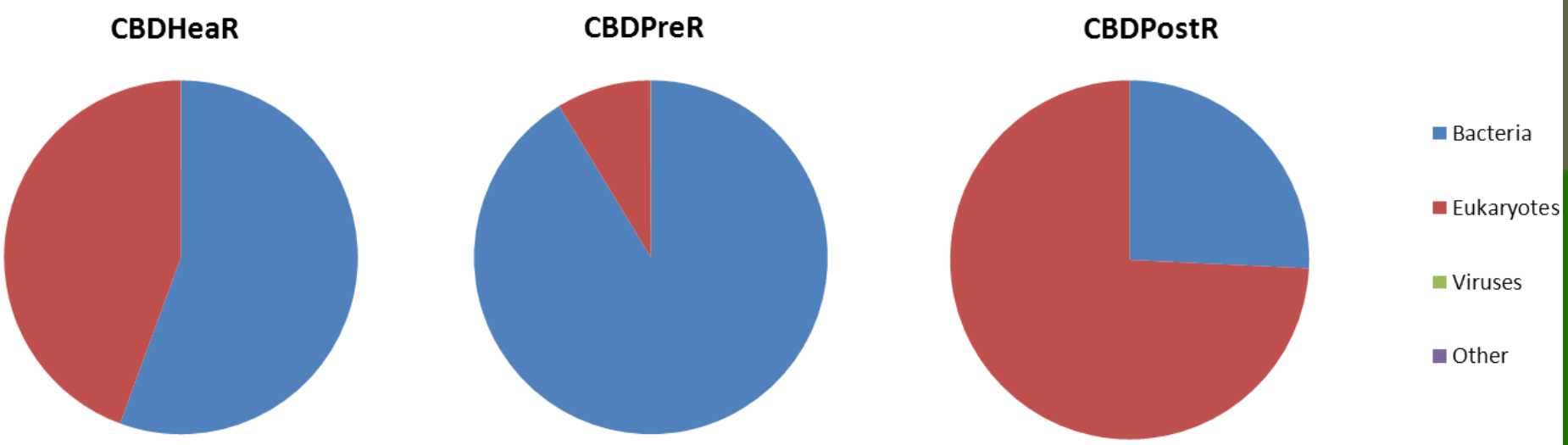
Preliminary investigation of faecal material from one diseased individual, compared to a pooled sample of material from eight non-diseased individuals

Sample	No. paired-end Illumina reads	No. reads assembled	No. contigs	No. singletons	N50 (bp)	Total consensus (bp)	Ave. coverage
(1) Pre-treatment	22,628,996 (250bp reads)	4,223,536	559,218	841	359	190,599,044	7.47
(2) Post-treatment	15,351,920 (250bp reads)	1,772,703	402,381	486	284	98,713,807	5.97
(3) Non-disease pool	20,266,624 (150bp reads)	3,118,309	329,022	1291	244	74,859,329	20.66

Exudative cloacitis in kakapo

Preliminary investigation of faecal material from one diseased individual, compared to a pooled sample of material from eight non-diseased individuals

Sample	No. paired-end Illumina reads	No. reads assembled	No. contigs	No. singletons	N50 (bp)	Total consensus (bp)	Ave. coverage
(1) Pre-treatment	22,628,996 (250bp reads)	4,223,536	559,218	841	359	190,599,044	7.47
(2) Post-treatment	15,351,920 (250bp reads)	1,772,703	402,381	486	284	98,713,807	5.97
(3) Non-disease pool	20,266,624 (150bp reads)	3,118,309	329,022	1291	244	74,859,329	20.66



Exudative cloacitis in kakapo

Viral agents related to diet detected, but no avian viruses detected.

However, very strong evidence for an *E. coli* phage present in the diseased bird, but not the non-diseased pool:

NCBI phage aligned to	No. contigs			Highest bit score	Identities	E-value	Accession number
	Pre-treatment	Post-treatment	Non-disease pool				
Escherichia phage TL-2011b	86	5	0	1335	744/748	0	JQ011317.1
Enterobacteria phage P1	2	0	11	1763	985/990	0	AF234173.1
Enterobacteria phage P7	8	0	0	333	187/189	<0.001	AF503408.1
Enterobacteria phage D6	8	0	0	374	298/357	<0.001	AY753669.1

Exudative cloacitis in kakapo

Viral agents related to diet detected, but no avian viruses detected.

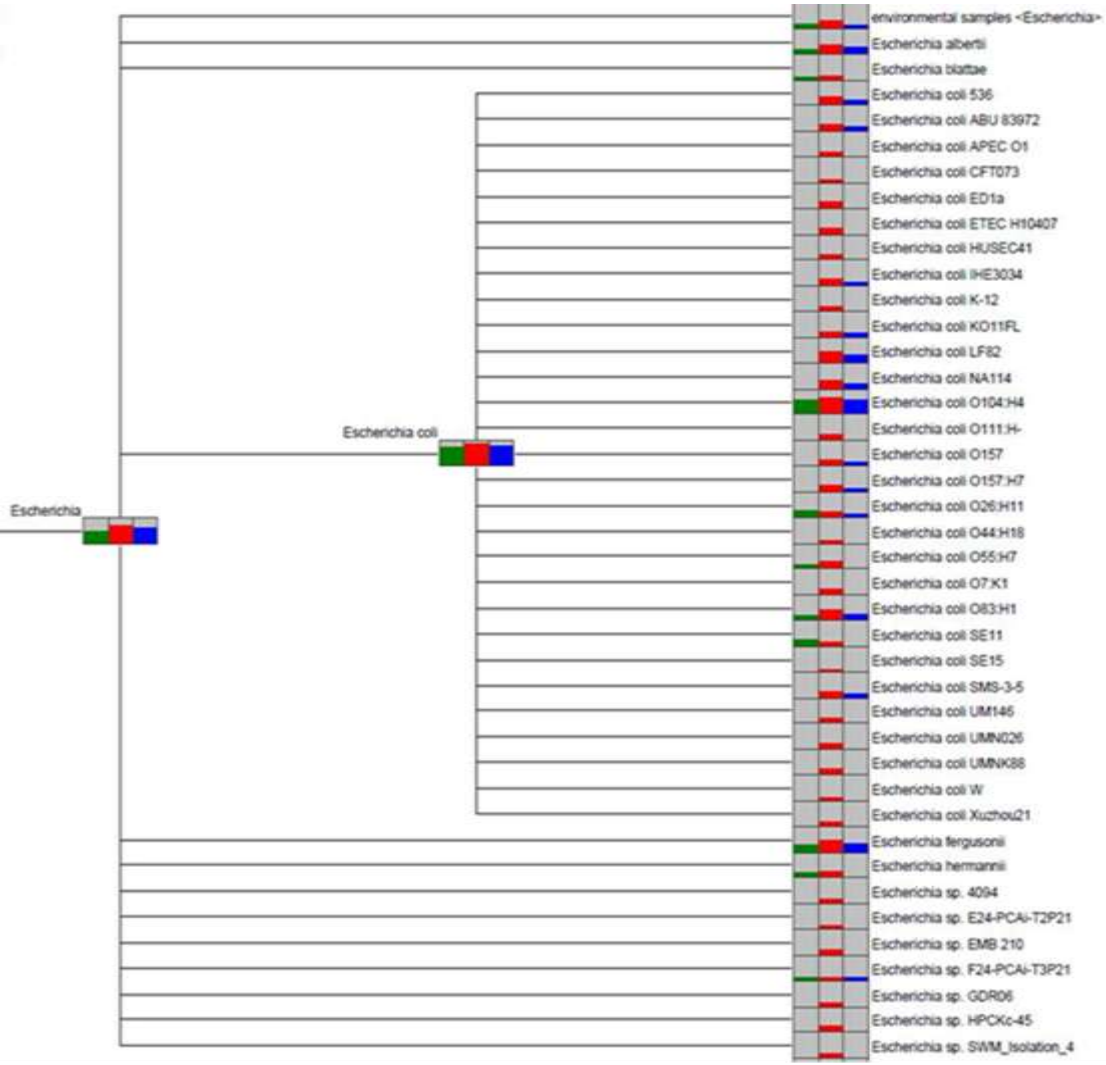
However, very strong evidence for an *E. coli* phage present in the diseased bird, but not the non-diseased pool:

NCBI phage aligned to	No. contigs			Highest bit score	Identities	E-value	Accession number
	Pre-treatment	Post-treatment	Non-disease pool				
Escherichia phage TL-2011b	86	5	0	1335	744/748	0	JQ011317.1
Enterobacteria phage P1	2	0	11	1763	985/990	0	AF234173.1
Enterobacteria phage P7	8	0	0	333	187/189	<0.001	AF503408.1
Enterobacteria phage D6	8	0	0	374	298/357	<0.001	AY753669.1

Escherichia phage TL-2011b is known to occur in *E. coli* causing food-borne disease outbreaks in people.

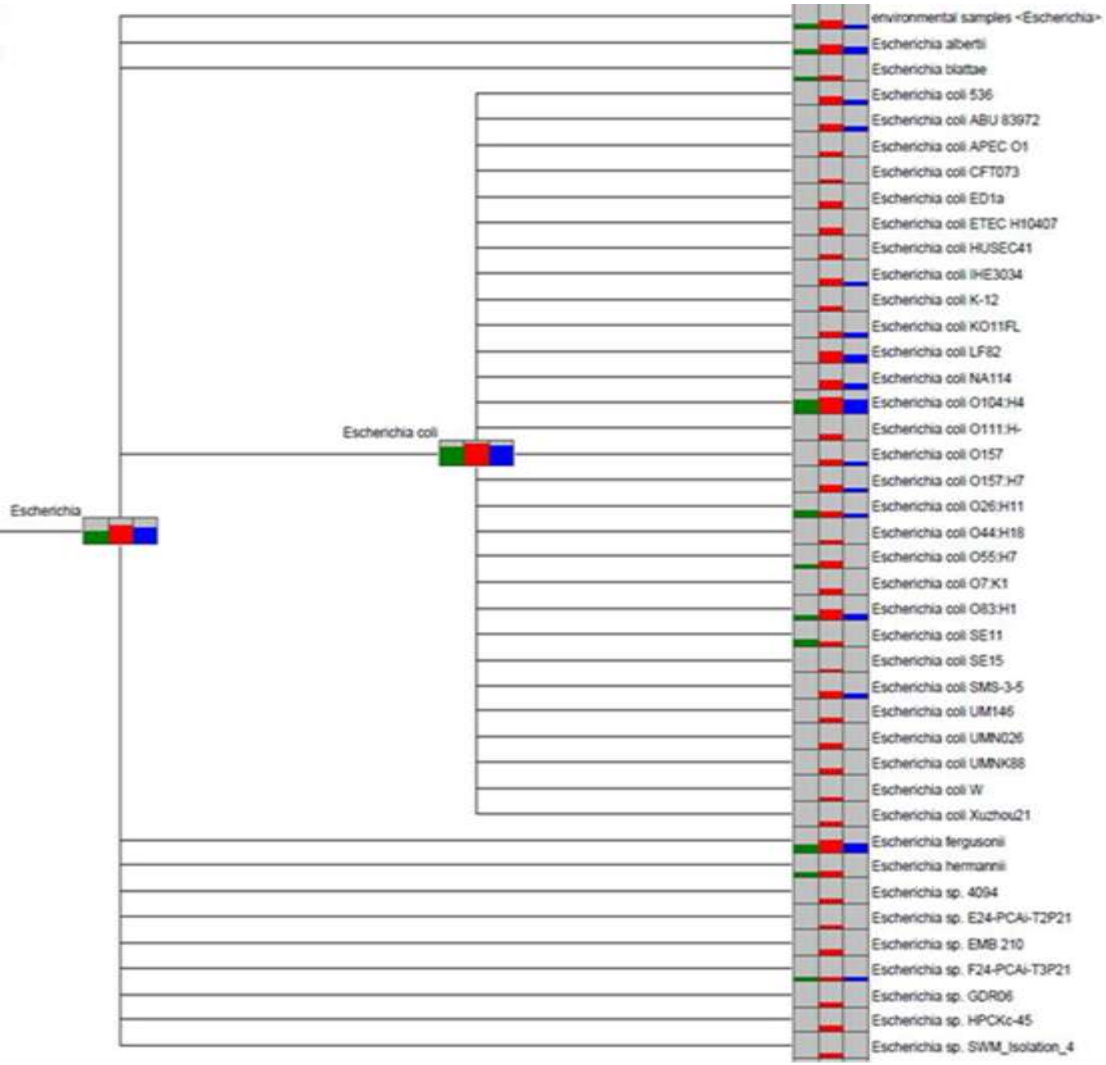
Exudative cloacitis in kakapo

Are there corresponding differences in genomic *E. coli* community structure?



Exudative cloacitis in kakapo

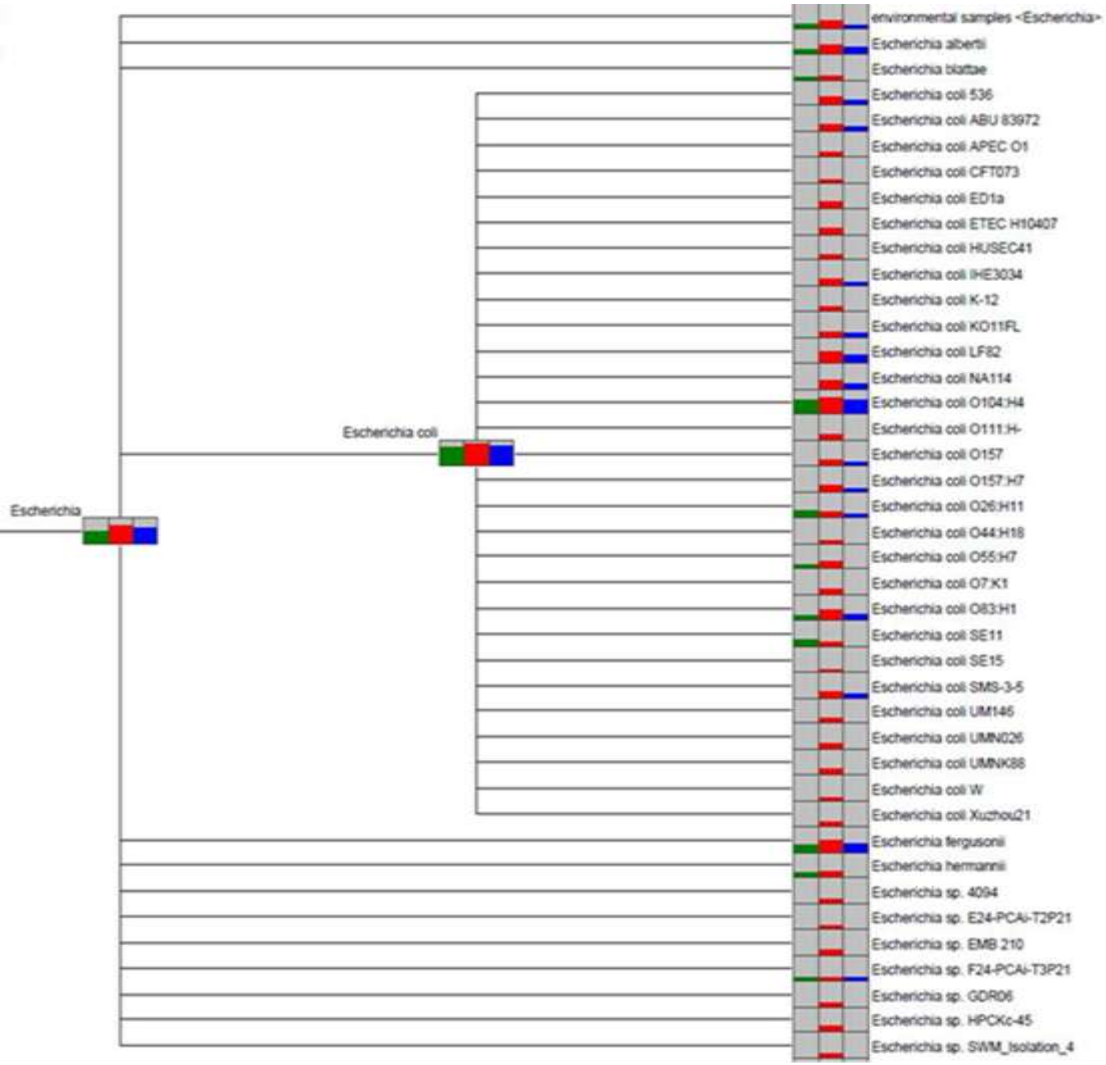
Are there corresponding differences in genomic *E. coli* community structure?



Several *E. coli* strains detected across all samples.

Exudative cloacitis in kakapo

Are there corresponding differences in genomic *E. coli* community structure?

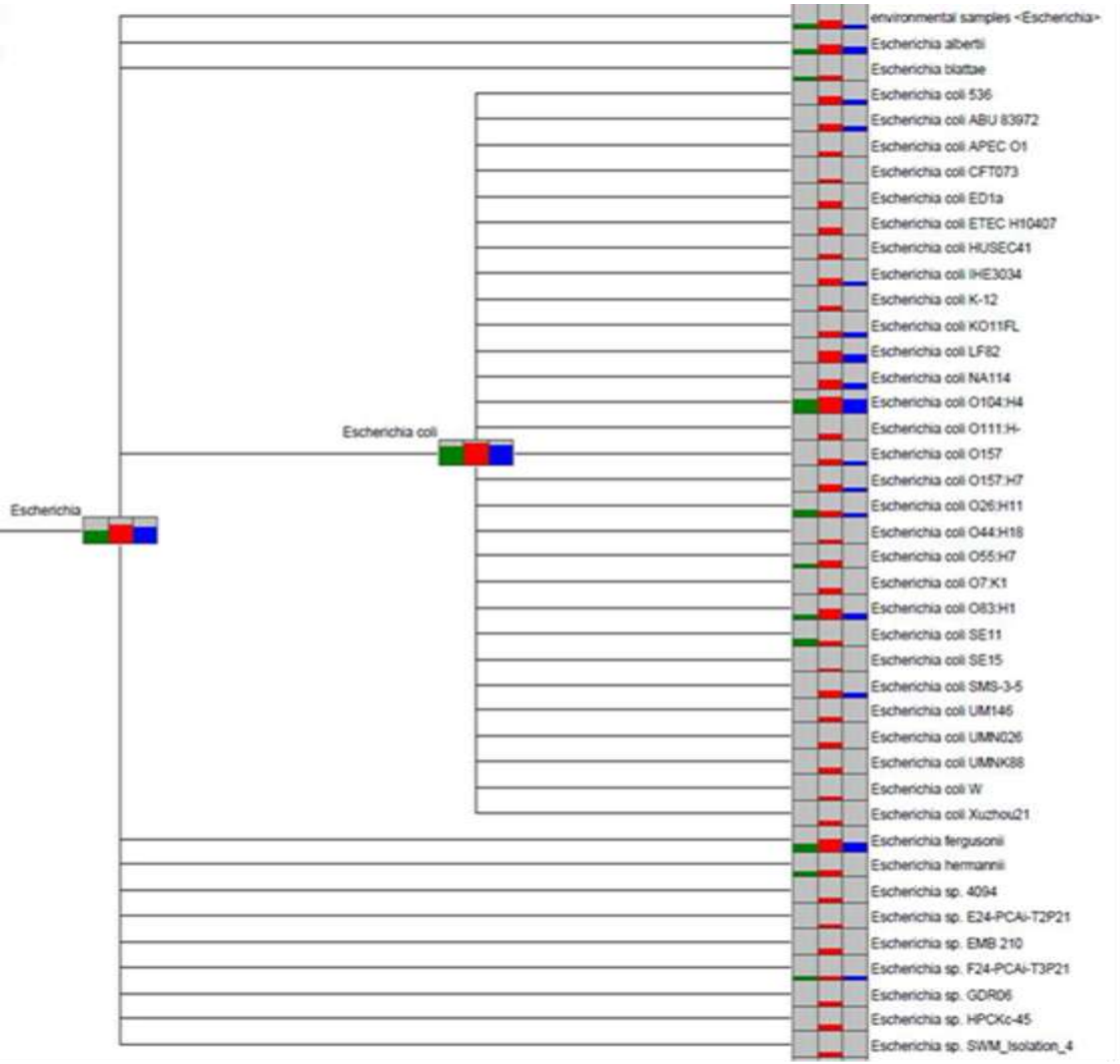


Several *E. coli* strains detected across all samples.

Very strong evidence for the presence of *E. coli* LF82 in the diseased bird, but not the non-diseased pool.

Exudative cloacitis in kakapo

Are there corresponding differences in genomic *E. coli* community structure?



Several *E. coli* strains detected across all samples.

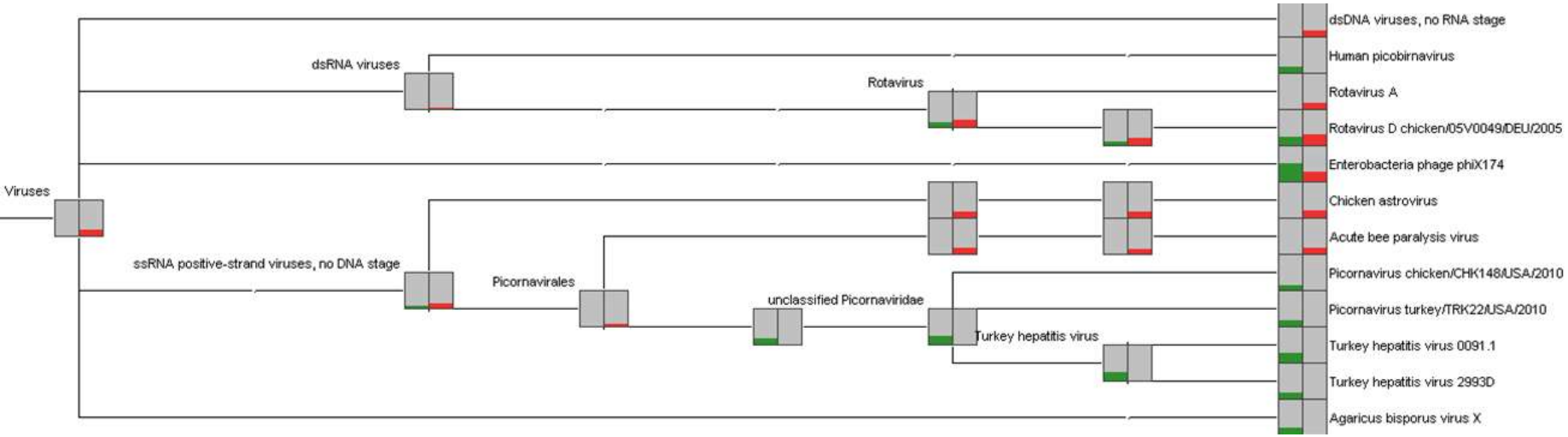
Very strong evidence for the presence of *E. coli* LF82 in the diseased bird, but not the non-diseased pool.

This strain is associated with inflammatory bowel disease (Crohn's disease) in people.

Adelie penguin feather loss

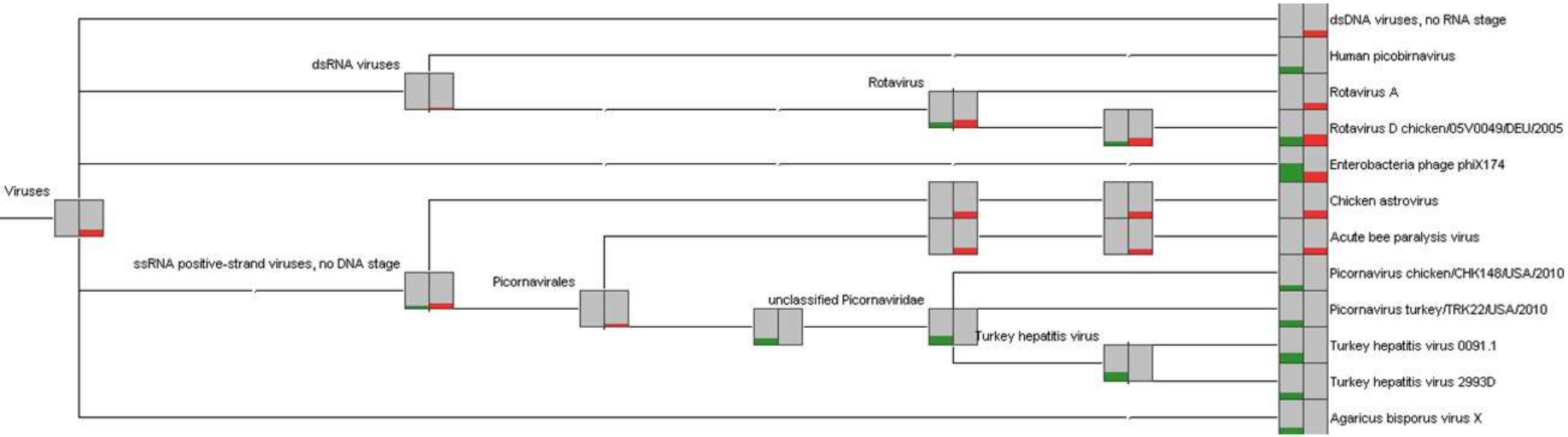
Adelie penguin feather loss

Viral community comparison between feather-loss (N = 12 individuals) and non-diseased (N = 12 individuals) pools of faecal material.



Adelie penguin feather loss

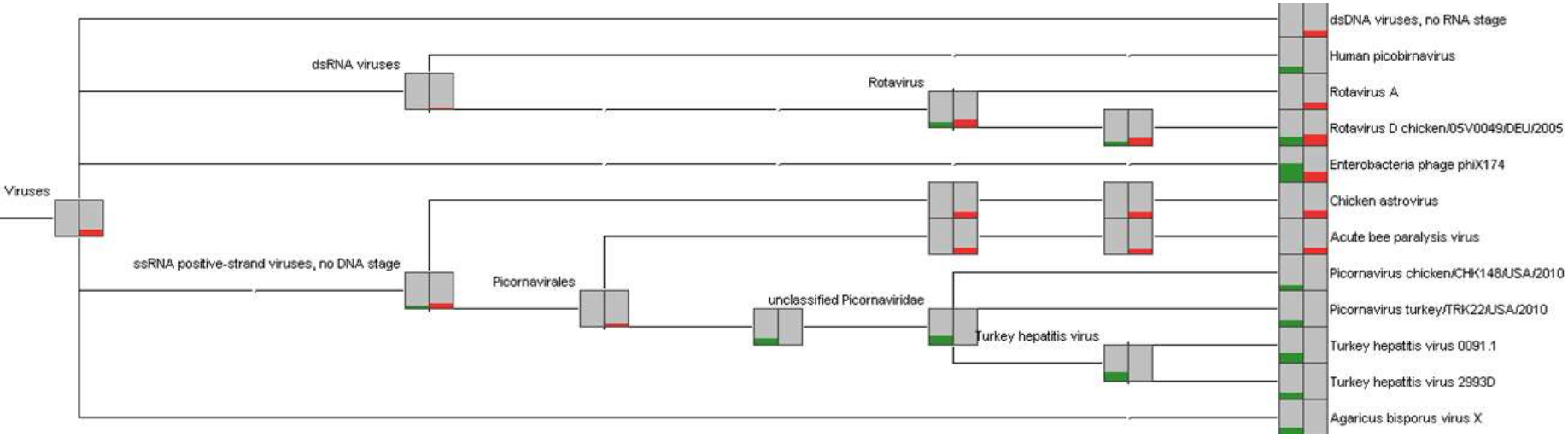
Viral community comparison between feather-loss (N = 12 individuals) and non-diseased (N = 12 individuals) pools of faecal material.



Potentially pathogenic rotavirus and hepatitis viruses detected in the pools of material from both the feather-loss and non-diseased individuals.

Adelie penguin feather loss

Viral community comparison between feather-loss (N = 12 individuals) and non-diseased (N = 12 individuals) pools of faecal material.

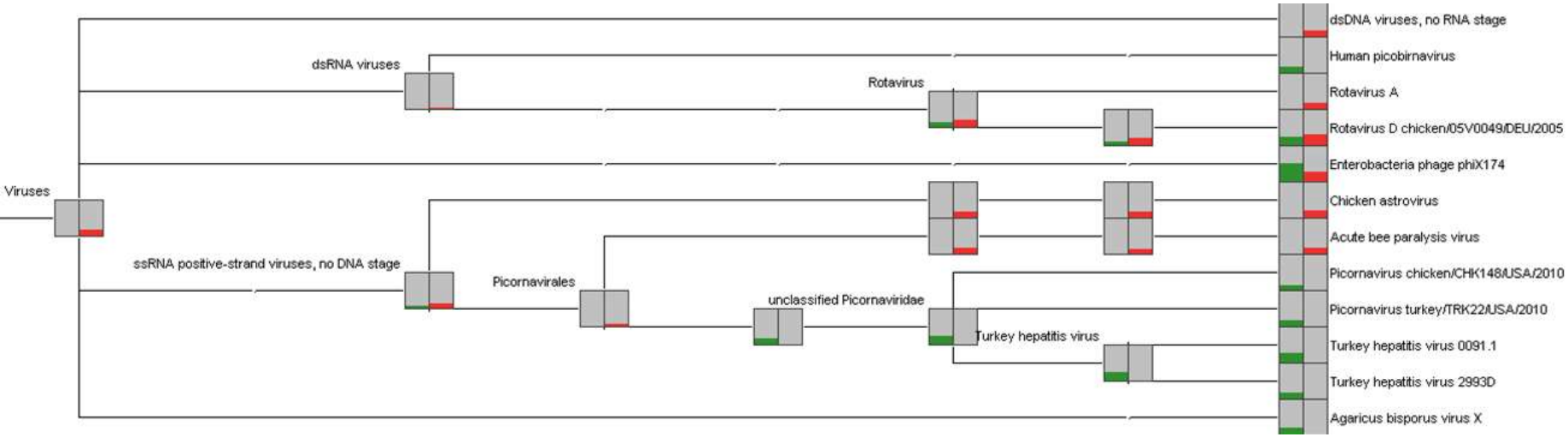


Potentially pathogenic rotavirus and hepatitis viruses detected in the pools of material from both the feather-loss and non-diseased individuals.

Strong evidence for the presence of an astrovirus in the feather-loss birds only (related to, but divergent from, Avian nephritis virus 2).

Adelie penguin feather loss

Viral community comparison between feather-loss (N = 12 individuals) and non-diseased (N = 12 individuals) pools of faecal material.

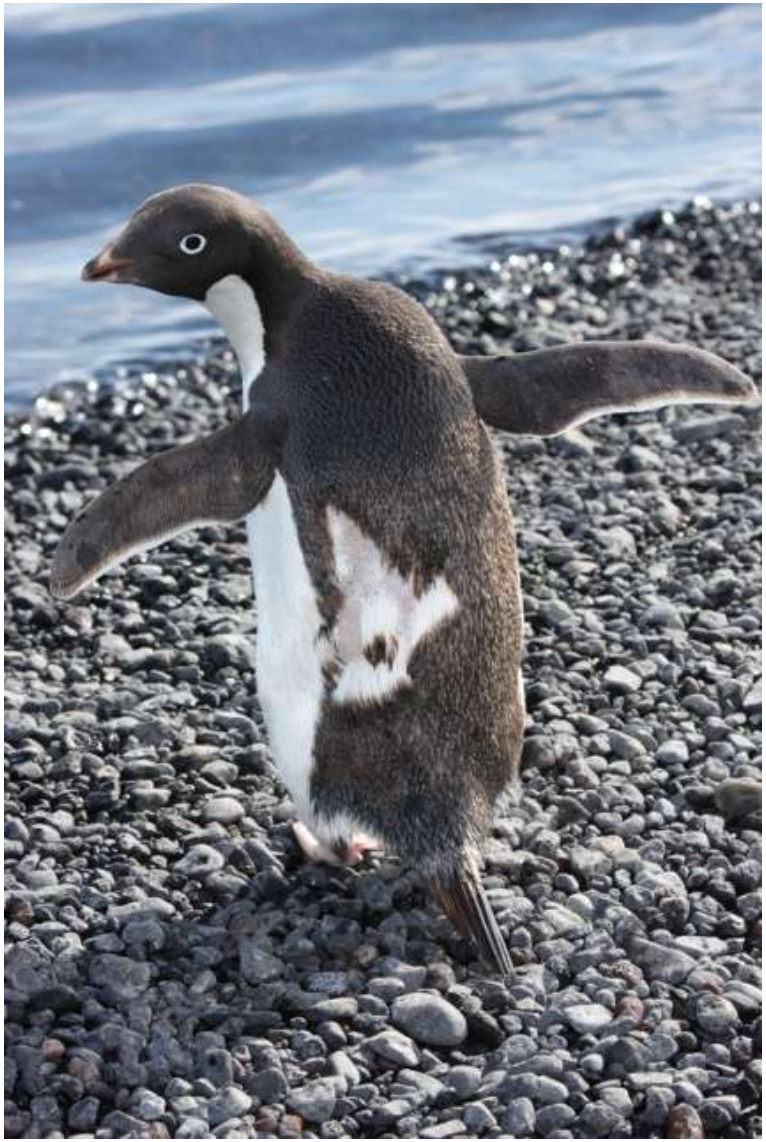


Potentially pathogenic rotavirus and hepatitis viruses detected in the pools of material from both the feather-loss and non-diseased individuals.

Strong evidence for the presence of an astrovirus in the feather-loss birds only (related to, but divergent from, Avian nephritis virus 2).

Mild cases in chickens - diarrhea, stunted growth and skin lesions.
Severe cases in immunosuppressed, under-nourished or stressed chicks – nephrosis, emaciation, and even sudden death.

Where to from here?



Where to from here?

Other applications?

- Application of capability to wildlife zoonoses (infections transmitted to people)

- Viral agents in New Zealand bat species?

Other applications?

- Application of capability to wildlife zoonoses (infections transmitted to people)

- Viral agents in New Zealand bat species?
- Serological evidence for Ross River Virus exposure to Australasian Gannets at Muriwai; are they also carrying the virus over from Australia?

Other applications?

- Application of capability to wildlife zoonoses (infections transmitted to people)



- Viral agents in New Zealand bat species?
- Serological evidence for Ross River Virus exposure to Australasian Gannets at Muriwai; are they also carrying the virus over from Australia?
- Rodent-borne infections – recent increase in human cases of murine typhus; are there other agents we should be concerned about?

Other applications?

- Application of capability to agricultural disease issues of concern

- Better understanding of Salmonella dynamics and causes of outbreaks?

Other applications?

- Application of capability to agricultural disease issues of concern

- Better understanding of Salmonella dynamics and causes of outbreaks?
- Viral component of Varroa mite impact on honey bees in New Zealand?

Other applications?

- Application of capability to agricultural disease issues of concern



- Better understanding of Salmonella dynamics and causes of outbreaks?
- Viral component of Varroa mite impact on honey bees in New Zealand?
- Import contamination – e.g. is palm kernel from Malaysia contaminated with livestock diseases?


SEARCHING PRINTS
STOKES
CSI:
CRIME SCENE INVESTIGATION
TO BE CONTINUED...
NO MATCH FOUND

E. coli strain	No. contigs	Highest bit score	Pathogenic association
536	97	875	community-acquired urinary tract infection (UTI)[1]
ABU 83972	51	681	asymptomatic, bacteriuria[5]
APEC O1	20	962	avian extraintestinal diseases, aerosacculitis, polyserositis, septicemia[7]
CFT073	5	910	uropathogenic, pyelonephritis[9]
ED1a	47	654	non-pathogenic
ETEC H10407	49	1961	enterotoxigenic, diarrhoea[10]
HUSEC41	17	621	haemolytic uraemic syndrome[11]
IHE3034	42	594	neonatal meningitis-associated[12]
K-12	16	641	non-pathogenic
KO11FL	20	410	non-pathogenic
LF82	730	991	Crohn's disease[4]
NA114	100	686	uropathogenic[6]
O111:H-	18	892	shiga-toxin producing, diarrhoea and haemorrhagic colitis[2]
O157	26	1229	diarrhoea, haemorrhagic colitis, and haemolytic uremic syndrome[3]
O157:H7	29	691	diarrhoea, haemorrhagic colitis, and haemolytic uremic syndrome[3]
O44:H18	5	1732	diarrhoea[8]
O7:K1	22	1779	urinary tract infection[13]
SE15	5	627	non-pathogenic
SMS-3-5	37	879	unknown
UM146	11	758	Crohn's disease[14]
UMN026	16	482	urinary tract infection, cystitis[15]
UMNK88	20	401	diarrhoea[16]
W	7	915	non-pathogenic
Xuzhou21	13	450	haemolytic uremic syndrome[17]

JQ011317.1: Escherichia phage TL-2011b, complete genome - Mozilla Firefox

File Edit View History Bookmarks Tools Help

NCBI Blast:CBDPreR_c308 | JQ011317.1: Escherichia phage TL-2011...

www.ncbi.nlm.nih.gov/nuccore/363498235?report=graph&rid=V2UWKCRP014&tracks=[key:sequence_track,name:Sequence,display_name:Sequence,id:STD1,category:Sequence

destructive synonym

NCBI Resources How To Sign in to NCBI

Nucleotide Nucleotide Search

Limits Advanced Help

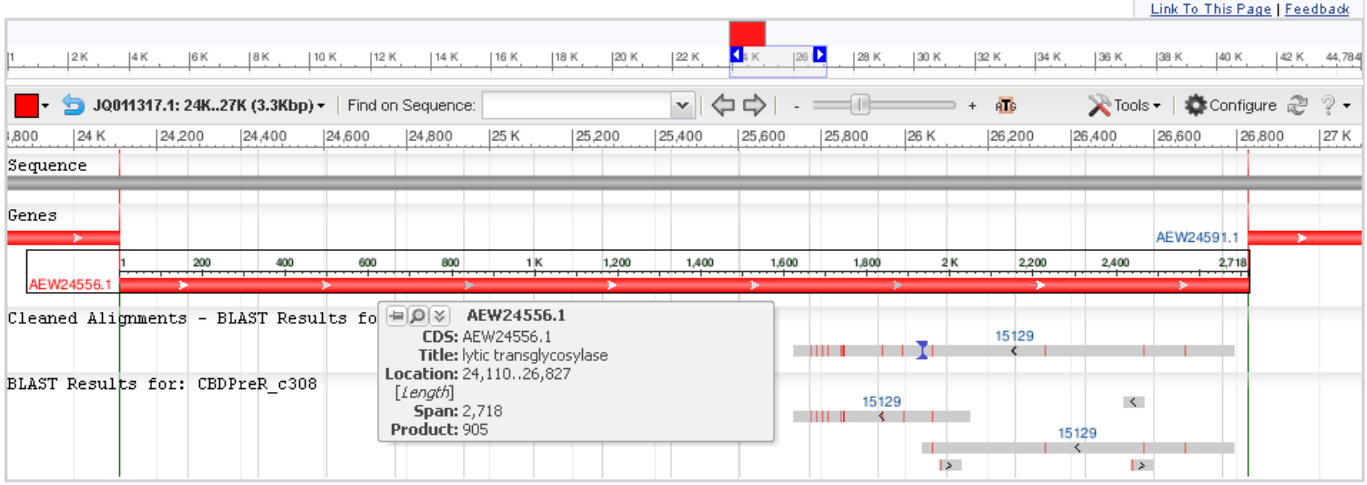
Display Settings: Graphics Send:

Escherichia phage TL-2011b, complete genome

GenBank: JQ011317.1

GenBank FASTA

Link To This Page | Feedback



Analyze this sequence

Run BLAST

Pick Primers

Highlight Sequence Features

LinkOut to external resources

REBASE enzyme M.Eph2011ORFAP
[REBASE - The Restriction Enzy...]

Related information

Related Sequences

Full text in PMC

Gene

Genome

Protein

PubMed

PubMed (Weighted)

Taxonomy

Recent activity

Turn Off Clear

db_logo_books

CBDPreR_c308

The BLAST Sequence Analysis Tool - The NCBI Handbook

See more...

You are here: NCBI > DNA & RNA > Nucleotide Database

Write to the Help Desk

GETTING STARTED	RESOURCES	POPULAR	FEATURED	NCBI INFORMATION
NCBI Education	Chemicals & Bioassays	PubMed	Genetic Testing Registry	About NCBI
NCBI Help Manual	Data & Software	Nucleotide	PubMed Health	Research at NCBI

CU651637.1: Escherichia coli LF82 chromosome, complete sequence - Mozilla Firefox

File Edit View History Bookmarks Tools Help

NCBI Blast:CBDPreR_rep_c3486... | New PubReader™ view of articles | CU651637.1: Escherichia coli LF... | Getting started - eResearch - U... | CU651637.1: Escherichia coli LF... | The BLAST Sequence Analysis T... | Euphyllophytina - Wikipedia, the...

www.ncbi.nlm.nih.gov/nuccore/222031834?report=graph&rid=UAPM3PXY01R&tracks=[key:sequence_track,name:Sequence,display_name:Sequence,id:STD1,category:Sequence,annots:Sequence,Show

Euphyllophyta

NCBI Resources How To Sign in to NCBI

Nucleotide Nucleotide Search

Limits Advanced Help

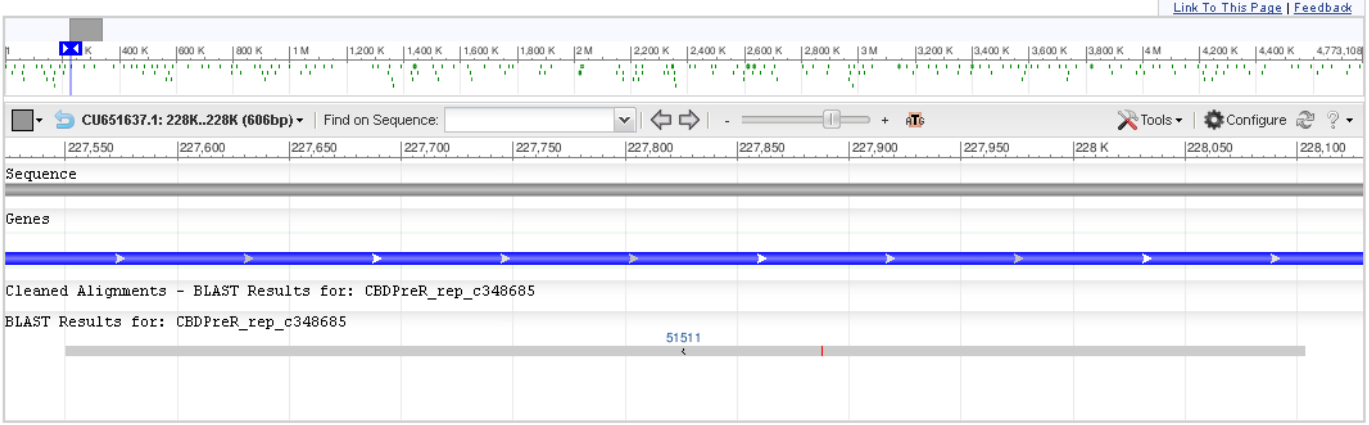
Display Settings: Graphics Send:

Escherichia coli LF82 chromosome, complete sequence

GenBank: CU651637.1

GenBank FASTA

Link To This Page | Feedback



Analyze this sequence

Run BLAST

Pick Primers

Highlight Sequence Features

LinkOut to external resources

REBASE enzyme M.EcoLF82DcmP [F [cpnDB: A Chaperonin Database]

REBASE enzyme EcoLF82McrBP

REBASE enzyme M.EcoLF82DamP

REBASE enzyme M.EcoLF82ORF738P

REBASE enzyme EcoLF82ORF719P

REBASE enzyme M.EcoLF82ORF408P

REBASE enzyme M.EcoLF82ORF719P

REBASE enzyme EcoLF82ORF738P

REBASE enzyme V.EcoLF82DcmP

REBASE enzyme S.EcoLF82ORF738P

REBASE enzyme M.EcoLF82ORF3263P

Ribosomal Database Project II

SILVA LSU Database

SILVA SSU Database

cpnDB: A Chaperonin Database

Find: score Next Previous Highlight all Match case