Introduction to NGS

Hubert Rehrauer
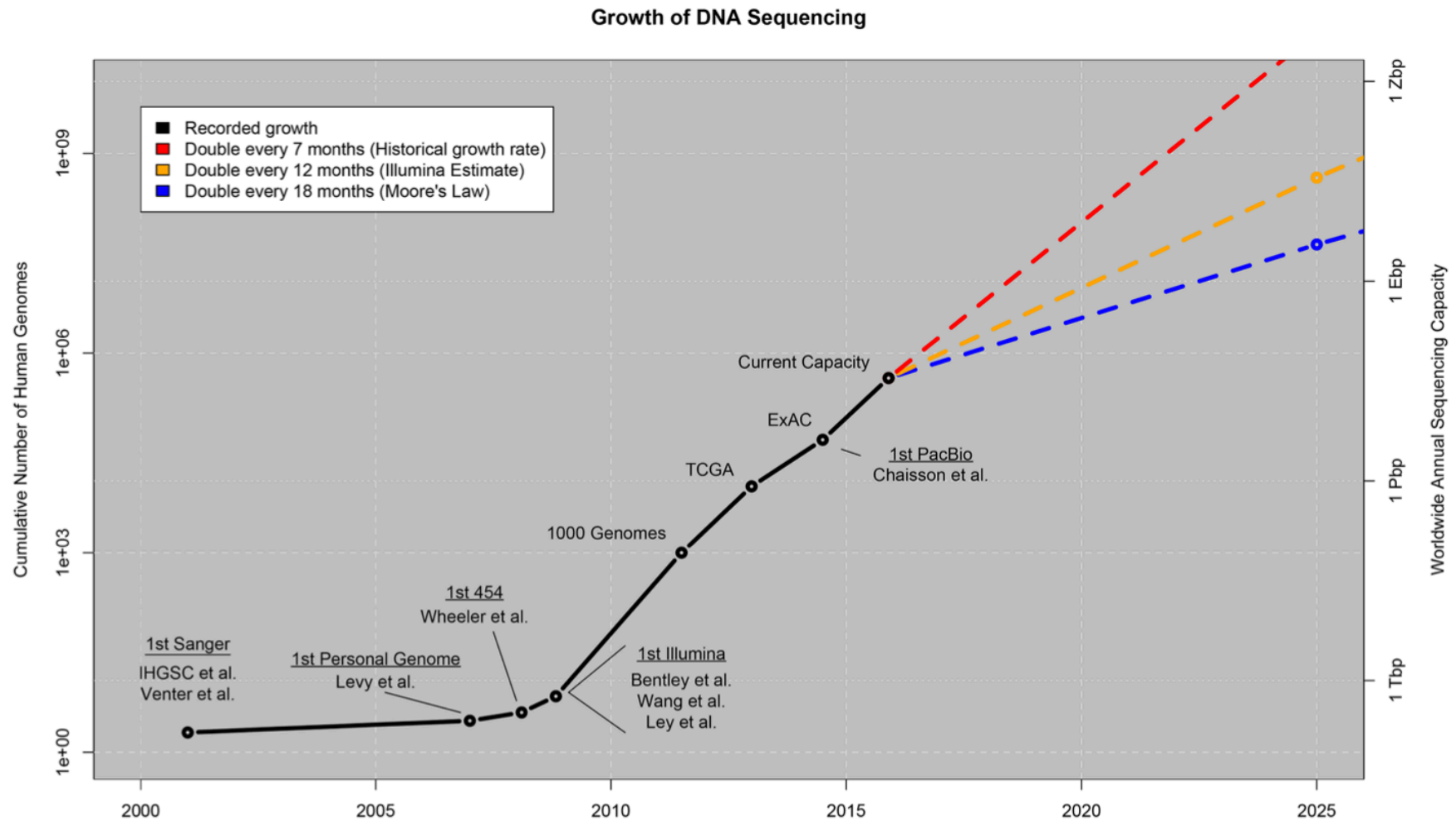
NGS Data Increase

Growth of DNA Sequencing

- NGS data increases faster than computer speed

Stephens ZD etal. (2015) Big Data: Astronomical or Genomical? PLoS Biol 13(7): e1002195.

Ingredients for the success

- Evolution has yielded DNA and RNA molecules for information storage and transfer. They have good properties to be read (**measured**)
- NGS technologies rely on
  - **massive parallelization**
  - measurement process is done by individual molecules (**cheap and fast**)

Illumina Flow cell
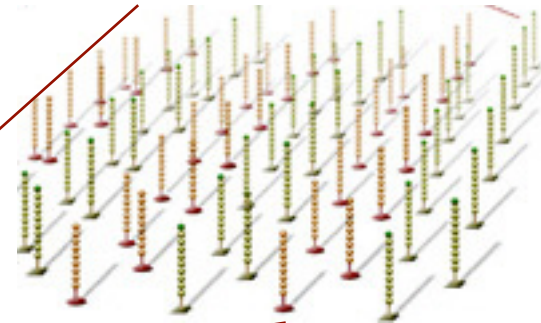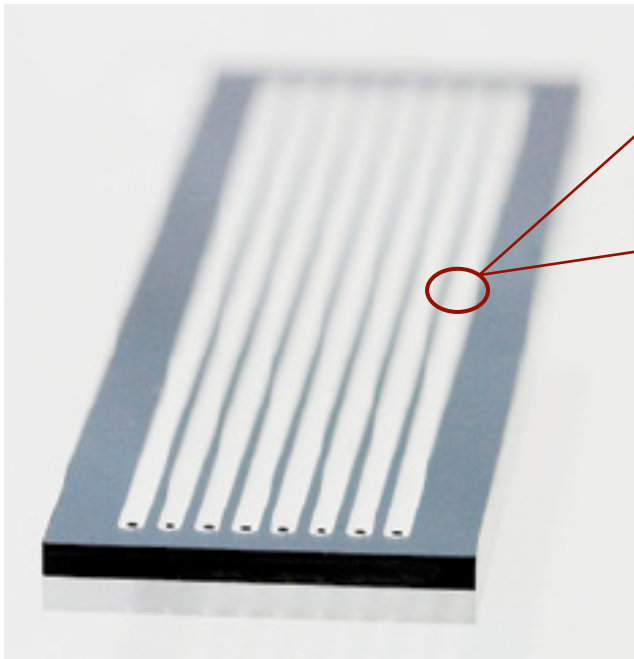
Cluster generation overview

Bind single DNA molecules to surface

Amplify on surface

~1000 molecules per ~ 1 µm cluster

annealing → elongation → denaturation

bridge amplification

annealing → elongation
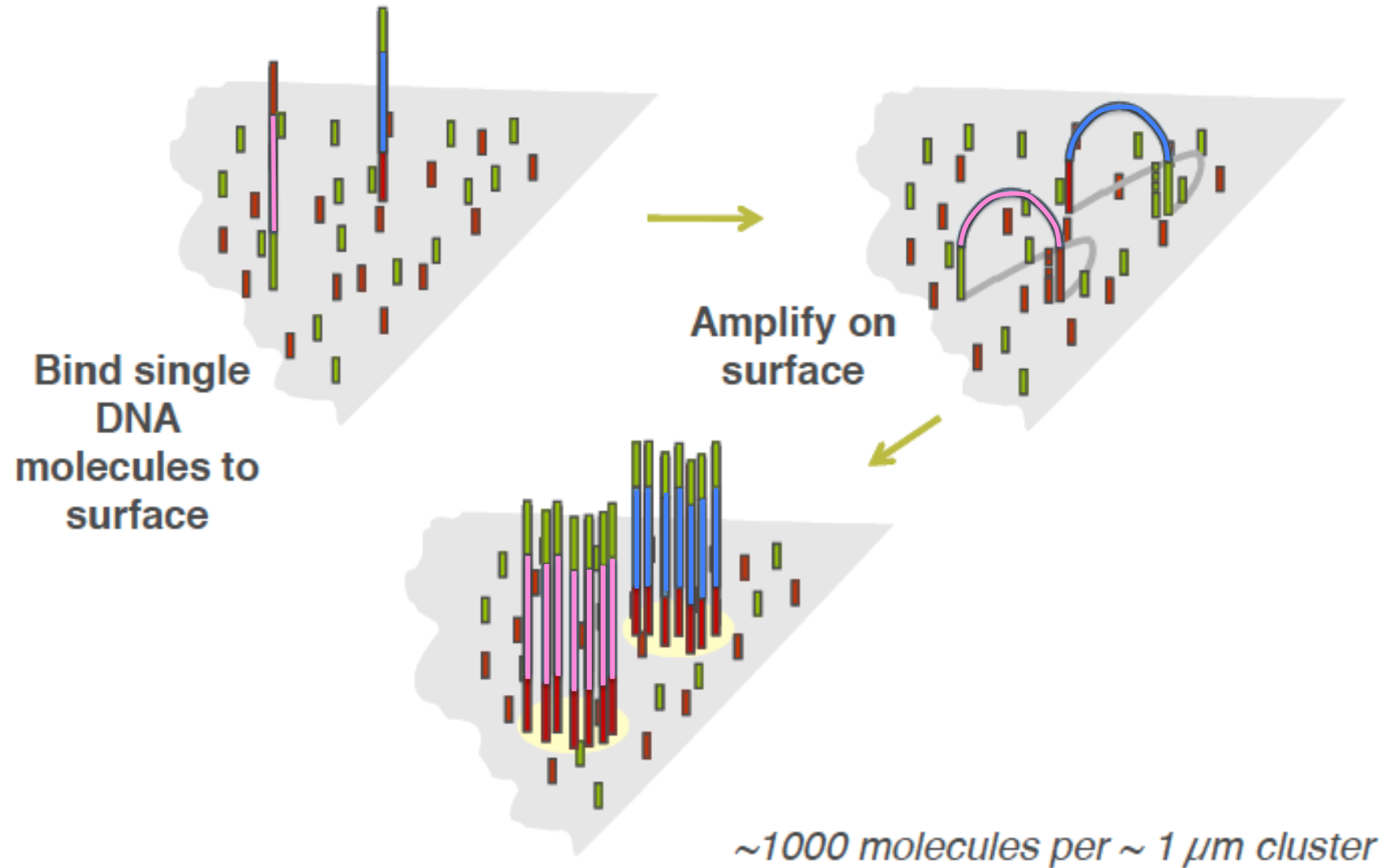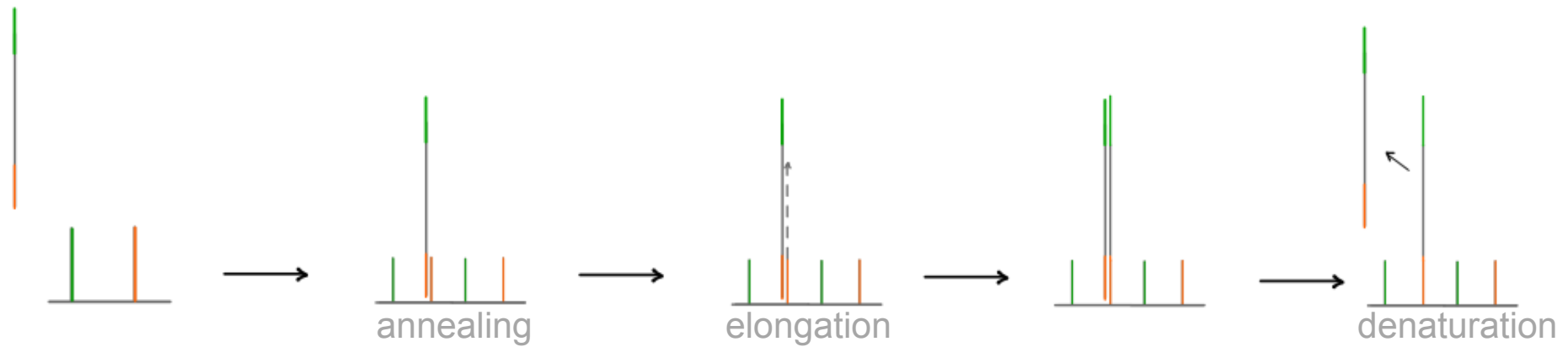
denaturation

Illumina Sequencing

C ⬤ A ⬤
T ⬤ G ⬤

Top: CATCGT
Bottom: CCCCCC

Laser
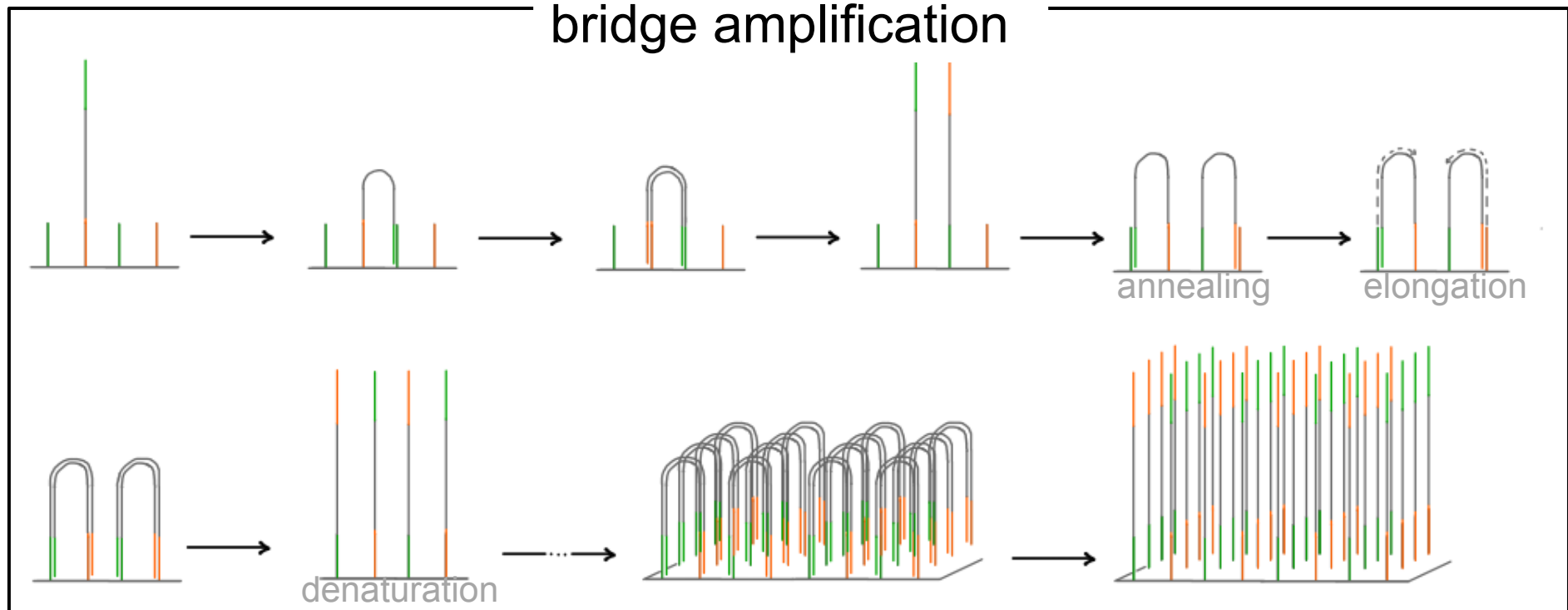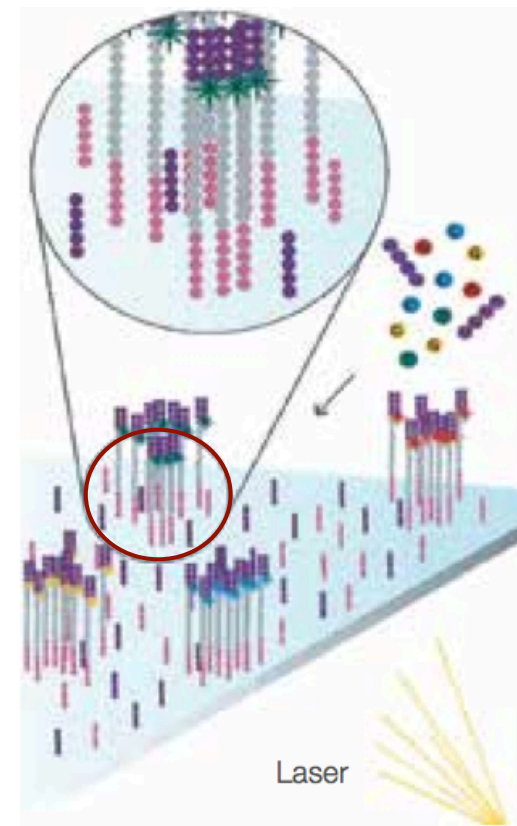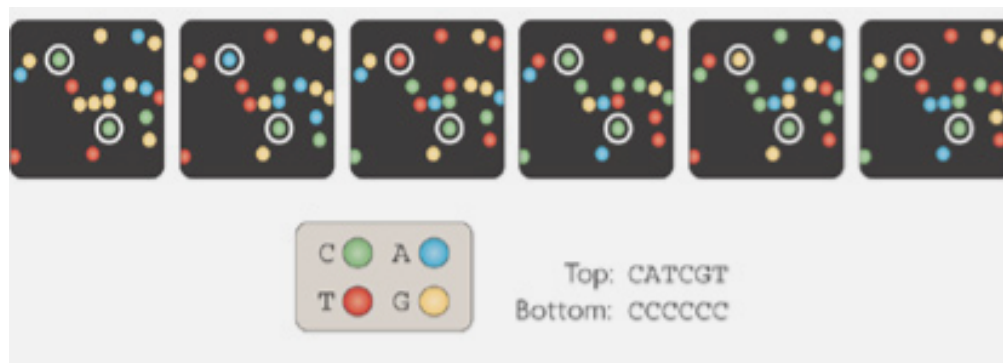
The first sequencing cycle begins by adding four labeled reversible terminators, primers, and DNA polymerase.

Cluster

Phred scores measure base call accuracy

- P
  - error probability of a given base call
- Q
  - $-10\log_{10}P$
- Assign to each base
- Range from 0-41

Phred Quality Score	Probability of Incorrect Base Call	Base Call Accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1,000	99.9%
40	1 in 10,000	99.99%
50	1 in 100,000	99.999%

Ewing B, Green P. 1998. Genome Res. 8(3):186-194.

http://en.wikipedia.org/wiki/Phred_quality_score

Phred scores are stored with sequences

- FASTQ
  - 4 lines:
    1. Header line for Read (starts with "@" and the sequence ID)
    2. Sequence
    3. Header line for Qualities (starts with "+")
    4. Quality score (represented in ASCII format)

```
@HWI-ST1034:40:C08PJACXX:2:1101:20681:1994 1:N:0:ATCACG

CTCGNAGACTGGCAACTTGTTCTGGTTTACTGCACCTTCTTTTAAAGGCAGAAAGGC

+

CCCF#2ADHHHGHJJJIJJHIIIJJHIJJJJJJJJJBGIIJJJJJJJJJJJJJJJIJ
```

Phred scores can be **ASCII encoded**

- Add an offset and convert the sum to ASCII

- Current format
    - **Illumina 1.9 (i.e. Sanger format)**
    - Phred scoring: 0-41;
    - Offset: 33
    - 41+33=74 (J)
    - All current sequencers

Dec	Hx	Oct	Char		Dec	Hx	Oct	Html	Chr	Dec	Hx	Oct	Html	Chr	Dec	Hx	Oct	Html	Chr
0	0	000	NUL	(null)	32	20	040	 	Space	64	40	100	@	@	96	60	140	`	`
1	1	001	SOH	(start of heading)	33	21	041	!	!	65	41	101	A	A	97	61	141	a	a
2	2	002	STX	(start of text)	34	22	042	"	"	66	42	102	B	B	98	62	142	b	b
3	3	003	ETX	(end of text)	35	23	043	#	#	67	43	103	C	C	99	63	143	c	c
4	4	004	EOT	(end of transmission)	36	24	044	$	$	68	44	104	D	D	100	64	144	d	d
5	5	005	ENQ	(enquiry)	37	25	045	%	%	69	45	105	E	E	101	65	145	e	e
6	6	006	ACK	(acknowledge)	38	26	046	&	&	70	46	106	F	F	102	66	146	f	f
7	7	007	BEL	(bell)	39	27	047	'	'	71	47	107	G	G	103	67	147	g	g
8	8	010	BS	(backspace)	40	28	050	(	(	72	48	110	H	H	104	68	150	h	h
9	9	011	TAB	(horizontal tab)	41	29	051	)	)	73	49	111	I	I	105	69	151	i	i
10	A	012	LF	(NL line feed, new line)	42	2A	052	*	*	74	4A	112	J	J	106	6A	152	j	j
11	B	013	VT	(vertical tab)	43	2B	053	+	+	75	4B	113	K	K	107	6B	153	k	k
12	C	014	FF	(NP form feed, new page)	44	2C	054	,	,	76	4C	114	L	L	108	6C	154	l	l
13	D	015	CR	(carriage return)	45	2D	055	-	-	77	4D	115	M	M	109	6D	155	m	m
14	E	016	SO	(shift out)	46	2E	056	.	.	78	4E	116	N	N	110	6E	156	n	n
15	F	017	SI	(shift in)	47	2F	057	/	/	79	4F	117	O	O	111	6F	157	o	o
16	10	020	DLE	(data link escape)	48	30	060	0	0	80	50	120	P	P	112	70	160	p	p
17	11	021	DC1	(device control 1)	49	31	061	1	1	81	51	121	Q	Q	113	71	161	q	q
18	12	022	DC2	(device control 2)	50	32	062	2	2	82	52	122	R	R	114	72	162	r	r
19	13	023	DC3	(device control 3)	51	33	063	3	3	83	53	123	S	S	115	73	163	s	s
20	14	024	DC4	(device control 4)	52	34	064	4	4	84	54	124	T	T	116	74	164	t	t
21	15	025	NAK	(negative acknowledge)	53	35	065	5	5	85	55	125	U	U	117	75	165	u	u
22	16	026	SYN	(synchronous idle)	54	36	066	6	6	86	56	126	V	V	118	76	166	v	v
23	17	027	ETB	(end of trans. block)	55	37	067	7	7	87	57	127	W	W	119	77	167	w	w
24	18	030	CAN	(cancel)	56	38	070	8	8	88	58	130	X	X	120	78	170	x	x
25	19	031	EM	(end of medium)	57	39	071	9	9	89	59	131	Y	Y	121	79	171	y	y
26	1A	032	SUB	(substitute)	58	3A	072	:	:	90	5A	132	Z	Z	122	7A	172	z	z
27	1B	033	ESC	(escape)	59	3B	073	;	;	91	5B	133	[	[	123	7B	173	{	{
28	1C	034	FS	(file separator)	60	3C	074	<	<	92	5C	134	\	\	124	7C	174	|		
29	1D	035	GS	(group separator)	61	3D	075	=	=	93	5D	135	]	]	125	7D	175	}	}
30	1E	036	RS	(record separator)	62	3E	076	>	>	94	5E	136	^	^	126	7E	176	~	~
31	1F	037	US	(unit separator)	63	3F	077	?	?	95	5F	137	_	_	127	7F	177	&#127;	DEL