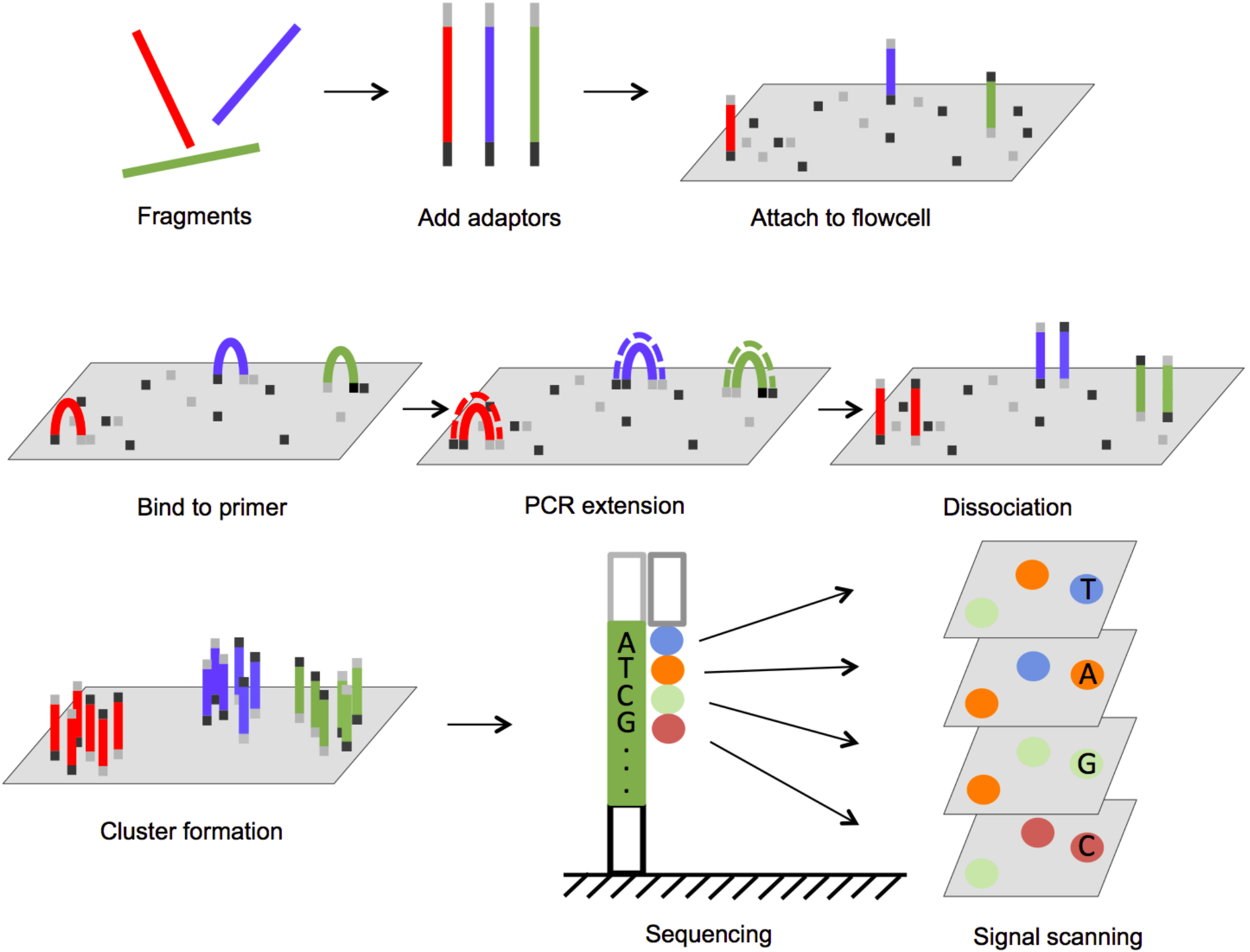
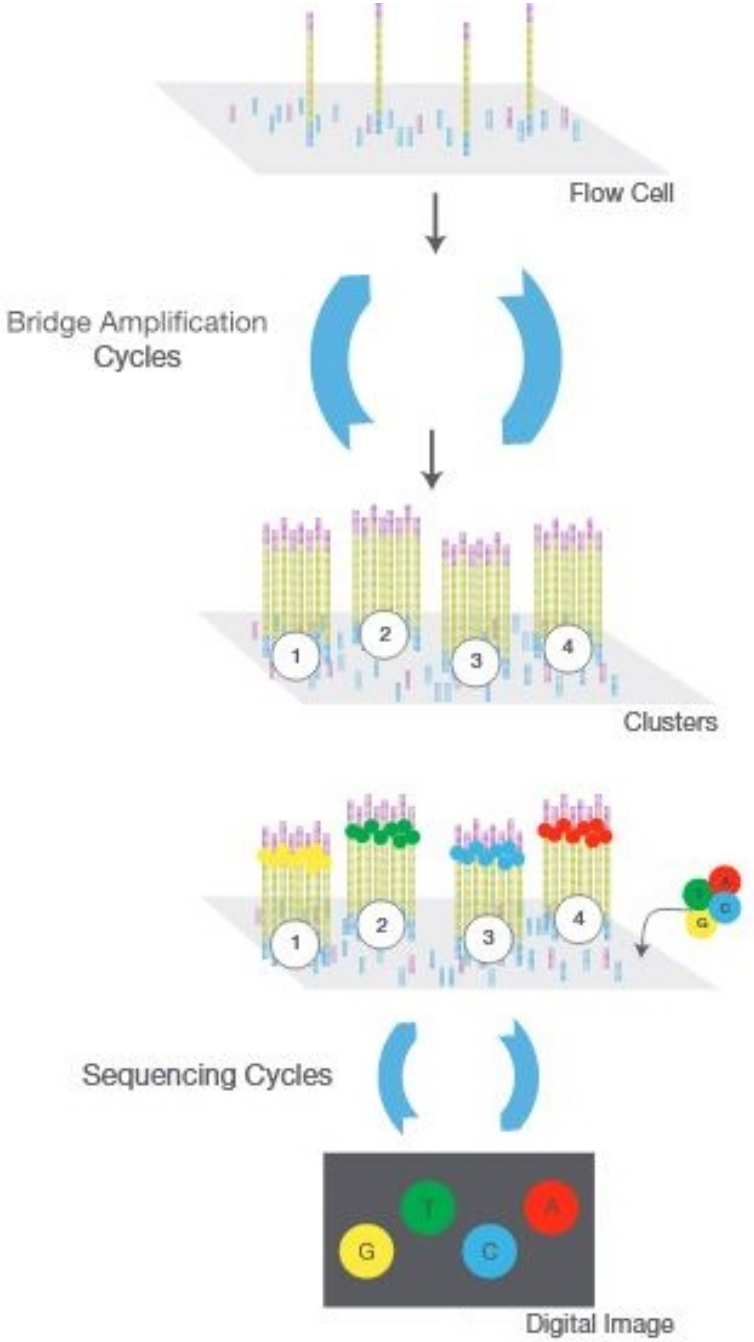
Grupos de trabajo

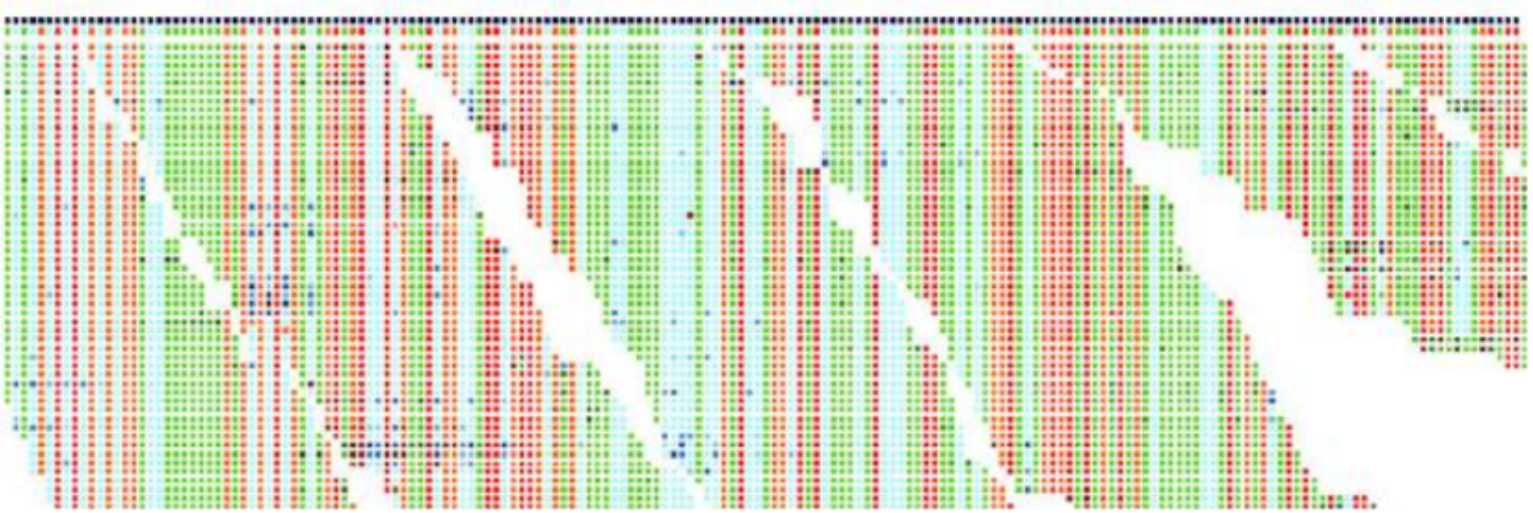
- Trabajarán por mucho tiempo (si es que siguen)
- Recomiendo trabajar en grupos de dos personas, pero pueden trabajar solos.
- Definir un nombre de usuario personal (corto)


Fragments
Add adaptors
Attach to flowcell
Bind to primer
PCR extension
Dissociation
Cluster formation
ATCG...
Sequencing
T
A
G
C
Signal scanning


Flow Cell
Bridge Amplification
Cycles
1
2
3
4
Clusters
1
2
3
4
Sequencing Cycles
G
T
A
C
Digital Image

The “secret” of massive DNA sequencing

To sequence in parallel a huge amount of relatively small fragments of a given DNA sample (i.e., whole genome, chromosome, metagenome, etc.)



But… you get a huge puzzle with thousands of pieces, hard to reconstruct completely and accurately

Read o lectura

- Formato fasta

  .fasta

  .fna

  .faa

  .fa

Fasta


Header
Sequence
Header
Sequence
Header
Sequence
>VIT_201s0011g03530.1
AATTAAGCATAAATACTCACTCTTACCCCCTTATTTTCTTATCTCTCATCACTTTTGGTGCGAAG
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>VIT_201s0011g03540.1
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AGCCTCTGAGACACCACCTCAAACCTTTCCACTTAAATACACATCCCTCACACCCTTTTCAATTC
>VIT_201s0011g03550.1
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GCCGAAATGGTAAAAGACTAAGGCTAGAAGTAGAATACCACTGTTCTTCTCATCACGTGGGCCCA

Fasta?

>NG_008679.1:5001-38170 Homo sapiens paired box 6 (PAX6)
ACCCTCTTTTCTTATCATTGACATTTAAACTCTGGGGCAGGTCCTCGCGTAGAACGCGGCTGTCAGATCT
GCCACTTCCCCTGCCGAGCGGCGGTGAGAAGTGTGGGAACCGGCGCTGCCAGGCTCACCTGCCTCCCCGC
CCTCCGCTCCCAGGTAACCGCCCGGGCTCCGGCCCCGGCCCGGCTCGGGGCCCGCGGGGCCTCTCCGCTG
CCAGCGACTGCTGTCCCCAAATCAAAGCCCGCCCCAAGTGGCCCCGGGGCTTGATTTTTGCTTTTAAAAG
GAGGCATACAAAGATGGAAGCGAGTTACTGAGGGAGGGATAGGAAGGGGGGTGGAGGAGGGACTTGTCTT
TGCCGAGTGTGCTCTTCTGCAAAAGTAGCAAAATGTTCCACTCCTAAGAGTGGACTTCCAGTCCGGCCCT
GAGCTGGGAGTAGGGGGCGGGAGTCTGCTGCTGCTGTCTGCTAAAGCCACTCGCGACCGCGAAAAATGCA
GGAGGTGGGGACGCACTTTGCATCCAGACCTCCTCTGCATCGCAGTTCACGACATCCACGCTTGGGAAAG
TCCGTACCCGCGCCTGGAGCGCTTAAAGACACCCTGCCGCGGGTCGGGCGAGGTGCAGCAGAAGTTTCCC
GCGGTTGCAAAGTGCAGATGGCTGGACCGCAACAAAGTCTAGAGATGGGGTTCGTTTCTCAGAAAGACGC

Fasta?

```
>gi|528476558|ref|NC_018928.2| Homo sapiens chromosome 17
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AGCACATCAGCTCTGAGGCCTCCACTCACTGAGACAATAGACCCAAG
TCACTGAGACAATAGACCCAAGCACATCAGCTCTGAGGCCTCCACTC
AACAGCTCTGAGGGCTCCACTCACTGAGACAACAGACCCAAGAACAA
>gi|528476670|ref|NC_018912.2| Homo sapiens chromosome 1
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
CCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAA
ACCCTAACCCTAACCCTAACCCTAACCCAACCCTAACCCTAACCCTAACCCTAACCCTA
TAACCCTAACCCTAAACCCTAAACCCTAACCCTAACCCTAACCCTAACCCTAACCCCAA
```

.faa

```
>sp|P11274|BCR_HUMAN Breakpoint cluster region protein OS=Homo sapiens
OX=9606 GN=BCR PE=1 SV=2
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TLLAKEKKSYDRQRWGFRRAAQAPDGASEPRASASRPQPAPADGADPPPAEEPEARPDGE
GSPGKARPGTARRPGAAASGERDDRGPPASVAALRSNFERIRKGHGQPGADAEKPFYVNV
EFHHERGLVKVNDKEVSDRISSLGSQAMQMERKKSQHGAGSSVGDASRPPYRGRSSESSC
GVDGDYEDAELNPRFLKDNLIDANGGSRPPWPPLEYQPYQSIYVGGMMEGEGKGPLLRSQ
STSEQEKRLTWPRRSYSPRSFEDCGGGYTPDCSSNENLTSSEEDFSSGQSSRVSPSPTTY
RMFRDKSRSPSQNSQQSFDSSSPPTPQCHKRHRHCPVVVSEATIVGVRKTGQIWPNDGEG
AFHGDADGSFGTPPGYGCAADRAEEQRRHQDGLPYIDDSPSSSPHLSSKGRGSRDALVSG
ALESTKASELDLEKGLEMRKWVLSGILASEETYLSHLEALLLPMKPLKAAATTSQPVLTS
QQIETIFFKVPELYEIHKEFYDGLFPRVQQWSHQQRVGDLFQKLASQLGVYRAFVDNYGV
AMEMAEKCCQANAQFAEISENLRARSNKDAKDPTTKNSLETLLYKPVDRVTRSTLVLHDL
LKHTPASHPDHPLLQDALRISQNFLSSINEEITPRRQSMTVKKGEHRQLLKDSFMVELVE
GARKLRHVFLFTDLLLCTKLKKQSGGKTQQYDCKWYIPLTDLSFQMVDELEAVPNIPLVP
DEELDALKIKISQIKNDIQREKRANKGSKATERLKKKLSEQESLLLLMSPSMAFRVHSRN
GKSYTFLISSDYERAEWRENIREQQKKCFRSFSLTSVELQMLTNSCVKLQTVHSIPLTIN
KEDDESPGLYGFLNVIVHSATGFKQSSNLYCTLEVDSFGYFVNKAKTRVYRDTAEPNWNE
EFEIELEGSQTLRILCYEKCYNKTKIPKEDGESTDRLMGKGQVQLDPQALQDRDWQRTVI
AMNGIEVKLSVKFNSREFSLKRMPSRKQTGVFGVKIAVVTKRERSKVPYIVRQCVEEIER
RGMEEVGIYRVSGVATDIQALKAAFDVNNKDVSVMMSEMDVNAIAGTLKLYFRELPEPLF
TDEFYPNFAEGIALSDPVAKESCMLNLLLSLPEANLLTFLFLLDHLKRVAEKEAVNKMSL
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KRQSILFSTEV
```

.fna

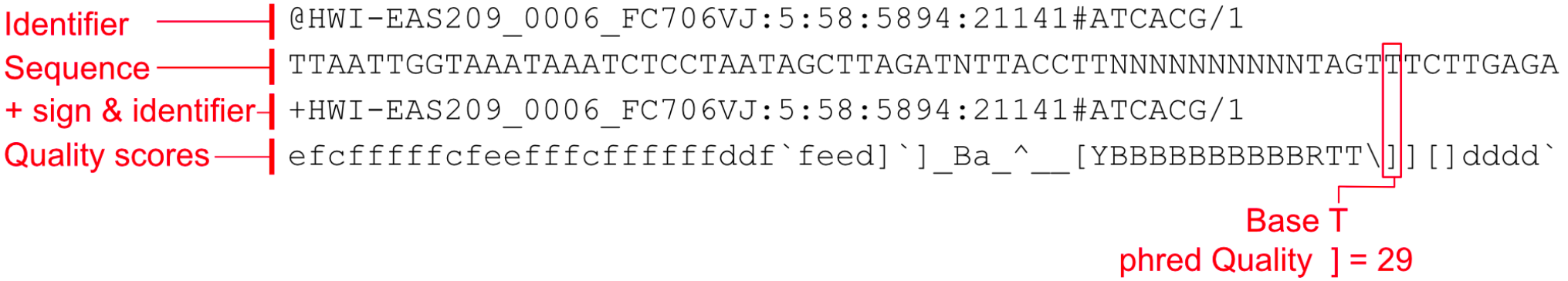
>NG_008679.1:5001-38170 Homo sapiens paired box 6 (PAX6)
ACCCTCTTTTCTTATCATTGACATTTAAACTCTGGGGCAGGTCCTCGCGTAGAACGCGGCTGTCAGATCT
GCCACTTCCCCTGCCGAGCGGCGGTGAGAAGTGTGGGAACCGGCGCTGCCAGGCTCACCTGCCTCCCCGC
CCTCCGCTCCCAGGTAACCGCCCGGGCTCCGGCCCCGGCCCGGCTCGGGGCCCGCGGGGCCTCTCCGCTG
CCAGCGACTGCTGTCCCCAAATCAAAGCCCGCCCCAAGTGGCCCCGGGGCTTGATTTTTGCTTTTAAAAG
GAGGCATACAAAGATGGAAGCGAGTTACTGAGGGAGGGATAGGAAGGGGGGTGGAGGAGGGACTTGTCTT
TGCCGAGTGTGCTCTTCTGCAAAAGTAGCAAAATGTTCCACTCCTAAGAGTGGACTTCCAGTCCGGCCCT
GAGCTGGGAGTAGGGGGCGGGAGTCTGCTGCTGCTGTCTGCTAAAGCCACTCGCGACCGCGAAAAATGCA
GGAGGTGGGGACGCACTTTGCATCCAGACCTCCTCTGCATCGCAGTTCACGACATCCACGCTTGGGAAAG
TCCGTACCCGCGCCTGGAGCGCTTAAAGACACCCTGCCGCGGGTCGGGCGAGGTGCAGCAGAAGTTTCCC
GCGGTTGCAAAGTGCAGATGGCTGGACCGCAACAAAGTCTAGAGATGGGGTTCGTTTCTCAGAAAGACGC

Read o lectura

- Formato fastq

  .fastq
- Incluye información sobre la calidad de la secuenciación

Fastq



Información sobre calidad codificada según el ASCII (American Standard Code for Information Interchange)

Error rates and quality values in next generation sequencing

Phred (Q) value: relates to the probability that the base has been incorrectly assigned.

The error usually comes from the acquisition of the signal or during basecalling

Quality value	Chance it is wrong	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%

- $Q = -10 \log_{10} P \iff P = 10^{-Q/10}$
  - Q = Phred quality score
  - P = probability of base call being incorrect

FastQ format

@SEQ_ID
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAG
TTT
+
!''*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>>CCCCCCC65

ASCII_BASE=33 Illumina, Ion Torrent, PacBio and Sanger

Q	P_error	ASCII	Q	P_error	ASCII	Q	P_error	ASCII	Q	P_error	ASCII
0	1.00000	33 !	11	0.07943	44 ,	22	0.00631	55 7	33	0.00050	66 B
1	0.79433	34 "	12	0.06310	45 -	23	0.00501	56 8	34	0.00040	67 C
2	0.63096	35 #	13	0.05012	46 .	24	0.00398	57 9	35	0.00032	68 D
3	0.50119	36 $	14	0.03981	47 /	25	0.00316	58 :	36	0.00025	69 E
4	0.39811	37 %	15	0.03162	48 0	26	0.00251	59 ;	37	0.00020	70 F
5	0.31623	38 &	16	0.02512	49 1	27	0.00200	60 <	38	0.00016	71 G
6	0.25119	39 '	17	0.01995	50 2	28	0.00158	61 =	39	0.00013	72 H
7	0.19953	40 (	18	0.01585	51 3	29	0.00126	62 >	40	0.00010	73 I
8	0.15849	41)	19	0.01259	52 4	30	0.00100	63 ?	41	0.00008	74 J
9	0.12589	42 *	20	0.01000	53 5	31	0.00079	64 @	42	0.00006	75 K
10	0.10000	43 +	21	0.00794	54 6	32	0.00063	65 A			

ASCII_BASE=64 Old Illumina

Q	P_error	ASCII	Q	P_error	ASCII	Q	P_error	ASCII	Q	P_error	ASCII
0	1.00000	64 @	11	0.07943	75 K	22	0.00631	86 V	33	0.00050	97 a
1	0.79433	65 A	12	0.06310	76 L	23	0.00501	87 W	34	0.00040	98 b
2	0.63096	66 B	13	0.05012	77 M	24	0.00398	88 X	35	0.00032	99 c
3	0.50119	67 C	14	0.03981	78 N	25	0.00316	89 Y	36	0.00025	100 d
4	0.39811	68 D	15	0.03162	79 O	26	0.00251	90 Z	37	0.00020	101 e
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7	0.19953	71 G	18	0.01585	82 R	29	0.00126	93]	40	0.00010	104 h
8	0.15849	72 H	19	0.01259	83 S	30	0.00100	94 ^	41	0.00008	105 i
9	0.12589	73 I	20	0.01000	84 T	31	0.00079	95 _	42	0.00006	106 j
10	0.10000	74 J	21	0.00794	85 U	32	0.00063	96 `			

DEC	OCT	HEX	BIN	Symbol	HTML Number	HTML Name	Description
32	040	20	00100000				Space
33	041	21	00100001	!	!		Exclamation mark
34	042	22	00100010	"	"	"	Double quotes (or speech marks)
35	043	23	00100011	#	#		Number
36	044	24	00100100	$	$		Dollar
37	045	25	00100101	%	%		Per cent sign
38	046	26	00100110	&	&	&	Ampersand
39	047	27	00100111	'	'		Single quote
40	050	28	00101000	(	(		Open parenthesis (or open bracket)
41	051	29	00101001	)	)		Close parenthesis (or close bracket)
42	052	2A	00101010	*	*		Asterisk
43	053	2B	00101011	+	+		Plus

ASCII_BASE=33 Illumina, Ion Torrent, PacBio and Sanger

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1	0.79433	65 A	12	0.06310	76 L	23	0.00501	87 W	34	0.00040	98 b
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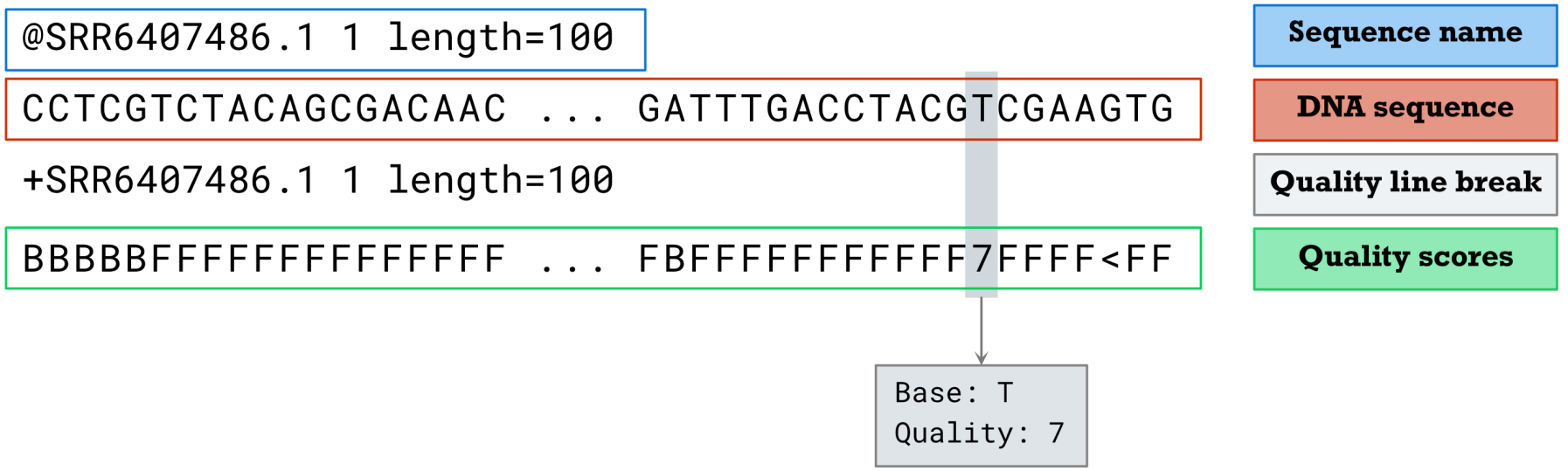
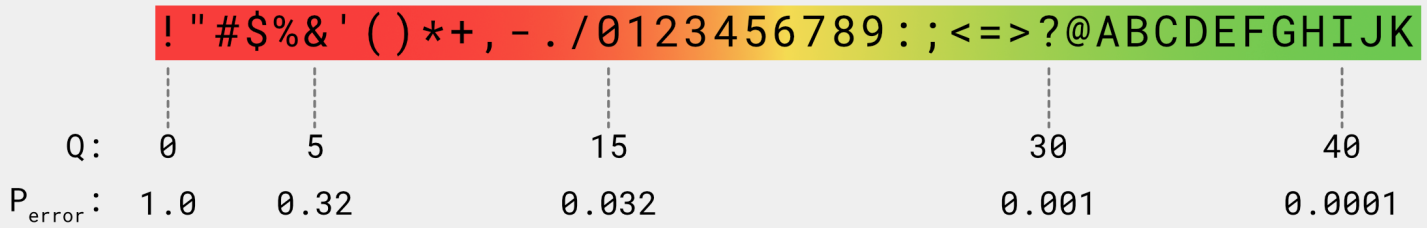
3	0.50119	67 C	14	0.03981	78 N	25	0.00316	89 Y	36	0.00025	100 d
4	0.39811	68 D	15	0.03162	79 O	26	0.00251	90 Z	37	0.00020	101 e
5	0.31623	69 E	16	0.02512	80 P	27	0.00200	91 [	38	0.00016	102 f
6	0.25119	70 F	17	0.01995	81 Q	28	0.00158	92 \	39	0.00013	103 g
7	0.19953	71 G	18	0.01585	82 R	29	0.00126	93]	40	0.00010	104 h
8	0.15849	72 H	19	0.01259	83 S	30	0.00100	94 ^	41	0.00008	105 i
9	0.12589	73 I	20	0.01000	84 T	31	0.00079	95 _	42	0.00006	106 j
10	0.10000	74 J	21	0.00794	85 U	32	0.00063	96 `			

FASTQ file sample:

```
@SRR6407486.1 1 length=100
CCTCGTCTACAGCGACAACGTCCAGACCCGCGAACGGGTGATGCGGGCCCTGGGCAAACGGTTGCACCCGGATCTGCCCGATTTGACCTACGTCGAAGTG
+SRR6407486.1 1 length=100
BBBBBFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF<FFFFFFFFFFFFFFFFFBFFFFFFFFFFFFFFFFFFFBFFFFFFFFFFF7FFFF<FF
```



Quality scores as ASCII characters:



$$Q = -10\log_{10} P_{error}$$

Fastq Paired-end files

- Dos archivos (R1 y R2)
- **El orden importa!**

mislecturas_R1.fastq

```
@M02286:19:000000000-AA549:1:1101:12677:1273 1:N:0:23
CCTACGGGTGGCAGCAGTGAGGAATATTGGTCAATGGACGGAAGTCT
+
ABC8C,:@F:CE8,B-,C,-6-9-C,CE9-CC--C-<-C++,,+;CE
@M02286:19:000000000-AA549:1:1101:15048:1299 1:N:0:23
CCTACGGGTGGCTGCAGTGAGGAATATTGGACAATGGTCGGAAGACT
+
ABC@CC77CFCEG;F9<F89<9--C,CE,--C-6C-,CE:++7:,CF
```

mislecturas_R2.fastq

```
@M02286:19:000000000-AA549:1:1101:12677:1273 2:N:0:23
CACTACCCGTGTATCTAATCCTGTTTGATACCCGCACCTTCGAGCTTA
+
--8A,CCE+,,;,<CC,,<CE@,CFD,,C,CFF+@+@CCEF,,,B+C,
@M02286:19:000000000-AA549:1:1101:15048:1299 2:N:0:23
CACTACCGGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGTCCATC
+
-6AC,EE@::CF7CFF<<FFGGDFFF,@FGGGG?F7FEGGGDEFF>FF
```

Grupos de trabajo

- Trabajarán por mucho tiempo (si es que siguen)
- Recomiendo trabajar en grupos de dos personas, pero pueden trabajar solos.
- Definir un nombre de usuario personal (corto)