

Raman-Spec(k)troscopy

Sequencing with LasOrz

Kevin Lamkiewicz

13.02.2018

33rd TBI Winterseminar in Bled

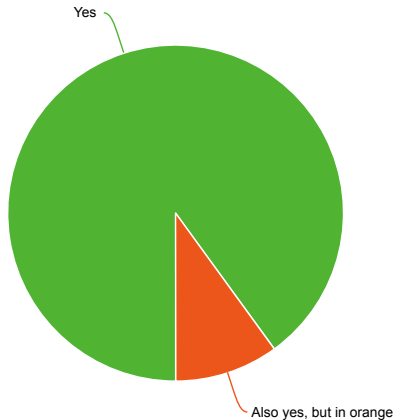


FRIEDRICH-SCHILLER-
UNIVERSITÄT
JENA

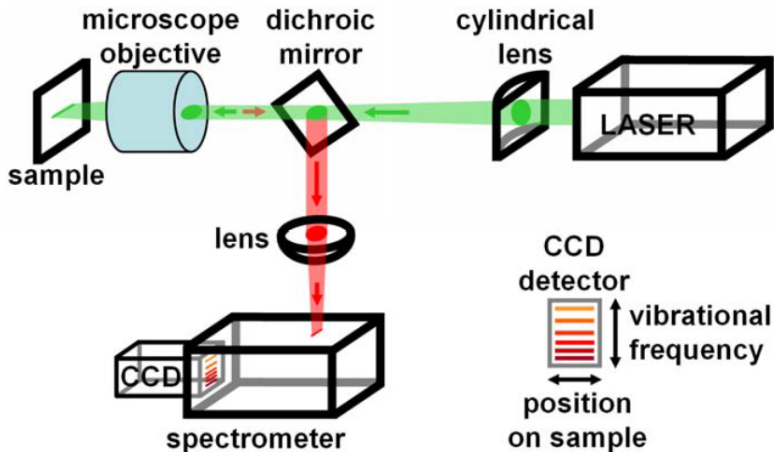
Raman, Speck – what's that all about?

POLL

Do you know Raman spectroscopy?



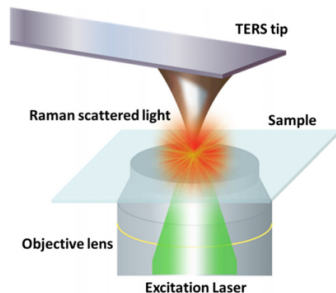
RAMAN SPECTROSCOPY



A. Downes, A. Elfick (2010): Raman spectroscopy and related techniques in biomedicine.

TERS – TIP-ENHANCED RAMAN-SPECTROSCOPY

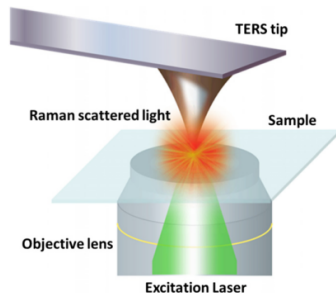
- ▶ proposed in 1895, first experiments in 2000
- ▶ usually silver or gold tips



N. Kumar et al. (2015): Tip-enhanced Raman spectroscopy: principles and applications

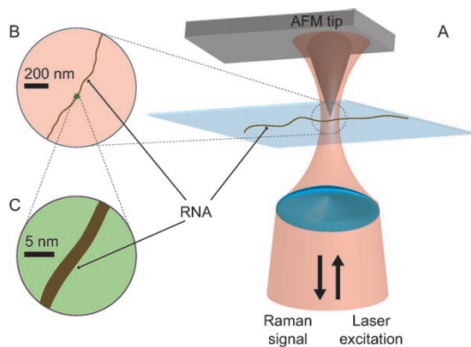
TERS – TIP-ENHANCED RAMAN-SPECTROSCOPY

- ▶ proposed in 1895, first experiments in 2000
- ▶ usually silver or gold tips
- ▶ provides higher resolution
- ▶ enhances the reflected raman signal
- ▶ expands the wavelengths ranges



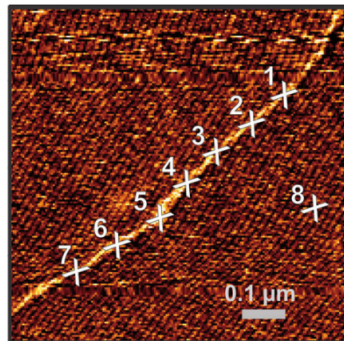
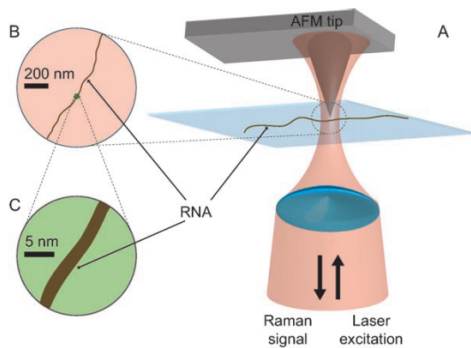
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TERS AND SEQUENCING



E. Bailo, V. Deckert (2008): Tip-Enhanced Raman Spectroscopy of Single RNA Strands: Towards a novel direct-sequencing method

TERS AND SEQUENCING



E. Bailo, V. Deckert (2008): Tip-Enhanced Raman Spectroscopy of Single RNA Strands: Towards a novel direct-sequencing method

Okay, fancy. Show me the sequences!

RAW DATA = RAMAN SPECTRA

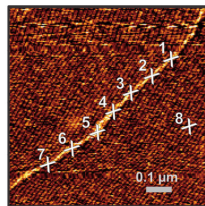
Raman Intensity	wave number
4895	330.793
4963	334.587
4978	338.380
4948	342.171
4941	345.960
5002	349.748
4841	353.534
4936	357.318
4861	361.101
4947	364.882
⋮	⋮

- ▶ for each tip-position one table
- ▶ wave numbers are identical for each tip-position

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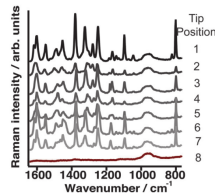
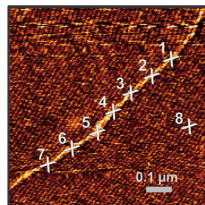


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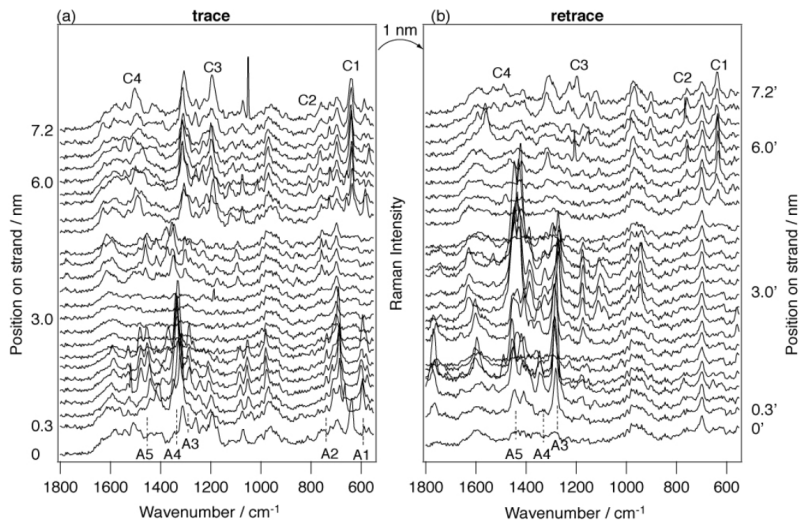
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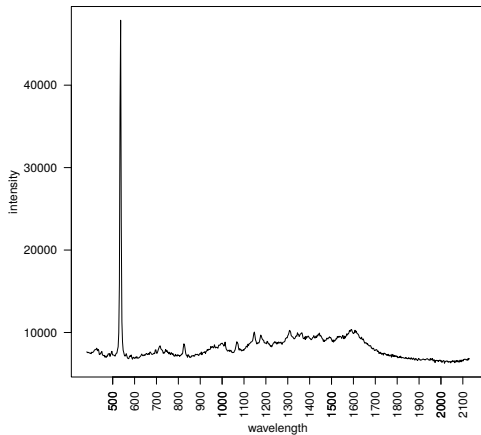
And what am I doing now?

CURRENT EXPERIMENT

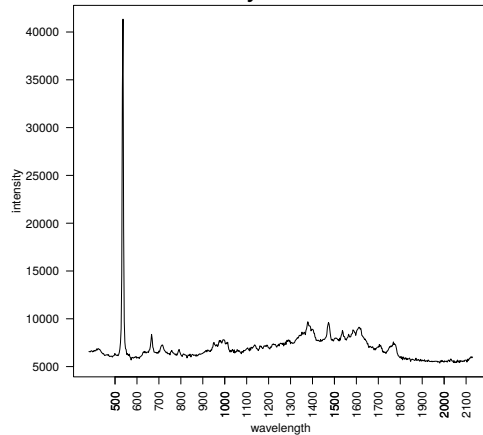


DERIVING NUCLEOTIDES FROM SPECTRA

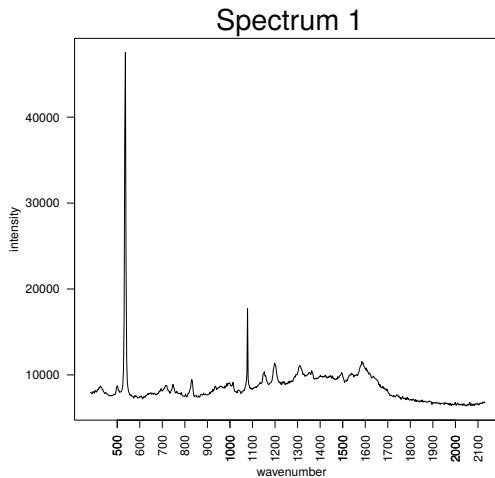
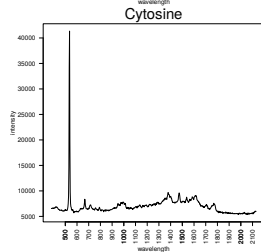
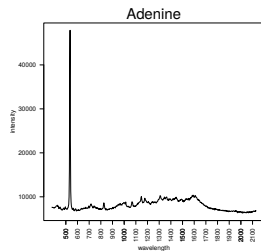
Adenine



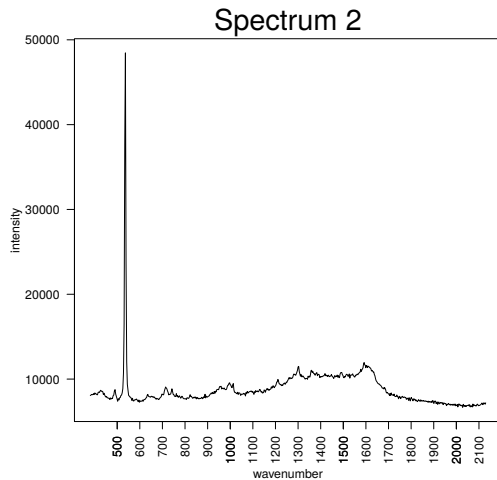
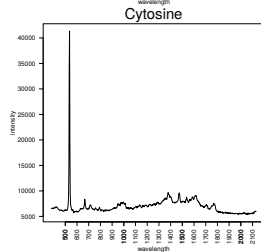
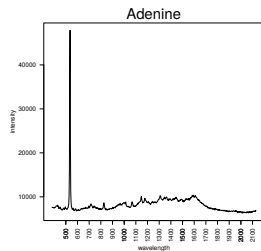
Cytosine



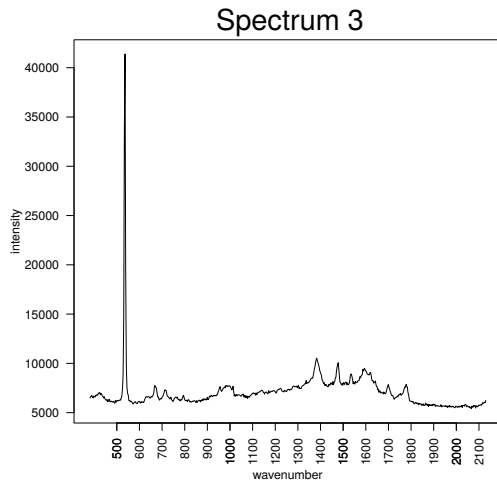
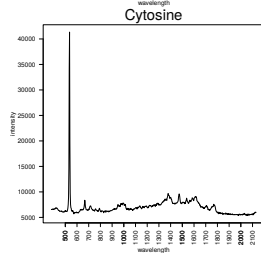
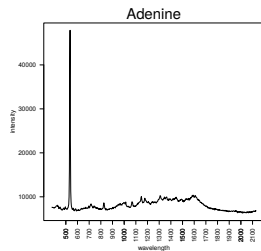
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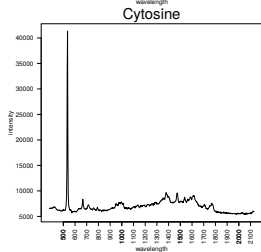
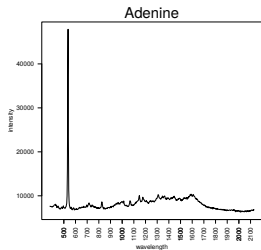
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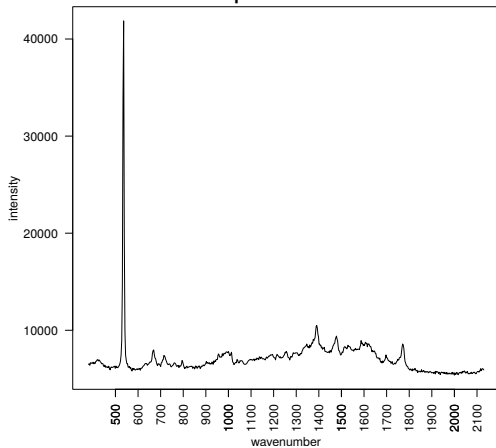
DERIVING NUCLEOTIDES FROM SPECTRA



DERIVING NUCLEOTIDES FROM SPECTRA



Spectrum 4



TEACHING MACHINES HOW TO INTERPRET SPECTRA

	Sample 1	Sample 2	...	Sample n
Gene 1	read count 1	read count 2	...	read count n
Gene 2	read count 1	read count 2	...	read count n
⋮	⋮	⋮	⋮	⋮
Gene n	read count 1	read count 2	...	read count n

TEACHING MACHINES HOW TO INTERPRET SPECTRA

	Sample 1	Sample 2	...	Sample n
Position 1	read count 1	read count 2	...	read count n
Position 2	read count 1	read count 2	...	read count n
⋮	⋮	⋮	⋮	⋮
Position n	read count 1	read count 2	...	read count n

TEACHING MACHINES HOW TO INTERPRET SPECTRA

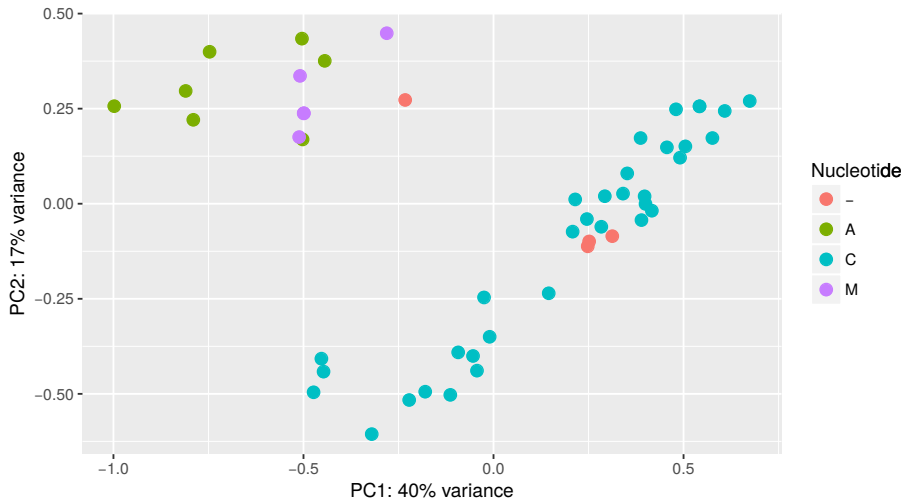
	wave number 1	wave number 2	...	wave number n
Position 1	read count 1	read count 2	...	read count n
Position 2	read count 1	read count 2	...	read count n
⋮	⋮	⋮	⋮	⋮
Position n	read count 1	read count 2	...	read count n

TEACHING MACHINES HOW TO INTERPRET SPECTRA

	wave number 1	wave number 2	...	wave number n
Position 1	spectrum 1	spectrum 2	...	spectrum n
Position 2	spectrum 1	spectrum 2	...	spectrum n
⋮	⋮	⋮	⋮	⋮
Position n	spectrum 1	spectrum 2	...	spectrum n

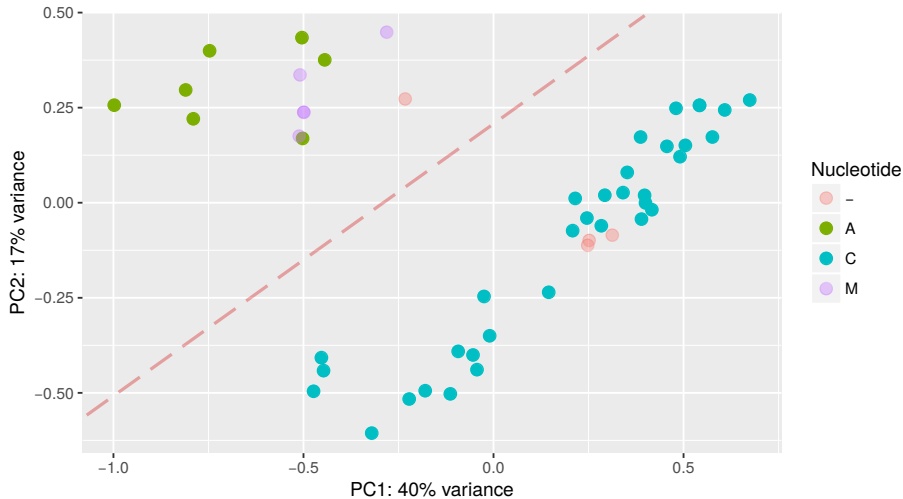
CLUSTERING THE DATA

PCA of first TERS dataset: grid7



MACHINE LEARNING?

PCA of first TERS dataset: grid7



But... we have sequencing methods! Why should anyone use TERS?

RAMAN SPECTROSCOPY CHANCES

- ▶ sequencing of DNA/RNA modifications
- ▶ direct aminoacid sequencing

RAMAN SPECTROSCOPY CHANCES

- ▶ sequencing of DNA/RNA modifications
- ▶ direct aminoacid sequencing
- ▶ viral fingerprint

CHALLENGES & DRAWBACKS

- ▶ no high-throughput method
- ▶ oxidation of silver tip
- ▶ thermal drift

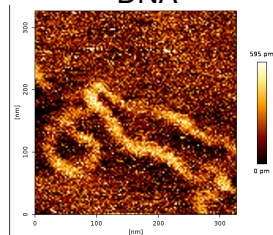
- ▶ no protocol for
RNA/aminoacid sequences

CHALLENGES & DRAWBACKS

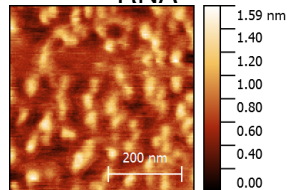
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DNA



RNA



**Acknowledgements:**

RNA Bioinformatics Group Jena
Deckert Gruppe

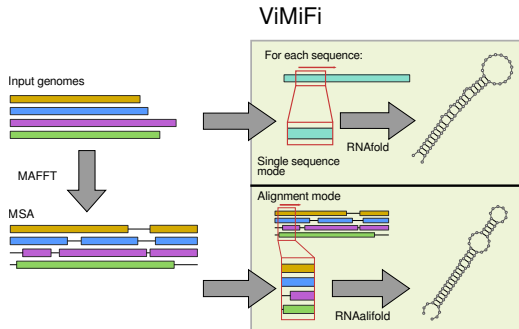
Funding:

BMBF InfectControl 2020
Project 03ZZ0820A

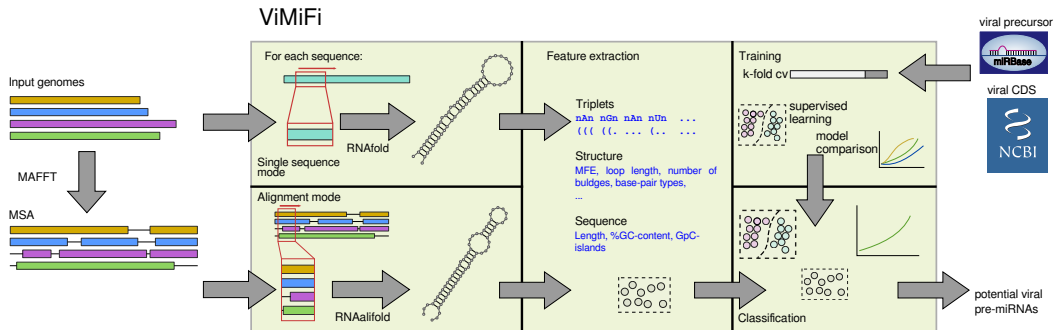
Thank you for your attention!

A short update of my viral research

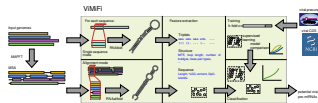
ViMiFi – VIRAL MICRORNA FINDER



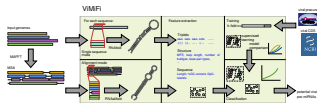
VIMiFi – VIRAL MICRORNA FINDER



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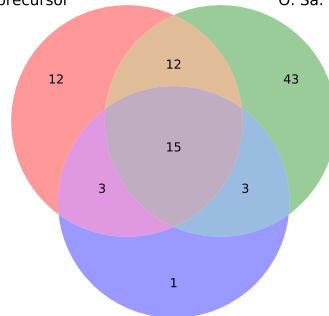
VIMIFI – VIRAL MICRORNA FINDER



Found candidates in Tombusviruses using different training sets

A. Th. precursor

O. Sa. precursor

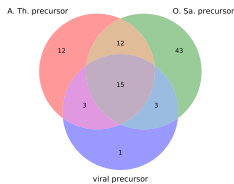


viral precursor

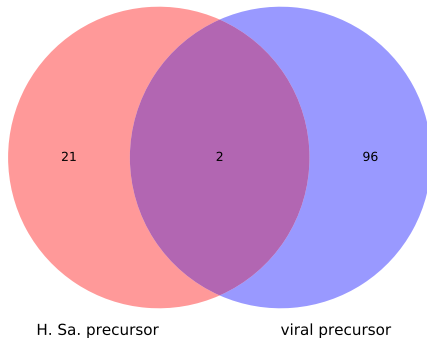
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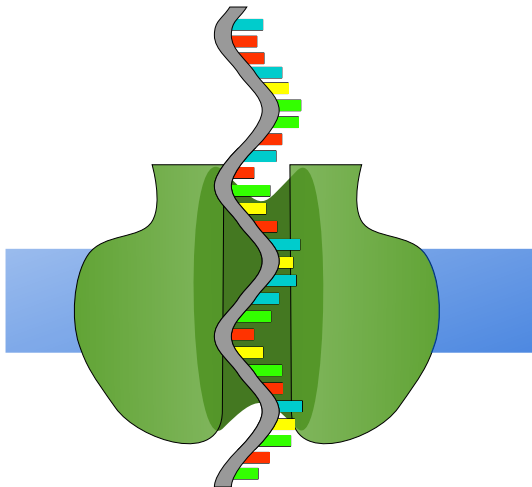
Found candidates in Tombusviruses using different training sets



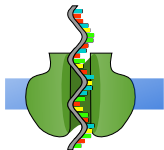
Found candidates in EBV using different training sets



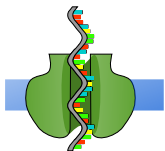
LOCATION – LONG COMPENSATORY MUTATIONS



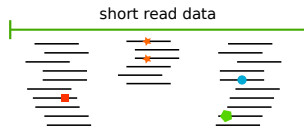
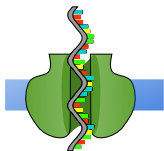
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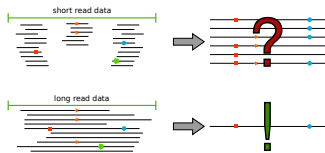
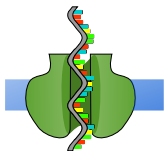
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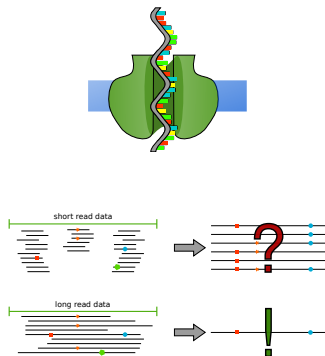
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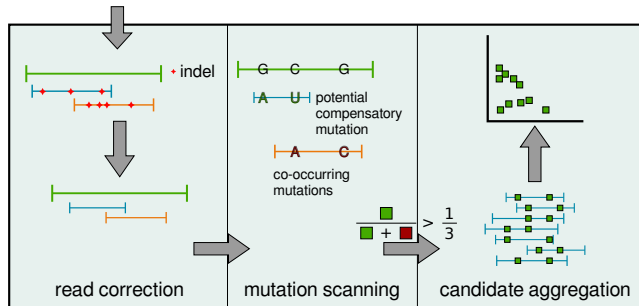
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reference
+
mapped reads



LOCATION – LONG COMPENSATORY MUTATIONS

