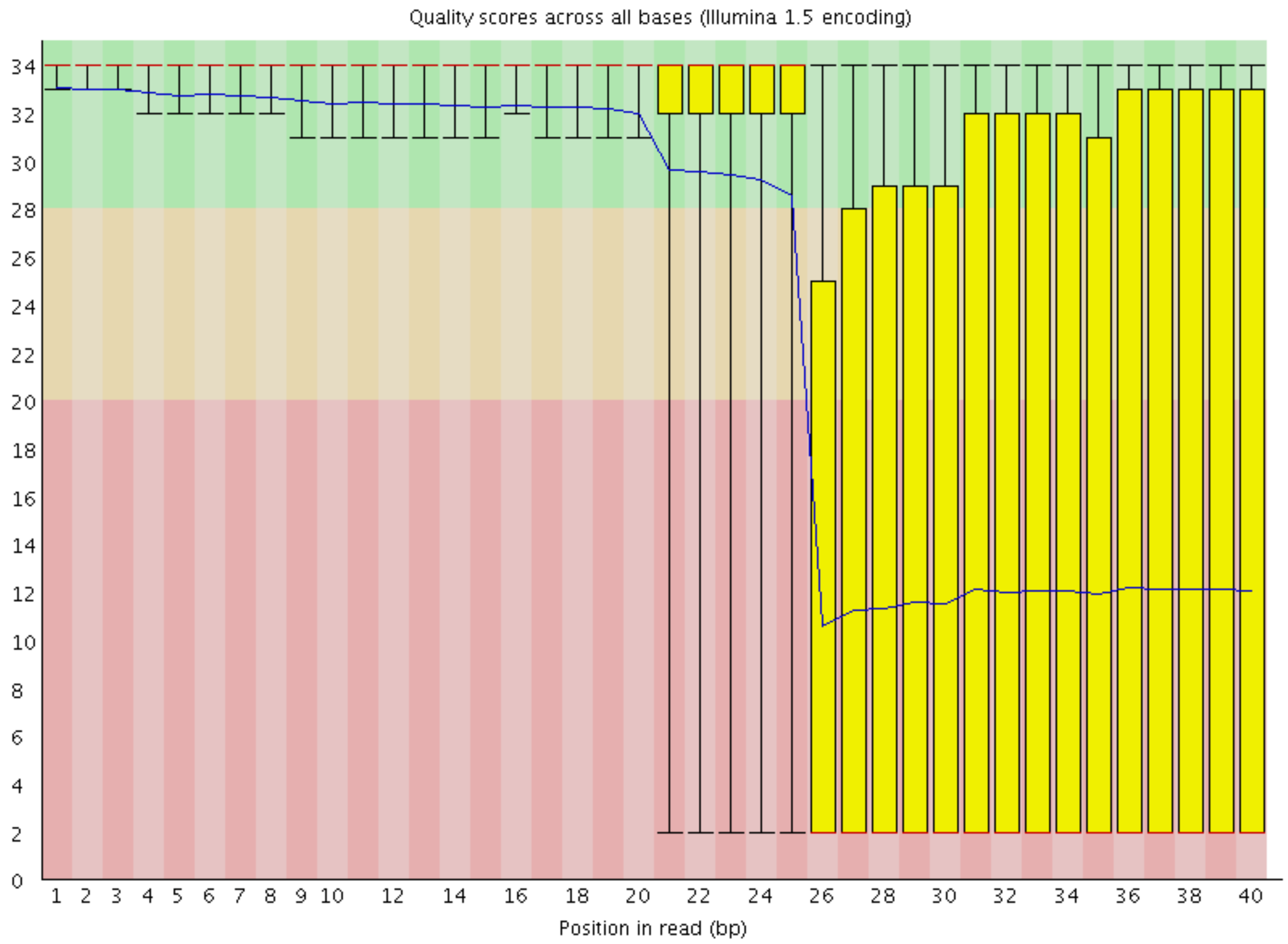
Error Profiles for Next-generation Sequencing Data

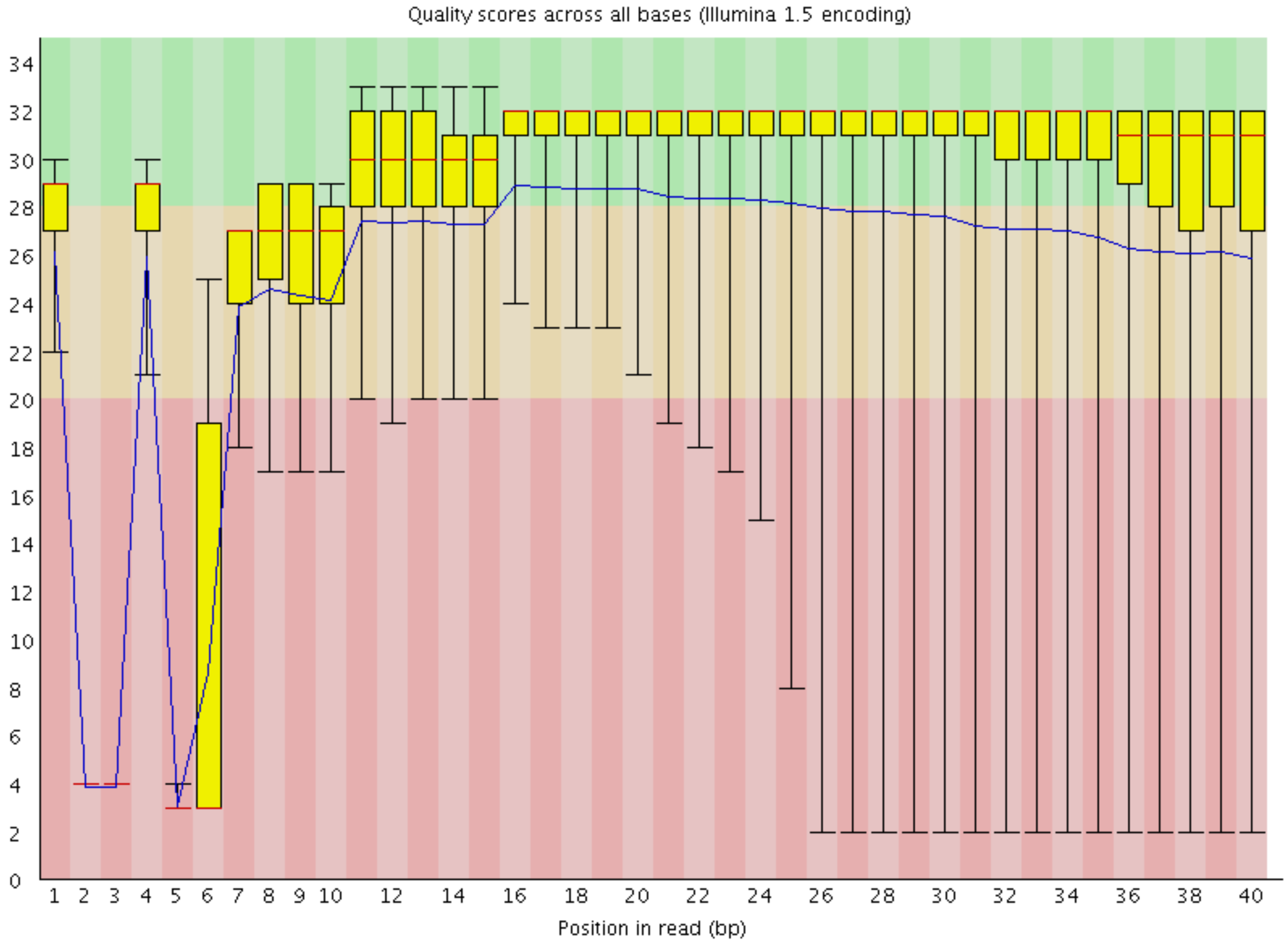
Technical Sequencer Problems

Manifold burst in cycle 26

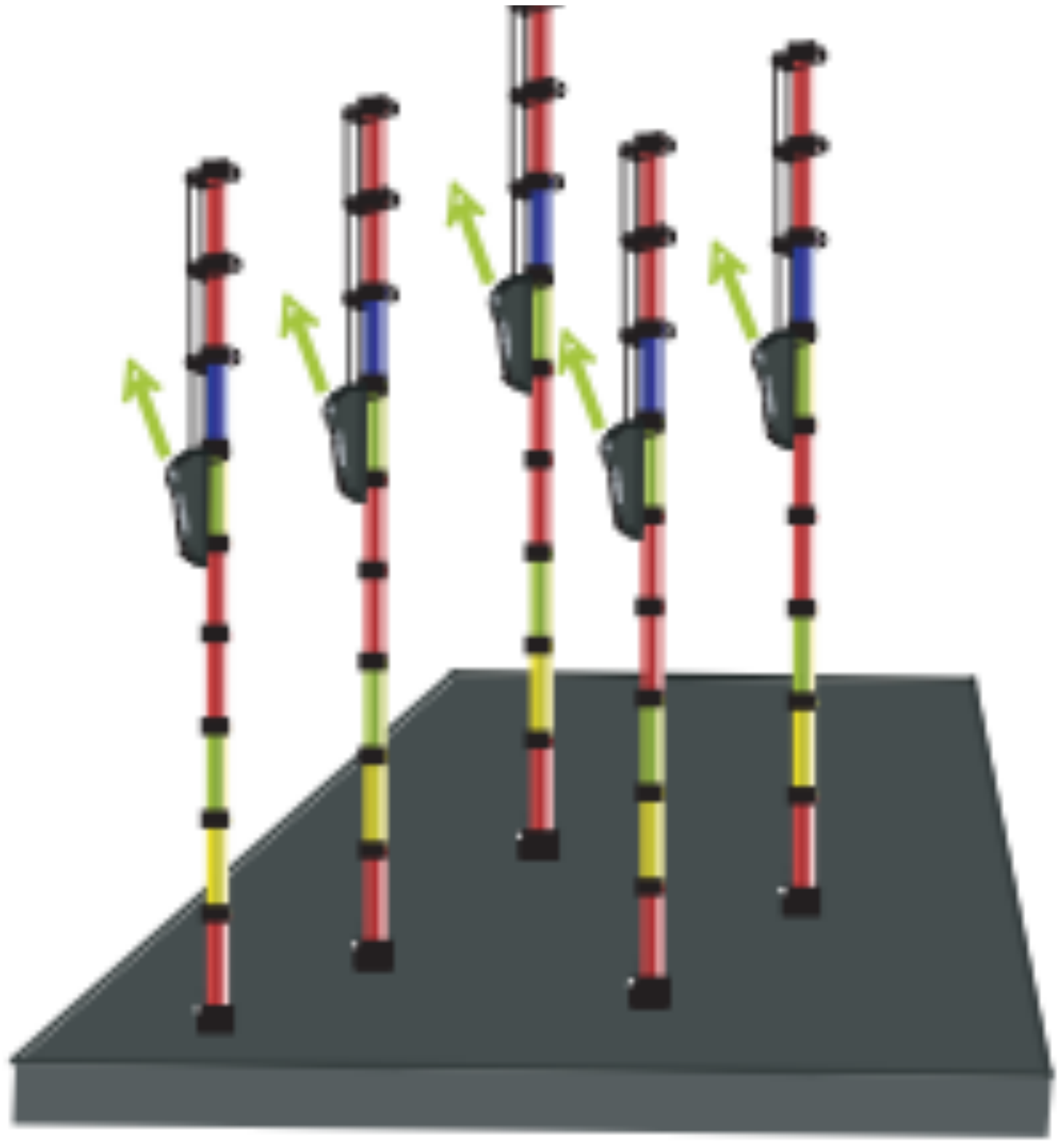


See http://bioinfo-core.org/index.php/9th_Discussion-28_October_2010 for more example

Specific cycles lost

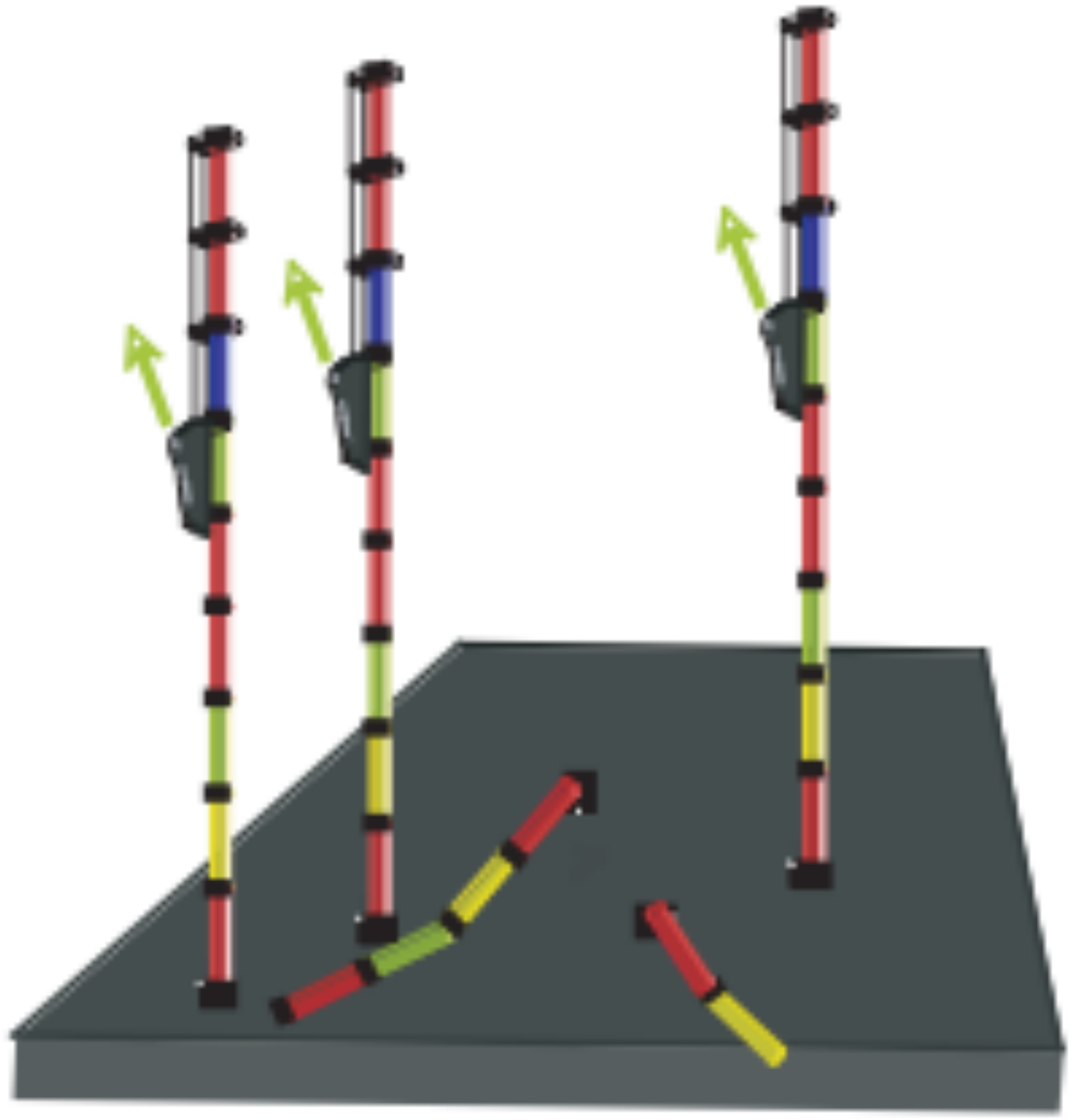


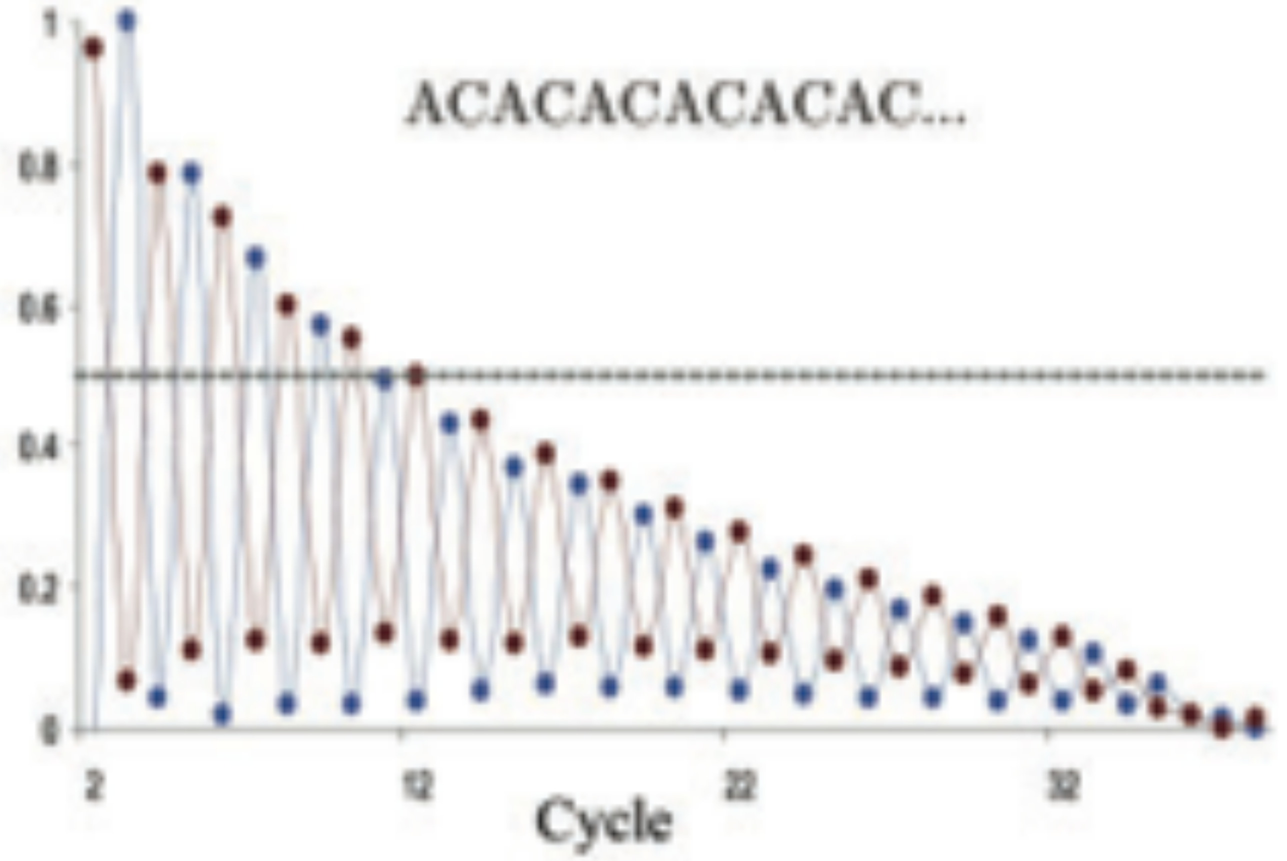
Error dependency on technology



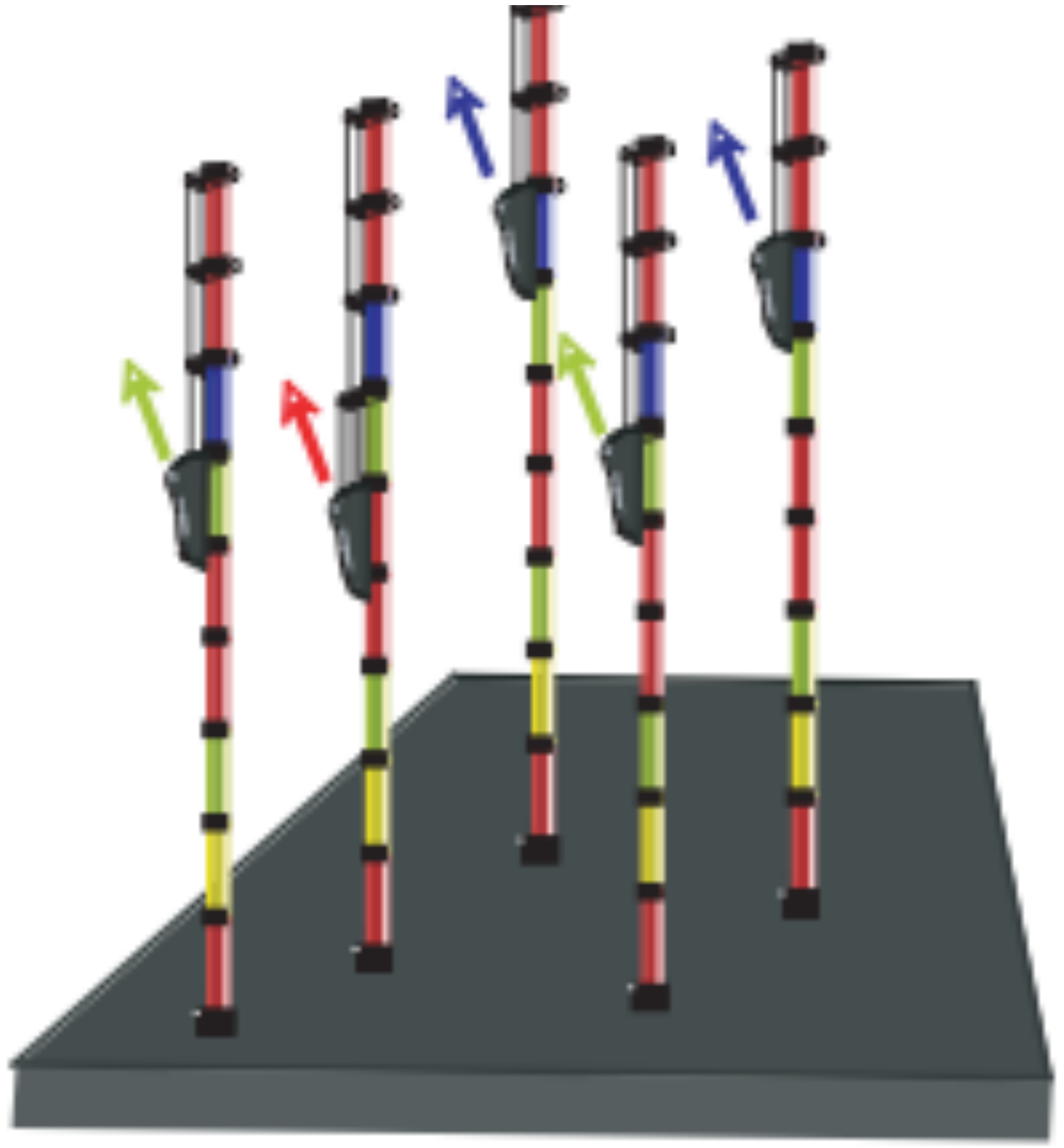
Illumina

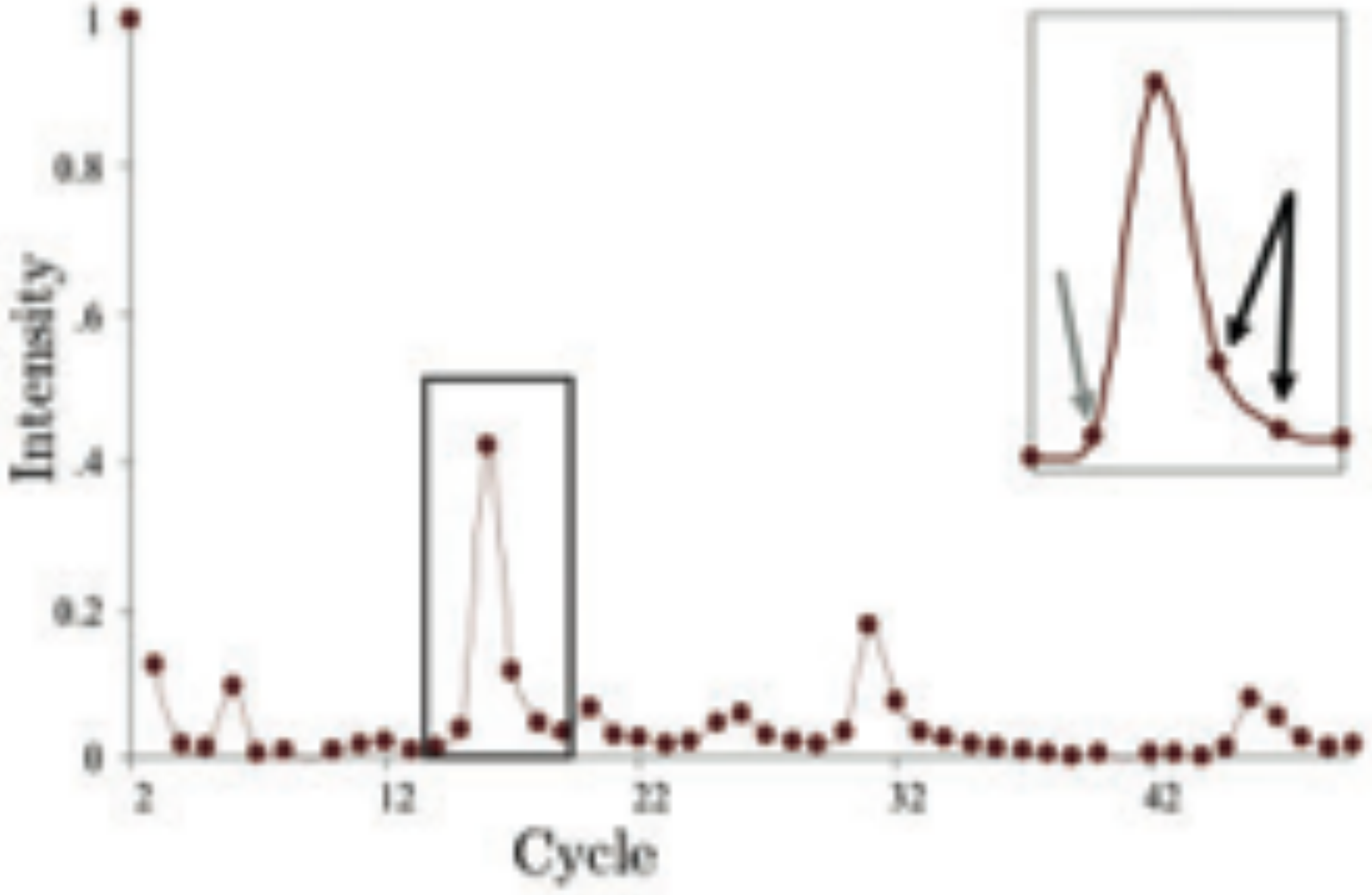
Base-calling for next-generation sequencing platforms. Brief Bioinform 2011, 12(5):489-497



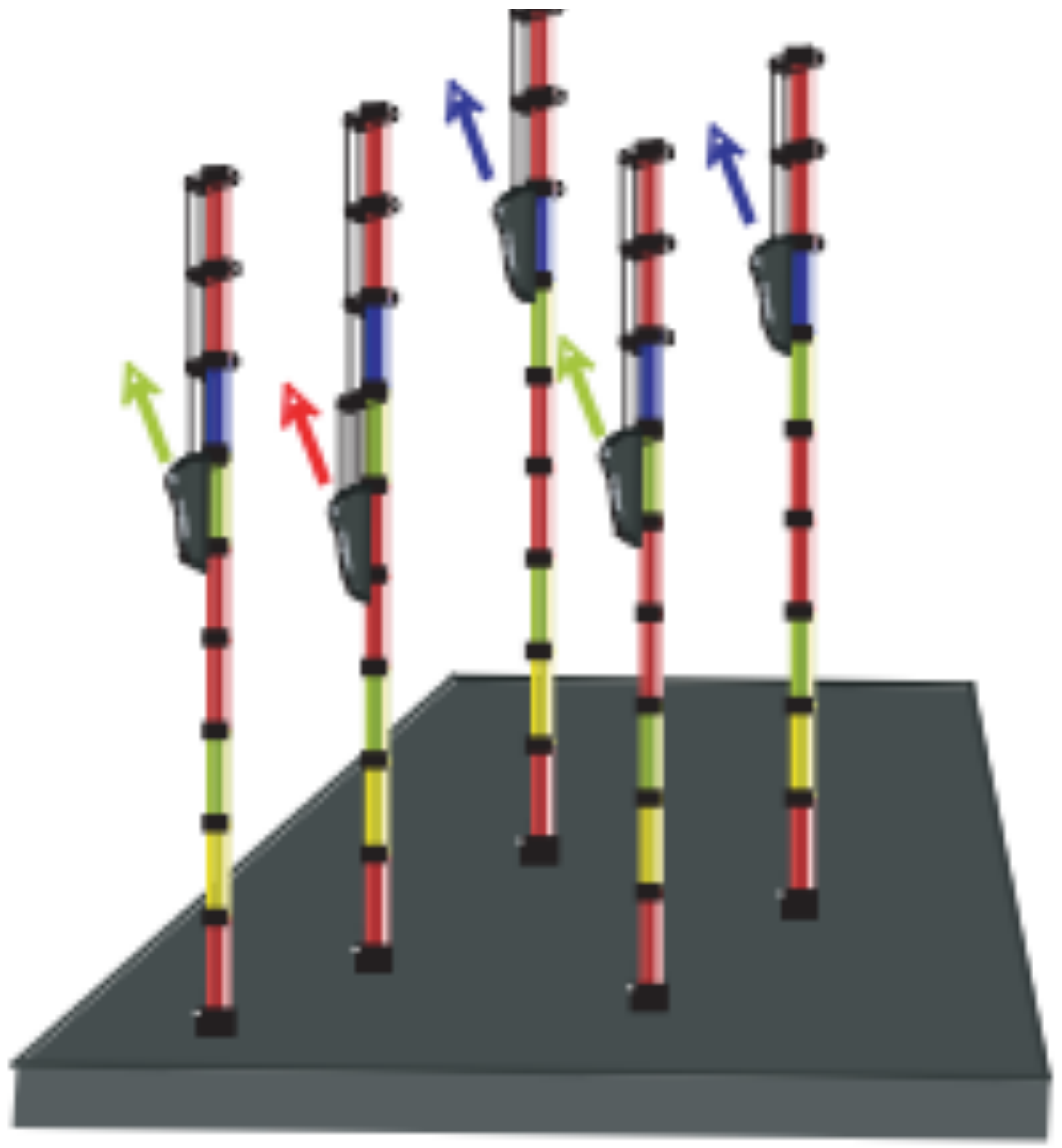


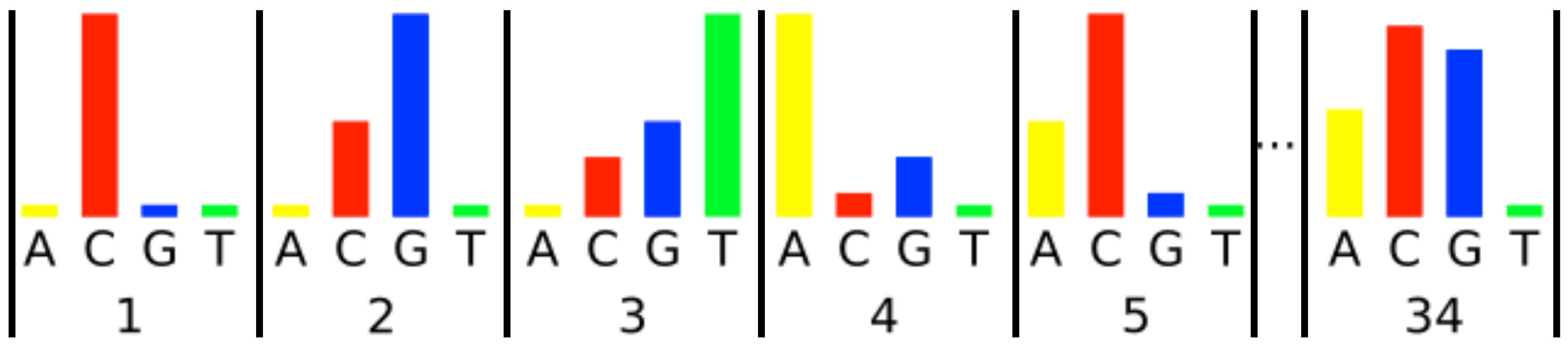
Illumina: signal decay



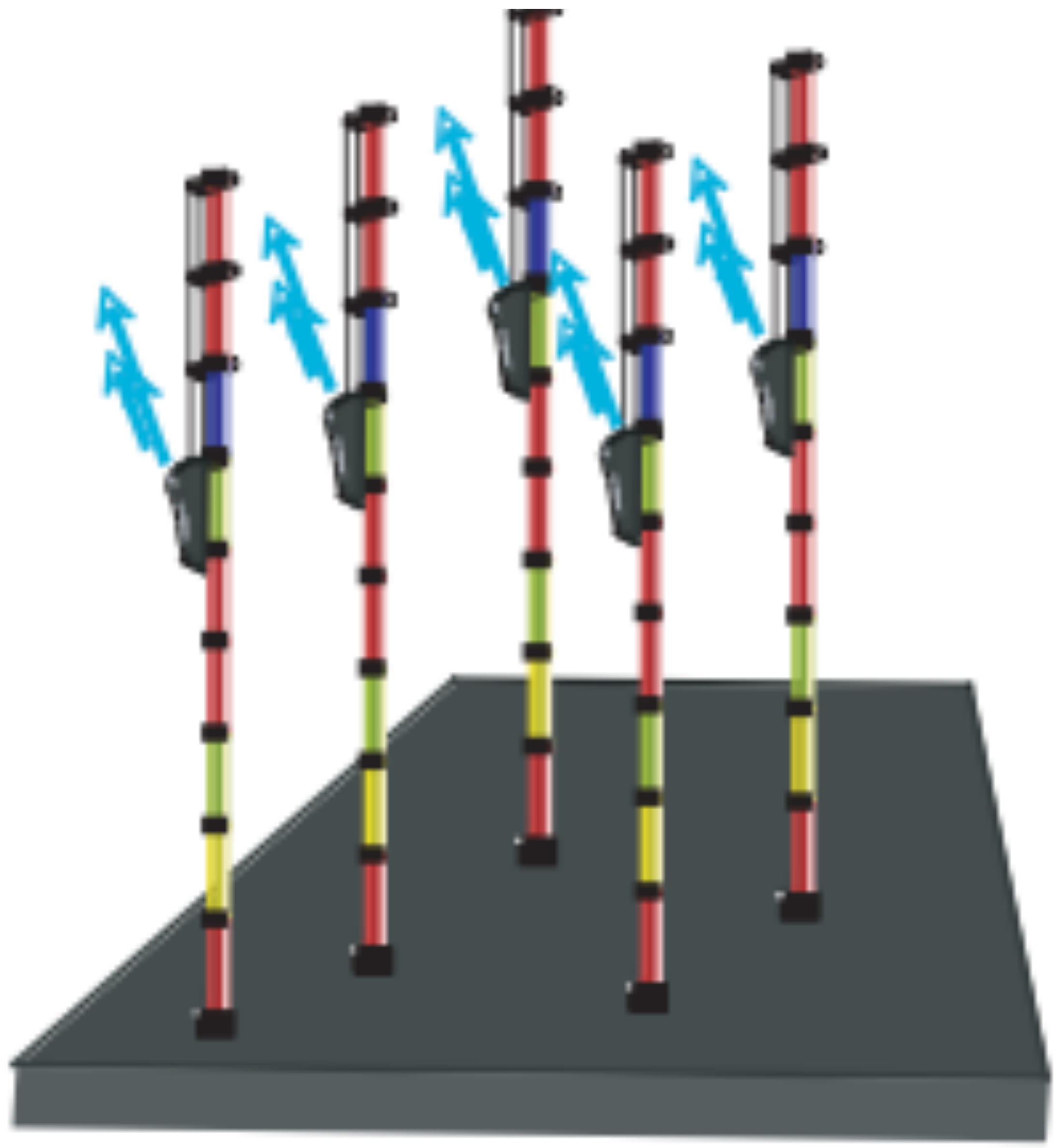


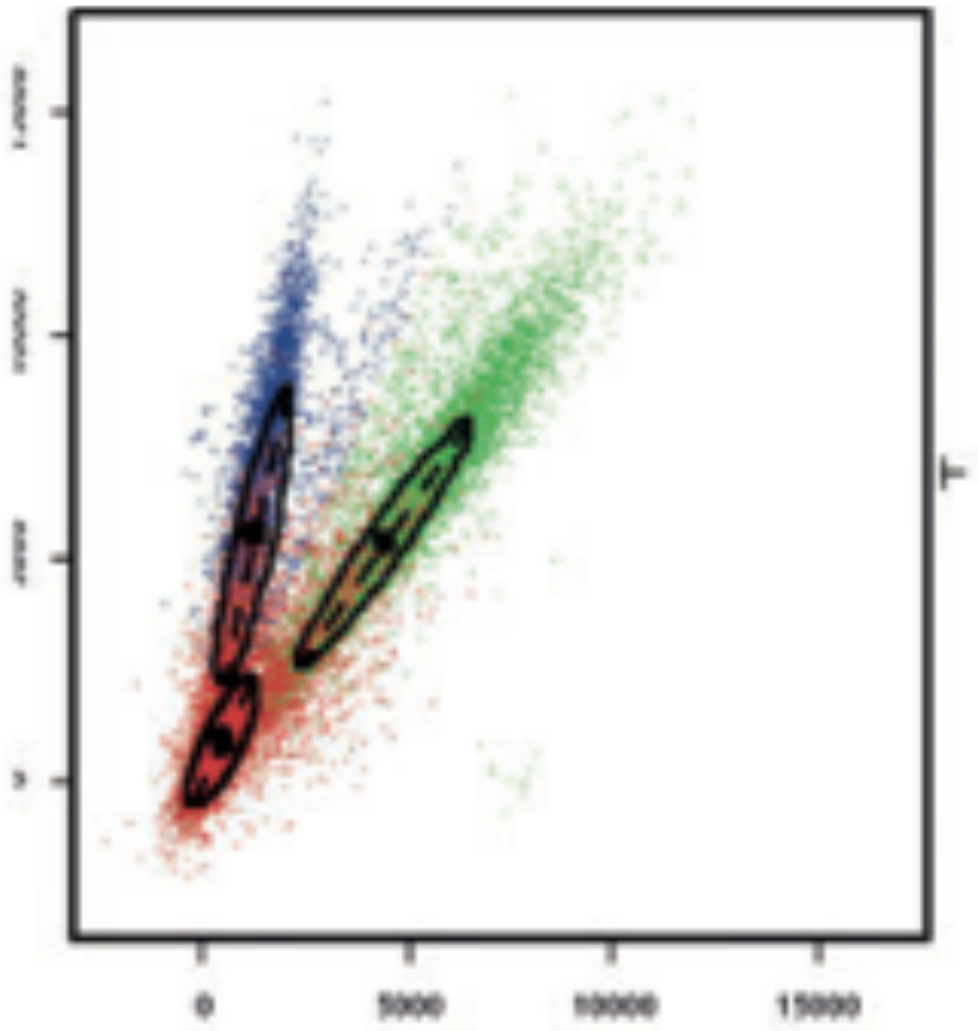
Illumina: phasing





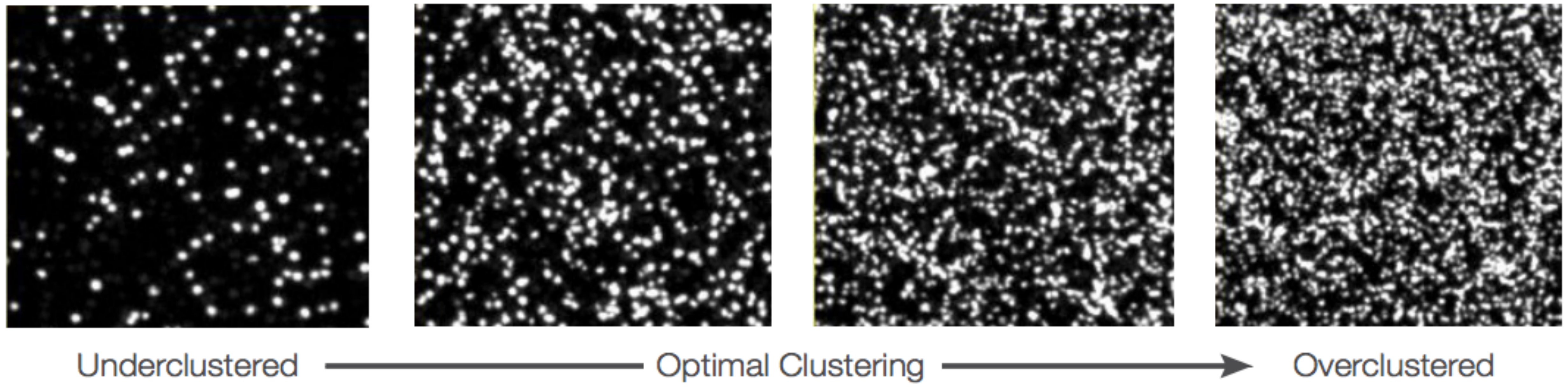
Illumina: phasing

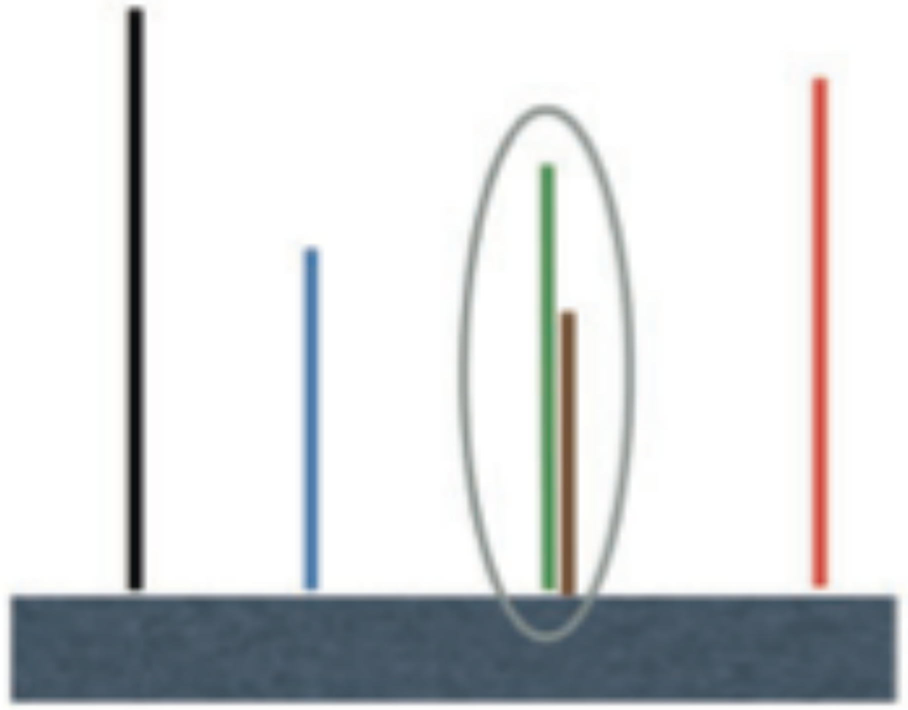


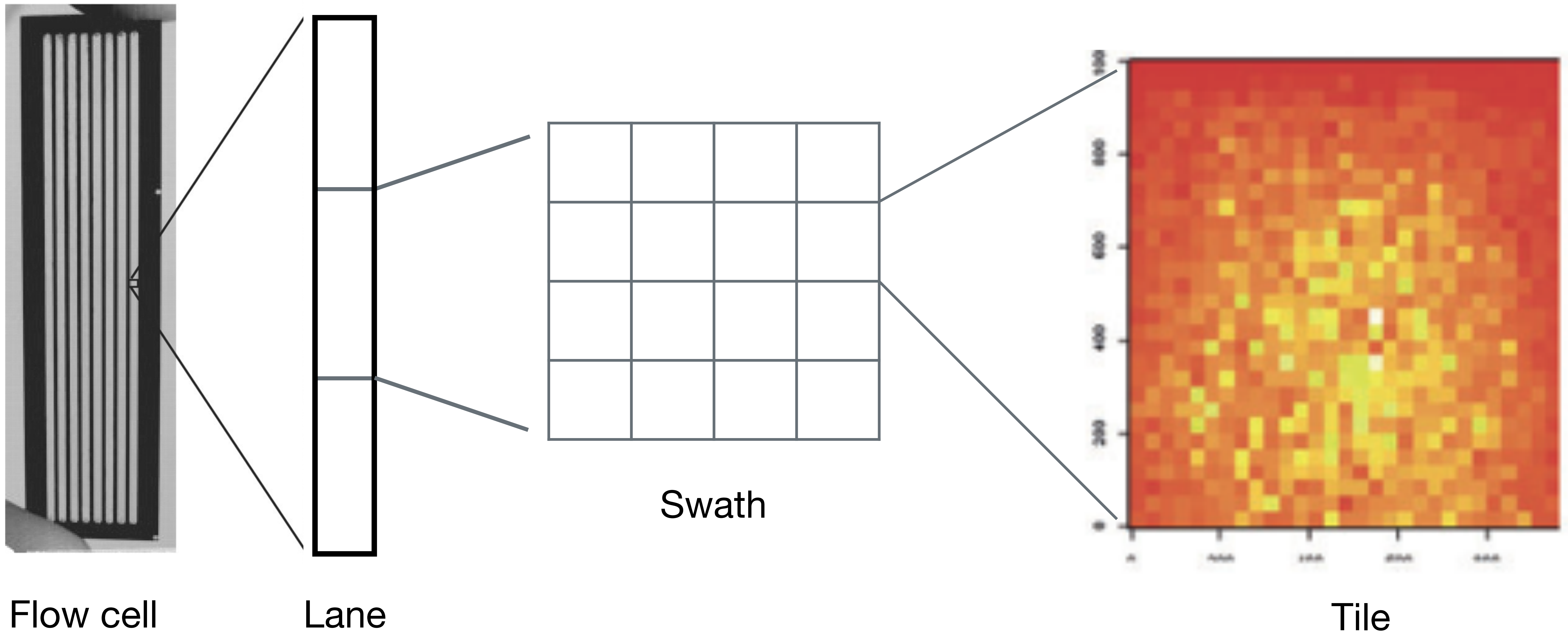


Illumina: cross-talk

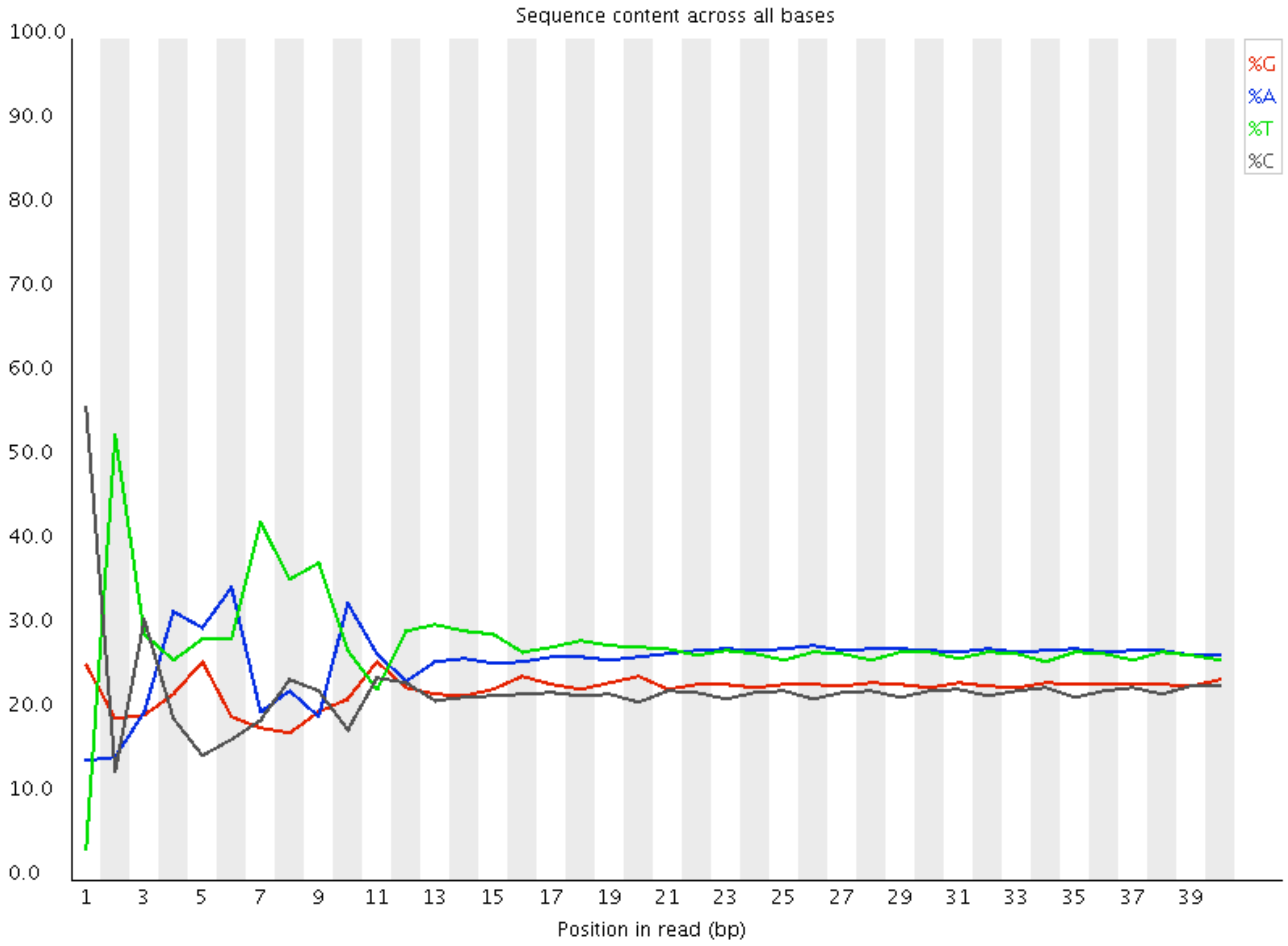
Illumina: flow cell clusters




mixed clusters



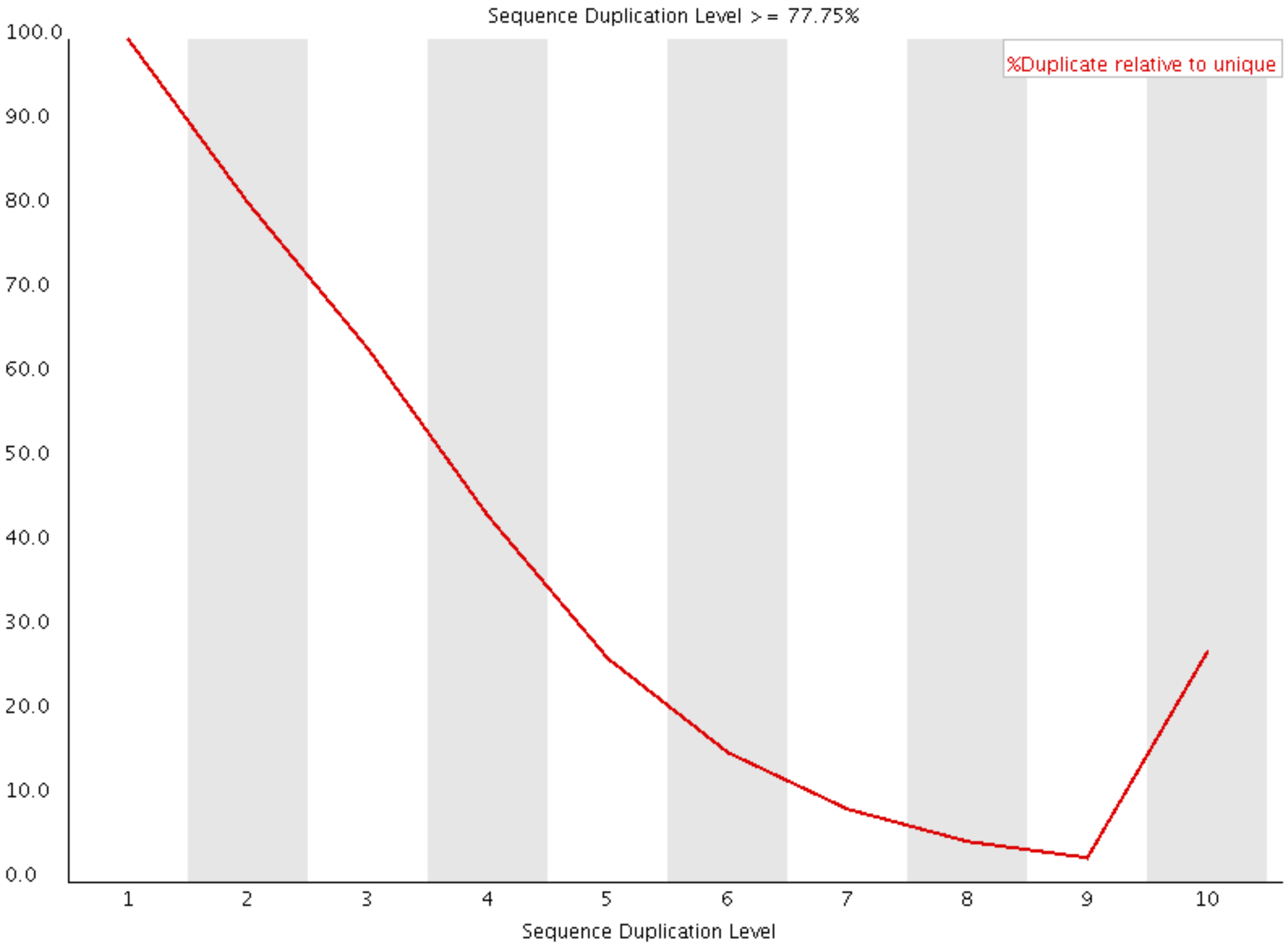
Illumina: optical effects



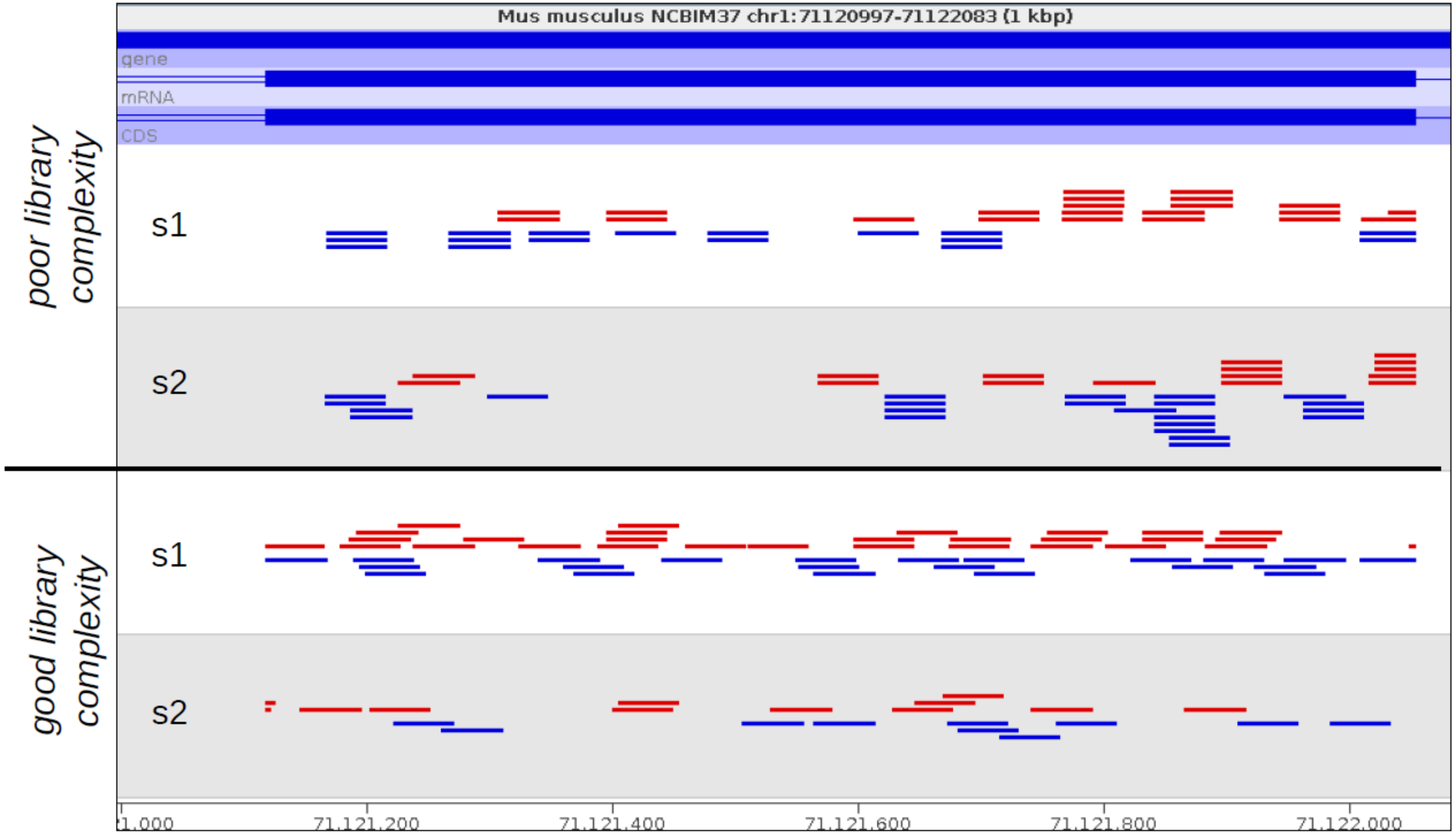
Positional sequence bias

See http://bioinfo-core.org/index.php/9th_Discussion-28_October_2010 for more examples

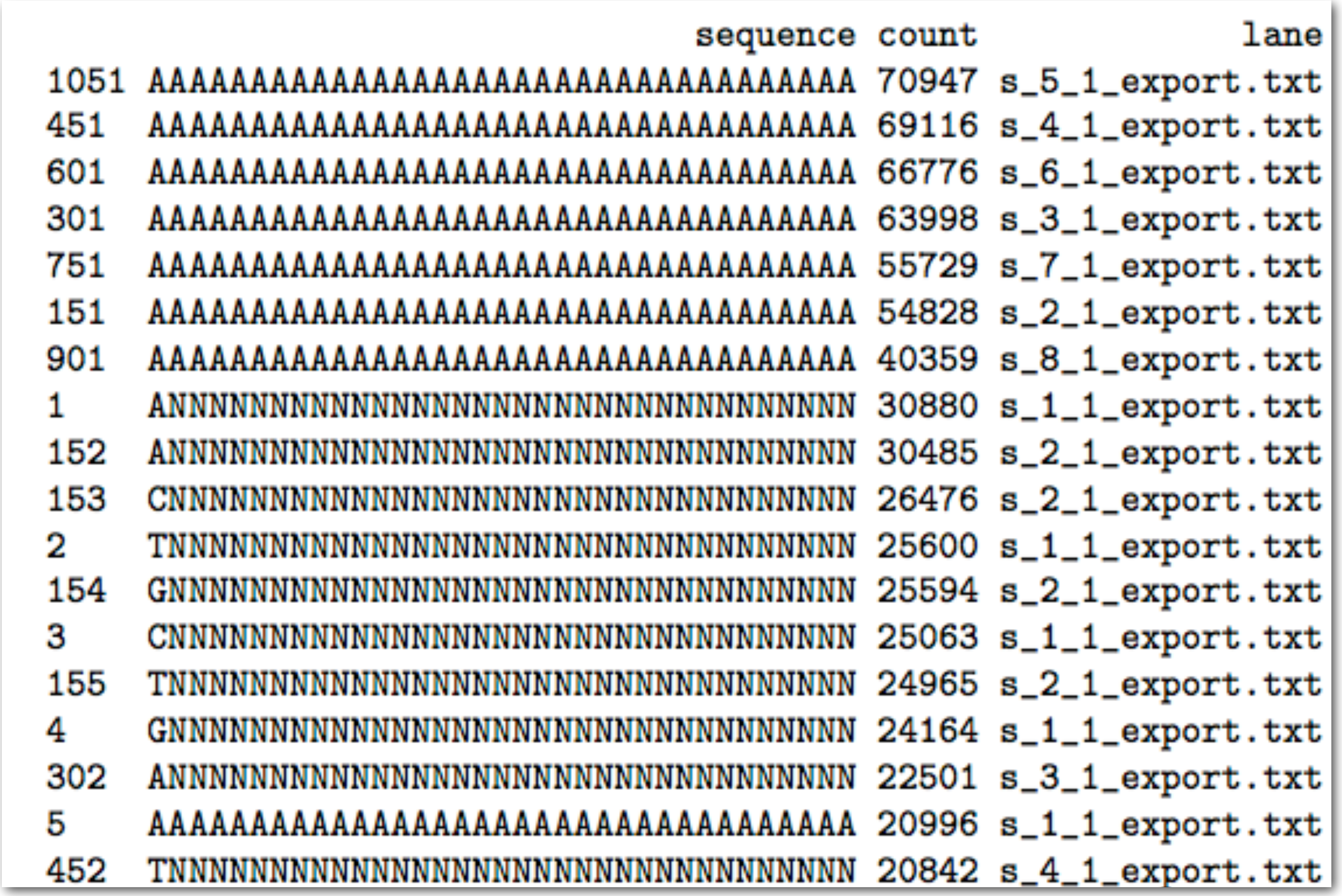
PCR Artifacts



Duplicated sequences

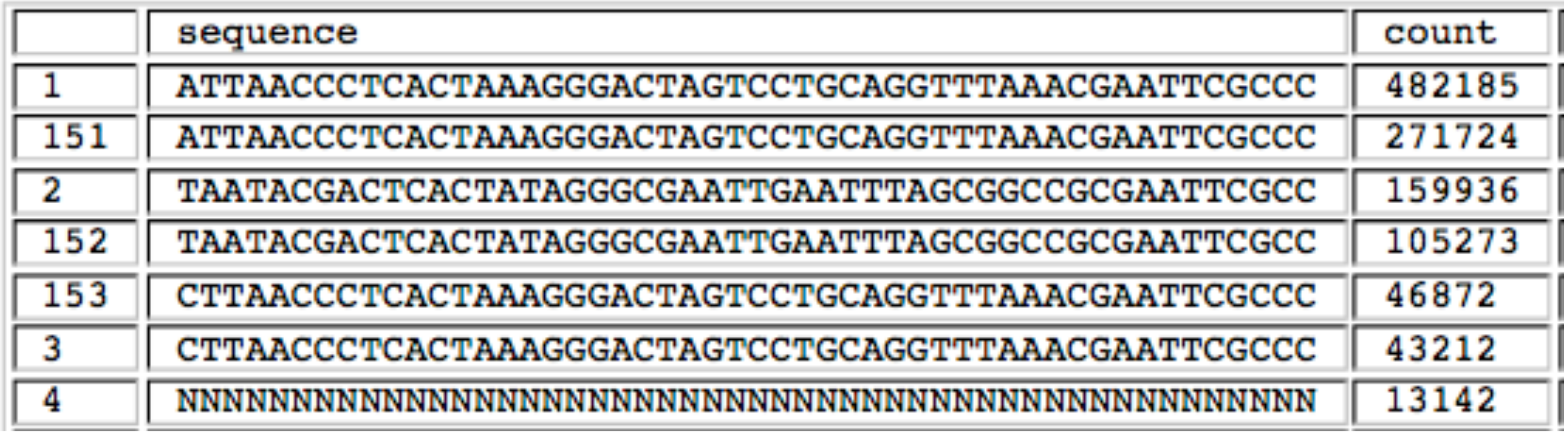

Mus musculus NCBIM37 chr1:71120997-71122083 (1 kbp)
gene
mRNA
CDS
poor library complexity
s1
s2
good library complexity
s1
s2
1.000
71.121.200
71.121.400
71.121.600
71.121.800
71.122.000

Over-represented sequences



```
                                sequence count             lane
1051 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 70947 s_5_1_export.txt
451  AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 69116 s_4_1_export.txt
601  AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 66776 s_6_1_export.txt
301  AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 63998 s_3_1_export.txt
751  AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 55729 s_7_1_export.txt
151  AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 54828 s_2_1_export.txt
901  AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 40359 s_8_1_export.txt
1    ANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 30880 s_1_1_export.txt
152  ANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 30485 s_2_1_export.txt
153  CNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 26476 s_2_1_export.txt
2    TNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 25600 s_1_1_export.txt
154  GNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 25594 s_2_1_export.txt
3    CNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 25063 s_1_1_export.txt
155  TNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 24965 s_2_1_export.txt
4    GNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 24164 s_1_1_export.txt
302  ANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 22501 s_3_1_export.txt
5    AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 20996 s_1_1_export.txt
452  TNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 20842 s_4_1_export.txt
```

Filtering



	sequence	count
1	ATTAACCCTCACTAAAGGGACTAGTCCTGCAGGTTTAAACGAATTCGCCC	482185
151	ATTAACCCTCACTAAAGGGACTAGTCCTGCAGGTTTAAACGAATTCGCCC	271724
2	TAATACGACTCACTATAGGGCGAATTGAATTTAGCGGCCGCGAATTCGCC	159936
152	TAATACGACTCACTATAGGGCGAATTGAATTTAGCGGCCGCGAATTCGCC	105273
153	CTTAACCCTCACTAAAGGGACTAGTCCTGCAGGTTTAAACGAATTCGCCC	46872
3	CTTAACCCTCACTAAAGGGACTAGTCCTGCAGGTTTAAACGAATTCGCCC	43212
4	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN	13142

Read Frequency Distribution

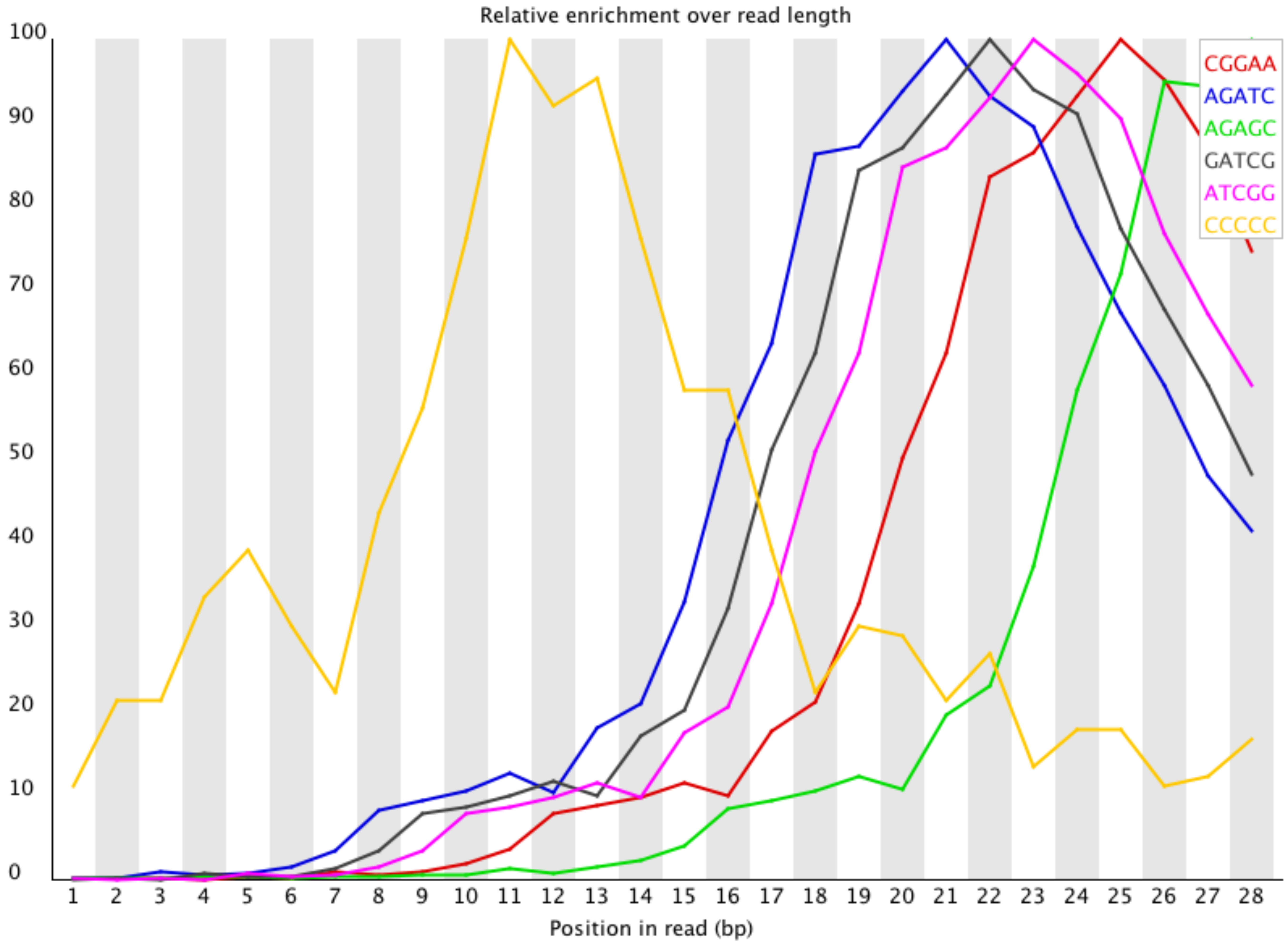
Contamination

```
> gnl|uv|NGB00105.1:1-219 pCR4-TOPO multiple cloning site
Length=219

 Score =  100 bits (50),  Expect = 9e-19
 Identities = 50/50 (100%), Gaps = 0/50 (0%)
 Strand=Plus/Plus

Query  1
ATTAACCCTCACTAAAGGGACTAGTCCTGCAGGTTTAAACGAATTCGCCC  50

||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  43
ATTAACCCTCACTAAAGGGACTAGTCCTGCAGGTTTAAACGAATTCGCCC  92
```



Adaptor contamination