n-gram analysis of biological sequences in R

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Introduction

biogram package

biogram: the **R** package for the n-gram analysis of biological sequences.

n-grams: features describing the sequence.

biogram workflow:

1. Extract n-grams.
2. Change an alphabet.
3. Filter n-grams.

Biological sequences

Long chains of amino acids (proteins) or nucleotides (RNA or DNA).

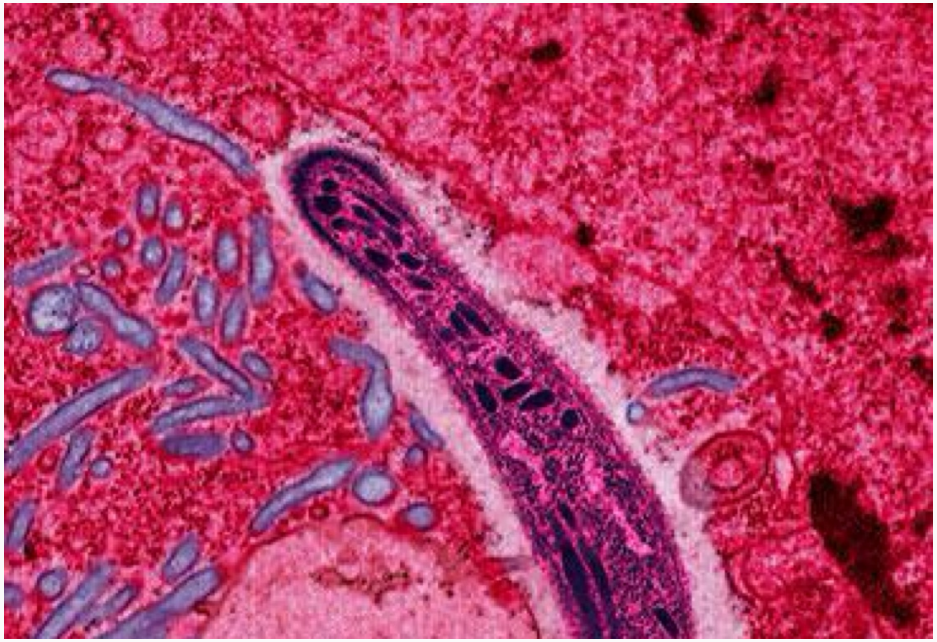
Sample protein sequence:

```
MRKLYCVLLLSAFEFTYMINFGRGQNYWEHPYQKSDVYHP

INEHREHPKEYQYPLHQEHTYQQEDSGEDENTLQHAYPID

HEGAEPAPQEQNLFSSIEIV...
```

Biological sequences



Plasmodium falciparum. Source: `http://www.protists.ensembl.org`

Protein of *Plasmodium falciparum*:

```
MRKLYCVLLLSAFEFTYMINFGRGQNYWEHPYQKSDVYHP

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```

Biological sequences

Signal peptide (red): n-terminal amino acid sequence directing proteins to the endomembrane system and next to extracellular localizations.

MRKLYCVLLLSAFEFTYMINFGRGQNYWEHPYQKSDVYHP

INEHREHPKEYQYPLHQEHTYQQEDSGEDENTLQHAYPID

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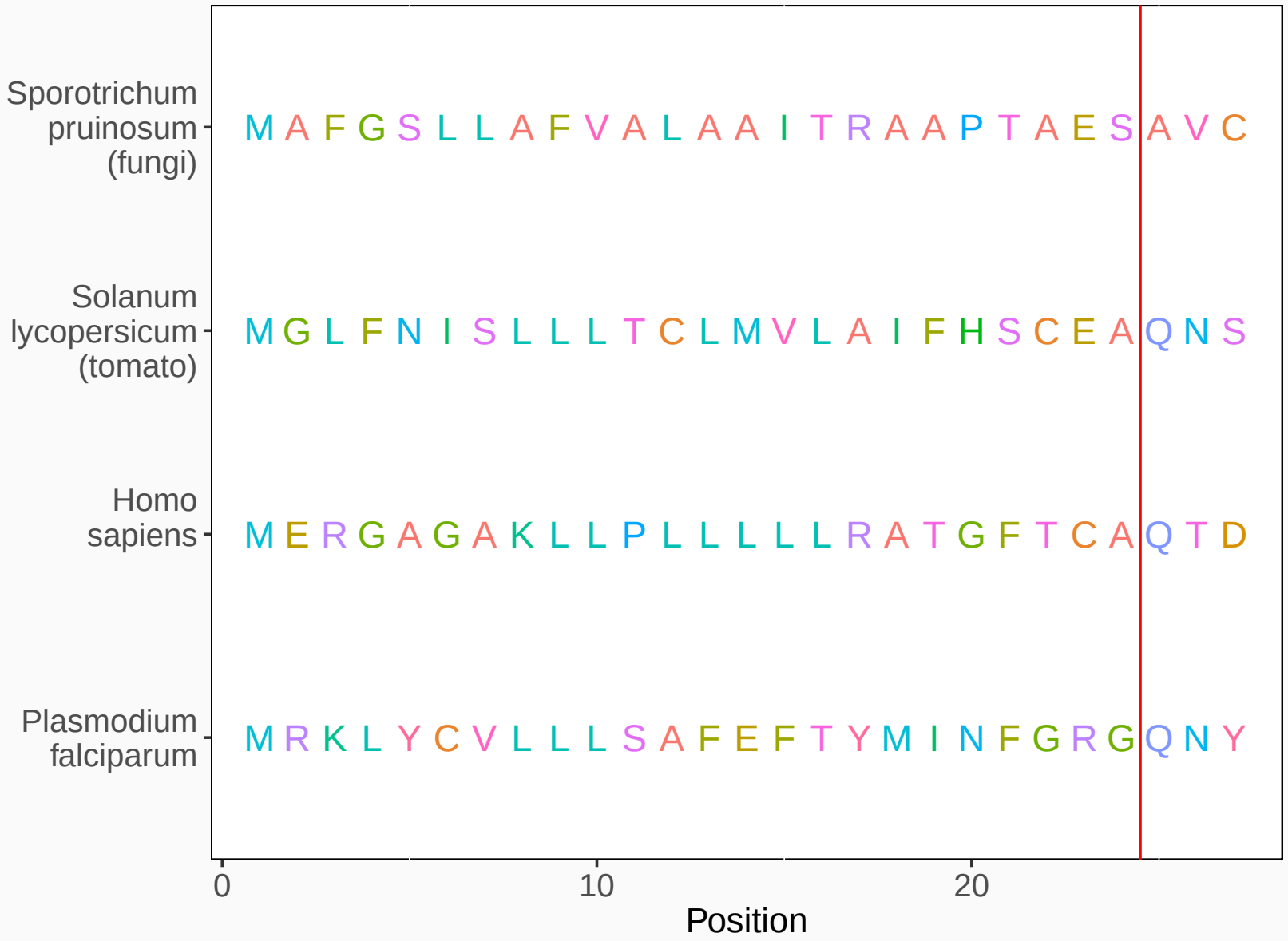
Biological sequences



n-grams

n-grams

n-grams (k-tuples) are vectors of n characters derived from input sequence(s).

	P1	P2	P3	P4	P5	P6	P7	P8	P9
PF	M	R	K	L	Y	C	V	L	L
HS	M	G	L	F	N	I	S	L	L
SL	M	A	F	G	S	L	L	A	F
SP	M	E	R	G	A	G	A	K	L

1-grams: M , M , M , M , R , G , A , E

n-grams

	P1	P2	P3	P4	P5	P6	P7	P8	P9
PF	M	R	K	L	Y	C	V	L	L
HS	M	G	L	F	N	I	S	L	L
SL	M	A	F	G	S	L	L	A	F
SP	M	E	R	G	A	G	A	K	L

2-grams: MR , MG , MA , ME , RK , GL , AF , ER

n-grams

	P1	P2	P3	P4	P5	P6	P7	P8	P9
PF	M	R	K	L	Y	C	V	L	L
HS	M	G	L	F	N	I	S	L	L
SL	M	A	F	G	S	L	L	A	F
SP	M	E	R	G	A	G	A	K	L

3-grams: MRK, MGL, MAF, MER, RKL, GLF, AFG, ERG

n-grams

	P1	P2	P3	P4	P5	P6	P7	P8	P9
PF	M	R	K	L	Y	C	V	L	L
HS	M	G	L	F	N	I	S	L	L
SL	M	A	F	G	S	L	L	A	F
SP	M	E	R	G	A	G	A	K	L

2-grams (with a single gap): M-K, M-L, M-F, M-R, R-L, G-F, A-G, E-G

n-grams

	P1	P2	P3	P4	P5	P6	P7	P8	P9
PF	M	R	K	L	Y	C	V	L	L
HS	M	G	L	F	N	I	S	L	L
SL	M	A	F	G	S	L	L	A	F
SP	M	E	R	G	A	G	A	K	L

3-grams (with gaps): M - K - - C , M - L - - I , M - F - - L ,
M - R - - G , R - L - - V , G - F - - S , A - G - - L , E - G - - A

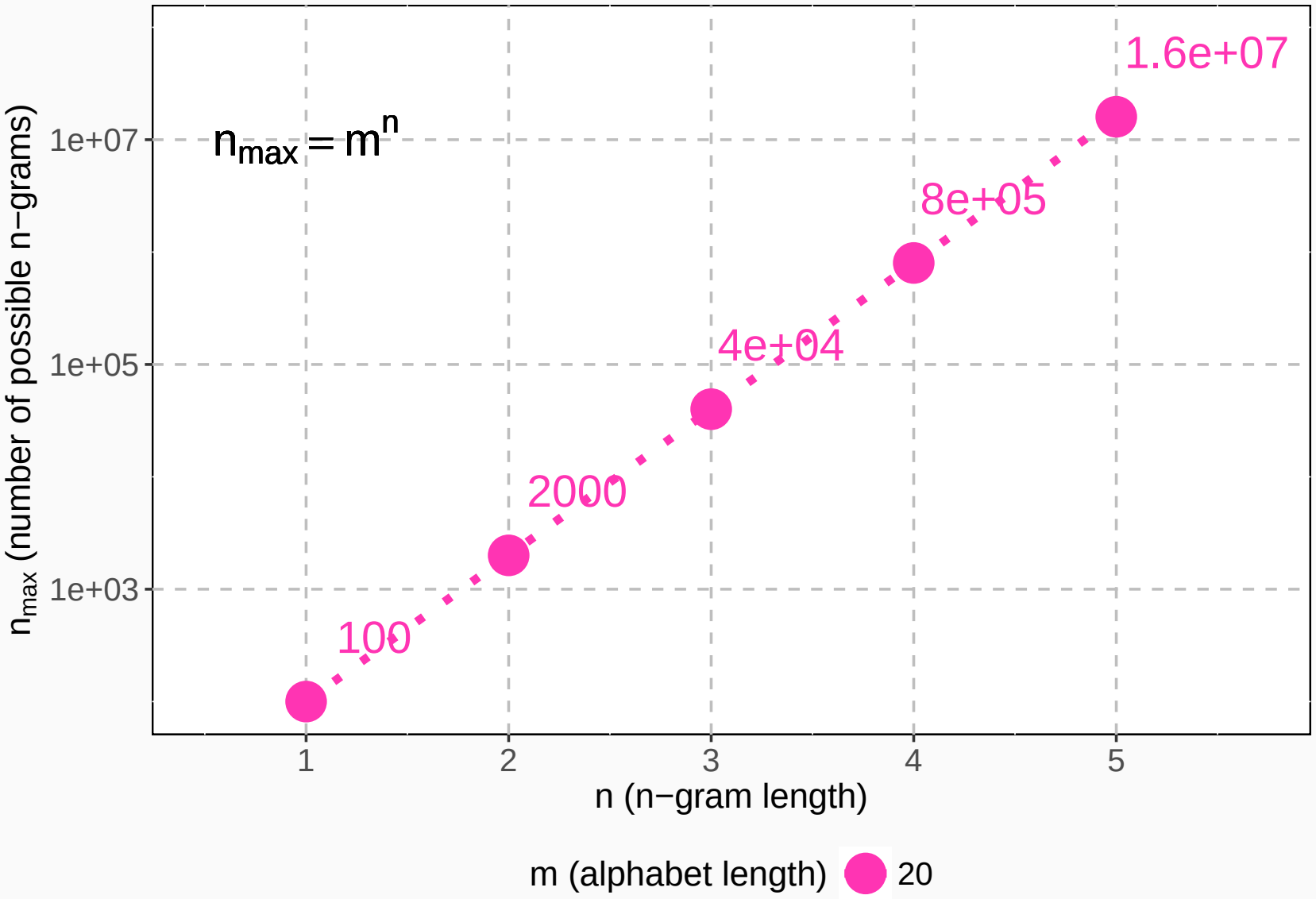
Proposed model

$$\mathrm{y} \sim \text{n-gram}_1 + \text{n-gram}_2 + \ldots + \text{n-gram}_n$$

Problems:

- large number of possible n-grams,
- the majority of n-grams is noninformative.

n-gram counts



n-gram counts

	A_0	C_0	D_0	E_0	F_0	...	L.F_0	M.F_0	N.F_0	...
PF	1	1	0	1	3	...	0	0	1	...
HS	2	2	0	1	2	...	1	0	0	...
SL	9	1	0	1	2	...	0	0	0	...
SP	4	1	1	1	1	...	0	0	0	...

The sparcity of the n-gram count matrix grows with n.

Sparse matrix representation

Sparse matrix with R

Packages:

- *Matrix*: S4, part of the base **R** distribution (499 depending or importing CRAN packages).
- *SparseM*: S4 (26 depending or importing CRAN packages).
- *slam*: S3, very small matrices (50 depending or importing CRAN packages).

All packages add their methods to common functions and operators for dense matrices.

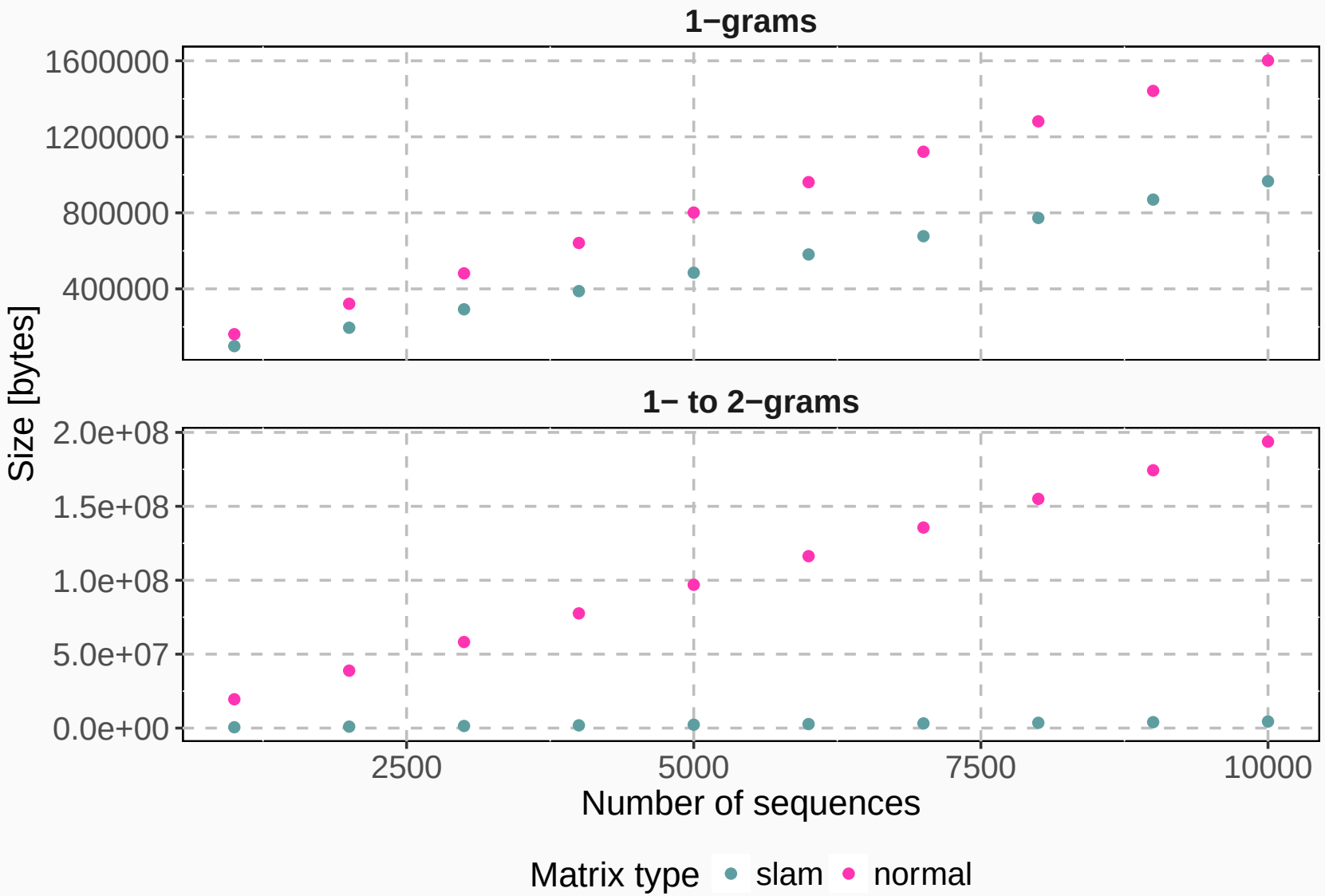
Sparse matrix with R

Packages:

- Matrix: S4, part of base R distribution, well-developed (499 depending or importing CRAN packages).
- SparseM: S4 (26 depending or importing CRAN packages).
- slam: S3, very small matrices (50 depending or importing CRAN packages, including *biogram*).

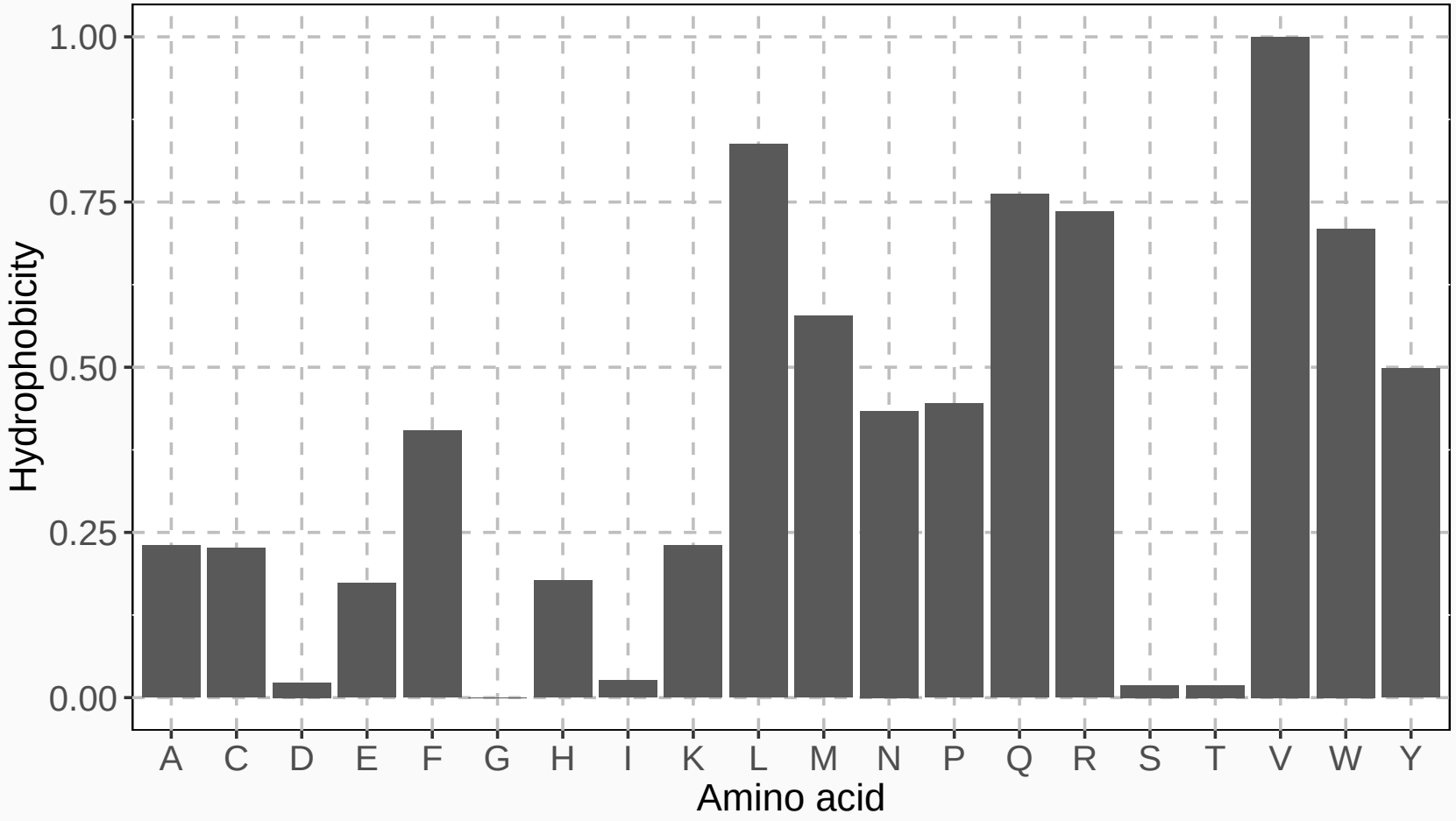
All packages add their methods to common functions and operators for dense matrices.

slam representation



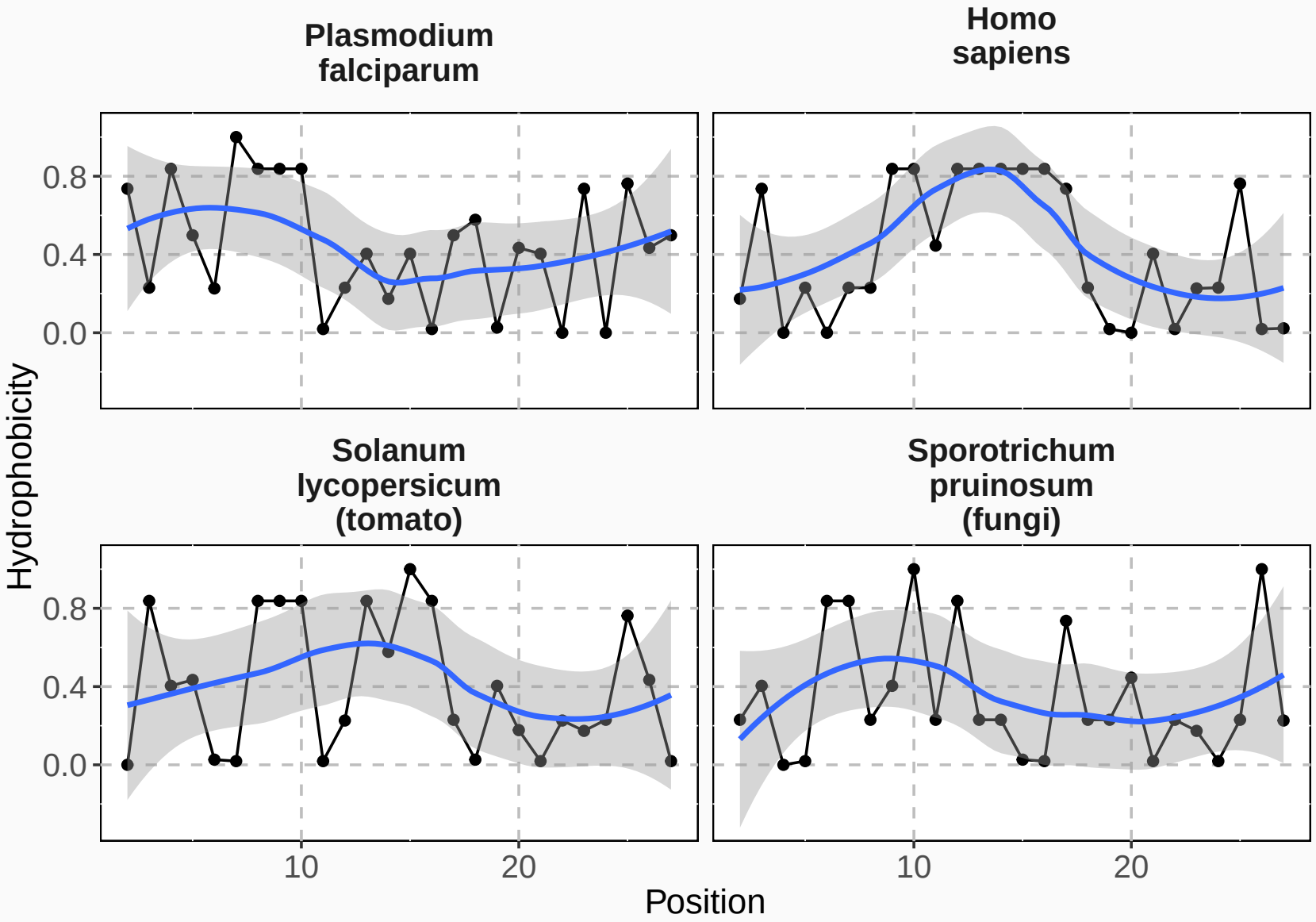
Reduction of the amino acid alphabet

Properites of amino acids

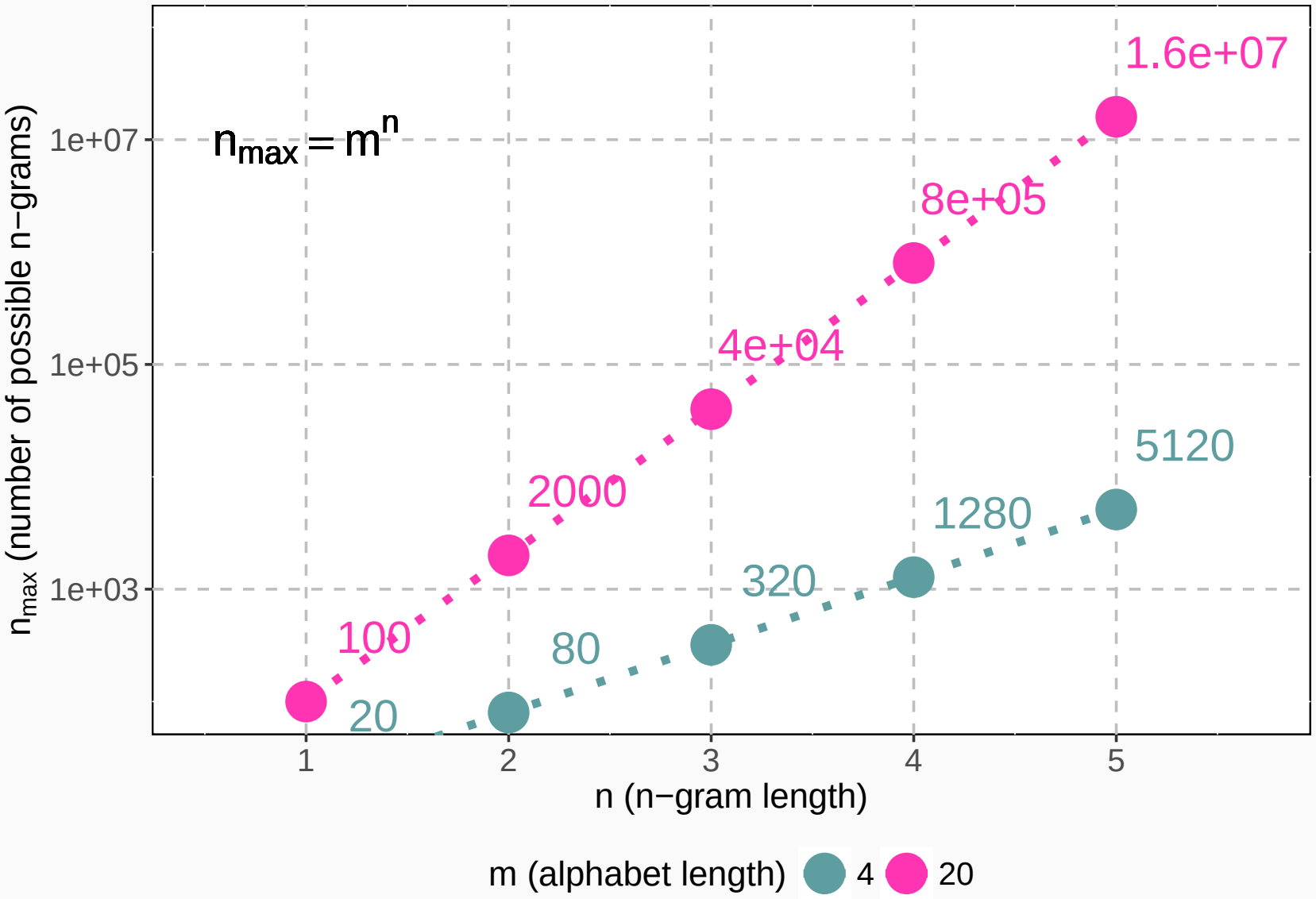


Amino acids may be described using their physicochemical properites.

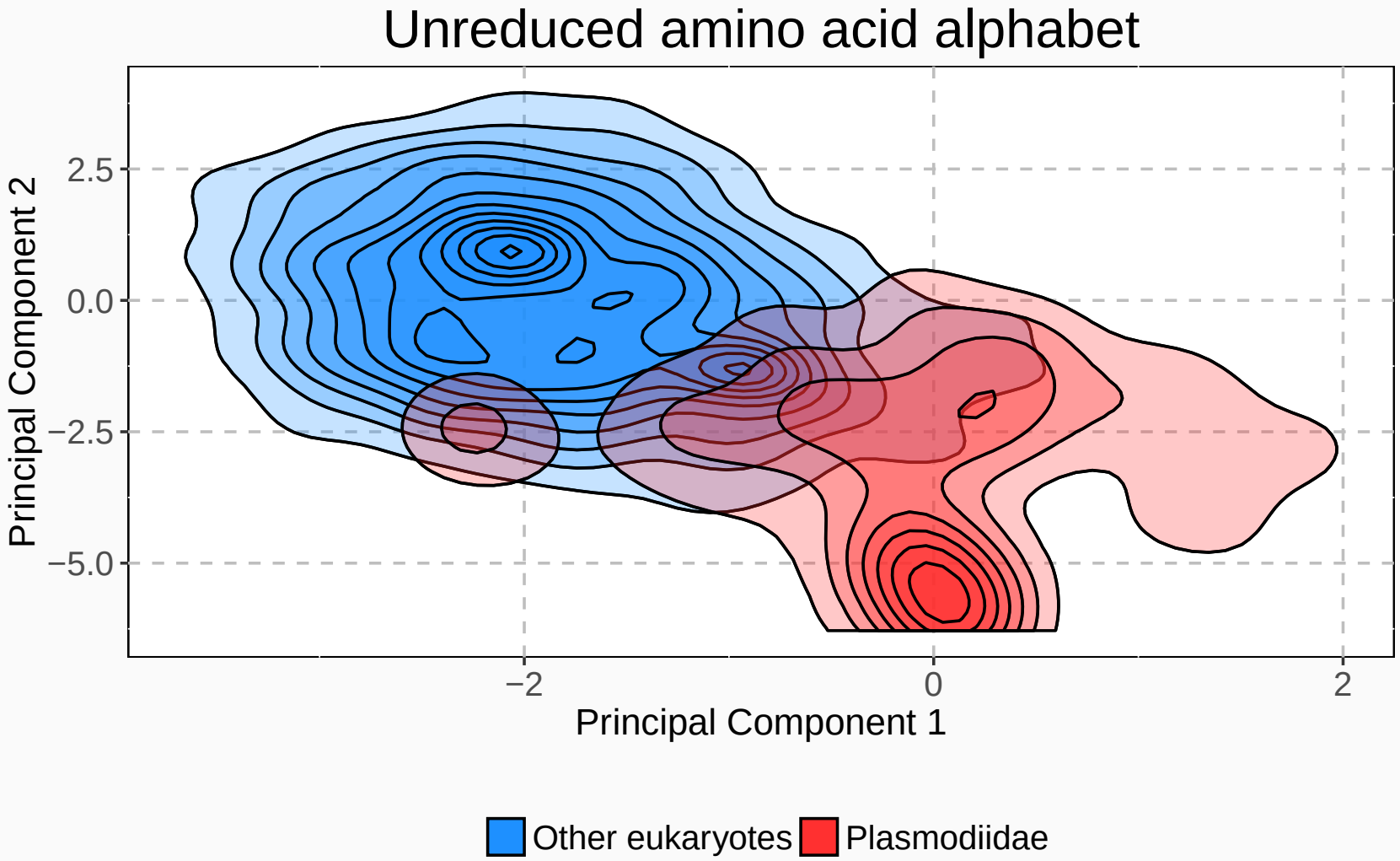
Properites of amino acids



Reduction of the alphabet



Reduction of the alphabet

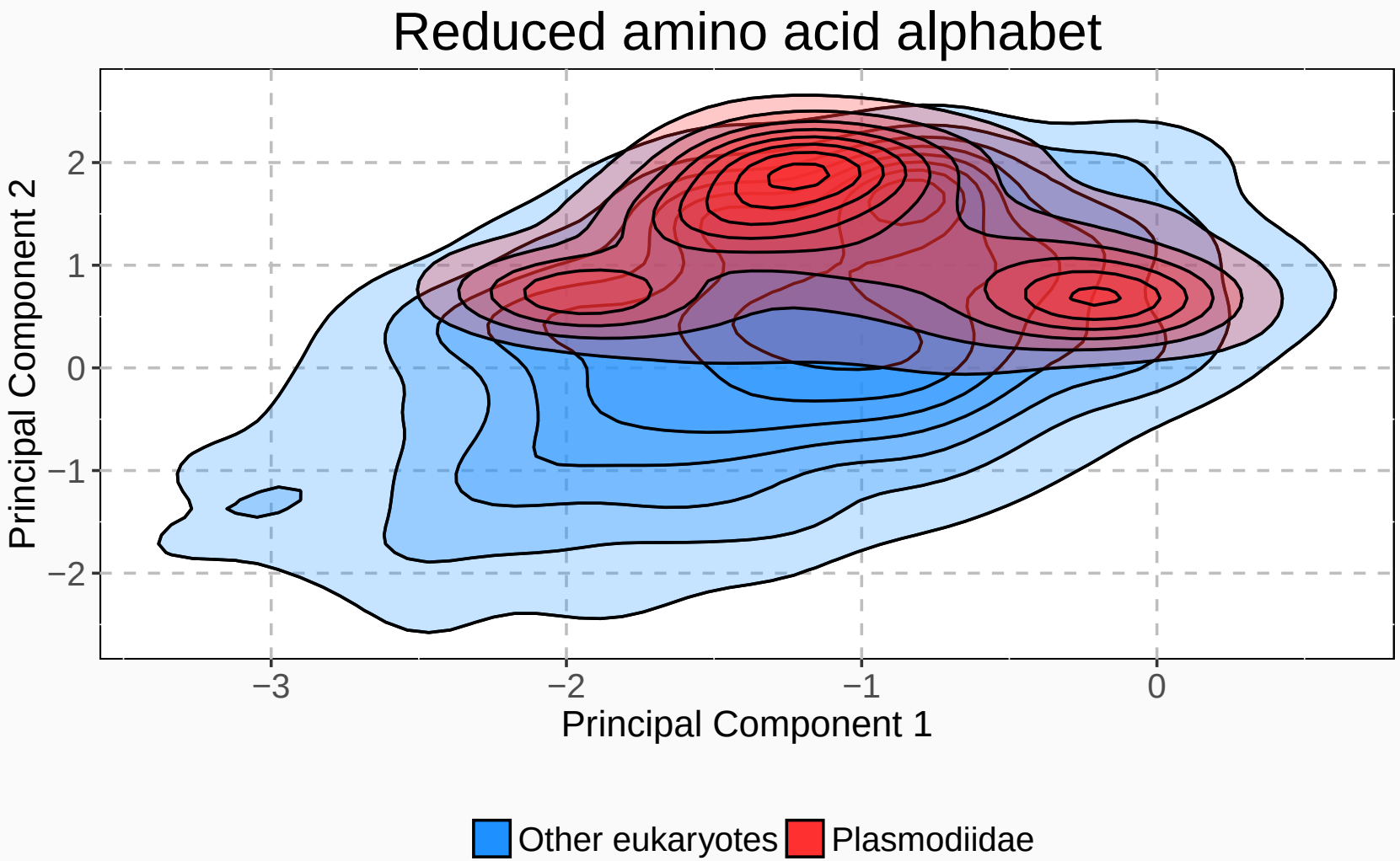


PCA analysis of amino acid frequency in signal peptides.

Reduction of the alphabet

Group	Amino acids
I	D, E, H, K, N, Q, R
II	G, P, S, T, Y
III	F, I, L, M, V, W
IV	A, C

Reduction of the alphabet



PCA analysis of amino acid frequency in signal peptides.

Filtering n-grams

Permutation Test

Informative n-grams are usually selected using permutation tests.

During a permutation test we shuffle randomly class labels and compute a defined statistic (e.g. information gain). Values of the statistic for permuted data are compared with the value of statistic for original data.

Permutation Test

target	Original data	Permuted data 1	Permuted data 2	...
0	0	1	1	...
0	1	0	1	...
0	1	1	1	...
1	0	0	0	...
1	1	1	0	...
1	0	0	0	...

$$\text{p-value} = \frac{N_{T_P > T_R}}{N}$$

$N_{T_P > T_R}$: number of cases, where T_P (permuted test statistic) has more extreme values than T_R (test statistic for original data).

N: number of permutations.

QuiPT

Quick **P**ermutation **T**est is a fast alternative to permutation tests for n-gram data. It computes a probability for a given contigency table providing the exact p-value for the specic value level of the test statistic.

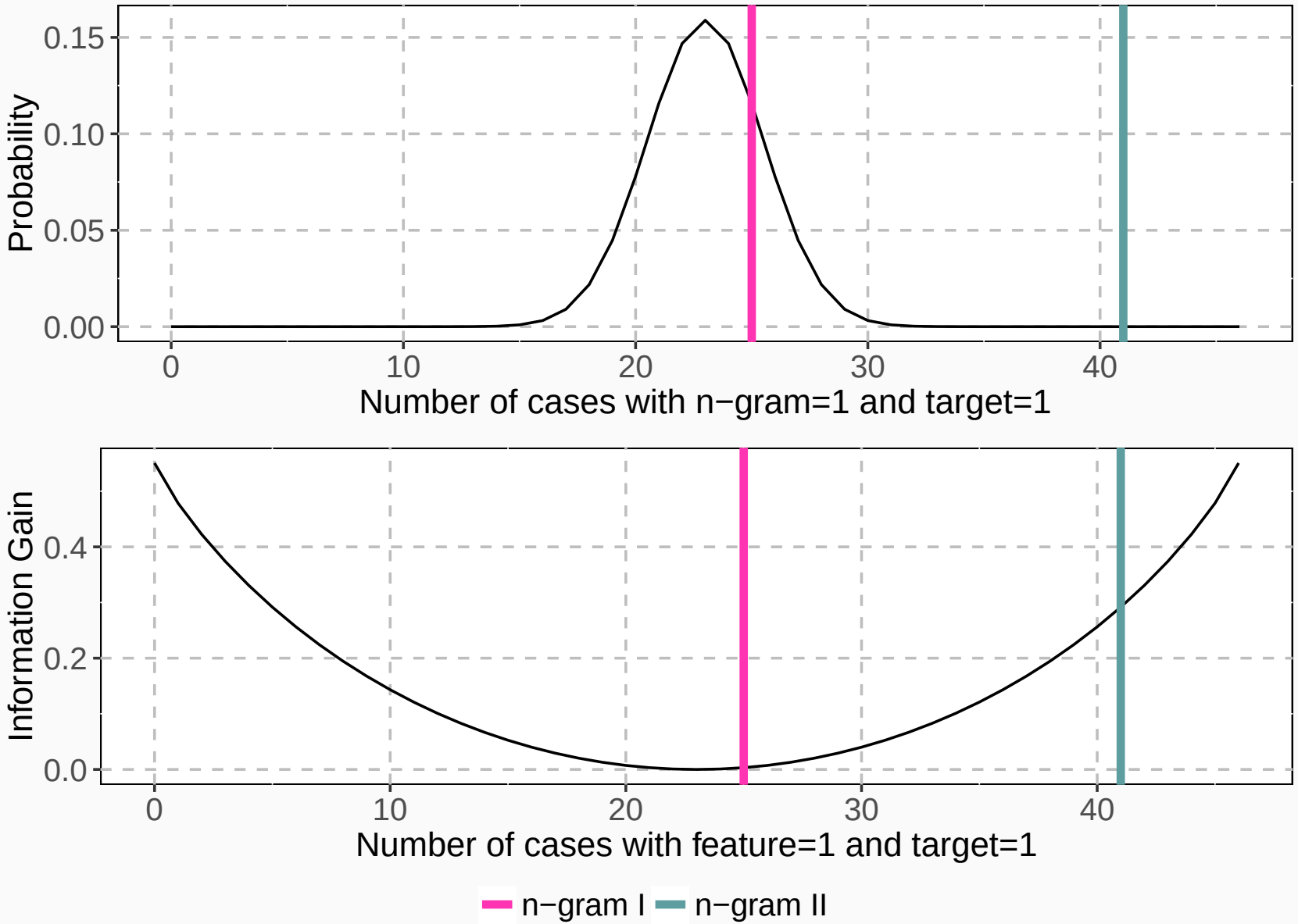
IG: 0.0032

Target	n-gram I	Count
0	0	29
1	0	25
0	1	21
1	1	25

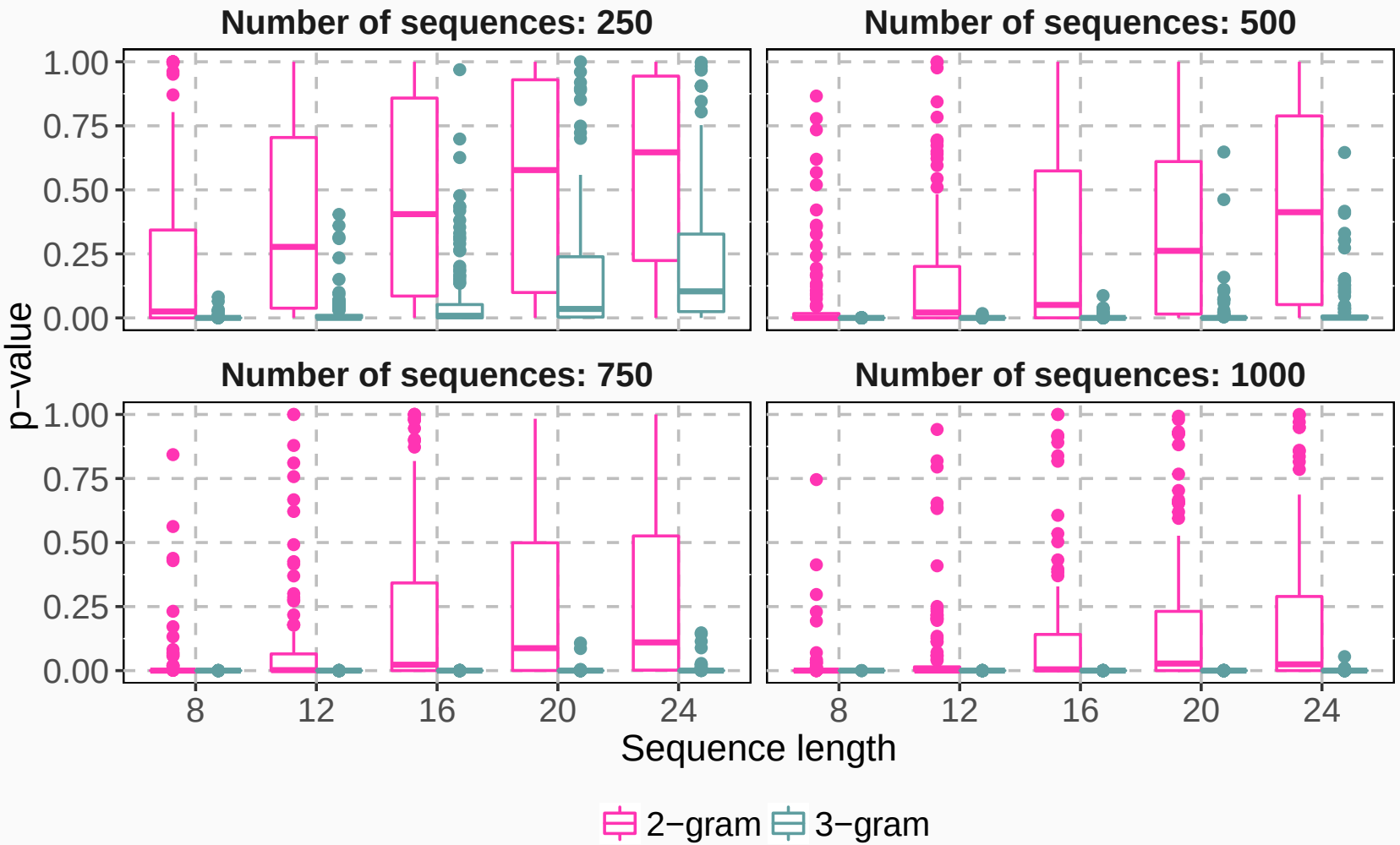
IG: 0.2917

Target	n-gram II	Count
0	0	45
1	0	9
0	1	5
1	1	41

QuiPT


Probability
0.15
0.10
0.05
0.00
0
10
20
30
40
Number of cases with n−gram=1 and target=1
Information Gain
0.4
0.2
0.0
0
10
20
30
40
Number of cases with feature=1 and target=1
n−gram I
n−gram II

Sensitivity of QuiPT



QuiPT works best for large data sets of short sequences.

Summary and conclusion

Summary

biogram: the **R** package for the n-gram analysis of biological sequences.

biogram workflow:

1. Extract n-grams (`count_ngrams()` and `count_multigrams()`).
2. Change an alphabet (`calc_ed()` and `calc_si()`).
3. Filter n-grams (`test_features()`).

Summary

biogram: the **R** package for the n-gram analysis of biological sequences.

`https://CRAN.R-project.org/package=biogram` (1.3)
`http://github.com/michbur/biogram` (1.4)

Case study

Burdukiewicz M, Sobczyk P, Rödiger S, Duda-Madej A, Mackiewicz P, Kotulska M. (2016) **Prediction of amyloidogenicity based on the n-gram analysis.** *PeerJ Preprints* 4:e2390v1 `https://doi.org/10.7287/peerj.preprints.2390v1`

A novel method of detecting amyloids, proteins involded in many neurodegenerative disorders, such as Alzheimer's or Creutzfeldt-Jakob's diseases based on n-grams used to train a random forest classifier (from the *ranger* package).

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- Paweł Mackiewicz,
- Małgorzata Kotulska,
- **biogram** package (`https://cran.r-project.org/package=biogram`):
  - Piotr Sobczyk,
  - Chris Lauber.