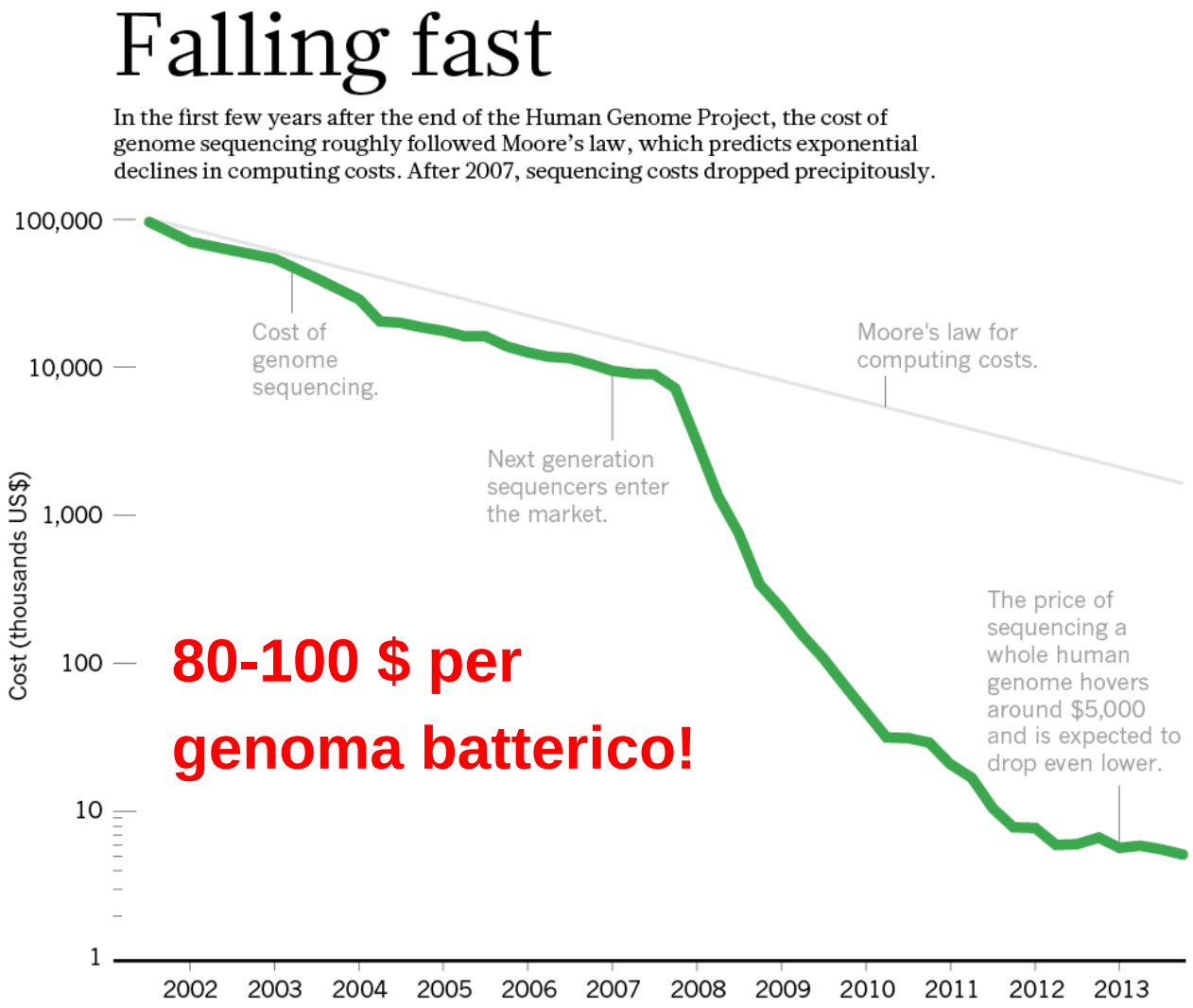
Next generation sequencing: uno strumento innovativo nella lotta ai microrganismi patogeni

Valeria Michelacci

Roma, 23 Ottobre 2018

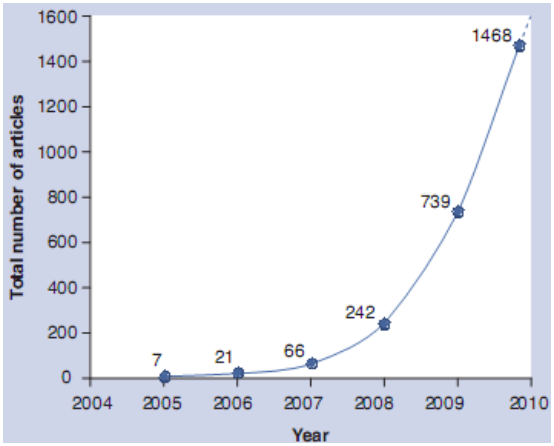
Costi associati al sequenziamento di DNA



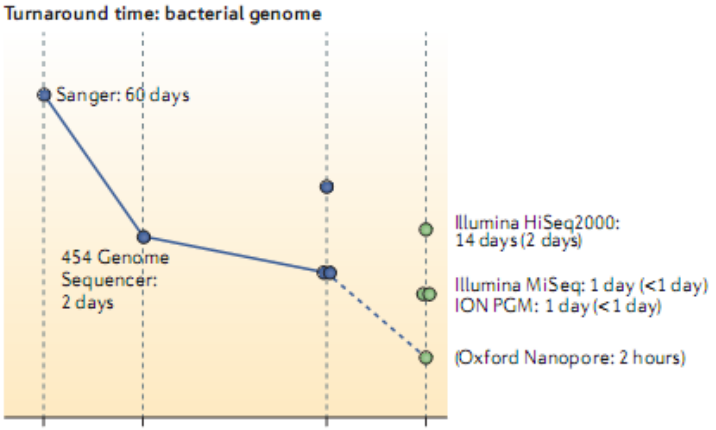
80-100 $ per genoma batterico!

Benefits from NGS

Massive sequence output & low cost/base



Su Z. et al Expert Rev Mol Diagn. 2011 Apr;11(3):333-43.



Didelot X et al. Nat Rev Genet. 2012 Aug 14;13(9):601-12.

NGS in Microbiology

Particularly attractive:

Ability to generate large quantity of **starting material**

Small microbial genomes

Application in microbiology:

Multiple resistance determinants

Epidemiological markers

Virulence factors

Typing based on the SNPs in the WGS

Metodi classici VS NGS

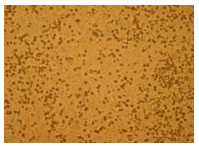
Isolamento del patogeno

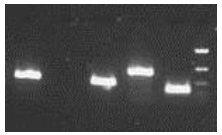
Identificazione della specie

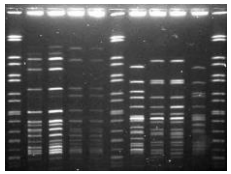
Sensibilità agli antimicrobici

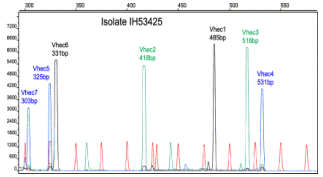
Determinazione del potenziale patogeno

Correlazione con altri ceppi patogeni della sua specie (PFGE, MLST, MLVA…)









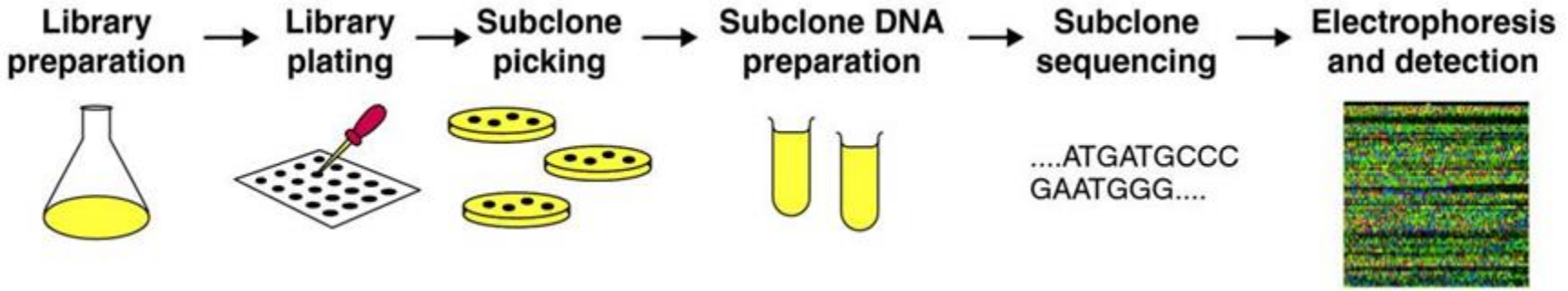
Processo multistep

giorni
---
settimane

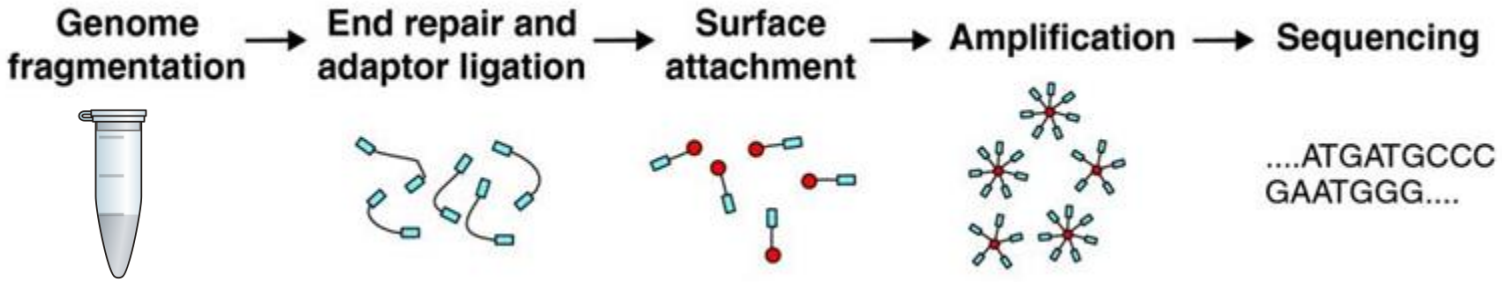
Possibilità di ottenere tutti i risultati con un solo esperimento di sequenziamento!

Sequenziamento convenzionale & NGS

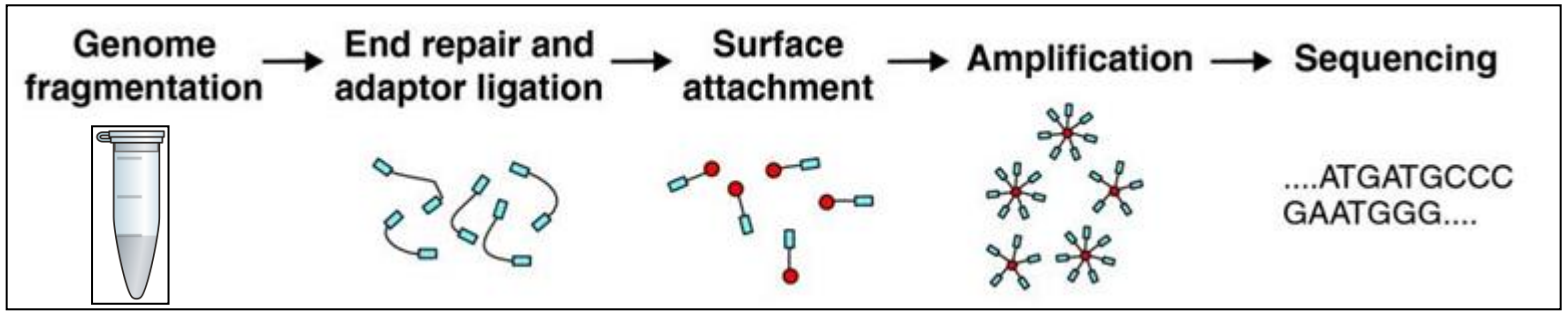
Convenzionale



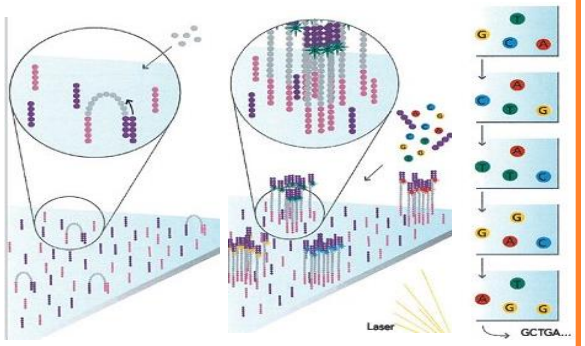
NGS



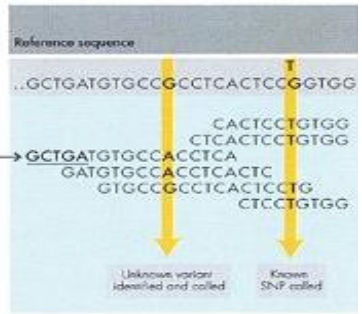
Next generation sequencing



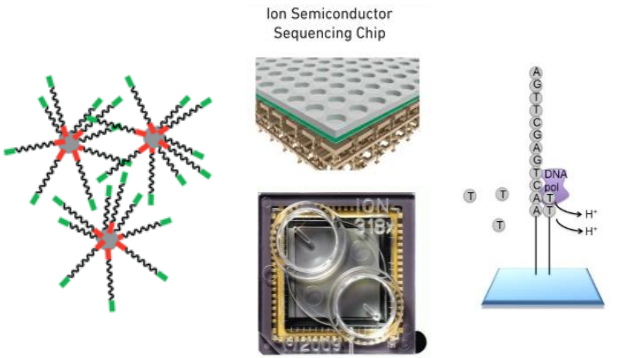
illumina MiSeq



Cattura dell'immagine
Registrazione della fluorescenza

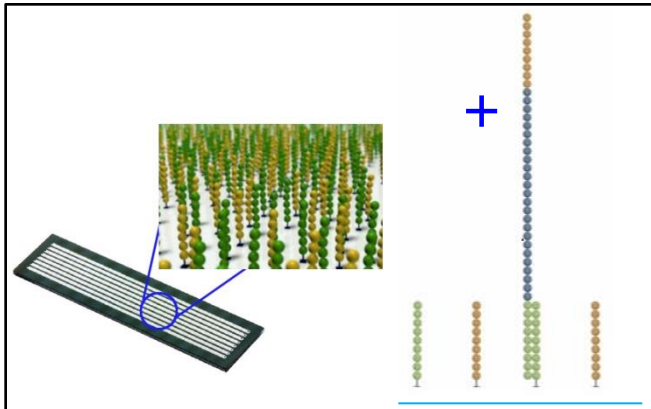


200bp-400bp short reads

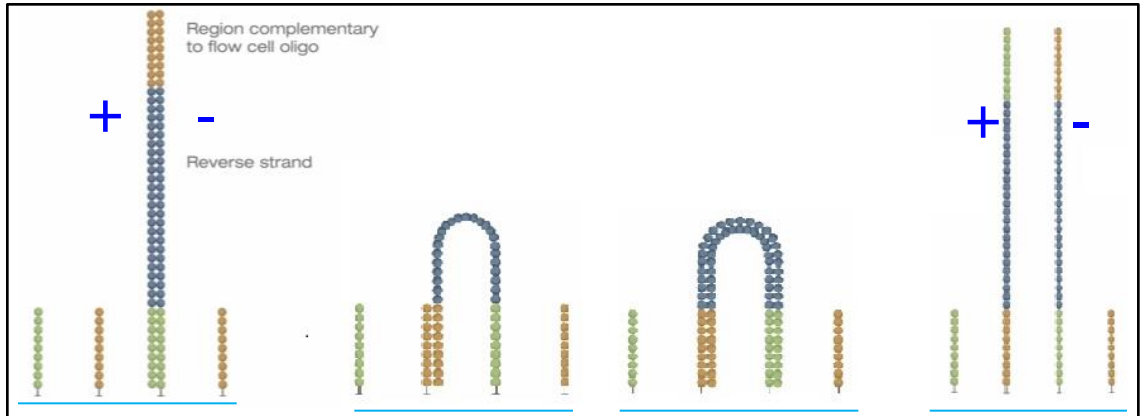


Variazione di pH per incorporazione di nt nel filamento crescente

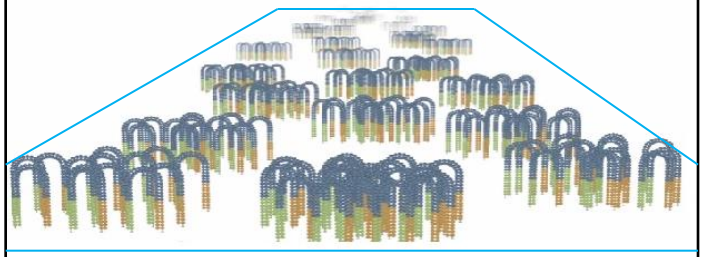
Illumina Sequencing by Synthesis/1



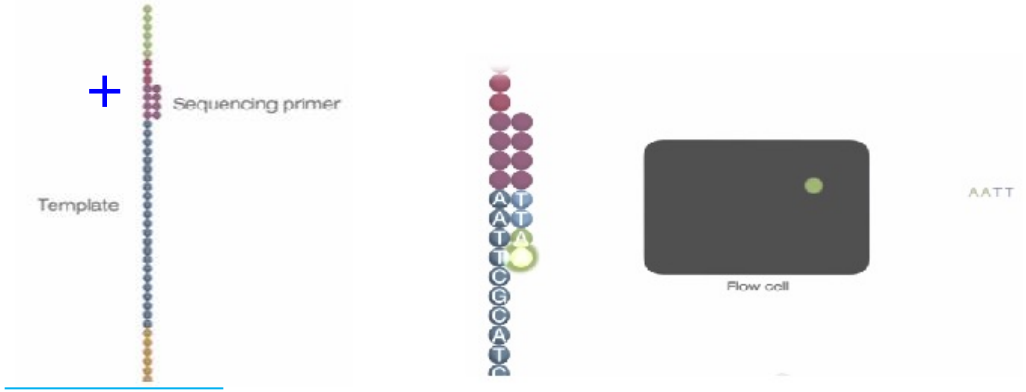
1 Flow cell ibridization



2 Cluster amplification in one position

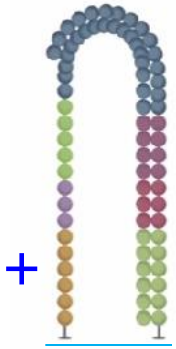


3 Cluster amplification throughout the flow cell



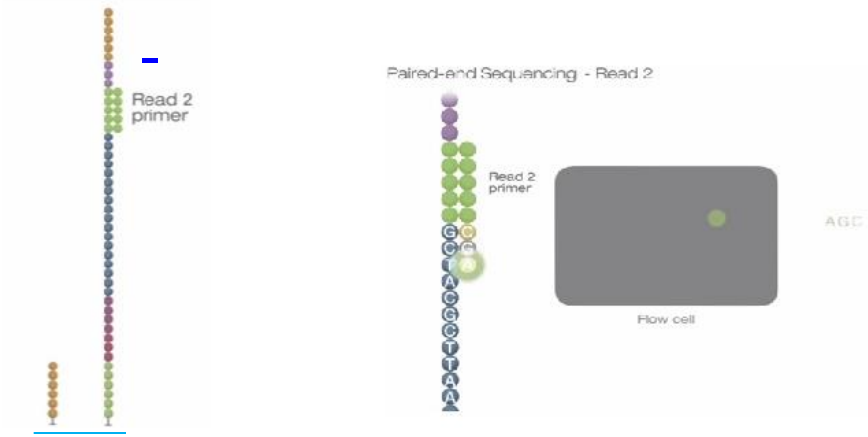
4 Read 1 sequencing (+); the sequenced strand is washed out

Illumina Sequencing by Synthesis/2



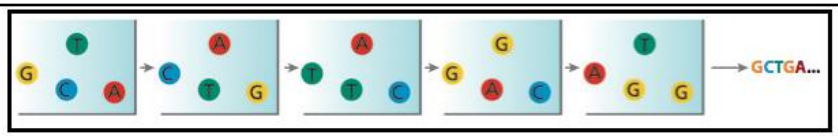
Synthesis of strand -. The strand + is cleaved and washed out

5



Read 2 sequencing (-)

6



strainname_R1.fastq

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+
BA1@CC7CBCCC9C8;B2@>C?B@B@B3=9?@B1:AB7B?B8B?B6B.7.
@read2
TTGGGCGGGATCTCCAGAAGCATATGGATGTGATCCACACAGCATTCTGC
+
?>?B@)<?@,AA7A@C<C?=@@B;+)?B5*@2=@+=BB,=B6C>AB@B24
@read3
TATGCTCAAGAAGGGGCTGATGAGTTGGTGTTTTACGATATCACTGCCTC
+
A3AB:B1:B;9/0BBBCBB<BB@AAO?BB9:BB<A@BB@7@6@<A@@@<3
```

strainname_R2.fastq

```
@read1
AGCTTATCCTCTGCTCACCCCCGGGTTAGCGCACTTGATGTATTCACAGC
+
BA1@CC7CBCCC9C8;B2@>C?B@B@B3=9?@B1:AB7B?B8B?B6B.7.
@read2
TTGGGCGGGATCTCCAGAAGCATATGGATGTGATCCACACAGCATTCTGC
+
?>?B@)<?@,AA7A@C<C?=@@B;+)?B5*@2=@+=BB,=B6C>AB@B24
@read3
TATGCTCAAGAAGGGGCTGATGAGTTGGTGTTTTACGATATCACTGCCTC
+
A3AB:B1:B;9/0BBBCBB<BB@AAO?BB9:BB<A@BB@7@6@<A@@@<3
```

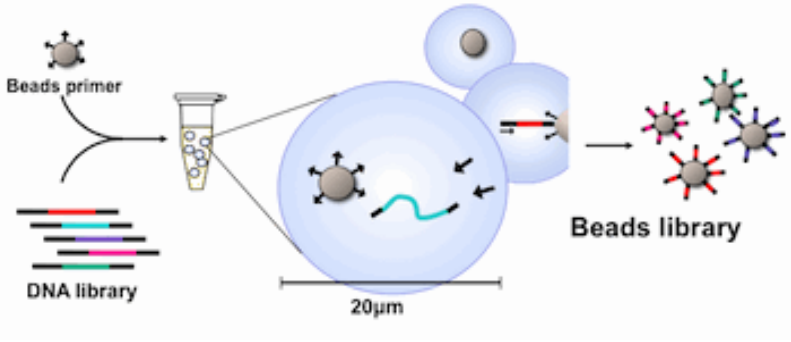
FastQ files compiling

7

Ion Torrent semiconductor sequencing/1

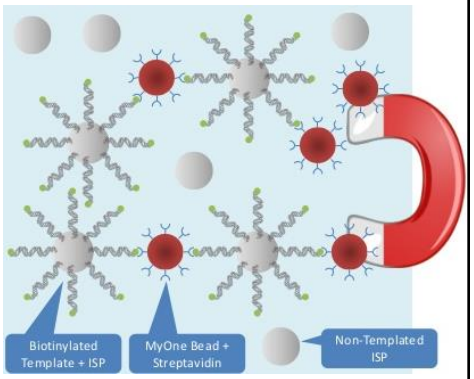
Shearing and adapter ligation

1



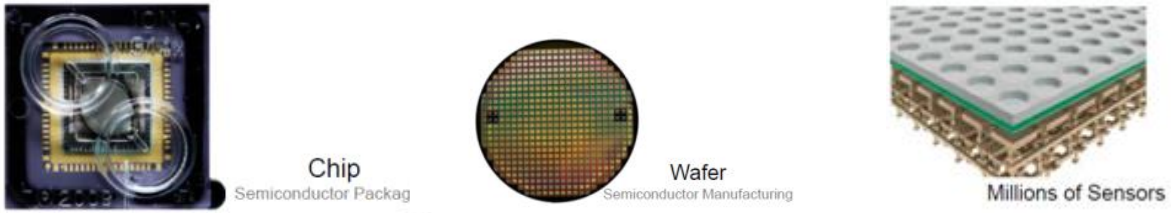
Equimolar emulsion PCR

2



Magnetic enrichment step for loaded spheres

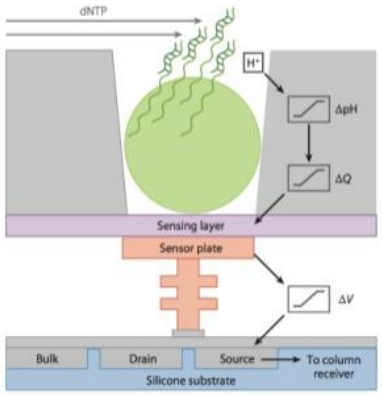
3

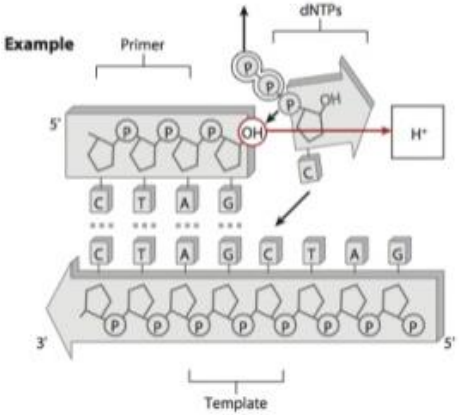


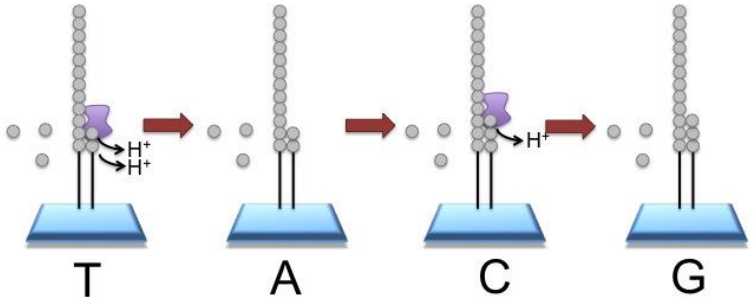
Chip loading

4

Ion Torrent semiconductor sequencing/2

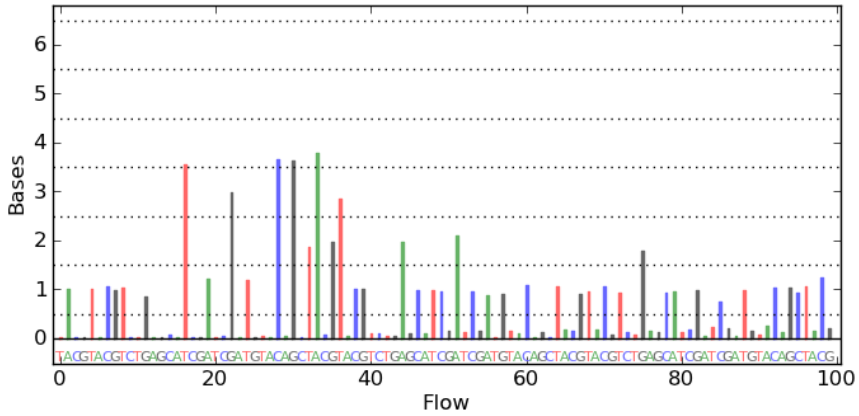






5

dNTPs flow and pH monitoring




```
@read1
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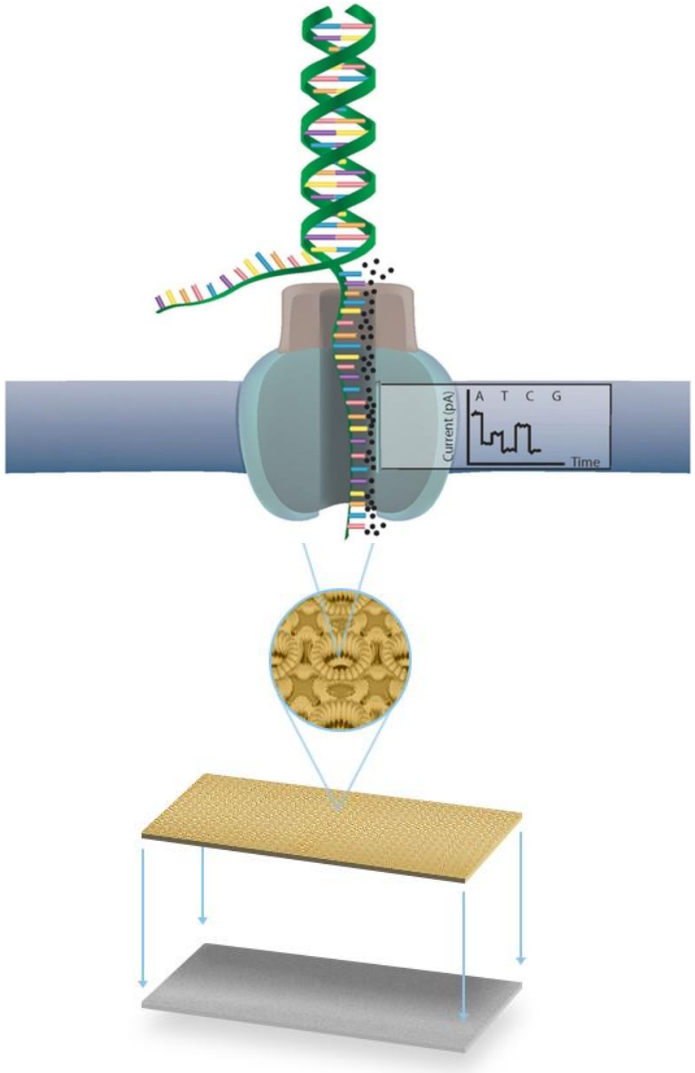
+
BA1@CC7CBCCC9C8;B2@>C?B@B@B3=9?@B1:AB7B?B8B?B6B.7.
@read2
TTGGGCGGGATCTCCAGAAGCATATGGATGTGATCCACACAGCATTCTGC
+
?>?B@)<?@,AA7A@C<C?=@@B;+)?B5*@2=@+=BB,=B6C>AB@B24
@read3
TATGCTCAAGAAGGGGCTGATGAGTTGGTGTTTTACGATATCACTGCCTC
+
A3AB:B1:B;9/0BBBCBB<BB@AA0?BB9:BB<A@BB@7@6@<A@@@<3
```

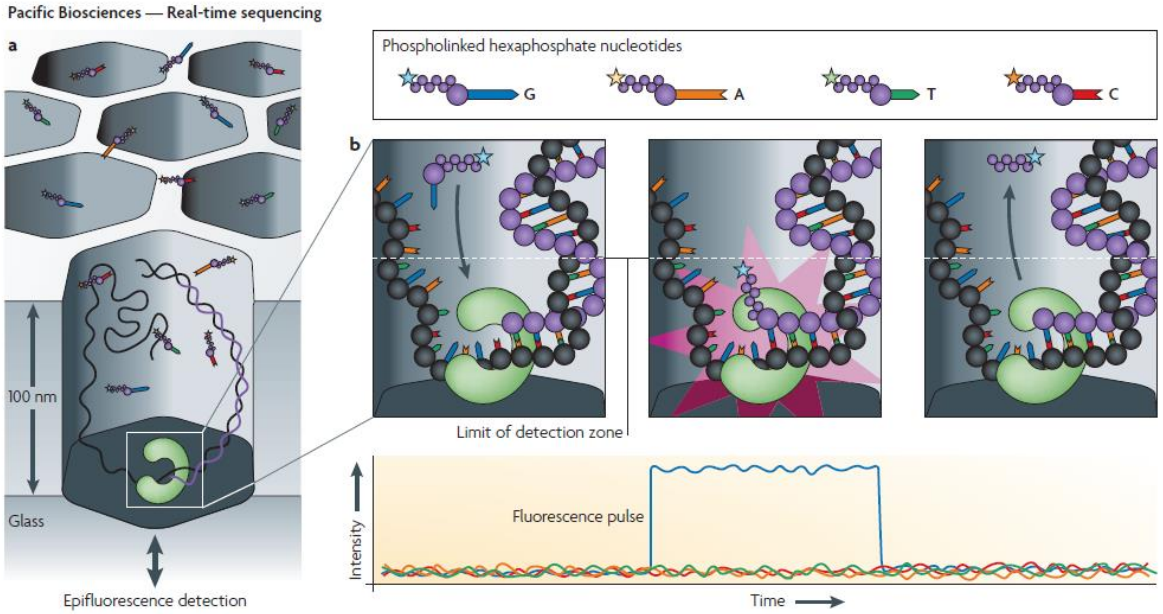
6

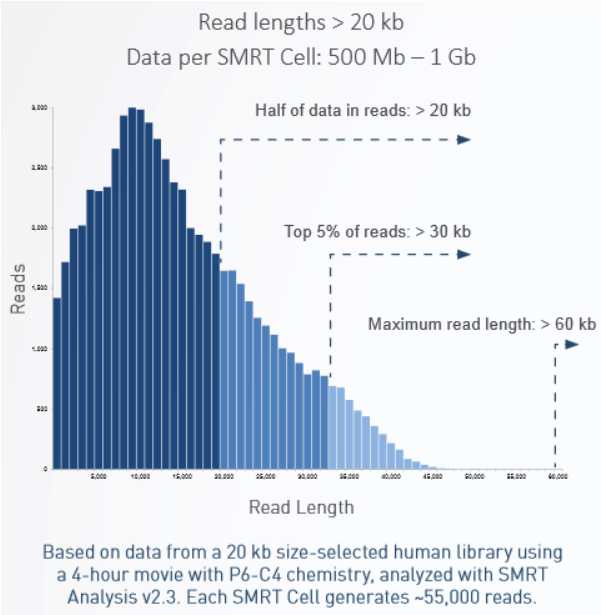
Flow diagram interpretation and fastQ file compiling

MinION - Oxford Nanopore Technologies

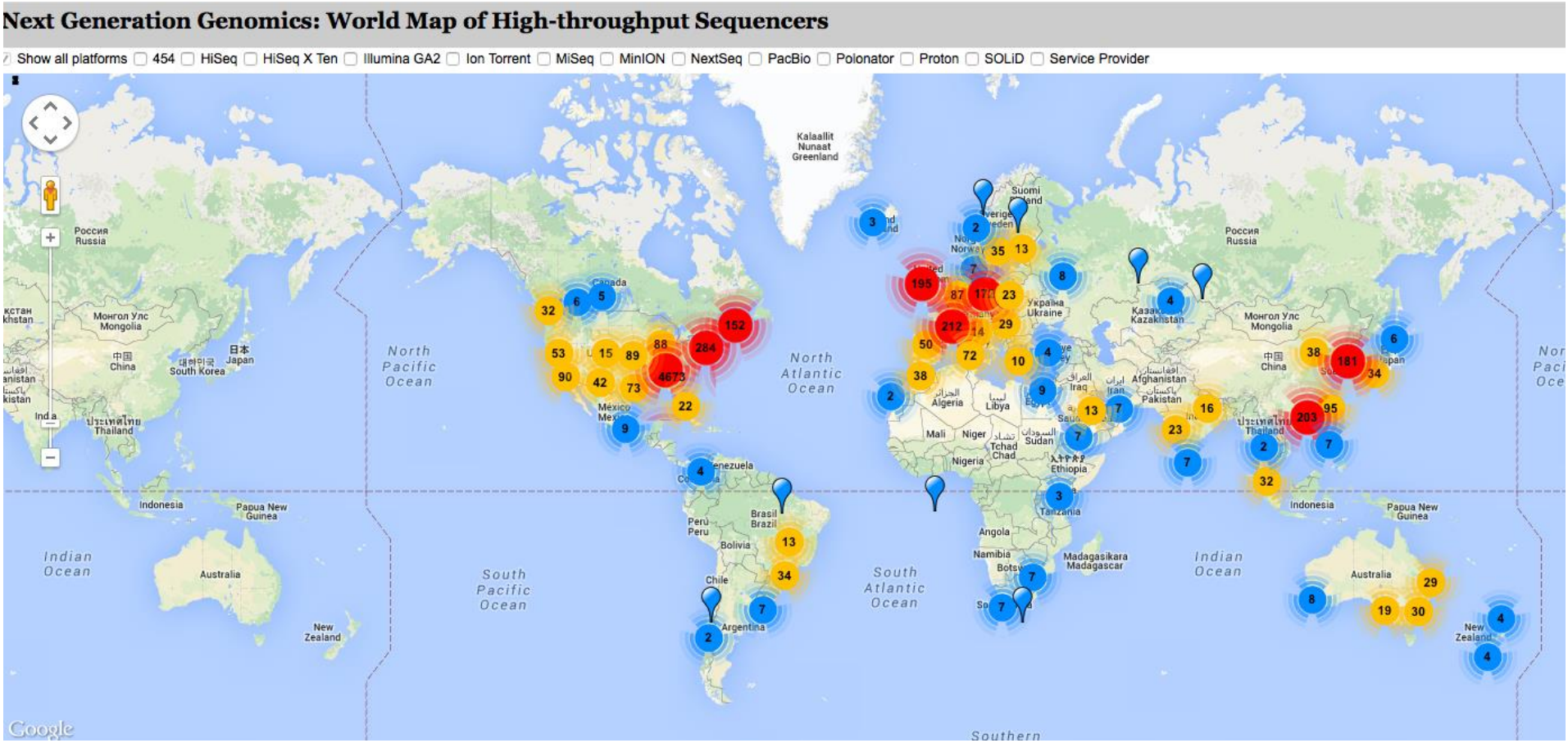


Pacific Biosciences



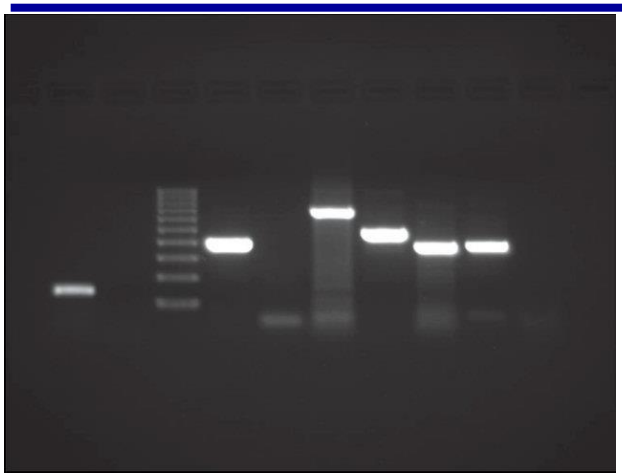


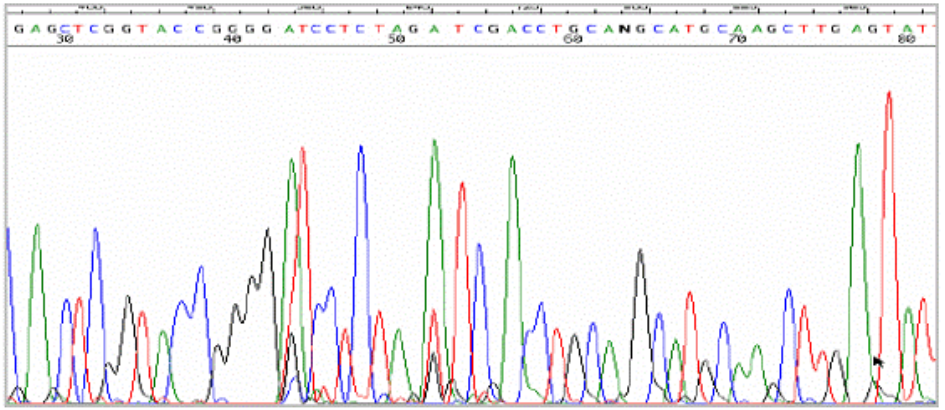
NGS equipments adoption

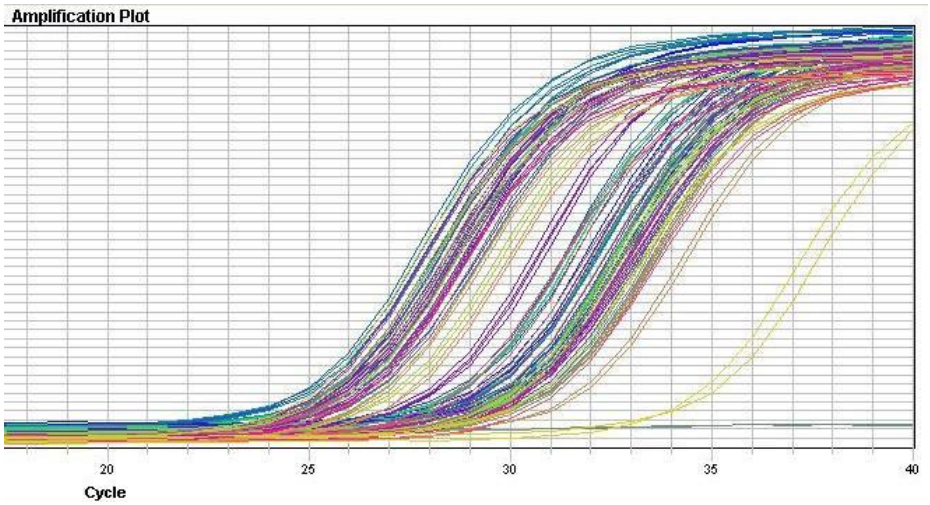


on: Omicsmap.com June, 17, 2015

Data Analysis: A new syntax







Mobio_16Run_400_hiq_Pool17062015.fastq

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+
BBCDAC6;;;/;CC8CCCCCC?>??C@CCEEDDD;;;;<<</<<<7<<<<<.;BAA@CCCADDABCDCCCCCCC@@@@@.;;
1;;?CACC???CCCCACCA?;;:=9=>CACE@CC@CC>CCCCC;>?CCACACAA=@@>B;::@288888:@=888?,82::?
D<BBB=CB@5828=CBBC?CC?;;>:>7<9B>=@@@A:@BBAA??;??
B@@8888*888*8<A=A2848888=:::@@=@@@CB?;;8;?BBBB@@@ABBD3:2:0171777000:008700*//*///
828<<4:;@?87
@C9IBY:03696:02678
CTTGGTGGTAATGGTGGTGTGGTTGGGCCGGAAGCCATCGCCGGGGGTTGGCGCTATTCTTGAAATGCTGCTCGGTGCAGCAGGTG
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GCCCTGTTGAGTGGTAAGGCGGGGGCGGTGATGCCGTCTTGAC
+
?@ACACCA????CACCA?;;:5:5:C/:5:<B=@B<;>CCCACDD:DADADDCCCFAB@?:::/;BBB>@?CD@CCCC>;;@<??
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CADDCACD9::?CC???@A=???B;BBC?A@@@A=AB?E:?>BBB3:;7;?BBBB>B???>B??
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CGGTGAACGTCACCATCCCGCTGTCCACCCTGCTCGGCCTGGACGACCACCCGCG
+
D@DDCCC@CCADDAC>;;;1;;;;D?D>?>=:::@@;B>??DDGEE5CCC>CAC;;8;>B>????A;;;?>:=>:?CCF?
D@CCD??>?CCAF??=BA5::::/:88::B4:::/:::AAC4:5;B><<000*00<*000
@C9IBY:02674:01311
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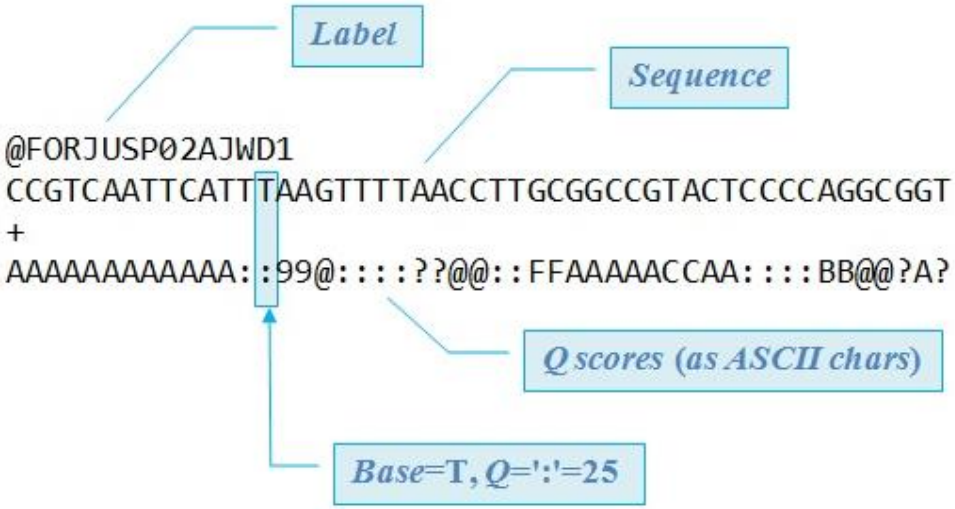
.fastq files



Each .fastq file covering a 5 Mb genome at 30X weights about **300 MB**

Phred quality scores are logarithmically linked to error probabilities

Phred Quality Score	Probability of incorrect base call	Base call accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%
40	1 in 10,000	99.99%
50	1 in 100,000	99.999%
60	1 in 1,000,000	99.9999%

Phred quality score

$$Q = -10 \log_{10} P$$

from 0 to 93 using ASCII characters 33 to 126

.fastq files

@

```
@X1L6C:01561:00672
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CCACCATTACCACCACCATCACCATTACCACAGGTAACGGTGCGGGCTGACGCGTACAGGAAACACAGAAAAAAAGCCCGCA
CCTGACAGTGCGGGCTTTTTTCGACCAAAGGTAACGAGGTAACAACCATGCG
+
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C:;;;-:9:BC<BBCCC9??<AA;AG<CB>GD@B;;;A<AE;AA<B??@9@C<BB<???BB;BBBAAAA:::BAB099/9>
@========(<<?)99997>>CCEBA>>=>2373333&3:99-33(3--717---43606704/47761
@X1L6C:01104:03031
AGAAGCTGCTATCAGACACTCTTTTTTTAATCCACACAGAGACATATTGCCCGTTGCAGTCAGAATGAAAAGCTGAAAAATA
CTTACTAAGGCGTTTTTTATTTGGTGATATTTTTTTCAATATCATGCAGCAAACGGTGCAACATTGCCGTGTCTCGTTGCTC
TAAAAGCCCCAGGCG
+
@AC=BCCC???B?@@CBB@???>>>>>*?8??>DAABEBCBABCAAA:@@>+9:8>;<;//.
98283988*44449;;9/88:?29:>>5;78333333&399298:6/./DCDDCC';>:ACBDAABB??9::+9<
1444@:?77-3<03368:8755888;:9833)3777'--'--
@X1L6C:03659:02717
GCTTCTGAACTGGTTACCTGCCGTGAGTAAATTAAAATTTTATTGACTTAGGTCACTAAATACTTTAACCAATATAGGCATA
GCGCACAGACAGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACCATTACCACCACCATCACCATTA
CCACAGGTAACGGTGCGGGCTGACGCGTACAGGAAACACAGAAAAAAGACCCGCCACTGACCAGTGCG
+
???9?BB@<CAA;A8@?:?@@5::BCCCEC;C=CCC8CEJ8DE;AACF>CC?DDCCCBB:B@???9?;B=B=CAA@?;?BCG
CCCCCCBABBBBCCDDAA2:4;@???CAB@AAA9@@AB?C:;;C;CDCCC>ECCAA<AC<CB>DC<AB=CD=C9::A4::>
CC;@@@A?CI@DDAFKDDD:A@CBCDC::::99199+8;4746@CA?)<444/3:4934333-3888//
@X1L6C:02011:02071
TTAAAATTTTATTGACTTAGGTCACTAAATACTTTAACCAATATAGGCATAGCGCACAGACAGATAAAAATTACAGAGTACA
CAACATCCATGAAACGCATTAGCACCACCATTACCACCACCATCACCATTACCACAGGTAACGGTGCGGGTGACGCGTACAG
GAAACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTCGACCAAAGTAACG
+
=0>>>19;;,;;7=CCDADC;?::::::,5;;==4>273:<@BBCF=CDH;@;MMFEED@?>>>:::::*5/55<
;::@:;:BC=BCBB<B@@@D<@@B:;3:::9@<BB=BD=AC;@B:??3::CAC=CD;;;=BBAB>CC;AA;BAAAA9AD@>>
>>?955>4?9499985555558&4<>2:;661499888...88/56666666$;6/.5:8(..+'++
@X1L6C:01333:03005
GCAATGCCAGGCAGGGCATGTACAGCTACGTACGTCTGAGCATCGATCGATGTACAGCTACGTACGTCTGAGCATCGATCGA
TGTACAGCTACGTACGTCTGAGCATCGATCGATGTACAGCTACG
+
555/55/(///(////(/8:9:<=>?<?@:98A??676<:;;@:5555555554444;=4443333;383338<68>>
68=333118311111111113933644588?==<76992---2+++0/
```

…and so on

Coverage

Reads mapped on a reference genome

Ref seq

COVERAGE

Mapped reads

Throughput of benchtop sequencers

	Read lenght	Total time	Total reads	Output
Illumina MiSeq	2 x 300bp	~56 hrs	44-50 M	13.2-15 Gb
Ion Torrent PGM	400bp	~19 hrs	4-5.5 M	1.6-2.2 Gb

e.g. *E. coli* multiplex genomes sequencing

Average length 5 Mb

Desired coverage 30X

150 Mb/strain

Assembly

Short sequencing reads

.fastq file

```
@HWI-ST700693:238:B0224ACXX:1:1101:1218:1982
NACACTTGCTTTGGTGACAGCGGGGCATCCTCAAGC
+
#1=DDDDDHAFF?GEFGIIIIIIIIIIIIIIIIIFI
@HWI-ST700693:238:B0224ACXX:1:1101:1161:1986
NGATTTTGACCTCTCCAGTTTCCTCTTAACACTTTG
+
#1:BDFFFGHHHGJJJIIJHIJJJJJJJJJJJJJJJ
@HWI-ST700693:238:B0224ACXX:1:1101:1193:1989
NTATCCAGCCTGCGGTGCTACTTGGTGGAAGAGGAT
+
#1=DDFFFHGHGGJJFGHJJIJJJIEGECHDFHCC?
@HWI-ST700693:238:B0224ACXX:1:1101:1440:1981
NTCAAGAATCCAAGTGGGGCCAGCATAATGTACGCT
+
#1=DDFFFHGHDFDAEGIIFGIICGGHGBFGEFDHI
```

Partially assembled genome (contigs)

.fasta file

```
>NODE_1_length_449_cov_4.835189
ATCTTTCGCGCCTTCCAGCTCCAGCCATTCGGAACCGTTCGCCAGAAAACGGGCGTAATC
GGGTAAGACATAGCGCGGTTTGTACGGCGCATGACCTTCAAACATATCGCAGATTACACC
TTCATCCAGCGCGCGGCGGGCTTCGGCAGGAAGCTGTGGGTAAGGCAGATTGTTTTCTGC
TTCCAGTGCCAGAAAATGGCGCTTCTGCTCCGGGCTAAGCACTGGGCTGGTGACAATTTG
CTGGCAACGTTGTTGCAGTGCATTTTCATGAGAAGTGGGCATCTTCTTTTCCTTTTATGC
CGAAGGTGATGCGCCATTGTAAGAAGTTTCGTGATGTTCACTTTGATCCTGATGCGTTTG
CCACCACTGACGCATTCATTTGAAAGTGAATTATTTGAACCAGATCGCATTACAGTGATG
CAAACTTGTAAGTAGATTTCCTTAATTGTGATGTGTATCGAAGTGTGTTGCGG
>NODE_2_length_309_cov_4.686084
ACTGGTCAGTGCGGGTATCCTTGGACAATGGCCGATTGGACGTCTGGCGGATAAGTTTGG
TCGACTGCTGGTGTTGCGTGTTCAGGTCTTTGTCGTCATTCTCGGCAGTATCGCGATGCT
TAGCCAGGCGGCGATGGCCCCAGCGTTATTCATCCTCGGTGCCGCTGGCTTTACGCTATA
TCCGGTGGCGATGGCATGGGCTTGCGAGAAAGTTGAACATCATCAACTGGTGGCGATGAA
CCAGGCCTTACTGTTGAGCTATACTGTGGGAAGTCTGCTTGGCCCGTCATTTACCGCTAT
GCTAATGCAGAATTTCTCCGATAATTTATTGTT
>NODE_3_length_101_cov_3.346535
AGCGCATGAGCGCGCAGCGCCGCCGTTACGTGGTGCATCAGCATGATGTTGGCCGGAGAG
TACAGAGACTCCCCTTCATCCATGATGCCCTCTTTCACCAGCAGTTCTTCAATCATCACC
```

FastqSize ≈ GenomeSize x Coverage x 2

At least 300 MB per genome

FastaSize for *E. coli* contigs

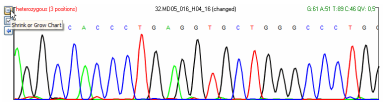
~10 MB

Need for long contigs to investigate the presence of interesting genetic features by blast analysis

7-genes MLST

Conventional Sanger sequencing

PCR, sequencing, electropherograms analysis



Uploading sequences on a webserver to obtain the corresponding alleles and STs

THE UNIVERSITY OF WARWICK

NGS-derived

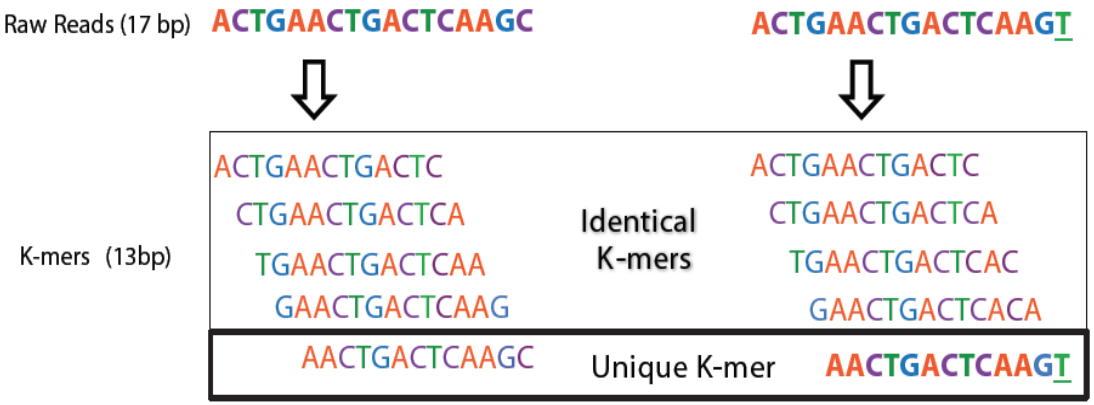
Direct upload of WGS contigs on a webserver
(e.g. ARIES or CGE)

Alleles are directly retrieved through blastn comparison with pre-installed database of alleles from University of Warwick with pre-compiled pipelines

Whole genome SNPs typing

Ref-based

Ref-free (kmers)



Escherichia coli

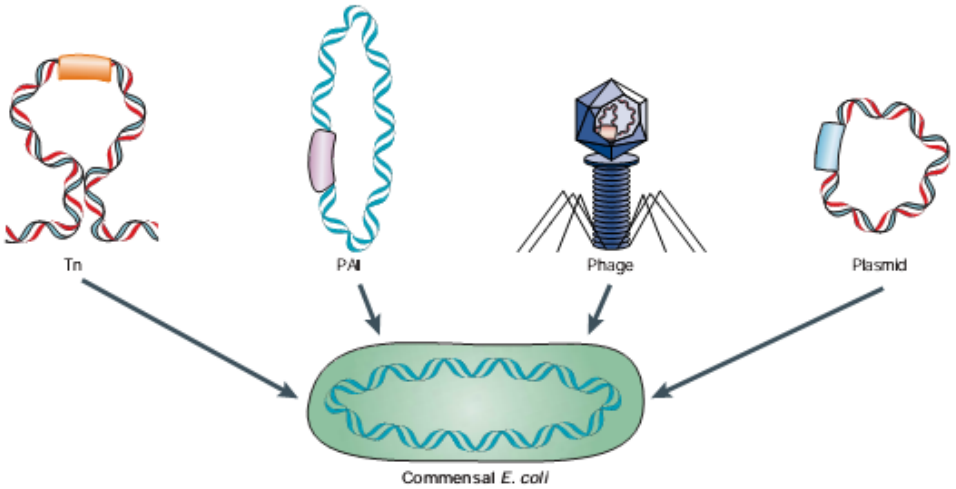
Ubiquitous
Commensal
Some cause infections

- Urinary tract infections
- Sepsis/meningitis
- **Diarrhoea-associated infections**

EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E. coli*
EIEC	Enteroinvasive *E. coli*
EAEC	Enteroaggregative *E. coli*
DAEC	Diffusely-Adherent *E. coli*
STEC	Shiga-toxin producing *E. coli*

Virulence determinants

- Toxins
- Factors involved in colonization

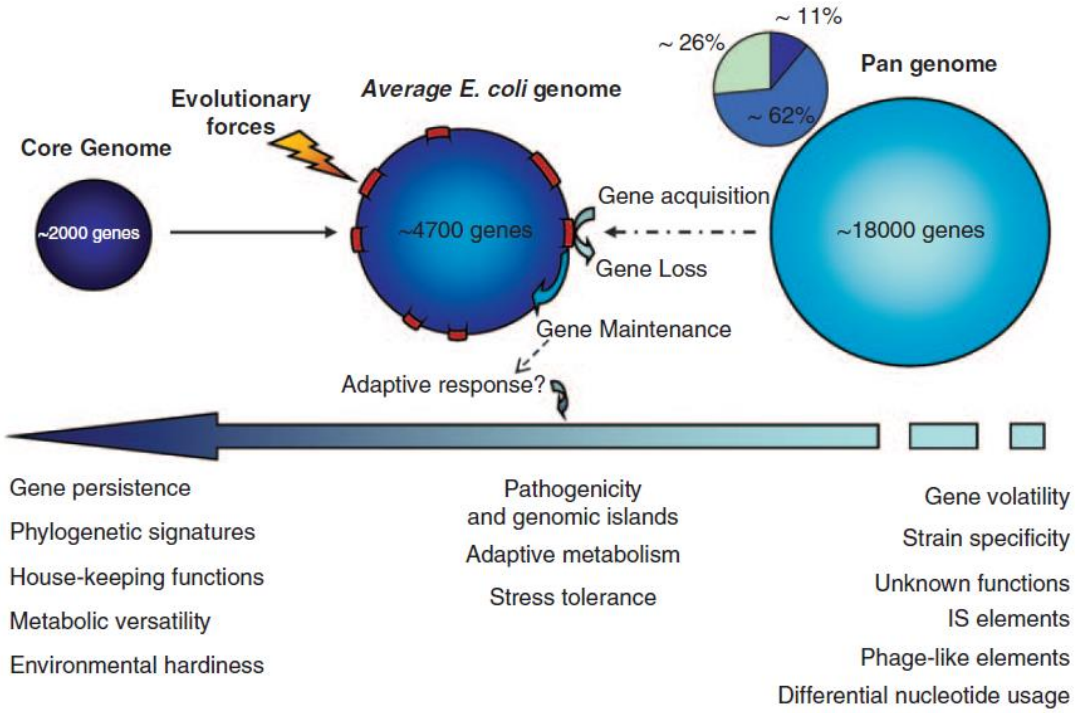


Kaper, Nataro *Nature Reviews* 2004

The *E. coli* pangenome

Genomic plasticity

Huge pangenome

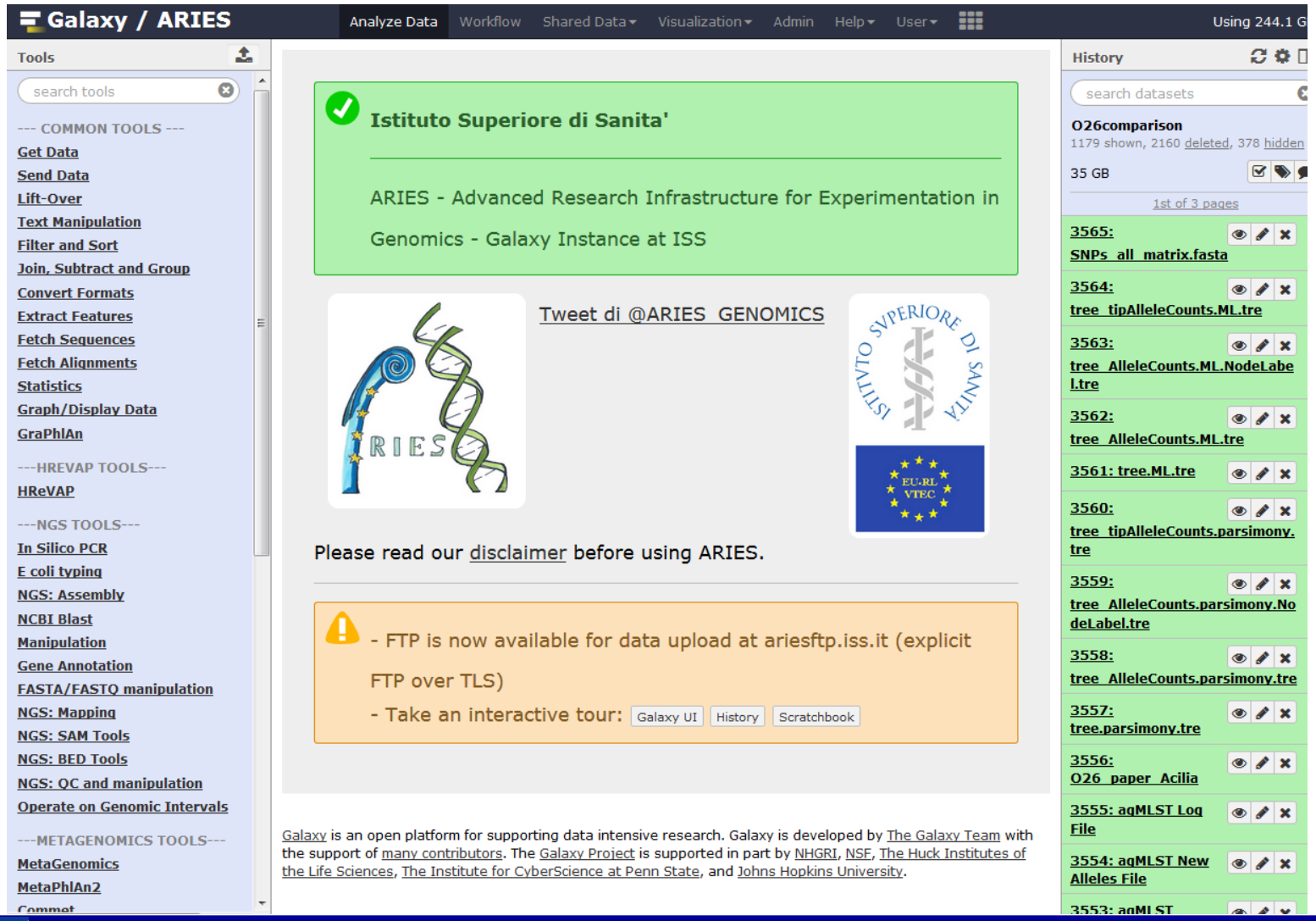


Van Elsas J.D. et al., 2011

Pangenome **Whole genome** **Core genome** **Accessory genome**

Housekeeping

ARIES: A Galaxy-based workspace for intensive data analyses



ARIES geographical spread

Total 76 users

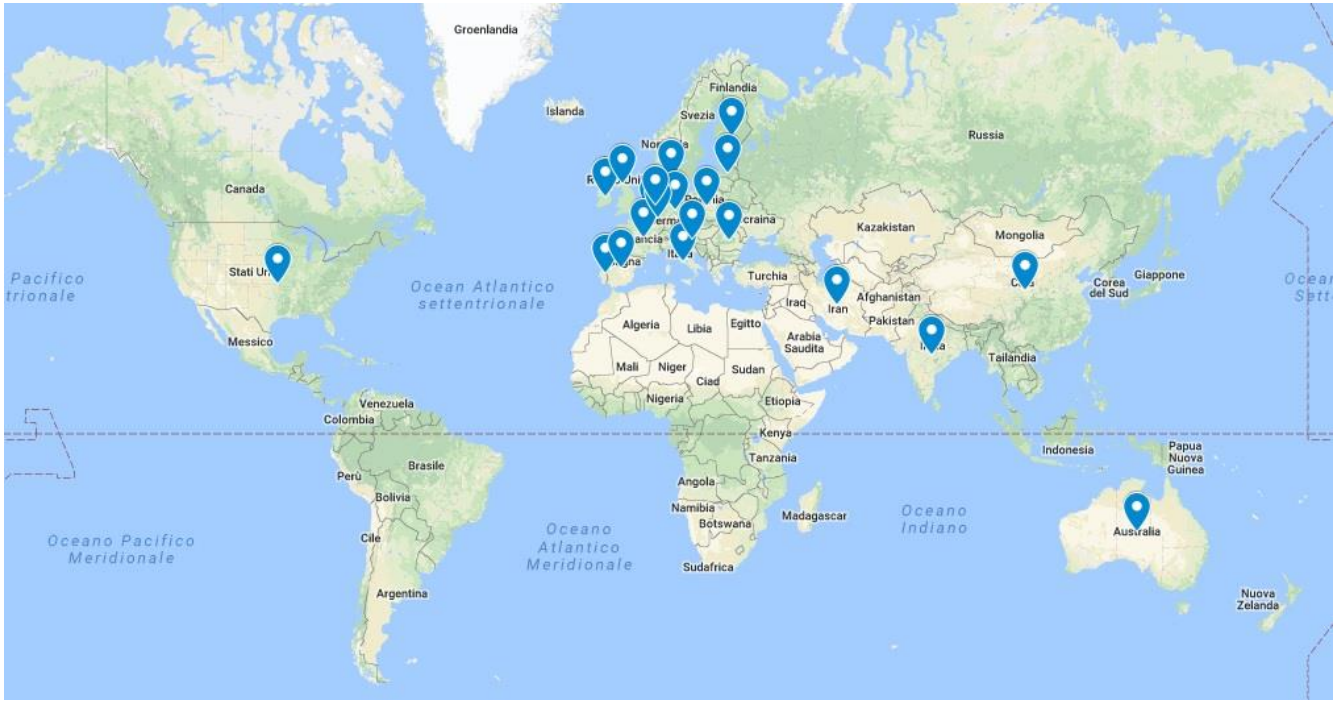
Including National Reference Laboratories from 13 Member States in EU

Belgium
Denmark
Finland
Germany
Ireland
Italy
Latvia
The Netherlands
Poland (2 NRLs)
Portugal
Slovenia
United Kingdom

Other users from Europe:
France, Luxemburgh, Romania, Spain

Users from outside Europe:

Australia, China, India, Iran, USA



NGS data analysis vision: user - friendly user interface

a5_pipeline.pl

```
Getopt::Long::Configure(qw{no_auto_abbrev no_ignore_case_always pass_through});
my $start = 1;
my $end = 5;
my $preproc = 0;
my $debug = 0;
my $metagenome = 0;
my $threads = 4;
my $adapter = dirname(abs_path($0))."/../adapter.fasta";
GetOptions( 'begin=i' => \$start,
            'end=i' => \$end,
                      'preprocessed' => \$preproc,
                      'debug' => \$debug,
                      'metagenome' => \$metagenome,
                      'threads=i' => \$threads,
                      'adapter=s' => \$adapter);

die $usage if (@ARGV < 2);

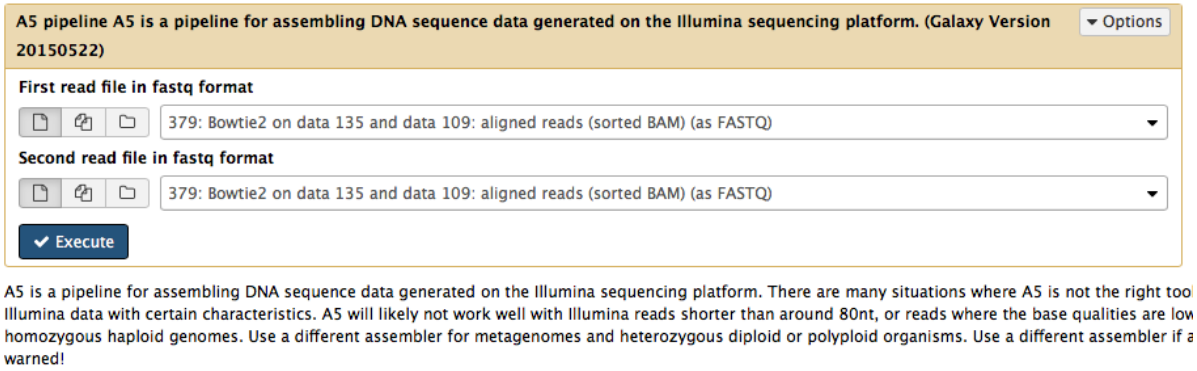
$AVAILMEM = get_availmem();

#
# Check that java is available and in the path
#
my $java = `which java`;
die "Unable to find a java runtime environment. The A5 pipeline requires java 6 or later. Please ensure that java is installed and in the \$PATH" unless
length($java)>1;
# OS compatibility checks
if($^O !~ /darwin/ && $^O !~ /inux/){
        die "Sorry, A5-miseq only works on Linux and OS X platforms\n";
}
if($^O =~ /darwin/){
        my $sga_test = system("$DIR/sga 2> /dev/null > /dev/null");
        if($sga_test){
                die "Sorry, A5-miseq does not work on your system.\nThe SGA component of A5-miseq requires OS X 10.6 or later\n";
        }
}

# check for spaces in file and path names -- these are not supported
my $message = "contains unsupported characters such as quotes, spaces, or &:%?*><\$";

my $libfile = $ARGV[0];
my $OUTBASE = $ARGV[1];

# check whether command-line input was FastQ files or a library file
if(@ARGV==3){
```



Esempi

- Caratterizzazione di un clone di *Escherichia coli* entoeroinvasivi (EIEC) circolante in Europa

- Caratterizzazione del ceppo STEC-EAEC O104:H4 responsabile dell'epidemia in Germania nel 2011

- Utilizzo dell'NGS in metagenomica

Analisi di un patogeno ri-emergente

Aumento di casi di infezioni da *Escherichia coli* entoeroinvasivi in Europa

- Epidemia in Italia nel 2012
- Caso isolato in Spagna nel 2012

Sequenza genomica di due isolati mediante Ion Torrent presso l'ISS

- Epidemia in UK nel 2014 → Sequenza genomica di un isolato mediante Illumina presso il PHE

Analisi del contenuto genico

Ricerca di determinanti genetici di interesse attraverso allineamenti blast

- Geni di virulenza
- Geni associati al sierotipo
- Geni parte degli schemi MLST
- Presenza di isole di patogenicità

Esistono database di riferimento per i geni di interesse?

Se non esistono: compilazione di databases ad hoc come liste di sequenze geniche in formato fasta

Bibliografia EIEC O96:H19

Characterization of an emergent clone of enteroinvasive *Escherichia coli* circulating in Europe.

Michelacci V, Prosseda G, Maugliani A, Tozzoli R, Sanchez S, Herrera-León S, Dallman T, Jenkins C, Caprioli A, Morabito S.

Clin Microbiol Infect. 2016 Mar;22(3):287.e11-9. doi: 10.1016/j.cmi.2015.10.025.
PMID: 26551840

Abstract

Enteroinvasive *Escherichia coli* (EIEC) cause intestinal illness indistinguishable from that caused by *Shigella*, mainly in developing countries. Recently an upsurge of cases of EIEC infections has been observed in Europe, with two large outbreaks occurring in Italy and in the United Kingdom. We have characterized phenotypically and genotypically the strains responsible for these epidemics together with an additional isolate from a sporadic case isolated in Spain. The three isolates belonged to the same rare serotype O96:H19 and were of sequence type ST-99, never reported before in EIEC or *Shigella*. The EIEC strains investigated possessed all the virulence genes harboured on the large plasmid conferring the invasive phenotype to EIEC and *Shigella* while showing only some of the known chromosomal virulence genes and none of the described pathoadaptative mutations. At the same time, they displayed motility abilities and biochemical requirements resembling more closely those of the non-pathogenic *E. coli* rather than the EIEC and *Shigella* strains used as reference. Our observations suggested that the O96:H19 strains belong to an emerging EIEC clone, which could be the result of a recent event of acquisition of the invasion plasmid by commensal *E. coli*.

Escherichia coli produttori di Shiga-tossina

Patogeni zoonotici

Trasmissione attraverso ingestione di alimenti contaminati

Manifestazioni cliniche intestinali: Assenza di sintomi – Diarrea - Colite emorragica

Manifestazioni cliniche sistemiche: Sindrome Emolitico Uremica (10%): causa principale di danno renale nell'infanzia. Richiede dialisi

Più di 180 sierogruppi circolanti. Solo alcuni sono associati frequentemente con casi di malattia grave (**TOP 5**: O157, O26, O111, O103, O145)

Grande variabilità dovuta alla grande plasticità genomica: geni ***stx*** veicolati da batteriofagi e **fattori di colonizzazione** fanno parte del genoma accessorio

La Shiga-tossina è anche detta Verocitotossina, quindi i geni *stx* sono anche detti *vtx* e gli STEC sono anche detti VTEC

Maggio 2011: epidemia da STEC-EAEC O104:H4

ALLARME

Paura in Germania: «germe killer» fa le prime vittime

Quattro morti e oltre 600 casi per un'epidemia causata dal batterio fecale, Escherichia Coli

CORRIERE DELLA SERA .it

Allarme epidemia

GERMANIA

L'E.coli adesso spaventa l'Ue
Oms: "Una variante mai vista prima"

la Repubblica.it

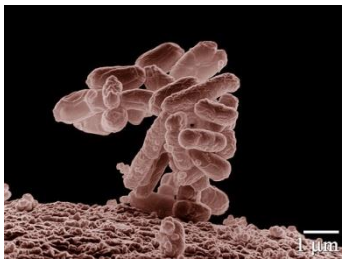
Identificato il ceppo: è nuovo, altamente tossico e resistente ad alcuni antibiotici. In Germania si parla di oltre 1500 casi, più i quasi 500 in altri Paesi Ue, ma è molto difficile fare stime precise. La Russia blocca le importazioni di verdura dall'Europa e Bruxelles protesta. Scagionati i cetrioli, la Spagna chiede i danni all'Unione europea

4033 casi

901 SEU

50 decessi

- Sierotipo **O104:H4** (raro)
- Adesione a pila di mattoni (typical of **EAEC**)

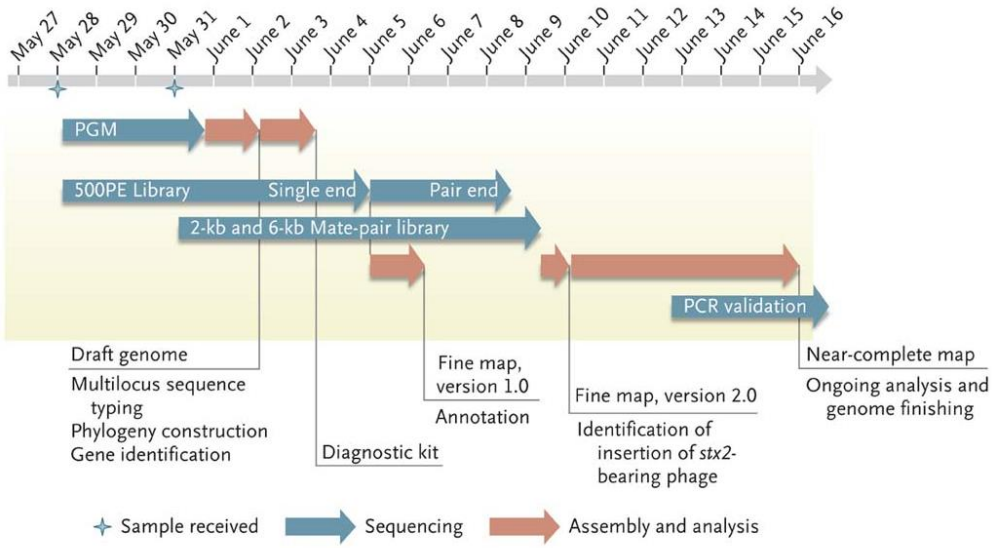


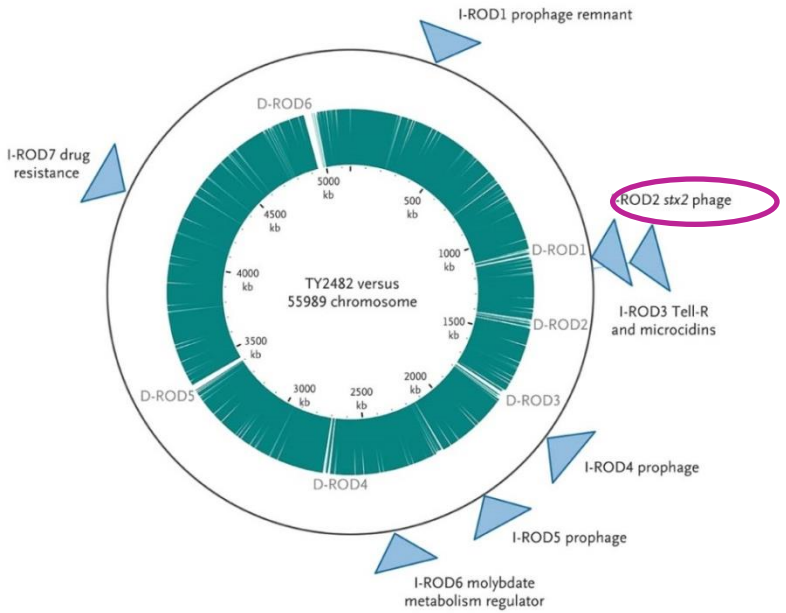
Enteroaggregative *E. coli*

Attaching/Effacing *E. coli*

Sequenziamento del genoma del ceppo O104:H4

Rohde H et al. N Engl J Med 2011;365:718-724

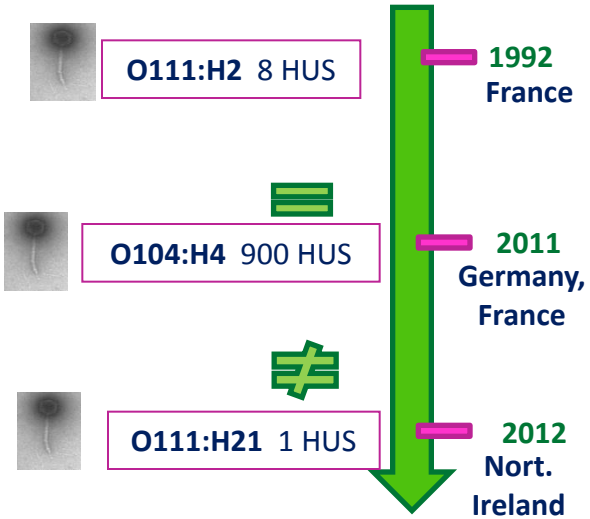


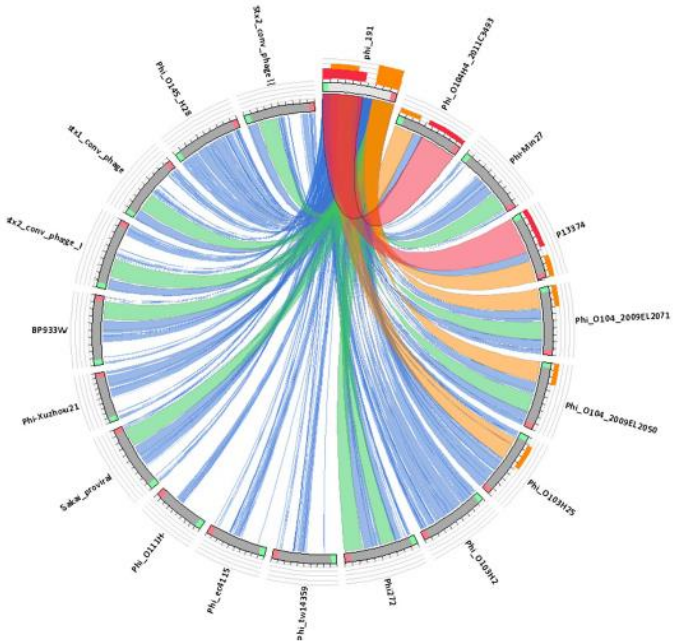


- Rapido sviluppo di metodi molecolari per identificarne la presenza (geni target: *stx*, wzx_{O104}, $fliC_{H4}$)
- Studio delle dinamiche evolutive che ne hanno permesso l'emergenza

Genetic Element	Notable Features or Functions	Size or 55989 Coordinates*
Plasmid		
pESBL TY2482	IncI1 plasmid, homologous to pEC_Bactec carrying *bla* CTX-M-15	88 kb
pAA TY2482	Plasmid encoding aggregative adherence fimbriae I	76 kb
pG2011 TY2482	Plasmid with no obvious phenotype	1.5 kb

Fagi veicolanti geni *stx* in ceppi EAEC



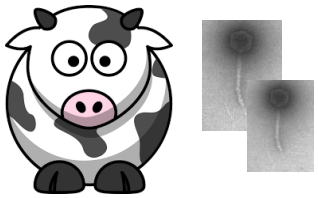


Stesso fago *stx2* conservato in due ceppi isolati a 20 anni di distanza

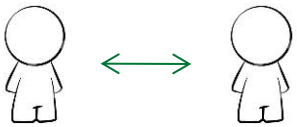
Almeno DUE fagi *stx2* sono in grado di infettare stabilmente ceppi EAEC

Un frammento genico codificante le fibre caudali è conservato in tutti i fagi *stx2* da EAEC

Ipotesi sull'emergenza di ceppo STEC-EAEC



Sorgente degli STEC e dei fagi *stx*



Sorgente degli EAEC

Sovrapposizione delle due sorgenti

Bibliografia STEC-EAEC O104:H4

Whole genome sequence comparison of vtx2-converting phages from Enteroaggregative Haemorrhagic *Escherichia coli* strains.

Grande L, **Michelacci V**, Tozzoli R, Ranieri P, Maugliani A, Caprioli A, Morabito S.

BMC Genomics. 2014 Jul 8;15:574. doi: 10.1186/1471-2164-15-574
PMID: 25001858

Abstract

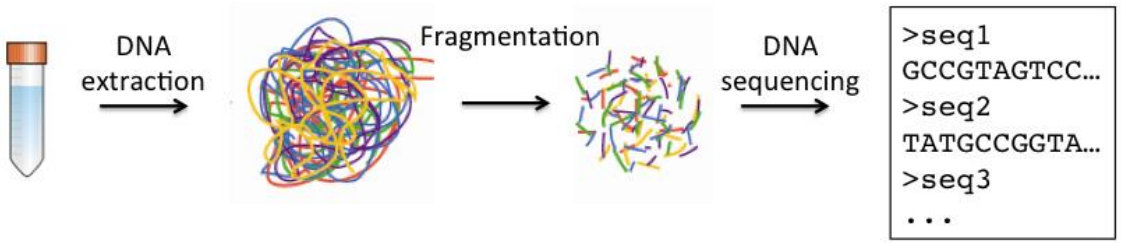
Background: Enteroaggregative Haemorrhagic *E. coli* (EAHEC) is a new pathogenic group of *E. coli* characterized by the presence of a *vtx2*-phage integrated in the genomic backbone of Enteroaggregative *E. coli* (EAggEC). So far, four distinct EAHEC serotypes have been described that caused, beside the large outbreak of infection occurred in Germany in 2011, a small outbreak and six sporadic cases of HUS in the time span 1992–2012. In the present work we determined the whole genome sequence of the *vtx2*-phage, termed Phi-191, present in the first described EAHEC O111:H2 isolated in France in 1992 and compared it with those of the *vtx*-phages whose sequences were available.

Results: The whole genome sequence of the Phi-191 phage was identical to that of the *vtx2*-phage P13374 present in the EAHEC O104:H4 strain isolated during the German outbreak 20 years later. Moreover, it was also almost identical to those of the other *vtx2*-phages of EAHEC O104:H4 strains described so far. Conversely, the Phi-191 phage appeared to be different from the *vtx2*-phage carried by the EAHEC O111:H21 isolated in the Northern Ireland in 2012. The comparison of the *vtx2*-phages sequences from EAHEC strains with those from the *vtx*-phages of typical Verocytotoxin-producing *E. coli* strains showed the presence of a 900 bp sequence uniquely associated with EAHEC phages and encoding a tail fiber.

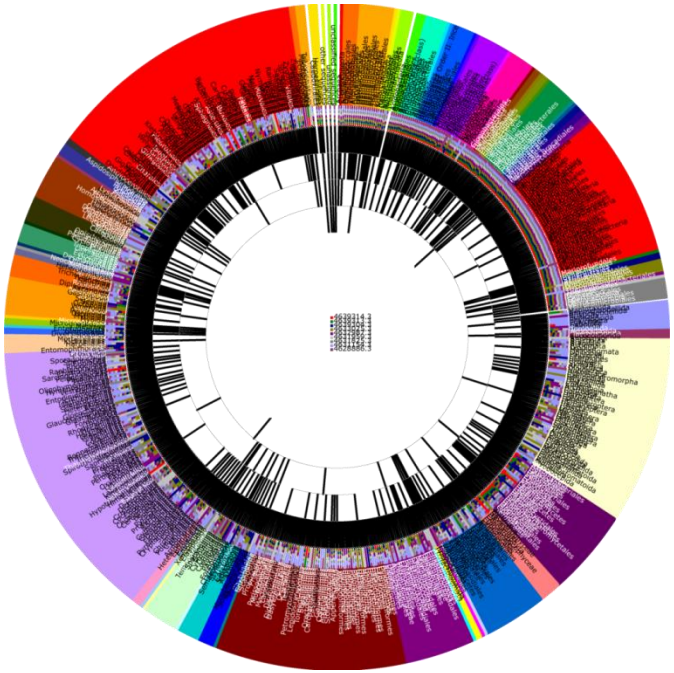
Conclusions: At least two different *vtx2*-phages, both characterized by the presence of a peculiar tail fiber-coding gene, intervened in the emergence of EAHEC. The finding of an identical *vtx2*-phage in two EAggEC strains isolated after 20 years in spite of the high variability described for *vtx*-phages is unexpected and suggests that such *vtx2*-phages are kept under a strong selective pressure.
The observation that different EAHEC infections have been traced back to countries where EAggEC infections are endemic and the treatment of human sewage is often ineffective suggests that such countries may represent the cradle for the emergence of the EAHEC pathotype. In these regions, EAggEC of human origin can extensively contaminate the environment where they can meet free *vtx*-phages likely spread by ruminants excreta.

Keywords: Enteroaggregative haemorrhagic *E. coli*, *vtx*-phages, Whole genome sequence, Tail fibers

Applicazioni dell'NGS in Metagenomica



Abbondanza tassonomica relativa (MG-RAST)



Resistenza agli antimicrobici (Diamond)

Bibliografia Metagenomica/1

Comparative analysis of metagenomes of Italian top soil improvers.
Gigliucci F, Brambilla G, Tozzoli R, **Michelacci V**, Morabito S.
Environ Res. 2017 May;155:108-115. doi: 10.1016/j.envres.2017.02.004.
PMID: 28214713

A B S T R A C T

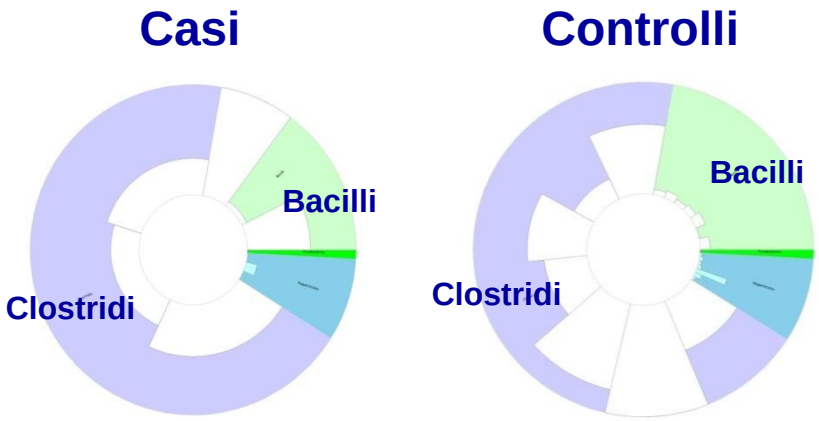
Biosolids originating from Municipal Waste Water Treatment Plants are proposed as top soil improvers (TSI) for their beneficial input of organic carbon on agriculture lands. Their use to amend soil is controversial, as it may lead to the presence of emerging hazards of anthropogenic or animal origin in the environment devoted to food production. In this study, we used a shotgun metagenomics sequencing as a tool to perform a characterization of the hazards related with the TSIs. The samples showed the presence of many virulence genes associated to different diarrheagenic *E. coli* pathotypes as well as of different antimicrobial resistance-associated genes. The genes conferring resistance to Fluoroquinolones was the most relevant class of antimicrobial resistance genes observed in all the samples tested. To a lesser extent traits associated with the resistance to Methicillin in *Staphylococci* and genes conferring resistance to Streptothricin, Fosfomycin and Vancomycin were also identified. The most represented metal resistance genes were cobalt-zinc-cadmium related, accounting for 15–50% of the sequence reads in the different metagenomes out of the total number of those mapping on the class of resistance to compounds determinants. Moreover the taxonomic analysis performed by comparing compost-based samples and biosolids derived from municipal sewage-sludges treatments divided the samples into separate populations, based on the microbiota composition. The results confirm that the metagenomics is efficient to detect genomic traits associated with pathogens and antimicrobial resistance in complex matrices and this approach can be efficiently used for the traceability of TSI samples using the microorganisms' profiles as indicators of their origin.

Studio del microbiota in un focolaio epidemico da STEC O26

Confronto con database di sequenze di geni di virulenza: conferma della presenza di STEC O26 *eae stx2a* nei campioni prelevati dai casi

A. 9	A. 4	A. 30	A. 40	A. 8	A. 16	A. 14	481-5	A. 32	A. 41
cif	epeA	senB	ehxA	cif	cba	cif		katP	mhcF
eae	espP		espP	eae	cma	eae		lpfA	tsh
efa1	gad		katP	efa1	gad	efa1			
ehxA	iha		mhcC	ehxA	ireA	ehxA			
espA			pic	espA	iss	espA			
espB			senB	espB	lpfA	espB			
espJ			toxB	espF	pic	espF			
espP				espJ	vat	espJ			
gad				espP		espP			
iha				gad		gad			
iss				iha		iha			
katP				iss		iroN			
lpfA				katp		iss			
nleA				lpfA		katP			
nleB				nleA		lpfA			
nleC				nleB		nleA			
stx2a				nleC		nleB			
tir				stx2a		nleC			
toxB				tir		stx2a			
				toxB		tir			
						toxB			

Studio della composizione tassonomica del microbiota intestinale nei casi VS i controlli



Identificazione di differenze nella rappresentatività di alcune classi di microrganismi che potrebbero svolgere un ruolo protettivo durante l'infezione

Bibliografia Metagenomica/2

Metagenomic Characterization of the Human Intestinal Microbiota in Fecal Samples from STEC-Infected Patients

Gigliucci F, von Meijenfeldt FAB, Knijn A, **Michelacci V**, Scavia G, Minelli F, Dutilh BE, Ahmad HM, Raangs GC, Friedrich AW, Rossen JWA, Morabito S.Front Cell Infect Microbiol. 2018 Feb 6;8:25. PMID: 29468143

Metagenomic Characterization of the Human Intestinal Microbiota in Fecal Samples from STEC-Infected Patients

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The human intestinal microbiota is a homeostatic ecosystem with a remarkable impact on human health and the disruption of this equilibrium leads to an increased susceptibility to infection by numerous pathogens. In this study, we used shotgun metagenomic sequencing and two different bioinformatic approaches, based on mapping of the reads onto databases and on the reconstruction of putative draft genomes, to investigate possible changes in the composition of the intestinal microbiota in samples from patients with Shiga Toxin-producing *E. coli* (STEC) infection compared to healthy and healed controls, collected during an outbreak caused by a STEC O26:H11 infection. Both the bioinformatic procedures used, produced similar result with a good resolution of the taxonomic profiles of the specimens. The stool samples collected from the STEC infected patients showed a lower abundance of the members of *Bifidobacteriales* and *Clostridiales* orders in comparison to controls where those microorganisms predominated. These differences seemed to correlate with the STEC infection although a flexion in the relative abundance of the Bifidobacterium genus, part of the *Bifidobacteriales* order, was observed also in samples from Crohn's disease patients, displaying a STEC-unrelated dysbiosis. The metagenomics also allowed to identify in the STEC positive samples, all the virulence traits present in the genomes of the STEC O26 that caused the outbreak as assessed through isolation of the epidemic strain and whole genome sequencing. The results shown represent a first evidence of the changes occurring in the intestinal microbiota of children in the course of STEC infection and indicate that metagenomics may be a promising tool for the culture-independent clinical diagnosis of the infection.

Conclusioni e prospettive

Il sequenziamento del genoma completo permette di ottenere dati utili alla caratterizzazione e tipizzazione dei microrganismi patogeni

- Base per lo **sviluppo di metodi** per l'identificazione di un patogeno
- Possibilità di estrarre informazioni utili per **capire l'origine e l'emergenza di sottotipi patogeni**
- Possibilità di confrontare ceppi patogeni ed identificarne le **relazioni filogenetiche**
  - attraverso studio degli SNPs nel genoma totale
  - per confronto del contenuto genico – necessità di database di geni e alleli
- Possibile applicazione della **metagenomica** nella diagnosi delle infezioni e nello studio di campioni complessi per investigare nuove possibili sorgenti di infezione

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