Next Generation Sequencing – The Role of New Sequence Technologies in Shaping the Future of Veterinary Science

Hosted by the RCVS Charitable Trust

RCVS charitable trust

Current “next generation” sequencing methodologies and what they can do

Neil hall

University of Liverpool

Technological Evolution: air travel

1950

1980

1950

Present

1970

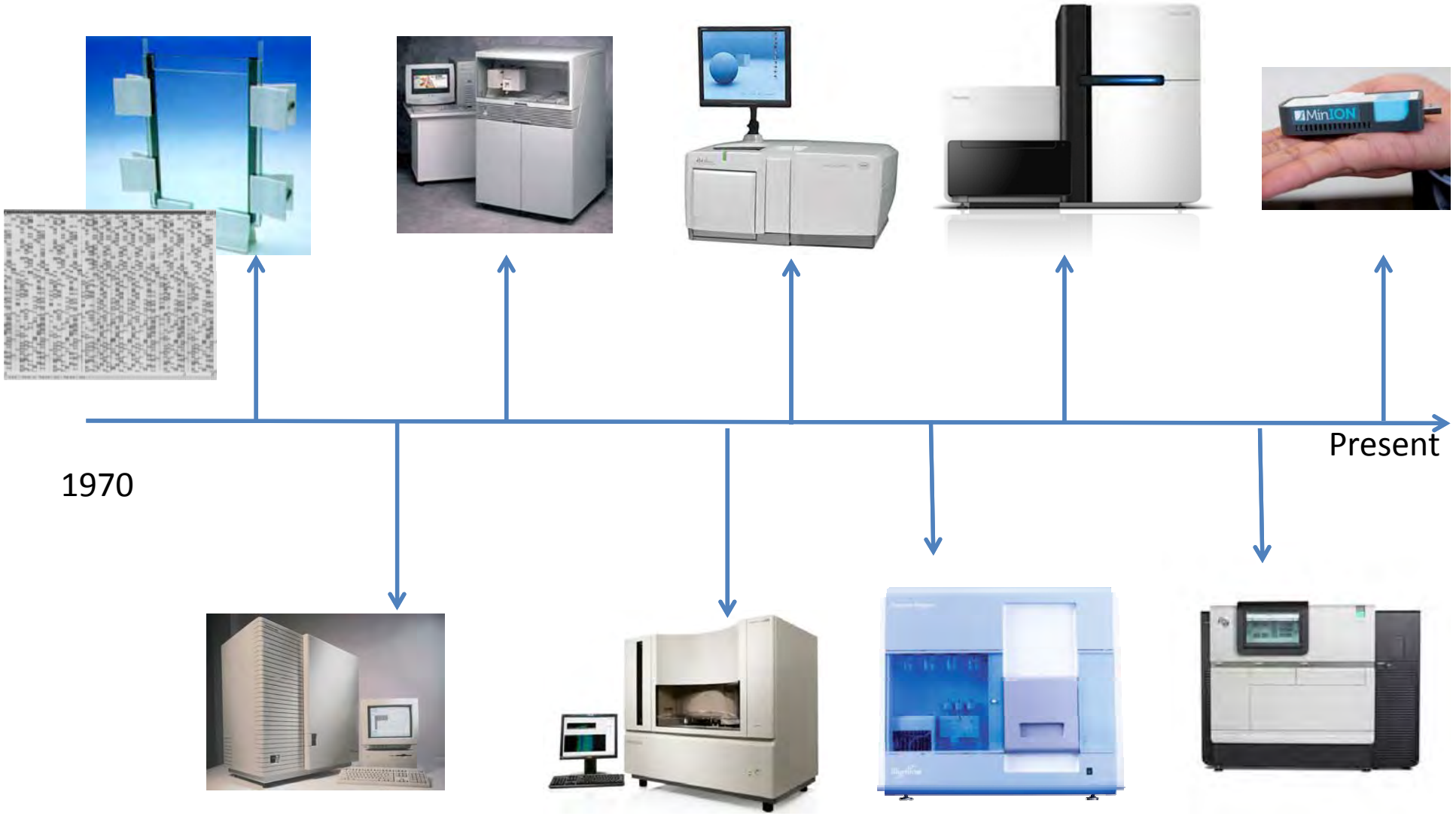
2012

Technological Evolution: mobile phones

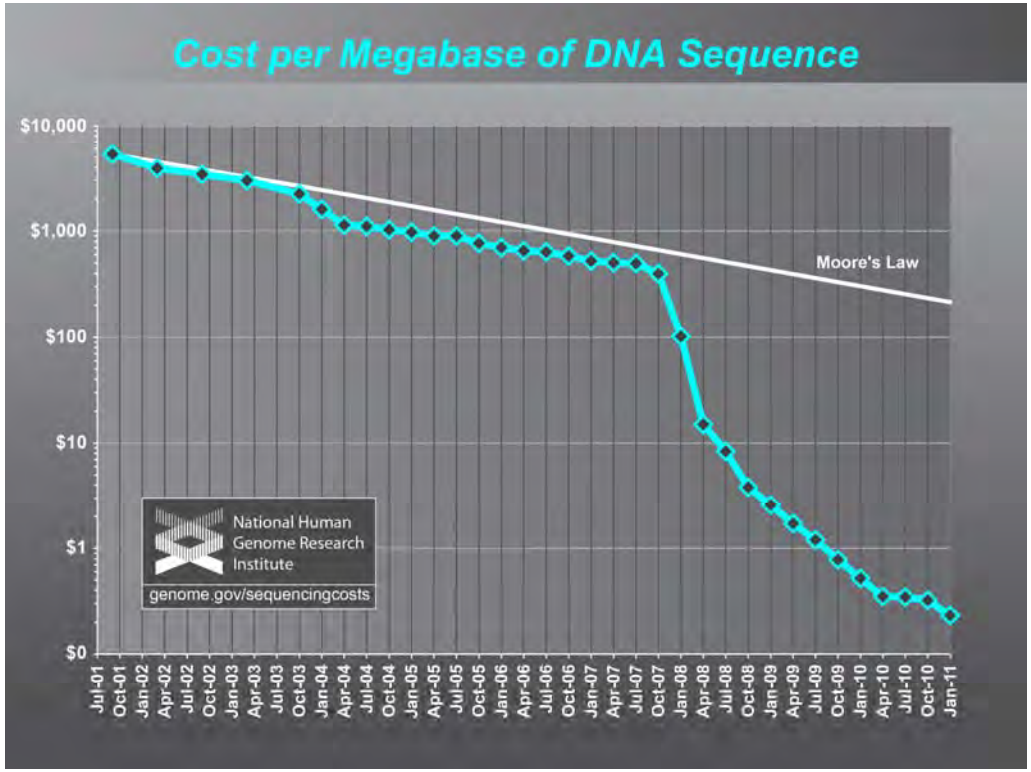
1940

present

Technological Evolution: DNA Sequencing



The road to the $100 genome



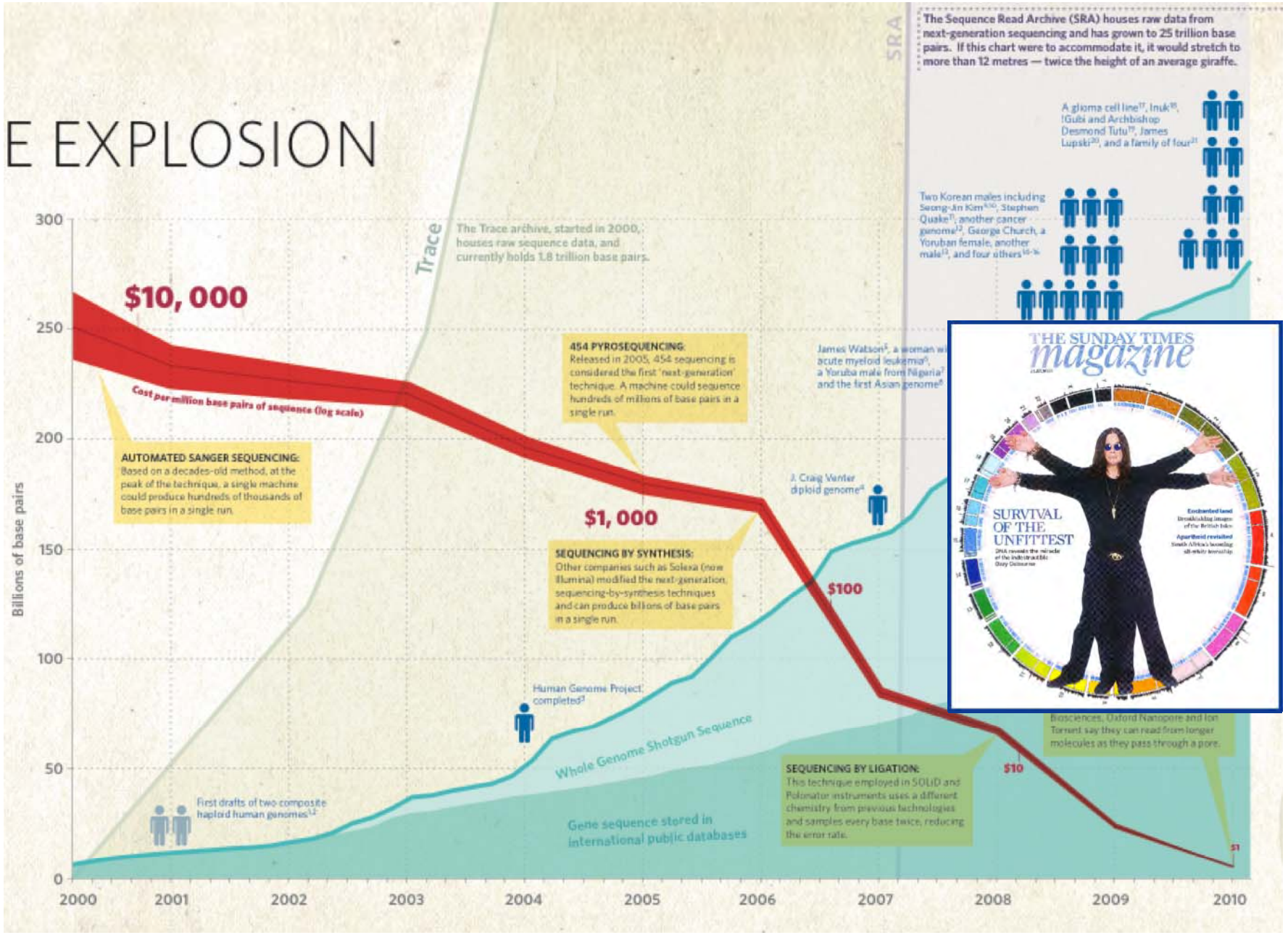
Current cost of a human genome sequence $4000

At the current rate of decrease a human genome will cost >$100 to sequence within 3 years

CGR

UNIVERSITY OF LIVERPOOL

E EXPLOSION


The Sequence Read Archive (SRA) houses raw data from next-generation sequencing and has grown to 25 trillion base pairs. If this chart were to accommodate it, it would stretch to more than 12 metres — twice the height of an average giraffe.
SRA
Trace
The Trace archive, started in 2000, houses raw sequence data, and currently holds 1.8 trillion base pairs.
A glioma cell line[17], Inuk[18], !Gubi and Archbishop Desmond Tutu[19], James Lupski[20], and a family of four[21]
Two Korean males including Seong-Jin Kim[9,10], Stephen Quake[11], another cancer genome[12], George Church, a Yoruban female, another male[13], and four others[14–16]
$10,000
Cost per million base pairs of sequence (log scale)
AUTOMATED SANGER SEQUENCING: Based on a decades-old method, at the peak of the technique, a single machine could produce hundreds of thousands of base pairs in a single run.
454 PYROSEQUENCING: Released in 2005, 454 sequencing is considered the first 'next-generation' technique. A machine could sequence hundreds of millions of base pairs in a single run.
James Watson[5], a woman with acute myeloid leukemia[6], a Yoruba male from Nigeria[7] and the first Asian genome[8]
J. Craig Venter diploid genome[4]
$1,000
SEQUENCING BY SYNTHESIS: Other companies such as Solexa (now Illumina) modified the next-generation, sequencing-by-synthesis techniques and can produce billions of base pairs in a single run.
$100
Human Genome Project completed[3]
Whole Genome Shotgun Sequence
Gene sequence stored in international public databases
First drafts of two composite haploid human genomes[1,2]
SEQUENCING BY LIGATION: This technique employed in SOLiD and Polonator instruments uses a different chemistry from previous technologies and samples every base twice, reducing the error rate.
$10
Biosciences, Oxford Nanopore and Ion Torrent say they can read from longer molecules as they pass through a pore.
$1
Billions of base pairs
300
250
200
150
100
50
0
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010

THE SUNDAY TIMES
magazine
SURVIVAL OF THE UNFITTEST

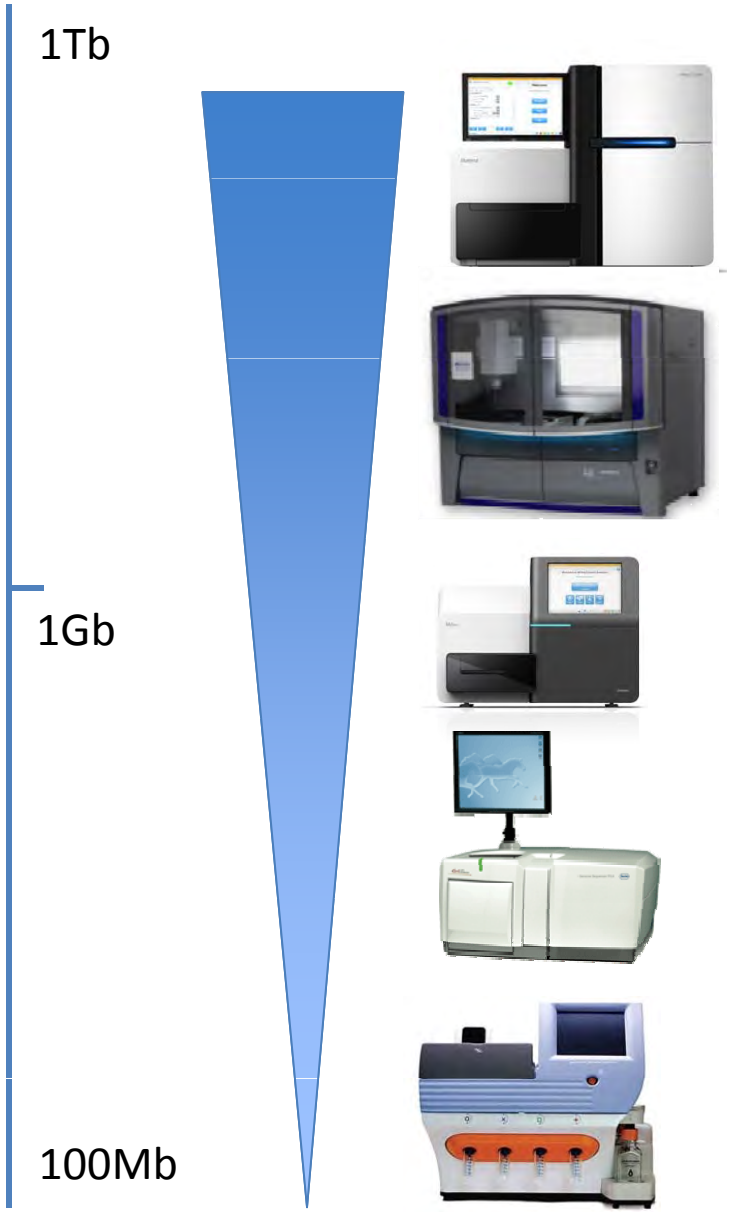
Illumina sequencing

- See separate file – Video for Slide 8

Sequencing chemistry and detection

	454	Ion Torrent	SOLiD	Illumina	PacBio	Helicos
Amplification	Emulsion PCR	Emulsion PCR	Emulsion PCR	Bridge Amplification	NA	NA
Extension	Controlled Non-competitive base extension	Controlled Non-competitive base extension	Controlled Ligation competitive oligo extension	Controlled Ligation competitive Base extension	Real-Time competitive base extension	Controlled Ligation competitive Base extension
Detection	Light emission	Charge	Fluorescent dye	Fluorescent dye	Real time video capture	Fluorescent dye

Platforms at the CGR



Illumina : de novo sequencing, resequencing

ABI-SOLiD : Resequencing, transcriptomics

Miseq : de novo, metagenomics

454 FLX+ : de novo, metagenomics, methylation

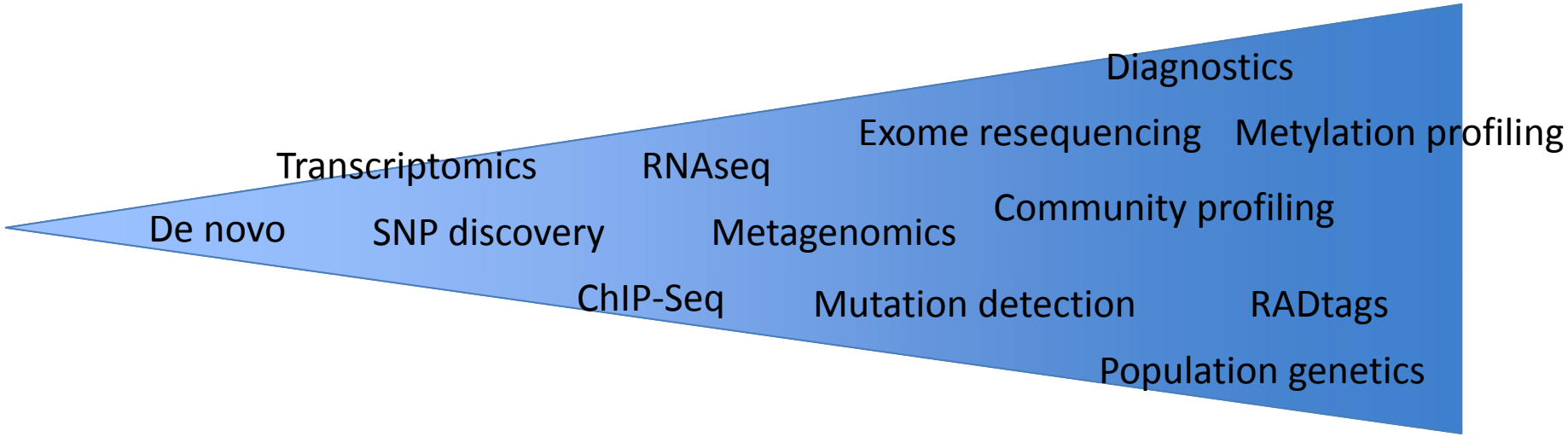
Ion torrent: de novo, amplicon

UNIVERSITY OF LIVERPOOL

Applications

10 years ago

NOW



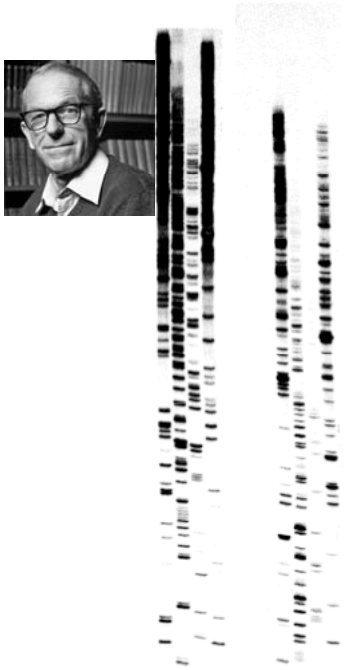
CGR

UNIVERSITY OF LIVERPOOL

Genomic Evolution

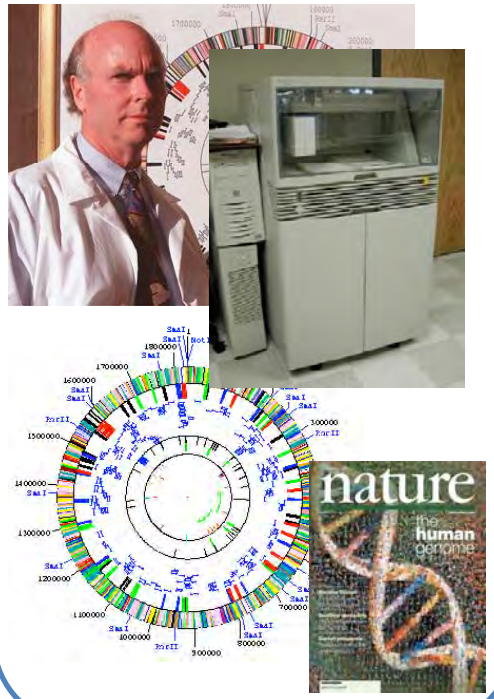
1975

Genes



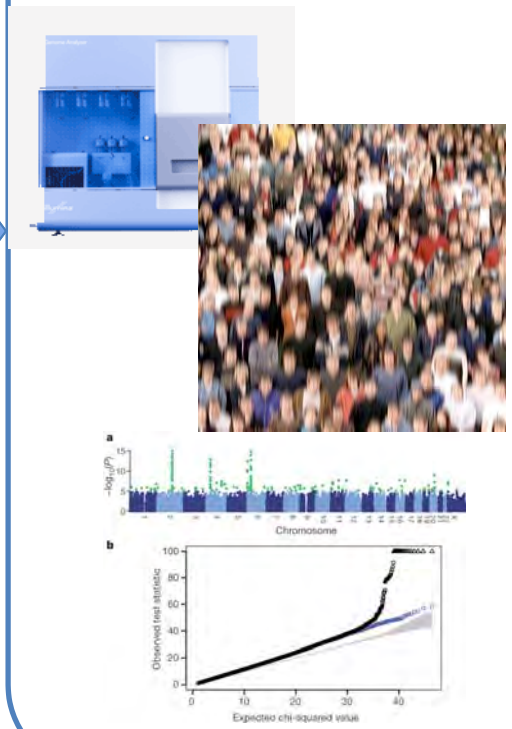
1995

Genomes



2007

Populations

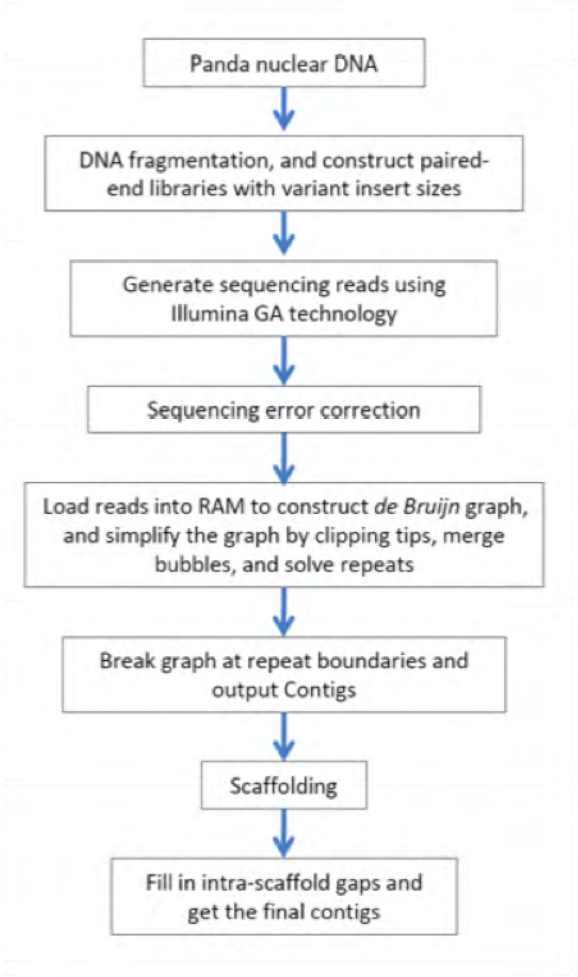


Applications

- De novo analysis
- Resequencing
- Pathogen discovery
- Metagenomics

Applications: Sequencing exotic megafauna

Li et al 2010 Nature 463:311



Applications: Pathogen de-novo

BRIEF REPORT

Open-Source Genomic Analysis of Shiga-Toxin–Producing *E. coli* O104:H4

Holger Rohde, M.D., Junjie Qin, Ph.D., Yujun Cui, Ph.D., Dongfang Li, M.E., Nicholas J. Loman, M.B., B.S., Moritz Hentschke, M.D., Wentong Chen, B.S., Fei Pu, B.S., Yangqing Peng, B.S., Junhua Li, B.E., Feng Xi, B.E., Shenghui Li, B.S., Yin Li, B.S., Zhaoxi Zhang, B.S., Xianwei Yang, B.S., Meiru Zhao, M.S., Peng Wang, B.M., Yuanlin Guan, B.E., Zhong Cen, M.E., Xiangna Zhao, B.S., Martin Christner, M.D., Robin Kobbe, M.D., Sebastian Loos, M.D., Jun Oh, M.D., Liang Yang, Ph.D., Antoine Danchin, Ph.D., George F. Gao, Ph.D., Yajun Song, Ph.D., Yingrui Li, B.S., Huanming Yang, Ph.D., Jian Wang, Ph.D., Jianguo Xu, M.D., Ph.D., Mark J. Pallen, M.D., Ph.D., Jun Wang, Ph.D., Martin Aepfelbacher, M.D., Ruifu Yang, M.D., Ph.D., and the *E. coli* O104:H4 Genome Analysis Crowd-Sourcing Consortium*

OPEN ACCESS Freely available online — PLoS one

Genome Sequence of *E. coli* O104:H4 Leads to Rapid Development of a Targeted Antimicrobial Agent against This Emerging Pathogen

Dean Scholl[1]*, Dana Gebhart[1], Steven R. Williams[1], Anna Bates[2], Robert Mandrell[2]

1 AvidBiotics Corporation, South San Francisco, California, United States of America, 2 Produce Safety and Microbiology Unit, Agricultural Research Service, Western Regional Research Center, United States Department of Agriculture, Albany, California, United States of America

Abstract

A recent widespread outbreak of *Escherichia coli* O104:H4 in Germany demonstrates the dynamic nature of emerging and re-emerging food-borne pathogens, particularly STECs and related pathogenic *E. coli*. Rapid genome sequencing and public availability of these data from the German outbreak strain allowed us to identify an O-antigen-specific bacteriophage tail spike protein encoded in the genome. We synthesized this gene and fused it to the tail fiber gene of an R-type pyocin, a phage tail-like bacteriocin, and expressed the novel bacteriocin such that the tail fiber fusion was incorporated into the bacteriocin structure. The resulting particles have bactericidal activity specifically against *E. coli* strains that produce the O104 lipopolysaccharide antigen, including the outbreak strain. This O-antigen tailspike-R-type pyocin strategy provides a platform to respond rapidly to emerging pathogens upon the availability of the pathogen's genome sequence.

In May 201
ing strain of *Escherichia coli* O104:H4 caused an outbreak of
hemolytic uremic syndrome in Germany. Although tradi-
tional culture and phenotypic tests can identify the outbreak

STEP 1
in silico
subtraction

OPEN ACCESS Freely available online — PLoS one

A Comparison of Shiga-Toxin 2 Bacteriophage from Classical Enterohemorrhagic *Escherichia coli* Serotypes and the German *E. coli* O104:H4 Outbreak Strain

Chad R. Laing[1,3], Yongxiang Zhang[1], Matthew W. Gilmour[2], Vanessa Allen[3], Roger Johnson[4], James E. Thomas[5], Victor P. J. Gannon[1]*

1 Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Lethbridge, Alberta, Canada, 2 National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada, 3 Ontario Agency for Health Protection and Promotion, Ontario, Canada, 4 Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, Ontario, Canada, 5 Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada

Abstract

Escherichia coli O104:H4 was associated with a severe foodborne disease outbreak originating in Germany in May 2011. More than 4000 illnesses and 50 deaths were reported. The outbreak strain was a typical enteroaggregative *E. coli* (EAEC) that acquired an antibiotic resistance plasmid and a Shiga-toxin 2 (Stx2)-encoding bacteriophage. Based on whole-genome phylogenies, the O104:H4 strain was most closely related to other EAEC strains; however, Stx2-bacteriophage are mobile, and do not necessarily share an evolutionary history with their bacterial host. In this study, we analyzed Stx2-bacteriophage from the *E. coli* O104:H4 outbreak isolates and compared them to all available Stx2-bacteriophage sequences. We also compared Stx2 production by an *E. coli* O104:H4 outbreak-associated isolate (ON-2011) to that of *E. coli* O157:H7 strains EDL933 and Sakai. Among the *E. coli* Stx2-phage sequences studied, that from O111:H- strain JB1-95 was most closely related phylogenetically to the Stx2-phage from the O104:H4 outbreak isolates. The phylogeny of most other Stx2-phage was largely concordant with their bacterial host genomes. Finally, O104:H4 strain ON-2011 produced less Stx2 than *E. coli* O157:H7 strains EDL933 and Sakai in culture; however, when mitomycin C was added, ON-2011 produced significantly more toxin than the *E. coli* O157:H7 strains. The Stx2-phage from the *E. coli* O104:H4 outbreak strain and the Stx2-phage from O111:H- strain JB1-95 likely share a common ancestor. Incongruence between the phylogenies of the Stx2-phage and their host genomes suggest the recent Stx2-phage acquisition by *E. coli* O104:H4. The increase in Stx2-production by ON-2011 following mitomycin C treatment may or may not be related to the high rates of hemolytic uremic syndrome associated with the German outbreak strain. Further studies are required to determine whether the elevated Stx2-production levels are due to bacteriophage or *E. coli* O104:H4 host related factors.

JCM

Real-Time Multiplex PCR for Detecting Shiga Toxin 2-Producing *Escherichia coli* O104:H4 in Human Stools

Wenlan Zhang,[a] Martina Bielaszewska,[a] Andreas Bauwens,[a] Angelika Fruth,[b] Alexander Mellmann,[a,c] and Helge Karch[a,c]

Institute for Hygiene and the National Consulting Laboratory for Hemolytic Uremic Syndrome, University of Münster, Münster, Germany[a]; National Reference Center for Salmonella and Other Bacterial Enteric Pathogens, Robert Koch Institute, Wernigerode, Germany[b]; and Interdisciplinary Center of Clinical Research (IZKF), University of Münster, Münster, Germany[c]

A real-time multiplex PCR targeting stx_2, wzy_{O104}, and $fliC_{H4}$ of enterohemorrhagic *Escherichia coli* (EHEC) O104:H4 correctly determined the presence or absence of these genes in 253 EHEC isolates and enrichment cultures of stool samples from 132 patients. It is a rapid, sensitive, and specific tool for detecting EHEC O104:H4 in human stools.

other serotypes. Genomewide comparisons were performed with the use of these enteroaggregative *E. coli* genomes, as well as those of 40 previously sequenced *E. coli* isolates.

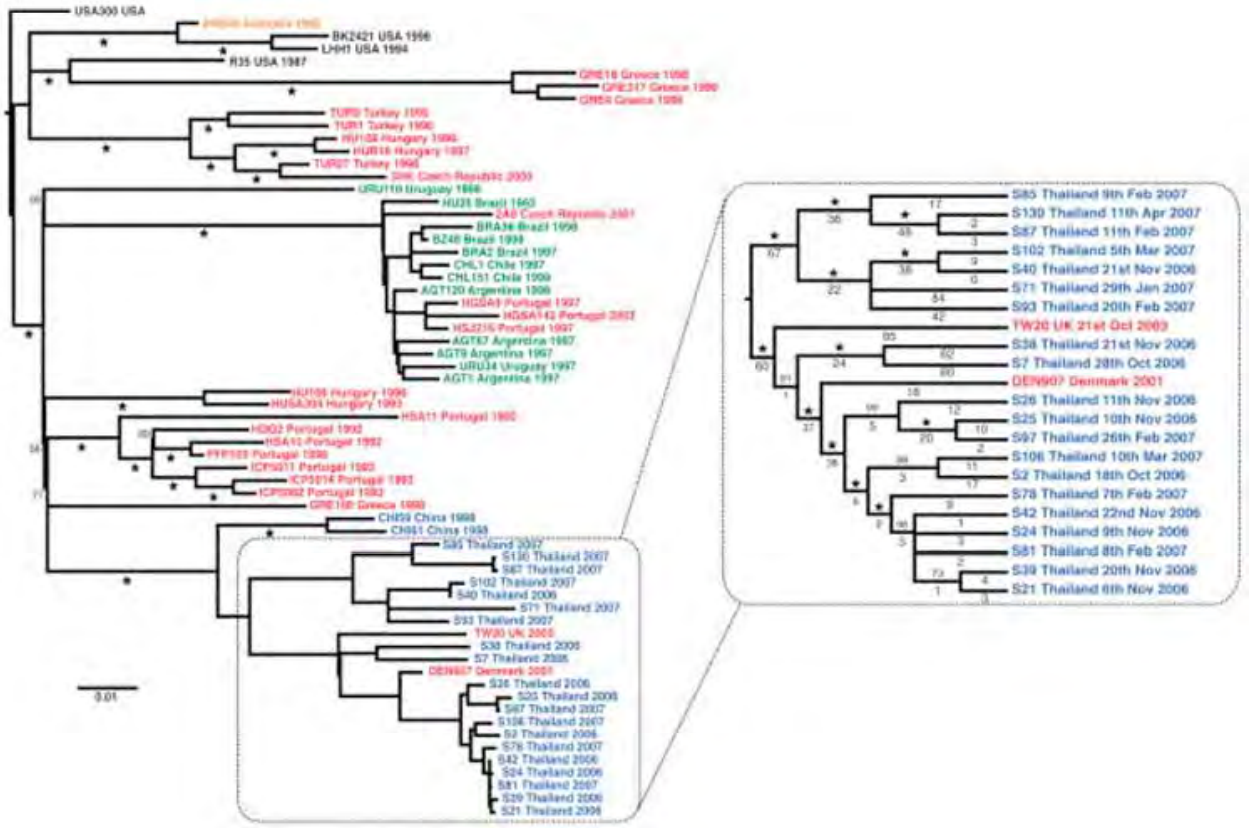
Applications: Pathogen re-sequencing

Evolution of MRSA During Hospital Transmission and Intercontinental Spread

Simon R. Harris,[1*] Edward J. Feil,[2*] Matthew T. G. Holden,[1] Michael A. Q
Emma K. Nickerson,[3,4] Narisara Chantratita,[3] Susana Gardete,[5,6] Ana Tav
Jodi A. Lindsay,[8] Jonathan D. Edgeworth,[9,10] Hermínia de Lencastre,[5,6] Ju
Sharon J. Peacock,[3,4] Stephen D. Bentley[1]†

Current methods for differentiating isolates of predominant lineages of path
do not provide sufficient resolution to define precise relationships. Here, we
throughput genomics approach that provides a high-resolution view of the e
microevolution of a dominant strain of methicillin-resistant *Staphylococcus a*
approach reveals the global geographic structure within the lineage, its inte
transmission through four decades, and the potential to trace person-to-perso
a hospital environment. The ability to interrogate and resolve bacterial popula
a range of infectious diseases, as well as microbial ecology.

The development of molecular typing techniques has been instrumental in studying the population structure and evolution of bacterial pathogens. Sequence-based approaches, such as multilocus sequence typing (MLST) (*1*), have resulted in large searchable databases of the most clinically important species. However, MLST defines variation within a very small sample of the genome and cann
closely related isolates. Ful
provides a complete inve
tionary changes, but this a
for large population sampl
generation sequencing te
Illumina Genome Analyze
mapping genome-wide si



Applications: metagenomics

Metagenomic Discovery of Biomass-Degrading Genes and Genomes from Cow Rumen

Matthias Hess,[1,2*] Alexander Sczyrba,[1,2*] Rob Egan,[1,2] Tae-Wan Kim,[3] Harshal Chokhawala,[3] Gary Schroth,[4] Shujun Luo,[4] Douglas S. Clark,[3,5] Feng Chen,[1,2] Tao Zhang,[1,2] Roderick I. Mackie,[6] Len A. Pennacchio,[1,2] Susannah G. Tringe,[1,2] Axel Visel,[1,2] Tanja Woyke,[1,2] Zhong Wang,[1,2] Edward M. Rubin[1,2]†

The paucity of enzymes that efficiently deconstruct plant polysaccharides represents a major bottleneck for industrial-scale conversion of cellulosic biomass into biofuels. Cow rumen microbes specialize in degradation of cellulosic plant material, but most members of this complex community resist cultivation. To characterize biomass-degrading genes and genomes, we sequenced and analyzed 268 gigabases of metagenomic DNA from microbes adherent to plant fiber incubated in cow rumen. From these data, we identified 27,755 putative carbohydrate-active genes and expressed 90 candidate proteins, of which 57% were enzymatically active against cellulosic substrates. We also assembled 15 uncultured microbial genomes, which were validated by complementary methods including single-cell genome sequencing. These data sets provide a substantially expanded catalog of genes and genomes participating in the deconstruction of cellulosic biomass.

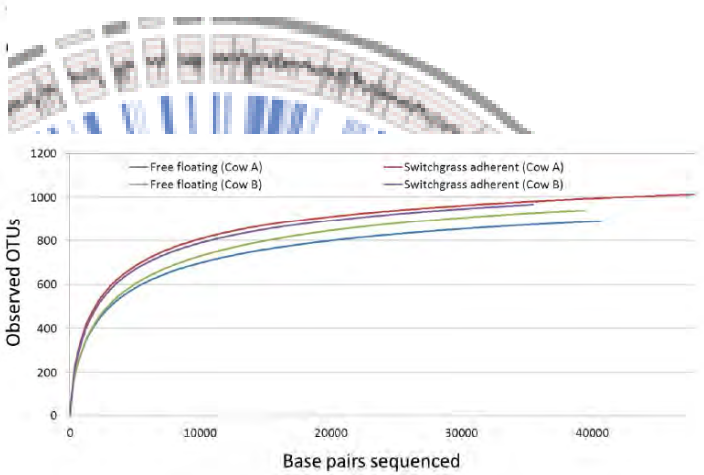
Biofuels derived from lignocellulosic plant material represent an important renewable energy alternative to transportation fossil fuels (*1*, *2*). A major obstacle to industrial-scale production of fuel from lignocellulose lies in the inefficient deconstruction of plant material, owing to the recalcitrant nature of the substrate toward enzymatic breakdown and the relatively low activity of currently available hydrolytic enzymes. Although the success of protein engineering to improve the performance of existing lignocellulose-degrading enzymes has been limited (*3*), retrieving enzymes from naturally evolved biomass-degrading microbial communities offers a promising strategy for the identification of new lignocellulolytic enzymes with potentially improved activities (*4*).

Metagenomics, the direct analysis of DNA from environmental samples, represents a strategy for discovering diverse enzymes encoded in nature (*5*, *6*). Although metagenomics has been used

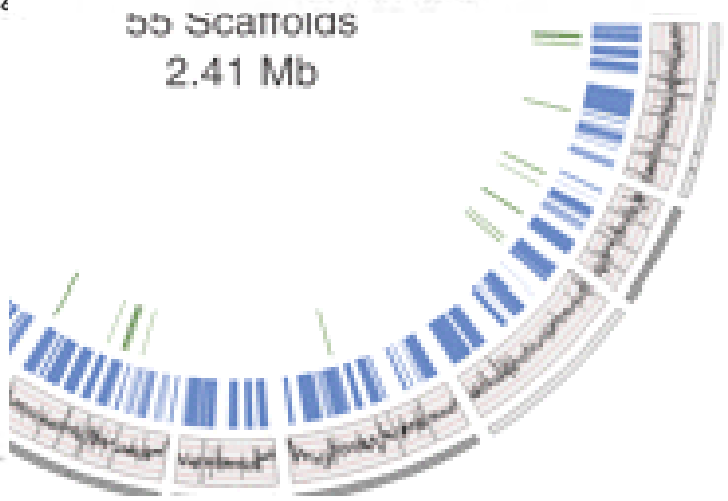
[1]Department of Energy, Joint Genome Institute, Walnut Creek, CA 94598, USA. [2]Genomics Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA. [3]Energy Biosciences Institute, University of California, Berkeley, CA 94720, USA. [4]Illumina Inc., Hayward, CA 94545, USA. [5]Department of Chemical and Biomolecular Engineering, University of California, Berkeley, CA 94720, USA. [6]Department of Animal Sciences, Institute for Genomic Biology and Energy Biosciences Institute, University of Illinois, Urbana, IL 61801, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: emrubin@lbl.gov

www.sciencemag.org SCIENCE VOL 331 28 JANUARY 2011 46



Hess et al 2001 Science 331:463



Applications: Pathogen discovery

OPEN ACCESS Freely available online

PLoS one

Discovery of DNA Viruses in Wild-Caught M
Using Small RNA High throughput Sequenc

Maijuan Ma[1], Yong Huang[1], Zhengda Gong[2], Lu Zhuang[1], Cun Li[1], Hong Yang[1], Y
Wuchun Cao[1]

1 State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing, China, 2 Yunnan In
Prevention, Dali, Yunnan, China

Abstract

Background: Mosquito-borne infectious diseases pose a severe threat to public health in many a
methods for pathogen detection and surveillance are usually dependent on prior knowledge
involved. Hence, efficient approaches are required for screening wild mosquito populations to de
pathogens.

Methodology/principal findings: In this study, we explored the use of Next Generation Sequenci
in wild-caught mosquitoes. We extracted total RNA from different mosquito species from Sou
length RNA molecule
Bioinformatic analyse
non-enveloped single
viral transcripts (.>80
obtained. The +/− st
mainly derived from
genome sequence. W
where no transcripts

OPEN ACCESS Freely available online

Newly Discovered Ebola Virus
Hemorrhagic Fever Outbreak

Jonathan S. Towner[1], Tara K. Sealy[1], Marina L. Khristova[2], César G. Albariño
Reeder[1], Phenix-Lan Quan[3], W. Ian Lipkin[3], Robert Downing[4], Jordan W. Ta
Julius Lutwama[6], Barnabas Bakamutumaho[6], John Kayiwa[6], James A. Come
Thomas G. Ksiazek[1], Stuart T. Nichol[1]*

1 Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, 2 Scie
Control and Prevention, Atlanta, Georgia, United States of America, 3 Center for Infection and Immunity, Mailman School of
New York, United States of America, 4 Global AIDS Program, Centers for Disease Control and Prevention, Entebbe, Ugan
Kampala, Uganda, 6 Uganda Virus Research Institute, Entebbe, Uganda

Abstract

Over the past 30 years, *Zaire* and *Sudan ebolaviruses* have been responsible for large hemorr
case fatalities ranging from 53% to 90%, while a third species, *Côte d'Ivoire ebolavirus*, cause
November 2007, HF cases were reported in Bundibugyo District, Western Uganda. Laborato
suspect-case blood specimens by classic methods (antigen capture, IgM and IgG ELISA) and
primed pyrosequencing approach quickly identified this to be an Ebola HF outbreak asso
ebolavirus species (*Bundibugyo ebolavirus*) distantly related to the *Côte d'Ivoire ebolavirus* fou
sequence divergence of this new virus relative to all previously recognized ebolaviruses,
implications for design of future diagnostic assays to monitor Ebola HF disease in humans a
to develop effective antivirals and vaccines.

Identification of a Severe Acute Respiratory Syndrome Coronavirus-Like Virus in a Leaf-Nosed Bat in Nigeria

Phenix-Lan Quan,[a] Cadhla Firth,[a] Craig Street,[a] Jose A. Henriquez,[a] Alexandra Petrosov,[a] Alla Tashmukhamedova,[a]
Stephen K. Hutchison,[b] Michael Egholm,[b] Modupe O. V. Osinubi,[c] Michael Niezgoda,[c] Albert B. Ogunkoya,[d] Thomas Briese,[a]
Charles E. Rupprecht,[c] and W. Ian Lipkin[a]

Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, New York, USA[a]; 454 Life Sciences, Branford, Connecticut, USA[b]; Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA[c]; and Department of Veterinary Surgery and Medicine, Ahmadu Bello University, Zaria, Nigeria[d]

ABSTRACT Bats are reservoirs for emerging zoonotic viruses that can have a profound impact on human and animal health, including lyssaviruses, filoviruses, paramyxoviruses, and severe acute respiratory syndrome coronaviruses (SARS-CoVs). In the course of a project focused on pathogen discovery in contexts where human-bat contact might facilitate more efficient interspecies transmission of viruses, we surveyed gastrointestinal tissue obtained from bats collected in caves in Nigeria that are frequented by humans. Coronavirus consensus PCR and unbiased high-throughput pyrosequencing revealed the presence of coronavirus sequences related to those of SARS-CoV in a Commerson's leaf-nosed bat (*Hipposideros commersoni*). Additional genomic sequencing indicated that this virus, unlike subgroup 2b CoVs, which includes SARS-CoV, is unique, comprising three overlapping open read
conjunction with thes

JVI
Journals.ASM.org

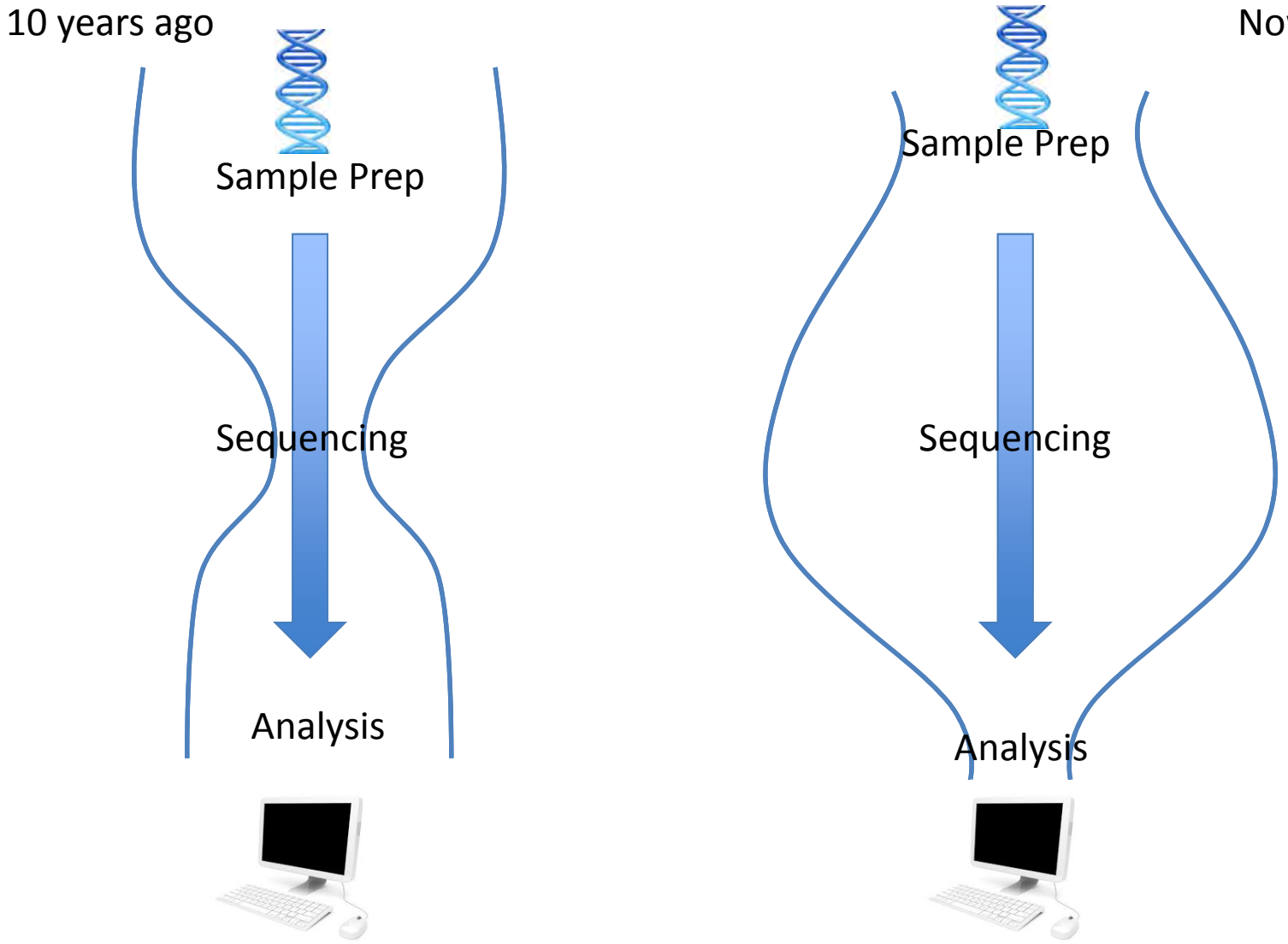
Identification of a Novel Feline Picornavirus from the Domestic Cat

Susanna K. P. Lau,[a,b,c,d] Patrick C. Y. Woo,[a,b,c,d] Cyril C. Y. Yip,[d] Garnet K. Y. Choi,[d] Ying Wu,[d] Ru Bai,[d] Rachel Y. Y. Fan,[d]
Kenneth K. Y. Lai,[d] Kwok-Hung Chan,[d] and Kwok-Yung Yuen[a,b,c,d]

State Key Laboratory of Emerging Infectious Diseases,[a] Research Centre of Infection and Immunology,[b] Carol Yu Centre for Infection,[c] and Department of Microbiology,[d] The University of Hong Kong, Hong Kong, Hong Kong

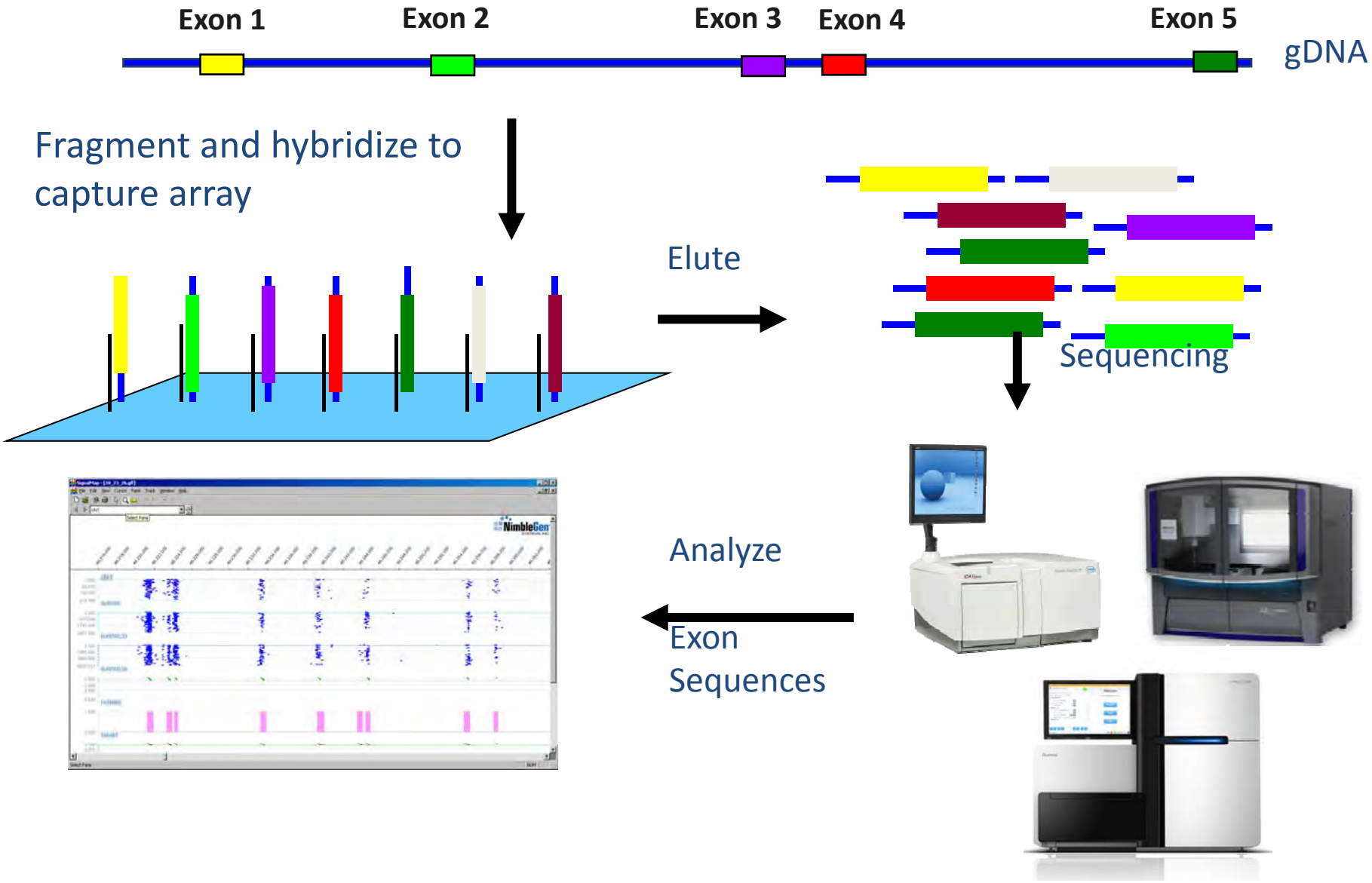
While picornaviruses are known to infect different animals, their existence in the domestic cat was unknown. We describe the discovery of a novel feline picornavirus (FePV) from stray cats in Hong Kong. From samples from 662 cats, FePV was detected in fecal samples from 14 cats and urine samples from 2 cats by reverse transcription-PCR (RT-PCR). Analysis of five FePV genomes revealed a distinct phylogenetic position and genomic features, with low sequence homologies to known picornaviruses especially in leader and 2A proteins. Among the viruses that belong to the closely related bat picornavirus groups 1 to 3 and the genus *Sapelovirus*, G+C content and sequence analysis of P1, P2, and P3 regions showed that FePV is most closely related to bat picornavirus group 3. However, FePV possessed other distinct features, including a putative type IV internal ribosome entry site/segment (IRES) instead of type I IRES in bat picornavirus group 3, protein cleavage sites, and H-D-C catalytic triad in $3C^{pro}$ different from those in sapeloviruses and bat picornaviruses, and the shortest leader protein among known picornaviruses. These results suggest that FePV may belong to a new genus in the family *Picornaviridae*. Western blot analysis using recombinant FePV VP1 polypeptide showed a high seroprevalence of 33.6% for IgG among the plasma samples from 232 cats tested. IgM was also detected in three cats positive for FePV in fecal samples, supporting recent infection in these cats. Further studies are important to understand the pathogenicity, epidemiology, and genetic evolution of FePV in these common pet animals.

Process bottlenecks have changed



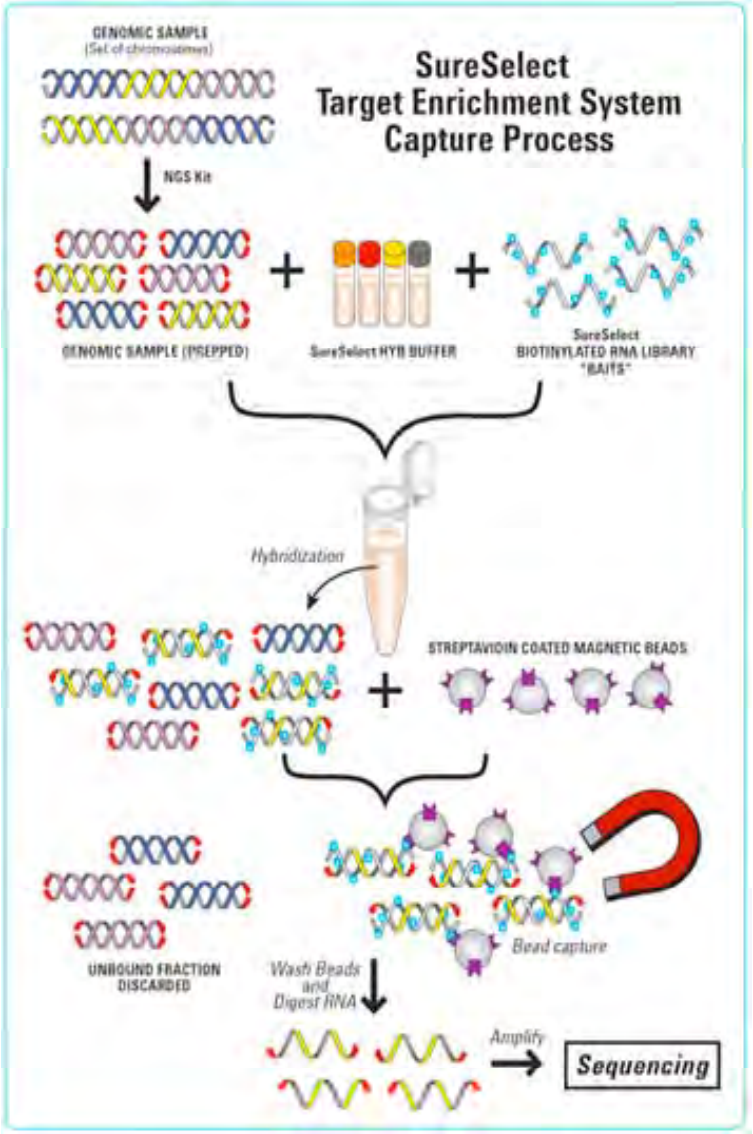
Targeted sequencing

Genome enrichment/ Exome Sequencing

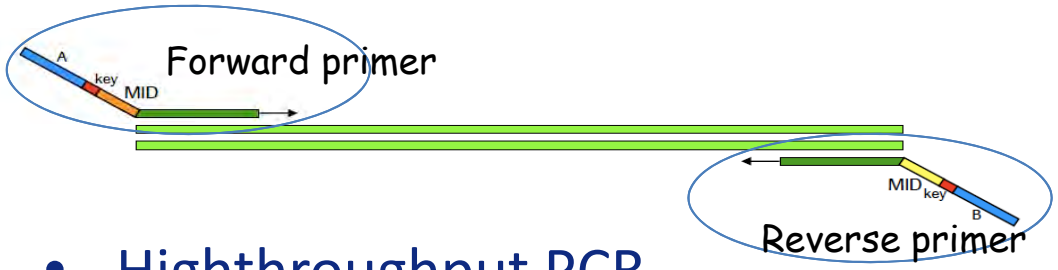


Solution based- Genome enrichment

- Biotinylated Probes (RNA or DNA) hybridies to target
- Removed on strepdavidin coated beads



Genome capture -PCR

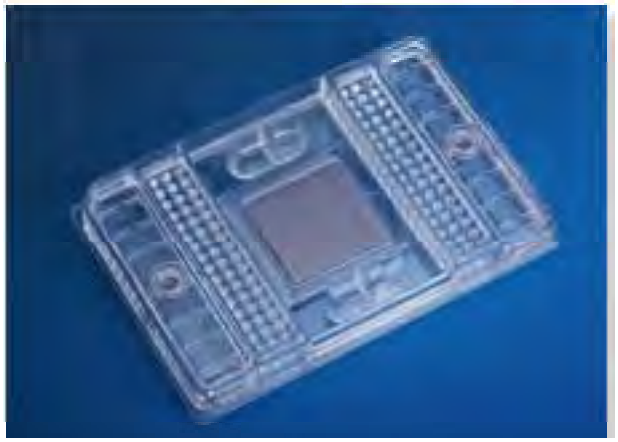


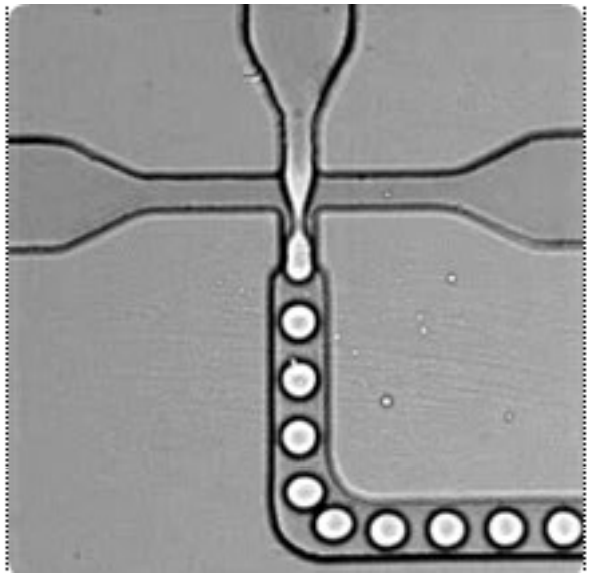
- Highthroughput PCR platforms
  - Fluidigm – 48 by 48 PCRs in a microfluidic array.

    http://www.fluidigm.com/

  - Raindance –microfluidic generation of primers in an emulsion allowing multiplexing in a single tube.

    http://www.raindancetechnologies.com/





Targeted sequencing: Fatal foal immunodeficiency in the fell pony

OPEN ACCESS Freely available online PLOS GENETICS

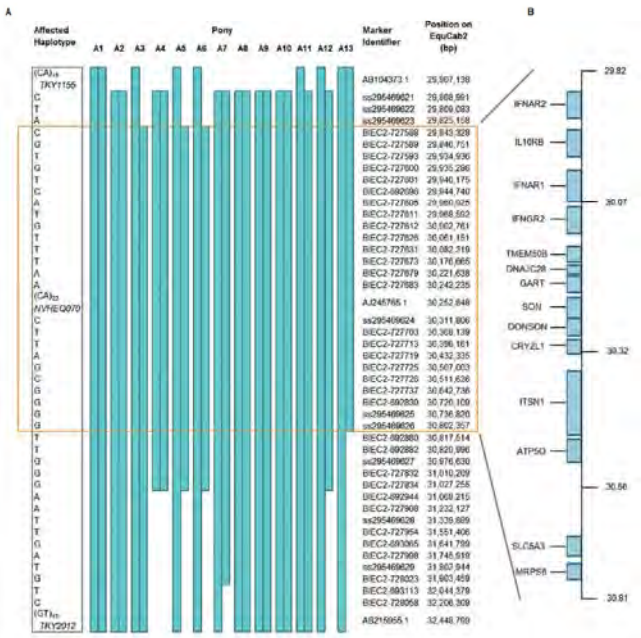
Identification of a Mutation Associated with Fatal Foal Immunodeficiency Syndrome in the Fell and Dales Pony

Laura Y. Fox-Clipsham[1], Stuart D. Carter[2], Ian Goodhead[3], Neil Hall[3], Derek C. Knottenbelt[4], Paul D. F. May[5], William E. Ollier[6], June E. Swinburne[1]*

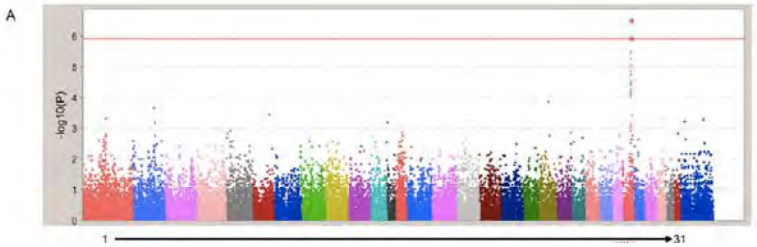
1 Animal Health Trust, Newmarket, Suffolk, United Kingdom, 2 Department of Infection Biology, School of Veterinary Science, University of Liverpool, Liverpool, United Kingdom, 3 Centre for Genomic Research, Institute of Integrative Biology, University of Liverpool, Liverpool, United Kingdom, 4 Department of Veterinary Clinical Science Equine Hospital, University of Liverpool, Liverpool, United Kingdom, 5 Townhead Veterinary Centre, Townhead Farm, Penrith, United Kingdom, 6 Genomic Medical Research, University of Manchester, Manchester, United Kingdom

Abstract

The Fell and Dales are rare native UK pony breeds at risk due to falling numbers, in-breeding, an... Specifically, the lethal Mendelian recessive disease Foal Immunodeficiency Syndrome (FIS), which manife... immunodeficiency and progressive anemia, is a substantial threat. A significant percentage (~10%) of ... each year dies from FIS, compromising the long-term survival of this breed. Moreover, the likely spre... breeds is of major concern. Indeed, FIS was identified in the Dales pony, a related breed, during the ... Using a stepwise approach comprising linkage and homozygosity mapping followed by haplotype anal... mutation using 14 FIS-affected, 17 obligate carriers, and 10 adults of unknown carrier status to a ~... 30.8 Mb) on chromosome (ECA) 26. A subsequent genome-wide association study identified two S... showed genome-wide significance after Bonferroni correction for multiple testing: BIEC2-692674 at 29... 693138 at 32.19 Mb. The associated region spanned 2.6 Mb from ~29.6 Mb to 32.2 Mb on ECA26. R... region identified a mutation in the sodium/myo-inositol cotransporter gene (*SLC5A3*); this causes a P446... protein. This gene plays a crucial role in the regulatory response to osmotic stress that is essential in ma... lymphoid tissues and during early embryonic development. We propose that the amino acid substitut... alters the function of SLC5A3, leading to erythropoiesis failure and compromise of the immune system... biological interest as it is unique and is caused by a gene not previously associated with a mamma... identified the associated gene, we are now able to eradicate FIS from equine populations by informed...



Identifying the Mutation in FIS



Orthologs

Horse	SLC5A3 (FIS)	QMYLYIQEVADYLTP**L**VAALFLLAIFWKRCN
Horse	SLC5A3 (wt)	QMYLYIQEVADYLTP**P**VAALFLLAIFWKRCN
Human	SLC5A3	QMYLYIQEVADYLTP**P**VAALFLLAIFWKRCN
Mouse	SLC5A3	QMYLYIQEVADYLTP**P**VAALFLLAIFWKRCN
Dog	SLC5A3	QMYLYIQEVADYLTP**P**VAALFLLAIFWKRCN
Cow	SLC5A3	QMYLYIQEVADYLTP**P**VAALFLLAIFWKRCN

Overview

- Sequencing technology is evolving incredibly rapidly
- Is being applied as an assay in many different types of study
- In veterinary research has applications in
  - Infection
    - Epidemiology
    - Diagnosis
    - discovery
  - Metagenomics
  - Genetic disease
- Bottlenecks are in sample prep and analysis
- Scale Up! Think of an impossible experiment and it may well be possible.

Next Generation Sequencing – The Role of New Sequence Technologies in Shaping the Future of Veterinary Science

Hosted by the RCVS Charitable Trust

RCVS charitable trust