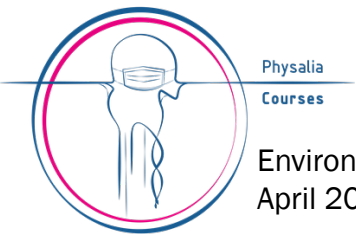


Environmental metagenomics

Quality control and trimming



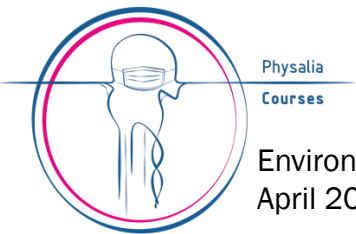
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April 2022

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Quality control

- Things to consider
 - Sequence quality
 - Adapter contamination



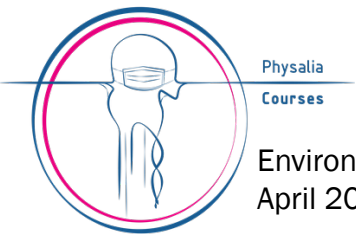
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Sequence quality

- PCR – what happens in PCR, stays in PCR



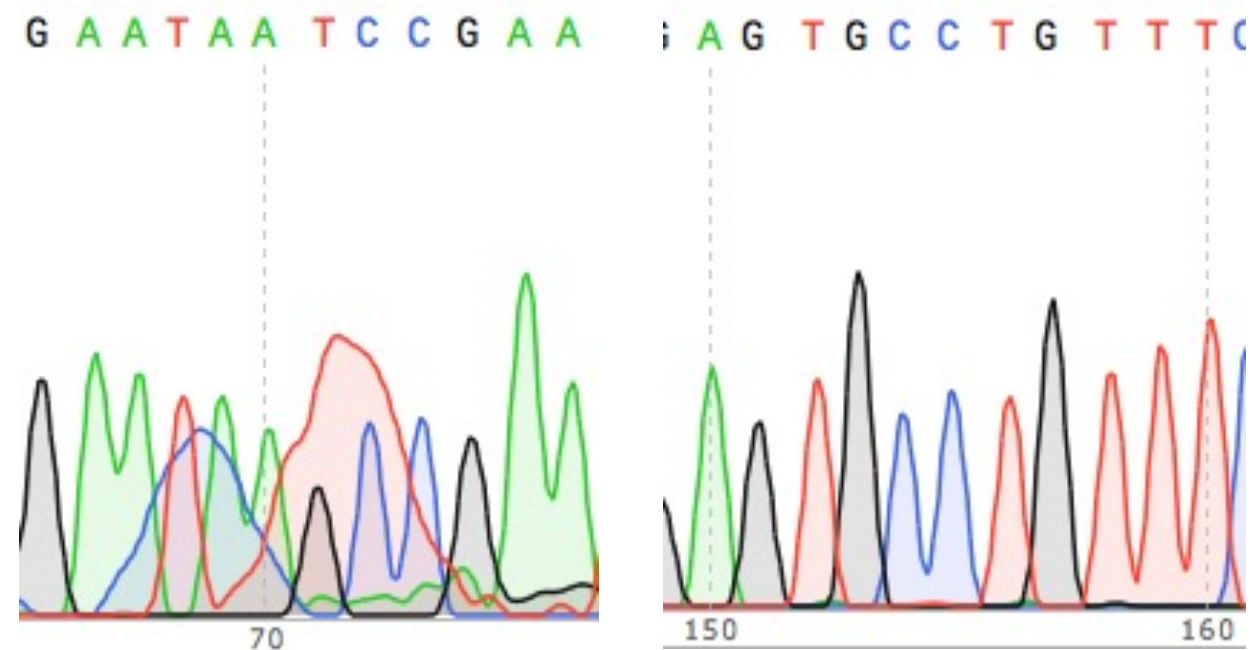
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Sequence quality

- PCR – what happens in PCR, stays in PCR
- Sequencing

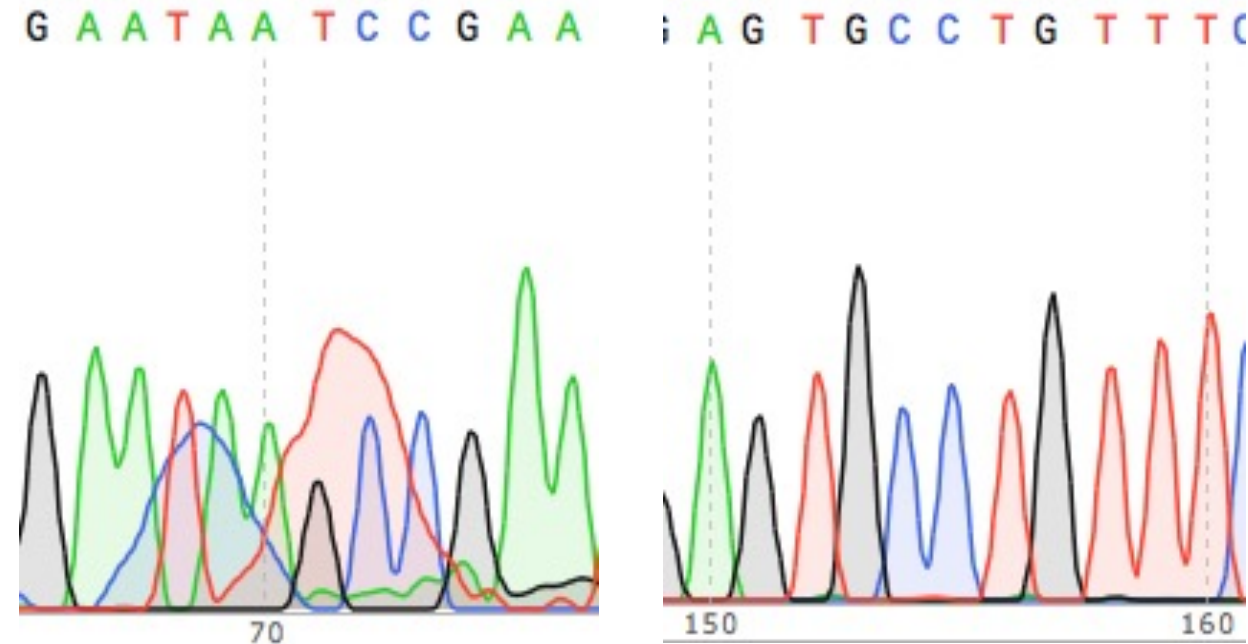


<https://bitesizebio.com/10709/how-to-analyze-dna-sequencing-results-properly/>

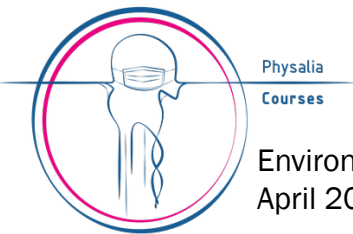
Sequence quality

- PCR – what happens in PCR, stays in PCR
- Sequencing

```
@A00464:69:H7L3KDSXX:1:1101:7726:1000 1:N:0:GTCTAATG+NGTGGTCA
GNATTGCCATCGGGTAAAGCGTCAGGAAGCCGAGCAGCAGGATCAGCAGGGCGATGCCGGCAG
GCCGGCCGAAGCGGCGGAGGCCGCGCGGCAGCGCAGCCGTCGGAAGAGTGACGGCAACCGTCA
TCGGCAGCCGCCCATCGTCAATCGT
+
F#FFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF:F
FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
```



<https://bitesizebio.com/10709/how-to-analyze-dna-sequencing-results-properly/>



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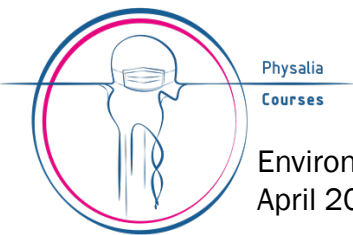
Sequence quality

- PCR – what happens in PCR, stays in PCR
- Sequencing

```
@A00464:69:H7L3KDSXX:1:1101:7726:1000 1:N:0:GTCTAATG+NGTGGTCA
GNATTGCCATCGGGTAAAGCGTCAGGAAGCCGAGCAGCAGGATCAGCAGGGCGATGCCGGCAG
GCCGGCCGAAGCGGCGGAGGCCGCGCGGCAGCGCAGCCGTCGGAAGAGTGACGGCAACCGTCA
TCGGCAGCCGCCCATCGTCAATCGT
+
F#FFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF:F
FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
```

ASCII TABLE

Decimal	Hex	Char	Decimal	Hex	Char	Decimal	Hex	Char	Decimal	Hex	Char
0	0	[NULL]	32	20	[SPACE]	64	40	@	96	60	`
1	1	[START OF HEADING]	33	21	!	65	41	A	97	61	a
2	2	[START OF TEXT]	34	22	"	66	42	B	98	62	b
3	3	[END OF TEXT]	35	23	#	67	43	C	99	63	c
4	4	[END OF TRANSMISSION]	36	24	\$	68	44	D	100	64	d
5	5	[ENQUIRY]	37	25	%	69	45	E	101	65	e
6	6	[ACKNOWLEDGE]	38	26	&	70	46	F	102	66	f
7	7	[BELL]	39	27	'	71	47	G	103	67	g
8	8	[BACKSPACE]	40	28	(72	48	H	104	68	h
9	9	[HORIZONTAL TAB]	41	29)	73	49	I	105	69	i
10	A	[LINE FEED]	42	2A	*	74	4A	J	106	6A	j
11	B	[VERTICAL TAB]	43	2B	+	75	4B	K	107	6B	k
12	C	[FORM FEED]	44	2C	,	76	4C	L	108	6C	l
13	D	[CARRIAGE RETURN]	45	2D	-	77	4D	M	109	6D	m
14	E	[SHIFT OUT]	46	2E	.	78	4E	N	110	6E	n
15	F	[SHIFT IN]	47	2F	/	79	4F	O	111	6F	o
16	10	[DATA LINK ESCAPE]	48	30	0	80	50	P	112	70	p
17	11	[DEVICE CONTROL 1]	49	31	1	81	51	Q	113	71	q
18	12	[DEVICE CONTROL 2]	50	32	2	82	52	R	114	72	r
19	13	[DEVICE CONTROL 3]	51	33	3	83	53	S	115	73	s
20	14	[DEVICE CONTROL 4]	52	34	4	84	54	T	116	74	t
21	15	[NEGATIVE ACKNOWLEDGE]	53	35	5	85	55	U	117	75	u
22	16	[SYNCHRONOUS IDLE]	54	36	6	86	56	V	118	76	v
23	17	[ENG OF TRANS. BLOCK]	55	37	7	87	57	W	119	77	w
24	18	[CANCEL]	56	38	8	88	58	X	120	78	x
25	19	[END OF MEDIUM]	57	39	9	89	59	Y	121	79	y
26	1A	[SUBSTITUTE]	58	3A	:	90	5A	Z	122	7A	z
27	1B	[ESCAPE]	59	3B	;	91	5B	[123	7B	{
28	1C	[FILE SEPARATOR]	60	3C	<	92	5C	\	124	7C	
29	1D	[GROUP SEPARATOR]	61	3D	=	93	5D]	125	7D	}
30	1E	[RECORD SEPARATOR]	62	3E	>	94	5E	^	126	7E	~
31	1F	[UNIT SEPARATOR]	63	3F	?	95	5F	_	127	7F	[DEL]



Sequence quality

- PCR – what happens in PCR, stays in PCR
- Sequencing

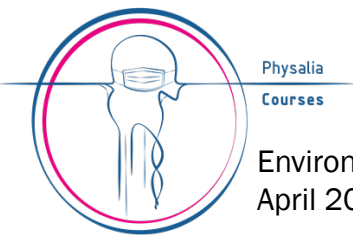
```
@A00464:69:H7L3KDSXX:1:1101:7726:1000 1:N:0:GTCTAATG+NGTGGTCA
GNATTGCCATCGGGTAAAGCGTCAGGAAGCCGAGCAGCAGGATCAGCAGGGCGATGCCGGCAG
GCCGGCCGAAGCGGCGGAGGCCGCGCGGCAGCGCAGCCGTCGGAAGAGTGACGGCAACCGTCA
TCGGCAGCCGCCCATCGTCAATCGT
+
F#FFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF:FFFF
FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
FFFFFFFFFFFFFFFFFFFFFFFF
```

Q = 0
E = 1

ASCII 33

ASCII TABLE

Decimal	Hex	Char	Decimal	Hex	Char	Decimal	Hex	Char	Decimal	Hex	Char
0	0	[NULL]	32	20	[SPACE]	64	40	@	96	60	`
1	1	[START OF HEADING]	33	21	!	65	41	A	97	61	a
2	2	[START OF TEXT]	34	22	"	66	42	B	98	62	b
3	3	[END OF TEXT]	35	23	#	67	43	C	99	63	c
4	4	[END OF TRANSMISSION]	36	24	\$	68	44	D	100	64	d
5	5	[ENQUIRY]	37	25	%	69	45	E	101	65	e
6	6	[ACKNOWLEDGE]	38	26	&	70	46	F	102	66	f
7	7	[BELL]	39	27	'	71	47	G	103	67	g
8	8	[BACKSPACE]	40	28	(72	48	H	104	68	h
9	9	[HORIZONTAL TAB]	41	29)	73	49	I	105	69	i
10	A	[LINE FEED]	42	2A	*	74	4A	J	106	6A	j
11	B	[VERTICAL TAB]	43	2B	+	75	4B	K	107	6B	k
12	C	[FORM FEED]	44	2C	,	76	4C	L	108	6C	l
13	D	[CARRIAGE RETURN]	45	2D	-	77	4D	M	109	6D	m
14	E	[SHIFT OUT]	46	2E	.	78	4E	N	110	6E	n
15	F	[SHIFT IN]	47	2F	/	79	4F	O	111	6F	o
16	10	[DATA LINK ESCAPE]	48	30	0	80	50	P	112	70	p
17	11	[DEVICE CONTROL 1]	49	31	1	81	51	Q	113	71	q
18	12	[DEVICE CONTROL 2]	50	32	2	82	52	R	114	72	r
19	13	[DEVICE CONTROL 3]	51	33	3	83	53	S	115	73	s
20	14	[DEVICE CONTROL 4]	52	34	4	84	54	T	116	74	t
21	15	[NEGATIVE ACKNOWLEDGE]	53	35	5	85	55	U	117	75	u
22	16	[SYNCHRONOUS IDLE]	54	36	6	86	56	V	118	76	v
23	17	[ENG OF TRANS. BLOCK]	55	37	7	87	57	W	119	77	w
24	18	[CANCEL]	56	38	8	88	58	X	120	78	x
25	19	[END OF MEDIUM]	57	39	9	89	59	Y	121	79	y
26	1A	[SUBSTITUTE]	58	3A	:	90	5A	Z	122	7A	z
27	1B	[ESCAPE]	59	3B	;	91	5B	[123	7B	{
28	1C	[FILE SEPARATOR]	60	3C	<	92	5C	\	124	7C	
29	1D	[GROUP SEPARATOR]	61	3D	=	93	5D]	125	7D	}
30	1E	[RECORD SEPARATOR]	62	3E	>	94	5E	^	126	7E	~
31	1F	[UNIT SEPARATOR]	63	3F	?	95	5F	_	127	7F	[DEL]



Sequence quality

- PCR – what happens in PCR, stays in PCR
- Sequencing

@m61415_211210_031838/561/ccs

```
AGGTGGAACCGGACGACCCAGCGCAGCGGCCCTGCGGGCCCAAGTTGCTGCGCCACGTCGCGACCGCCATCAATGCGCGGATTGCAACACGCGGACGAAACGCTTGGTCGACATGATCAAACGCCCAATGCGGAATGTGGTGGCTGC
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GTCCAGTGCAGCGGTGAAATCGCGCTCGTCAAAATCAGAACGGGCTCTTACCGCAAGCTCCATGCTGAACTTTTGAGGCGCTCGCGCTTGCACGATTTCGTTGCCGTCGCGCTCGATCCGTCGAACGAAATCGCACGACGTCGCGCTGTTTGCAC
AGCGGCTCACCCGCTCTTTTCGTAAGCGGTGACGACGTTTCAAGACGCGCGGGAATGCCCGCTCAAGCGCAATTCAACAAGGCGCGCAGCGCTCATCGCGACAGTTTCGCTCATCTTCAATACCGCGCTATTGCCGAACGCGAGACAGGGCGCGG
TCTTCCACGTCGCGGTGATGAATGGCAGCTTCCACGGACTAATCAATCGCGCACACCGGACAGGATGAAACAGCGTGTAGTTCAAGTGCCTAGGCGTTGGATAGGTGTGGCCATCCACCGCGTGCACATTTTCGCGAAGTAATAGAAGTTGTCGCGTGC
GCGCGGATGAGTTGCTTGCCAGTTTGCAGGATGACTTGGCGCGAGTCCATCGTTTCGGTGCAGGAAATTTCCGGCACATGCTGCGCGATCAAATCGCGGAGTTTGCACAAAGGCGCGCGCTCCGTCGCGGGCAGCCCGCCACTTTGGGAACGCA
GCTTTCGCGGCGGCGACGCGCGCTTCACTTCCGCTTCTCCACCGGAGCGACTTTCGCGTTCGCGGTTGATGCTCTCGAAGTAATCACGTCCTGAACTGCTTTGCCGTCATCAGATGTTTCGATGCGCATGGGATGCCCTTGTAC
+
```

```
~z~~Xu@uq~l~~~|~b~~~~~`~y~c~~~s~i~~~e~(~p~v~n~o~~~\~~~f~~|y~~cb~~P~~~~~Q~~~~~q~~~~~x~~~~~f~~z}w~~~~~|~~p~S~~~v~~~{~~~~~y~~m~r~~N
~s`~n~~~~~k~~~~~v~~~~~^~R~~~~~x~~~~~?~~~x~t~~~y~~4~~~s~~~f~~~~~z~m~h~~~}~~~~~r~~`^~~~\~~~i~~~t~~~g~me~j~Y~~~~~{~~~~~}~~~~~x~~~~~|~~~w~~~~~|~~~y~~~~~O~~~~~L~~~{
~~~~~|~~~~~|~~~~~G~~~~~Z~~~~~y~~~~~s~~~~~u~~~~~|~~~~~t~~~~~v~~~|~~~~~s~~~~~}~~~~~R~~~~~x~~~~~y~~~~~{~A~~~~~}~~~~~n~~~~~a~~~~~z~~~~~{~X~k~~~l~~~u~~~~~f~~~~~yOz~~~~~|~|
~t~gp~~~~~t~~~~~l~~~K~~~~~e~~~~~Z~~~~~v~~~d~~~~~v~e~~~A~~~~~m~~~~~g~~~~~Rw}~~~A~~~~~r~~~~~j~~~~~qgh~}~va~~~V~~~yX~~~~~l~~~t~~~Z~~~~~z~~~}~~~~~W~~~~~V~~~t
~f~n~~~{~~~~~a~~~~~|b~t~{~~~~~=~~~~~}h~~~f~|~z~~~~~F~~~~~t~~~~~c~~~l~~~r~~~x~~~~~u~P~~~~~w~g~~~~~}~~~~~\~~~Z~~~~~c~~~~~w~~~~~e~~~x~~~{~~~O~~~
~y~~~~~|~~~~~i~o~~~~~}~~~~~S~~~~~x~~~~~c~~~y~~~y~~~~~e~~~~~i~~~~~k~~~~~u~z~~~~~s~~~~~z~~~~~{~~~v~t~~~~~w~g~q~~~I~~~~~j~~~~~5~~~~~=~~~~~|i~~~i~~~v~~~~~|~~~g~~~v~~~~~m~~~V|~
~z~d~~~~~W~~~~~wm~~~~~{~~~~~vp~~~~~o~~~~~m~~~~~wh~o~p~Z{~~~}~~~r~~~~~k~~~~~mm~~~~~i~~~~~k~~~~~x~~~~~t~~~~~x~~~~~D~~~~~
~s~~~~~e~~~~~{~~~~~i~~~~~v~~~}~~~~~_Y~~~~~G~~~~~D~wt~~~~~s~~~~~6~~~~~o~t~~~|~{~~~~~l~n~~~nS~~~I~~~~~^~~~~~y~~~~~
~z~~~~~}~~~~~u~~~~~t~~~~~e~~~~~p~~~~~c~~~\~~~v~W~r~~~~~t~~~~~}~~~~~oq~~~~~h~~~~~w~m~~~~~mznKRa~~~l~~~q~~~~~o~t~a~~~~~u~~~~~
```

Decimal	Hex	Char	Decimal	Hex	Char	Decimal	Hex	Char
32	20	[SPACE]	64	40	@	96	60	`
33	21	!	65	41	A	97	61	a
34	22	"	66	42	B	98	62	b
35	23	#	67	43	C	99	63	c
36	24	\$	68	44	D	100	64	d
37	25	%	69	45	E	101	65	e
38	26	&	70	46	F	102	66	f
39	27	'	71	47	G	103	67	g
40	28	(72	48	H	104	68	h
41	29)	73	49	I	105	69	i
42	2A	*	74	4A	J	106	6A	j
43	2B	+	75	4B	K	107	6B	k
44	2C	,	76	4C	L	108	6C	l
45	2D	-	77	4D	M	109	6D	m
46	2E	.	78	4E	N	110	6E	n
47	2F	/	79	4F	O	111	6F	o
48	30	0	80	50	P	112	70	p
49	31	1	81	51	Q	113	71	q
50	32	2	82	52	R	114	72	r
51	33	3	83	53	S	115	73	s
52	34	4	84	54	T	116	74	t
53	35	5	85	55	U	117	75	u
54	36	6	86	56	V	118	76	v
55	37	7	87	57	W	119	77	w
56	38	8	88	58	X	120	78	x
57	39	9	89	59	Y	121	79	y
58	3A	:	90	5A	Z	122	7A	z
59	3B	;	91	5B	[123	7B	{
60	3C	<	92	5C	\	124	7C	
61	3D	=	93	5D]	125	7D	}
62	3E	>	94	5E	^	126	7E	~
63	3F	?	95	5F	_	127	7F	[DEL]



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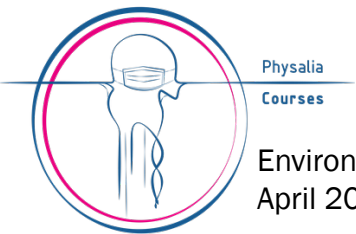
Igor S. Pessi & Antti Karkman, University of Helsinki

Sequence quality

- PCR – what happens in PCR, stays in PCR
- Sequencing
- Illumina:
 - Occurrence: substitutions > indels
 - Quality scores: substitutions < indels
 - Overall quality: R1 > R2; beginning > end

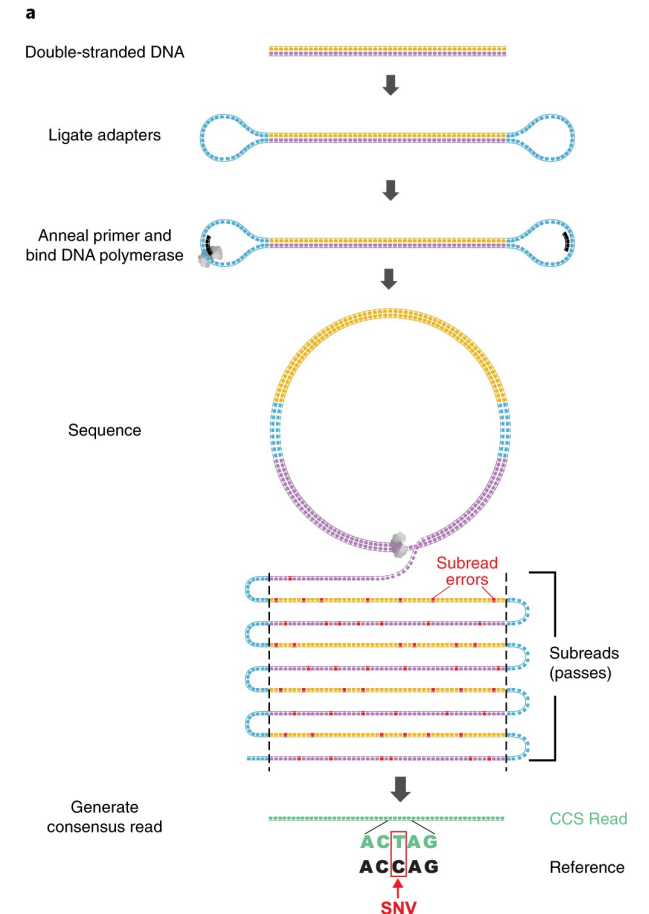
”G seems to be preferentially incorporated if an A, C or T is sequenced and if G is sequenced a T is falsely incorporated for the majority of substitutions.”

Schirmer et al. 2016. BMC Bioinformatics

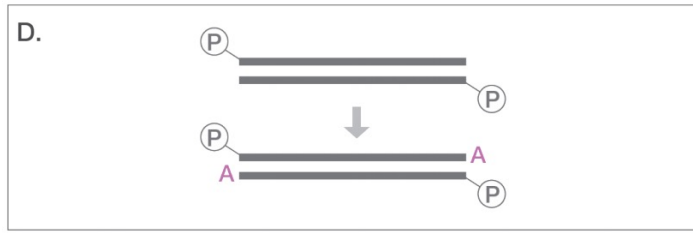


Sequence quality

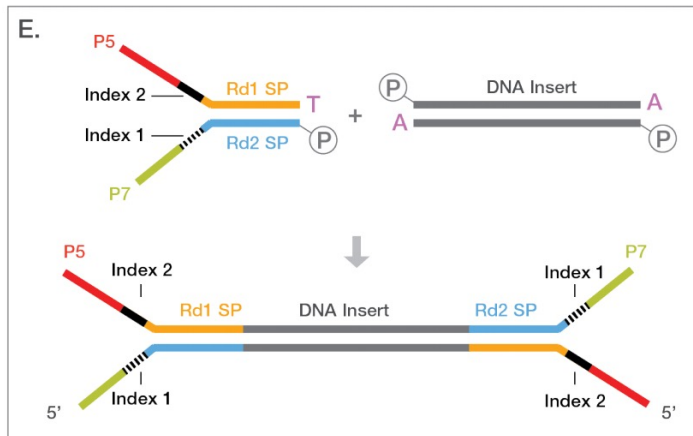
- PCR – what happens in PCR, stays in PCR
- Sequencing
- Illumina:
 - Occurrence: substitutions > indels
 - Quality scores: substitutions < indels
 - Overall quality: R1 > R2; beginning > end
- Pacbio: substitutions



Adapter contamination

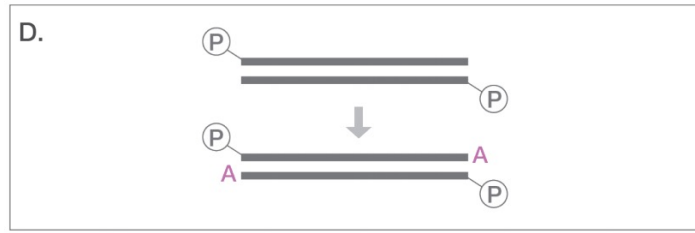


A-base is added.

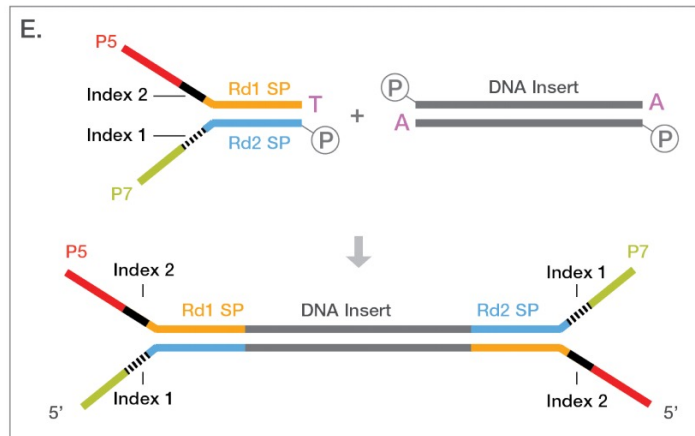


Dual-index adapters are ligated to the fragments* and final product is ready for cluster generation.

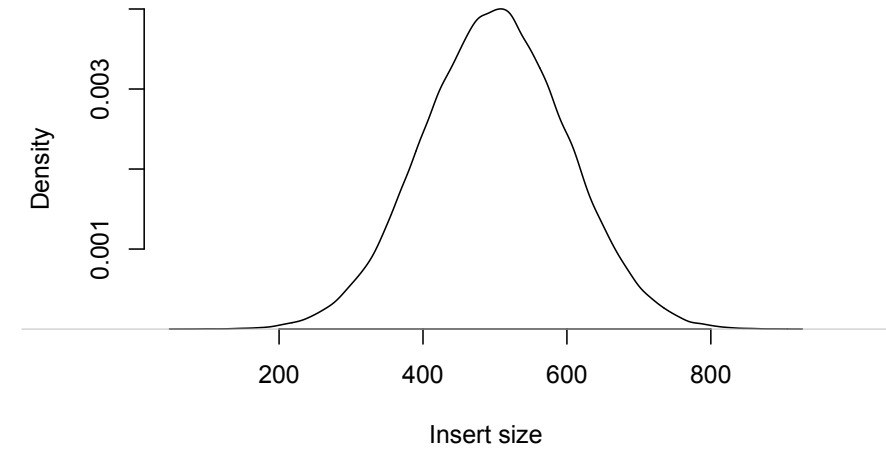
Adapter contamination



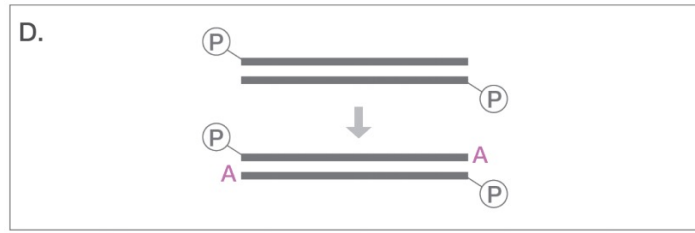
A-base is added.



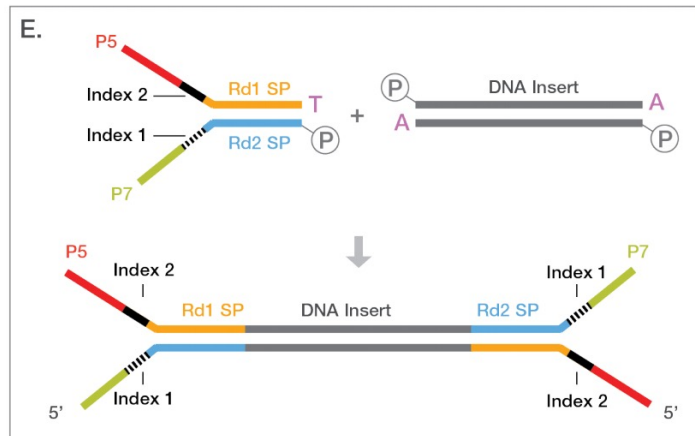
Dual-index adapters are ligated to the fragments* and final product is ready for cluster generation.



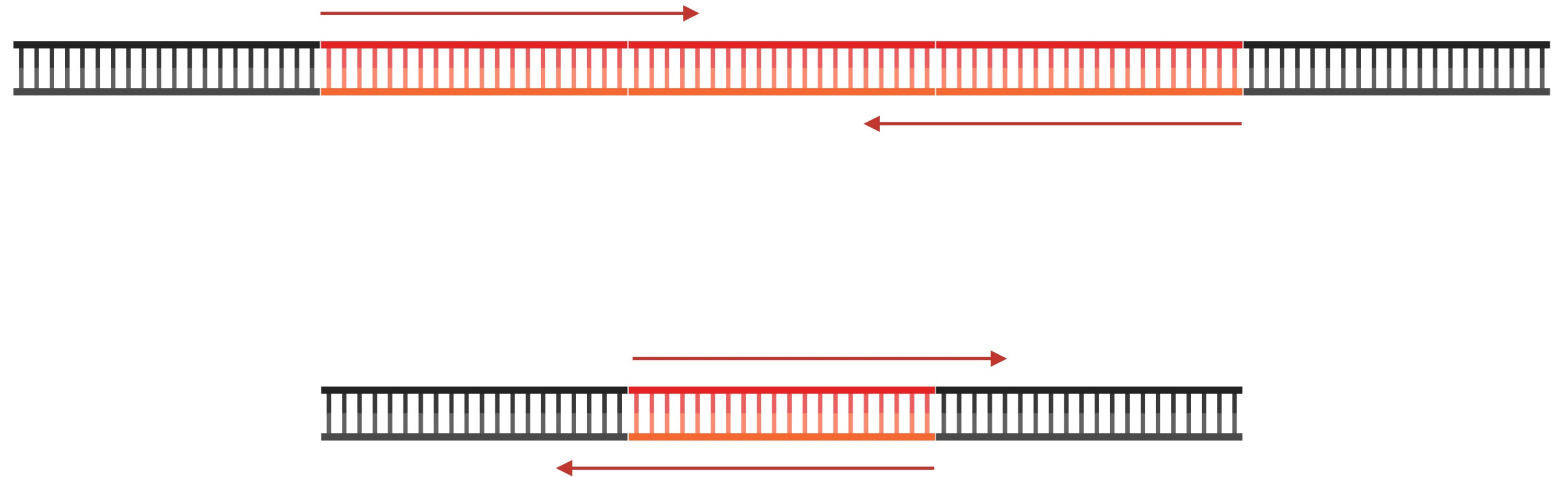
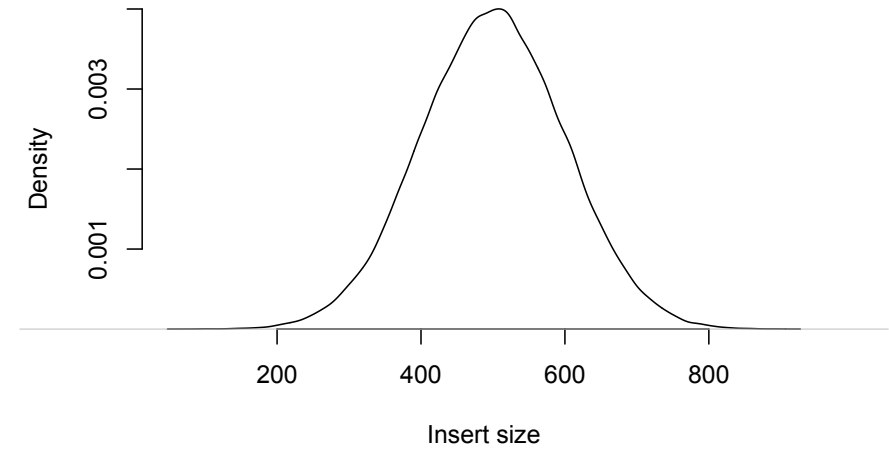
Adapter contamination



A-base is added.

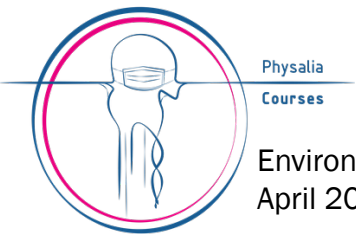


Dual-index adapters are ligated to the fragments* and final product is ready for cluster generation.



Trimming

- What to do then?



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