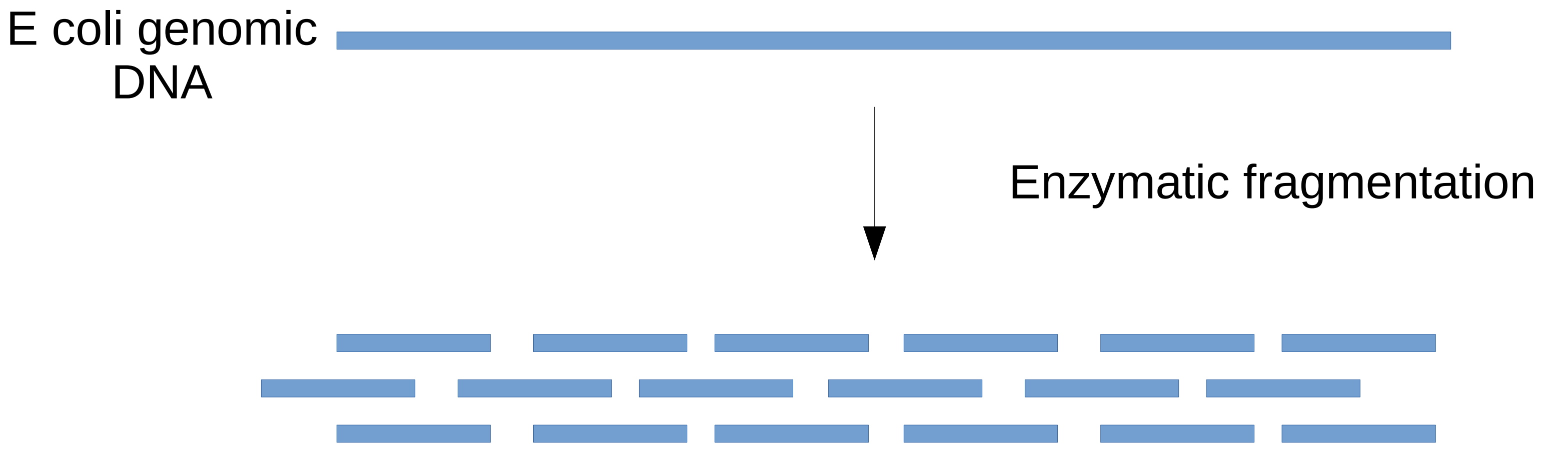
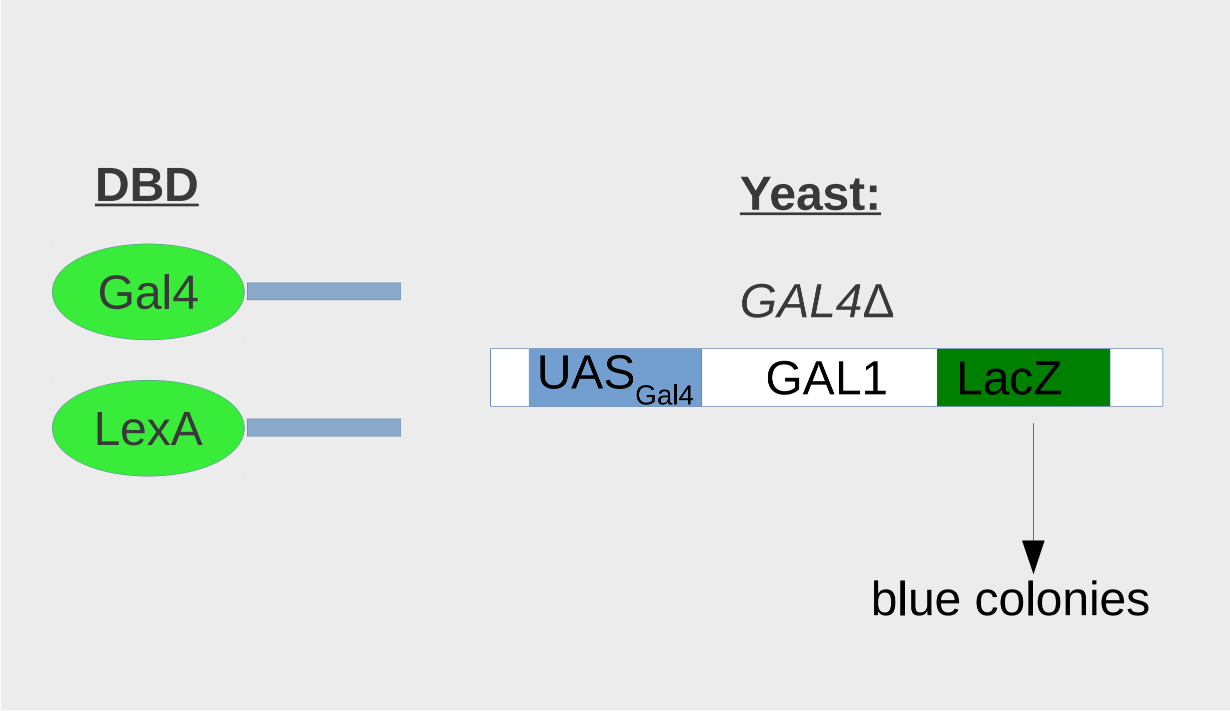
Initial “*motif search*” on TADs



E coli genomic DNA

Enzymatic fragmentation



DBD

Gal4

LexA

Yeast:

GAL4Δ

UAS$_{Gal4}$ GAL1 LacZ

blue colonies

- 15000 transformants
- 0.1-1% function as TA.
- 12 to 81 aa
- Negative charge

Ma & Ptashne, 1987 (Cell)
Ruden et al., 1991 (Nature)

Small molecules mimicking TADs

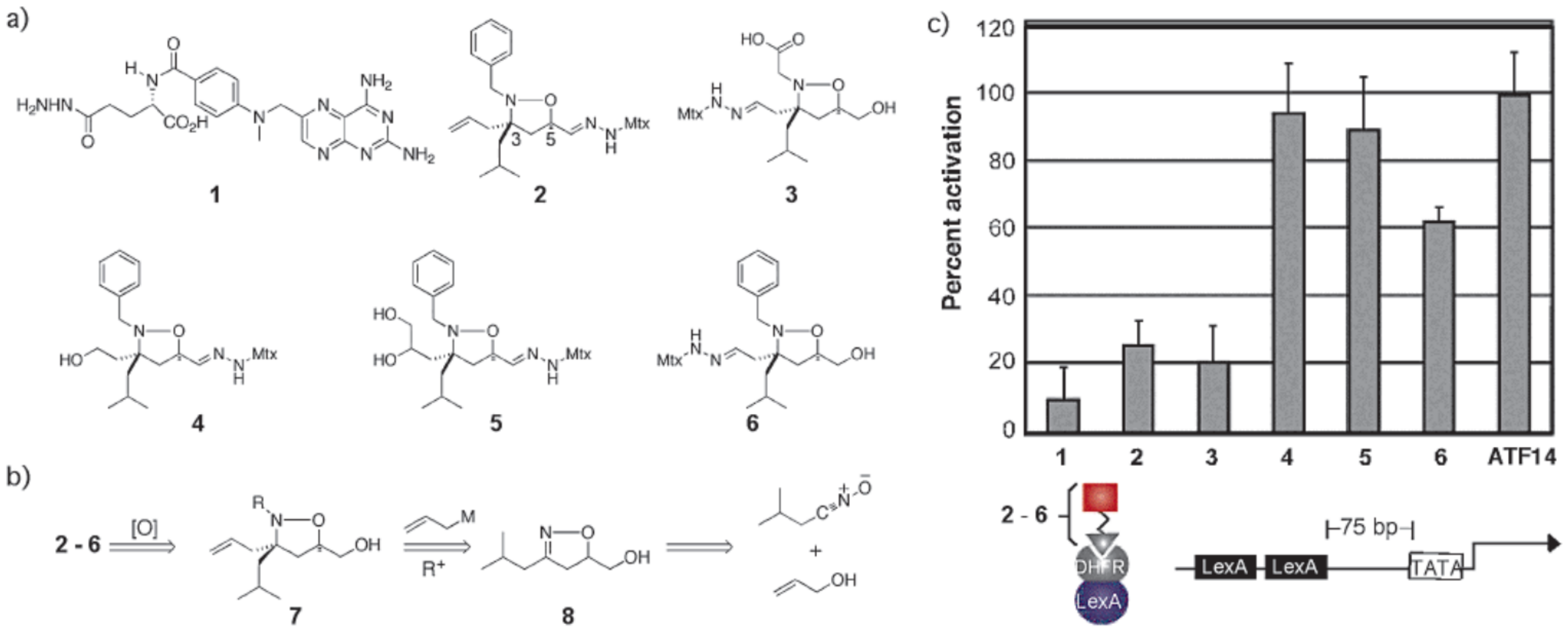
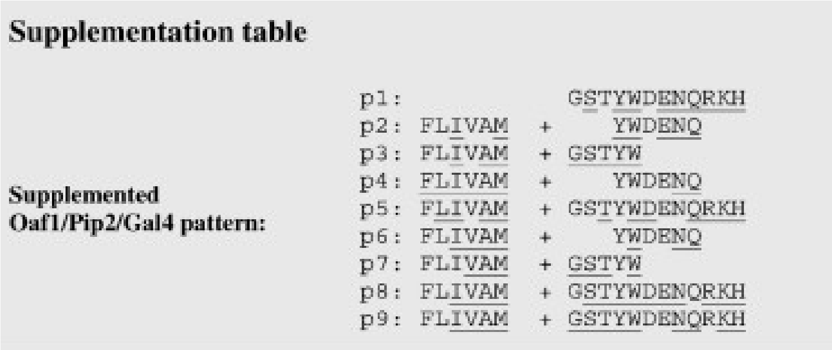


Figure 2. Isoxazolidine-based activation domains. (a) Five isoxazolidines (**2**−**6**) bearing functional groups commonly found in natural activation domains were targeted. (b) Synthetic strategy used to prepare isoxazolidines. (c) Results from in vitro transcription assays. The activity of each compound represents the average of at least three individual experiments with the indicated error (SDOM). For details see the Supporting Information.

Minter et al., 2004 (JACS)

TAD motif?

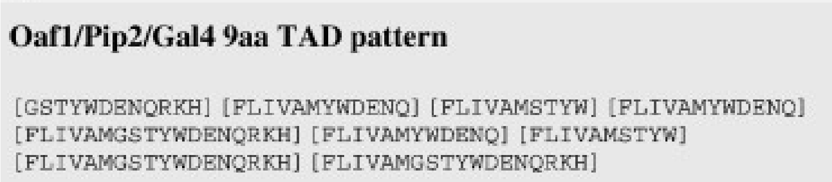


Supplementation table

Supplemented Oaf1/Pip2/Gal4 pattern:

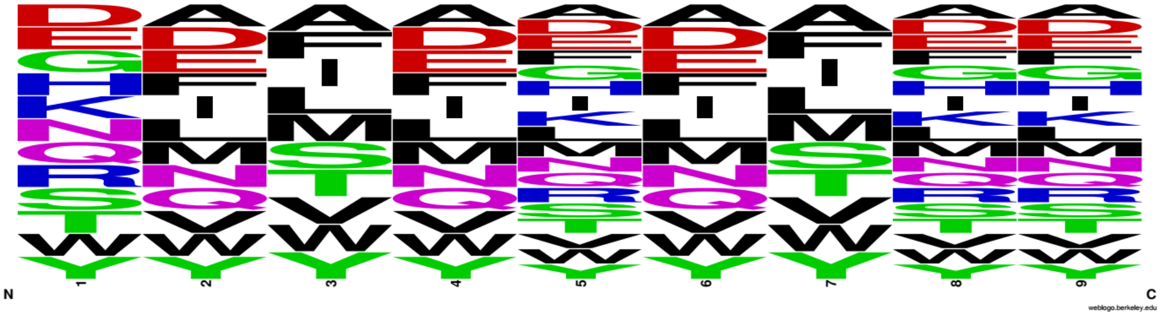
```
p1:                GSTYWDENQRKH
p2: FLIVAM   +        YWDENQ
p3: FLIVAM   +     GSTYW
p4: FLIVAM   +        YWDENQ
p5: FLIVAM   +     GSTYWDENQRKH
p6: FLIVAM   +        YWDENQ
p7: FLIVAM   +     GSTYW
p8: FLIVAM   +     GSTYWDENQRKH
p9: FLIVAM   +     GSTYWDENQRKH
```

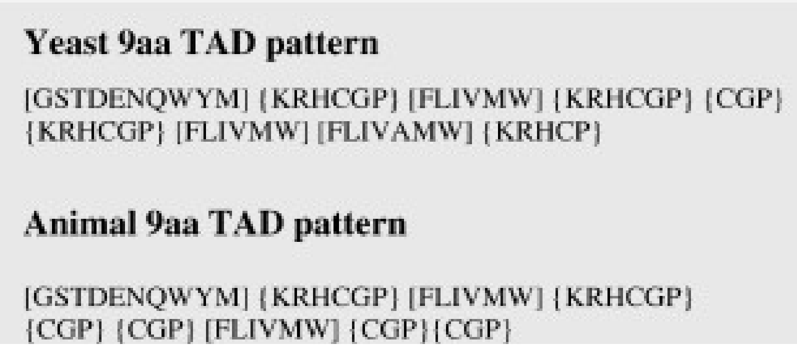
D



Oaf1/Pip2/Gal4 9aa TAD pattern

[GSTYWDENQRKH] [FLIVAMYWDENQ] [FLIVAMSTYW] [FLIVAMYWDENQ] [FLIVAMGSTYWDENQRKH] [FLIVAMYWDENQ] [FLIVAMSTYW] [FLIVAMGSTYWDENQRKH] [FLIVAMGSTYWDENQRKH]



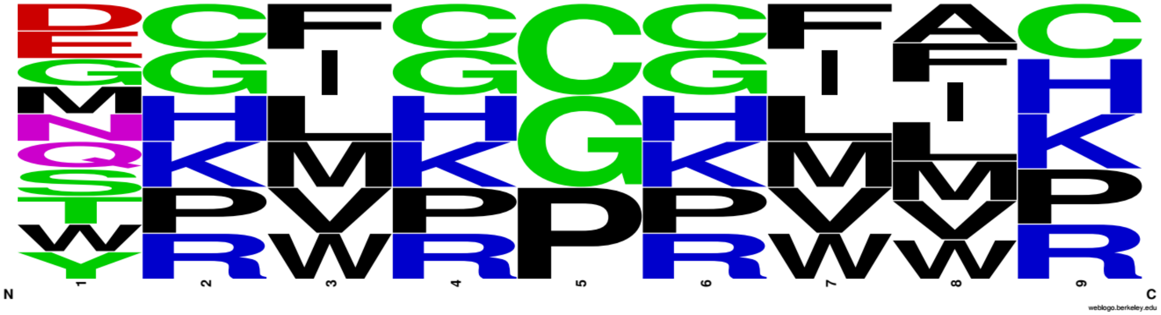


Yeast 9aa TAD pattern

[GSTDENQWYM] {KRHCGP} [FLIVMW] {KRHCGP} {CGP} {KRHCGP} [FLIVMW] [FLIVAMW] {KRHCP}

Animal 9aa TAD pattern

[GSTDENQWYM] {KRHCGP} [FLIVMW] {KRHCGP} {CGP} {CGP} [FLIVMW] {CGP}{CGP}



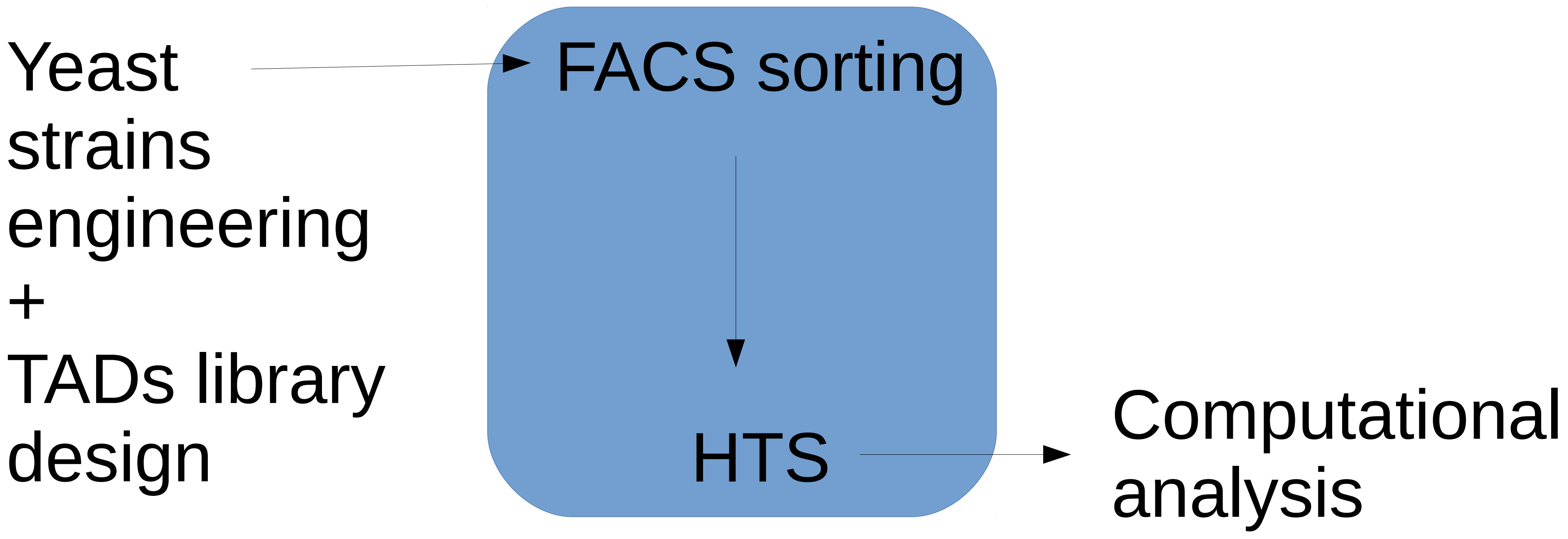
Piskacek et al., 2007 (Genomics)

And yet… it’s not clear what TADs have in common.

- **Assumption:**
  There are patterns or motifs common to all TADs.

- **Plan:**
  Use *state of the art* experimental and computational methodologies to analyze a big combinatorial space of TAD sequences to find motifs or patterns.

Workflow



Yeast strains engineering
+
TADs library design

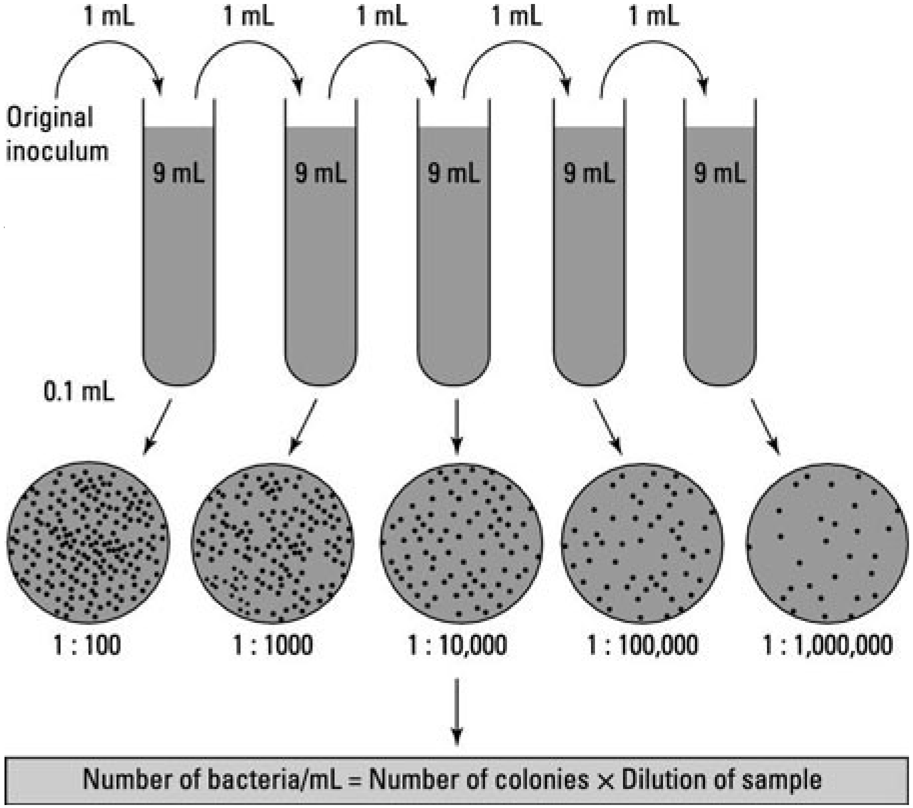
FACS sorting

HTS

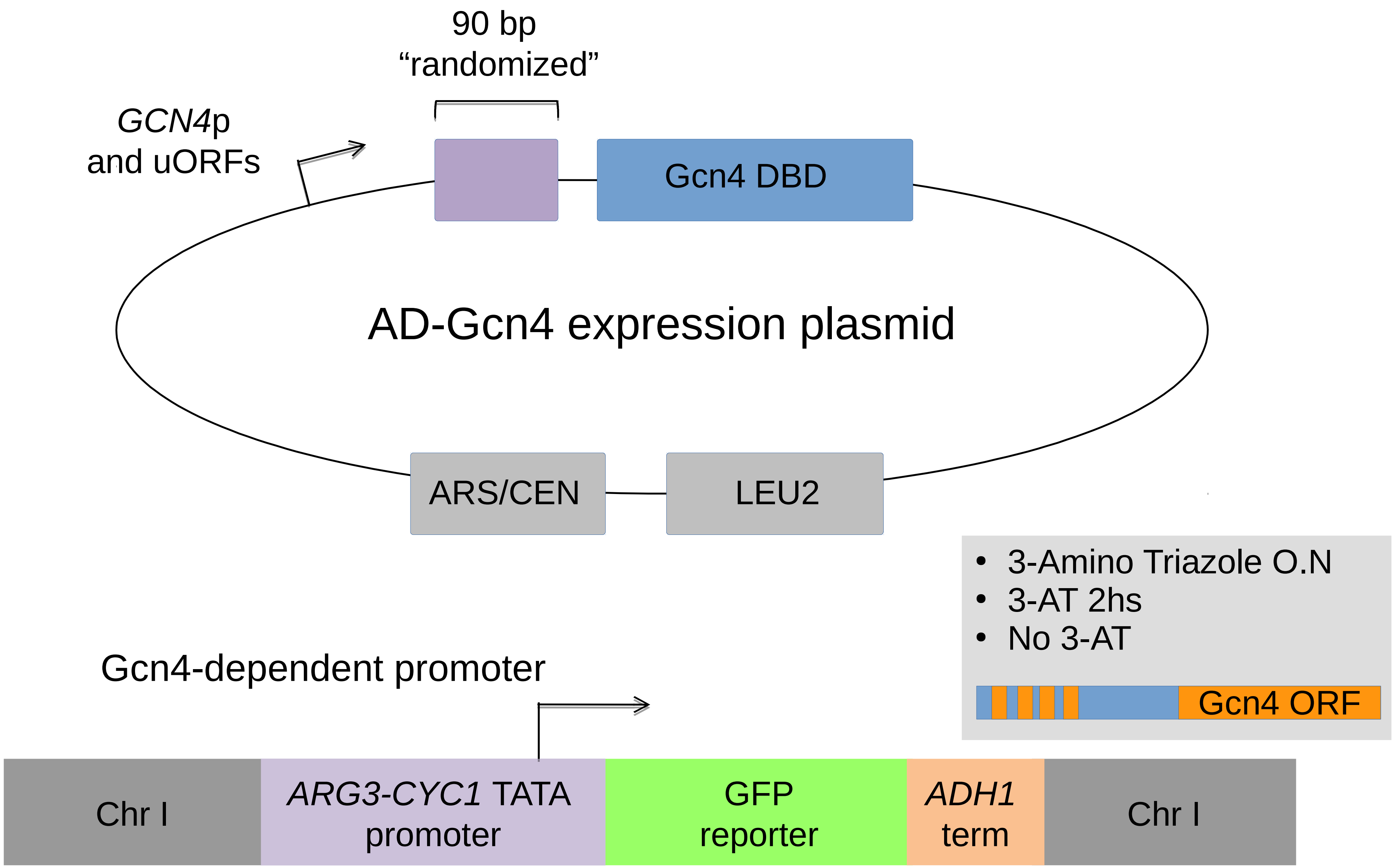
Computational analysis

Bottleneck is yeast transformation

~10^7 **CFU**



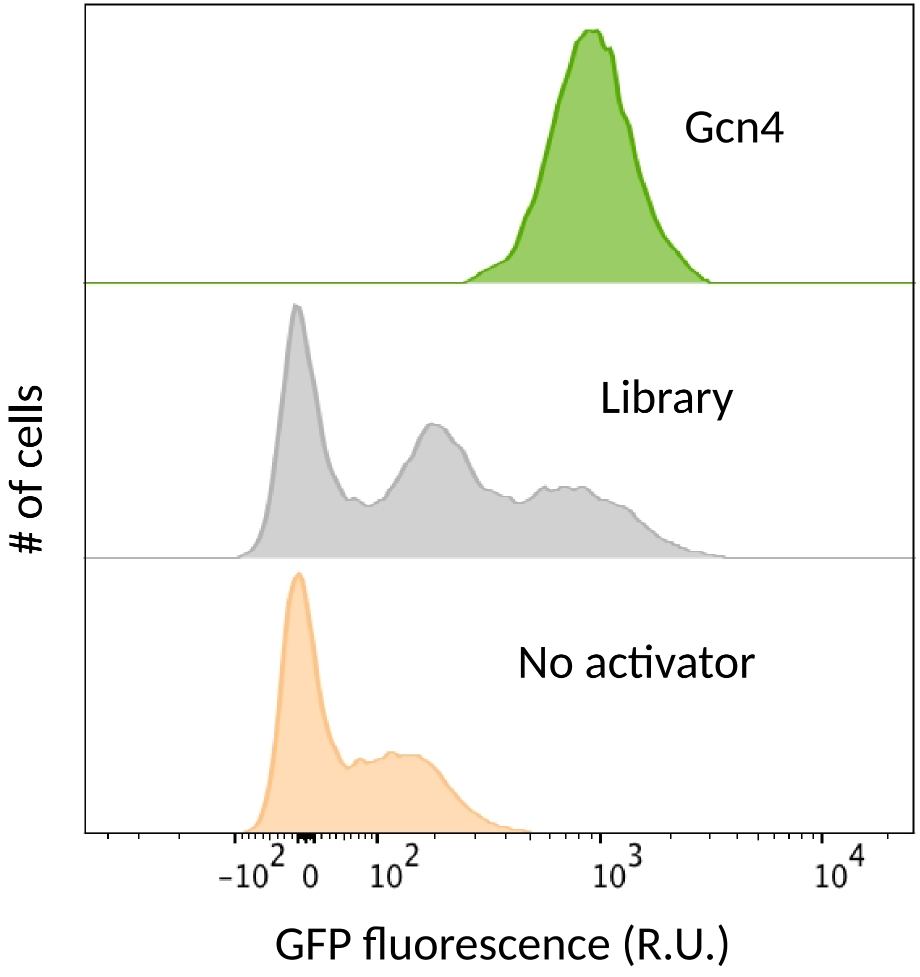
Strategy for high throughput isolation of activation domains



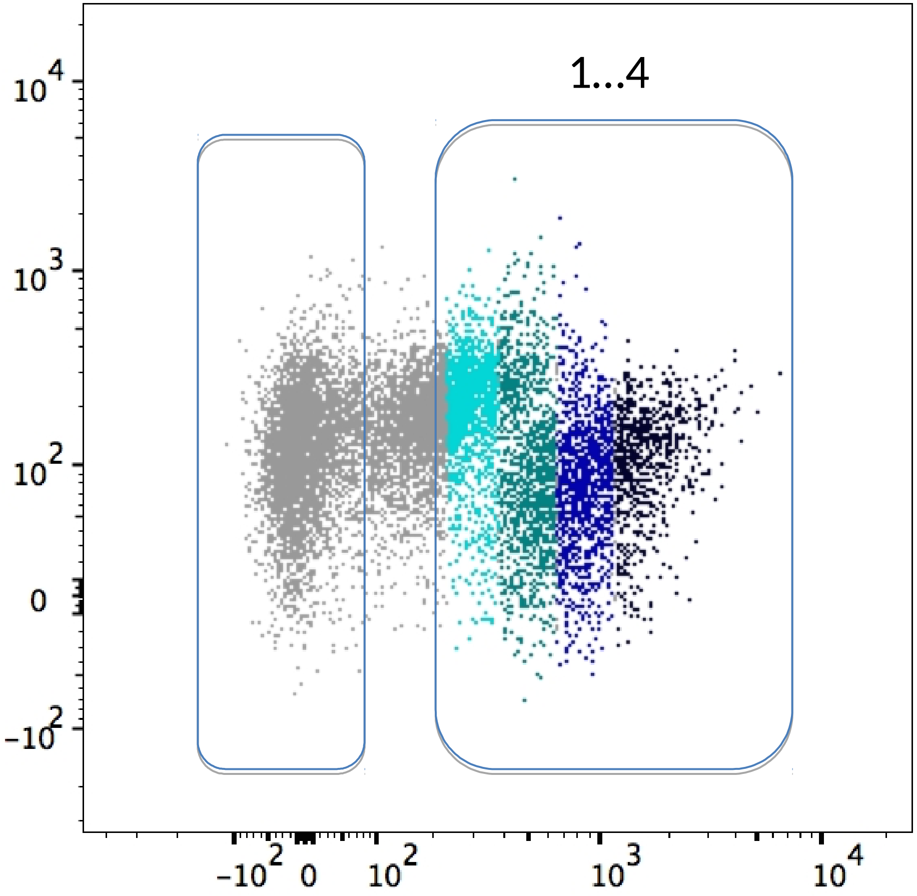
- 3-Amino Triazole O.N
- 3-AT 2hs
- No 3-AT

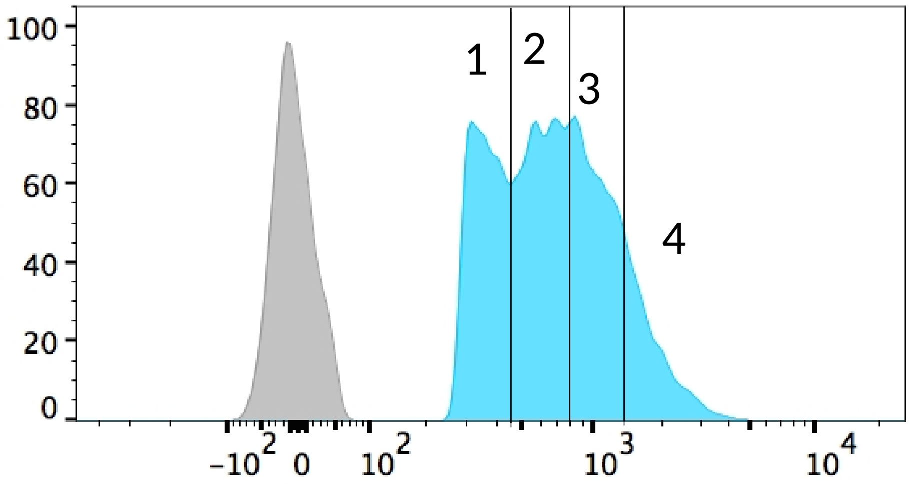
Linda Warfield, Ariel, Erijman, Steven Petesch

FACS selection of TAD libraries



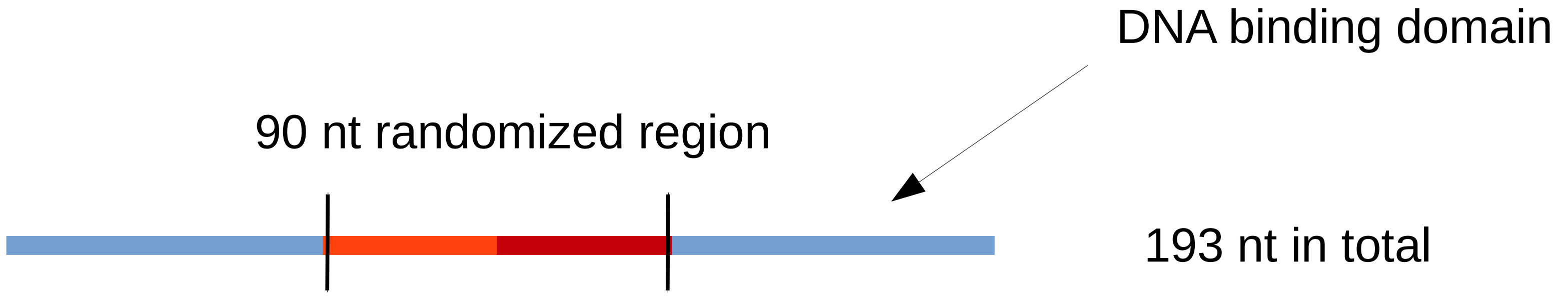
Splitting novel ADs on their strength

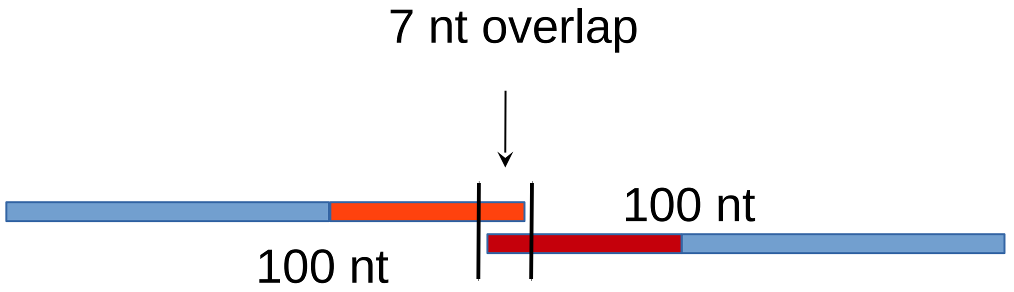




Library sequencing

Platform: HiSeq Illumina (paired end) - 100nt reads with 7nt overlap





HT-seq analysis

- **PAIR READS** (FLASH, PMID: 21903629)
- **TRANSLATION TO AMINO ACID** (custom script)
- **CLUSTER SEQUENCES** (USEARH, pmid: 20709691) - sequences are redundant, probably due to random techinical errors. Clusters allow up to 6 mismatches (20%).
- **SCORE SEQUENCES** based on number or reads/bin
- **PREDICT PHYSICOCHEMICAL PROPERTIES OF 30mers** (*intrinsic disorder*: IUPred, *Secondary Structure*: PSIPRED, *GRAVY scores*: custom scripts)

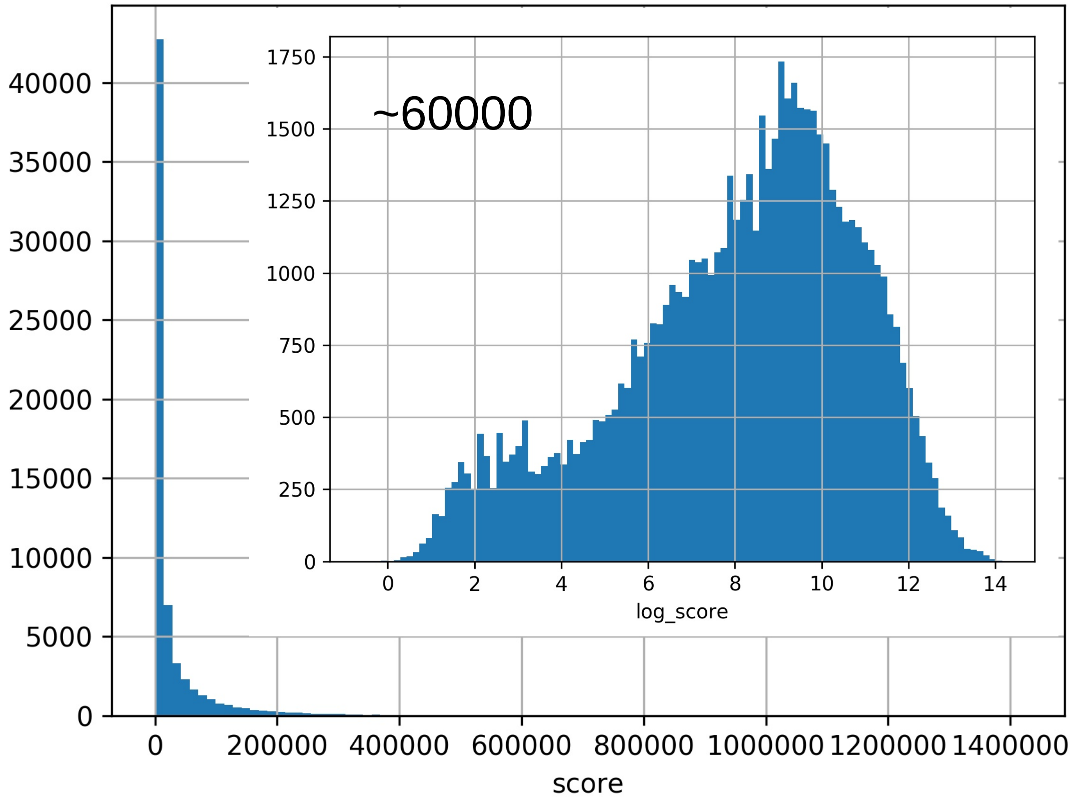
- **DEEP LEARNING…** you?
- Tried MEME and Gibs sampler without success...

Translation to Amino-acids

(inspect the raw reads in FastaQC program followed by custom scripts – Qual offset=33, HiSeq Illumina v. >1.8)

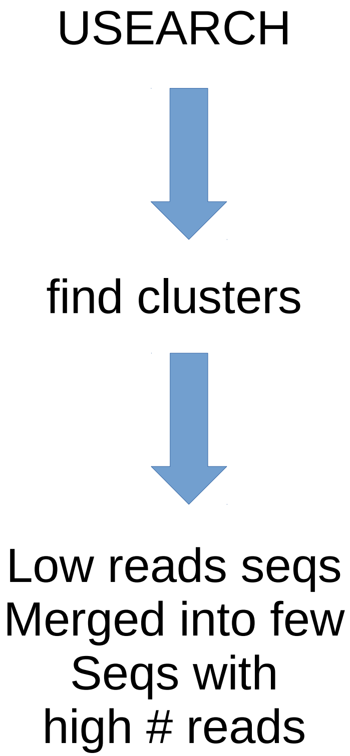
- Include filters for:
  - Early-stop (0.9 – 40%)
  - no5-primer (~3%)
  - no3-primer (5-25%)
  - Frame-shift (~0.3%)
  - Low-quality$_{\text{Phred=30}}$ (15-35%)
  - Short-seq (0.2-20%)

<5 reads (all bins) → discarded
Positives: bins(3,4) > 2*bins(0,1,2) ~20000
Negatives: bins(0) > 2*bins(2,3,4) ~20000



~60000

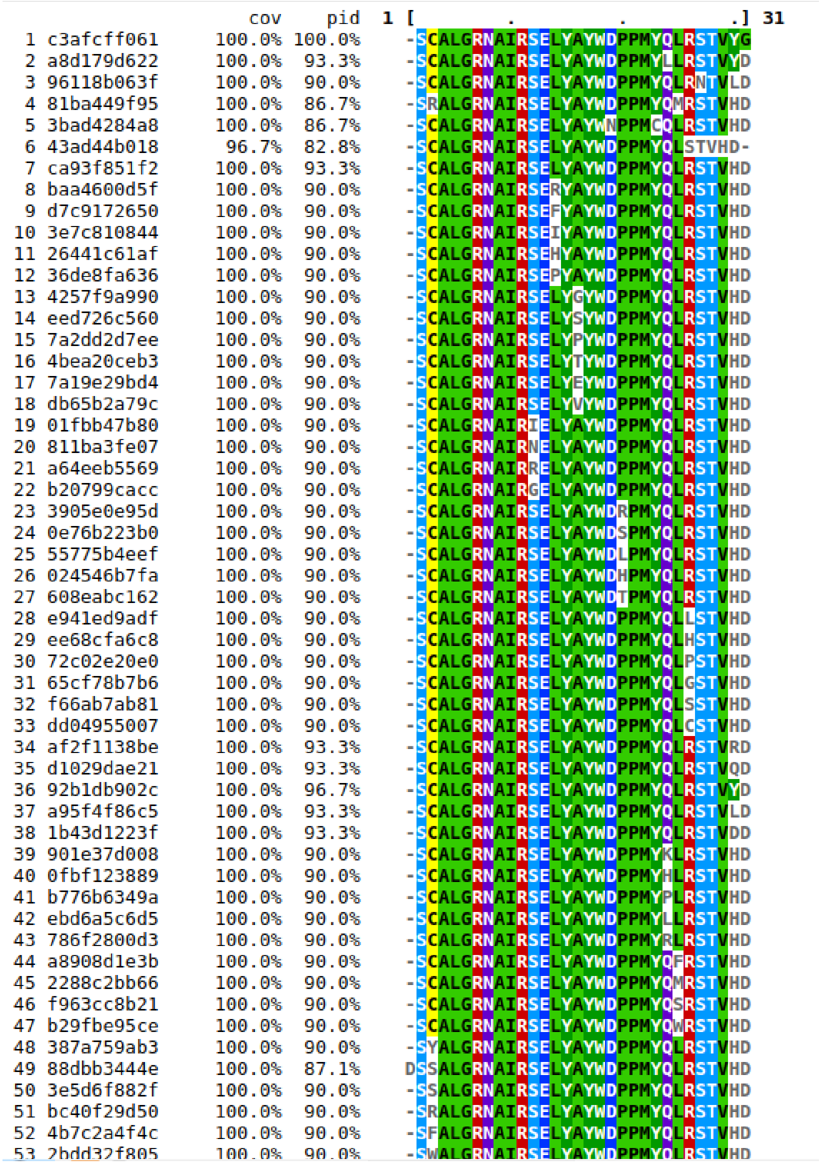
Remove sequence redundancy that might arise from technical errors



USEARCH

find clusters

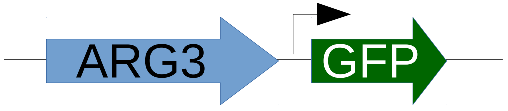
Low reads seqs
Merged into few
Seqs with
high # reads

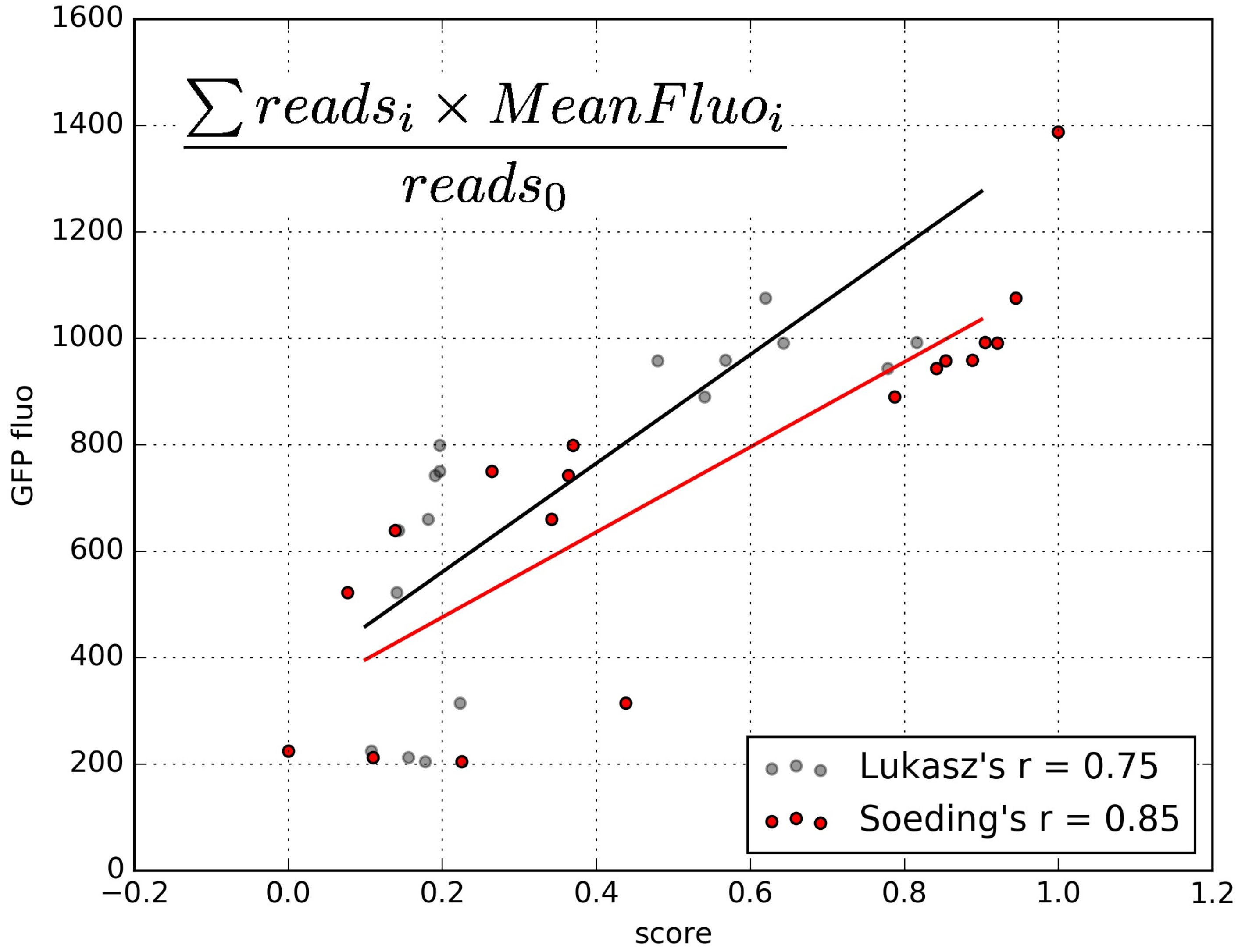


```
                       cov     pid  1 [          .          .        .] 31
 1 c3afcff061       100.0% 100.0%    -SCALGRNAIRSELYAYWDPPMYQLRSTVYG
 2 a8d179d622       100.0%  93.3%    -SCALGRNAIRSELYAYWDPPMYLLRSTVYD
 3 96118b063f       100.0%  90.0%    -SCALGRNAIRSELYAYWDPPMYQLRNTVLD
 4 81ba449f95       100.0%  86.7%    -SRALGRNAIRSELYAYWDPPMYQMRSTVHD
 5 3bad4284a8       100.0%  86.7%    -SCALGRNAIRSELYAYWNPPMCQLRSTVHD
 6 43ad44b018        96.7%  82.8%    -SCALGRNAIRSELYAYWDPPMYQLSTVHD-
 7 ca93f851f2       100.0%  93.3%    -SCALGRNAIRSELYAYWDPPMYQLRSTVHD
 8 baa4600d5f       100.0%  90.0%    -SCALGRNAIRSERYAYWDPPMYQLRSTVHD
 9 d7c9172650       100.0%  90.0%    -SCALGRNAIRSEFYAYWDPPMYQLRSTVHD
10 3e7c810844       100.0%  90.0%    -SCALGRNAIRSEIYAYWDPPMYQLRSTVHD
11 26441c61af       100.0%  90.0%    -SCALGRNAIRSEHYAYWDPPMYQLRSTVHD
12 36de8fa636       100.0%  90.0%    -SCALGRNAIRSEPYAYWDPPMYQLRSTVHD
13 4257f9a990       100.0%  90.0%    -SCALGRNAIRSELYGYWDPPMYQLRSTVHD
14 eed726c560       100.0%  90.0%    -SCALGRNAIRSELYSYWDPPMYQLRSTVHD
15 7a2dd2d7ee       100.0%  90.0%    -SCALGRNAIRSELYPYWDPPMYQLRSTVHD
16 4bea20ceb3       100.0%  90.0%    -SCALGRNAIRSELYTYWDPPMYQLRSTVHD
17 7a19e29bd4       100.0%  90.0%    -SCALGRNAIRSELYEYWDPPMYQLRSTVHD
18 db65b2a79c       100.0%  90.0%    -SCALGRNAIRSELYVYWDPPMYQLRSTVHD
19 01fbb47b80       100.0%  90.0%    -SCALGRNAIRIELYAYWDPPMYQLRSTVHD
20 811ba3fe07       100.0%  90.0%    -SCALGRNAIRNELYAYWDPPMYQLRSTVHD
21 a64eeb5569       100.0%  90.0%    -SCALGRNAIRRELYAYWDPPMYQLRSTVHD
22 b20799cacc       100.0%  90.0%    -SCALGRNAIRGELYAYWDPPMYQLRSTVHD
23 3905e0e95d       100.0%  90.0%    -SCALGRNAIRSELYAYWDRPMYQLRSTVHD
24 0e76b223b0       100.0%  90.0%    -SCALGRNAIRSELYAYWDSPMYQLRSTVHD
25 55775b4eef       100.0%  90.0%    -SCALGRNAIRSELYAYWDLPMYQLRSTVHD
26 024546b7fa       100.0%  90.0%    -SCALGRNAIRSELYAYWDHPMYQLRSTVHD
27 608eabc162       100.0%  90.0%    -SCALGRNAIRSELYAYWDTPMYQLRSTVHD
28 e941ed9adf       100.0%  90.0%    -SCALGRNAIRSELYAYWDPPMYQLLSTVHD
29 ee68cfa6c8       100.0%  90.0%    -SCALGRNAIRSELYAYWDPPMYQLHSTVHD
30 72c02e20e0       100.0%  90.0%    -SCALGRNAIRSELYAYWDPPMYQLPSTVHD
31 65cf78b7b6       100.0%  90.0%    -SCALGRNAIRSELYAYWDPPMYQLGSTVHD
32 f66ab7ab81       100.0%  90.0%    -SCALGRNAIRSELYAYWDPPMYQLSSTVHD
33 dd04955007       100.0%  90.0%    -SCALGRNAIRSELYAYWDPPMYQLCSTVHD
34 af2f1138be       100.0%  93.3%    -SCALGRNAIRSELYAYWDPPMYQLRSTVRD
35 d1029dae21       100.0%  93.3%    -SCALGRNAIRSELYAYWDPPMYQLRSTVQD
36 92b1db902c       100.0%  96.7%    -SCALGRNAIRSELYAYWDPPMYQLRSTVYD
37 a95f4f86c5       100.0%  93.3%    -SCALGRNAIRSELYAYWDPPMYQLRSTVLD
38 1b43d1223f       100.0%  93.3%    -SCALGRNAIRSELYAYWDPPMYQLRSTVDD
39 901e37d008       100.0%  90.0%    -SCALGRNAIRSELYAYWDPPMYKLRSTVHD
40 0fbf123889       100.0%  90.0%    -SCALGRNAIRSELYAYWDPPMYHLRSTVHD
41 b776b6349a       100.0%  90.0%    -SCALGRNAIRSELYAYWDPPMYPLRSTVHD
42 ebd6a5c6d5       100.0%  90.0%    -SCALGRNAIRSELYAYWDPPMYLLRSTVHD
43 786f2800d3       100.0%  90.0%    -SCALGRNAIRSELYAYWDPPMYRLRSTVHD
44 a8908d1e3b       100.0%  90.0%    -SCALGRNAIRSELYAYWDPPMYQFRSTVHD
45 2288c2bb66       100.0%  90.0%    -SCALGRNAIRSELYAYWDPPMYQMRSTVHD
46 f963cc8b21       100.0%  90.0%    -SCALGRNAIRSELYAYWDPPMYQSRSTVHD
47 b29fbe95ce       100.0%  90.0%    -SCALGRNAIRSELYAYWDPPMYQWRSTVHD
48 387a759ab3       100.0%  90.0%    -SYALGRNAIRSELYAYWDPPMYQLRSTVHD
49 88dbb3444e       100.0%  87.1%    DSSALGRNAIRSELYAYWDPPMYQLRSTVHD
50 3e5d6f882f       100.0%  90.0%    -SSALGRNAIRSELYAYWDPPMYQLRSTVHD
51 bc40f29d50       100.0%  90.0%    -SRALGRNAIRSELYAYWDPPMYQLRSTVHD
52 4b7c2a4f4c       100.0%  90.0%    -SFALGRNAIRSELYAYWDPPMYQLRSTVHD
53 2bdd32f805       100.0%  90.0%    -SWALGRNAIRSELYAYWDPPMYQLRSTVHD
```

Scoring the sequences

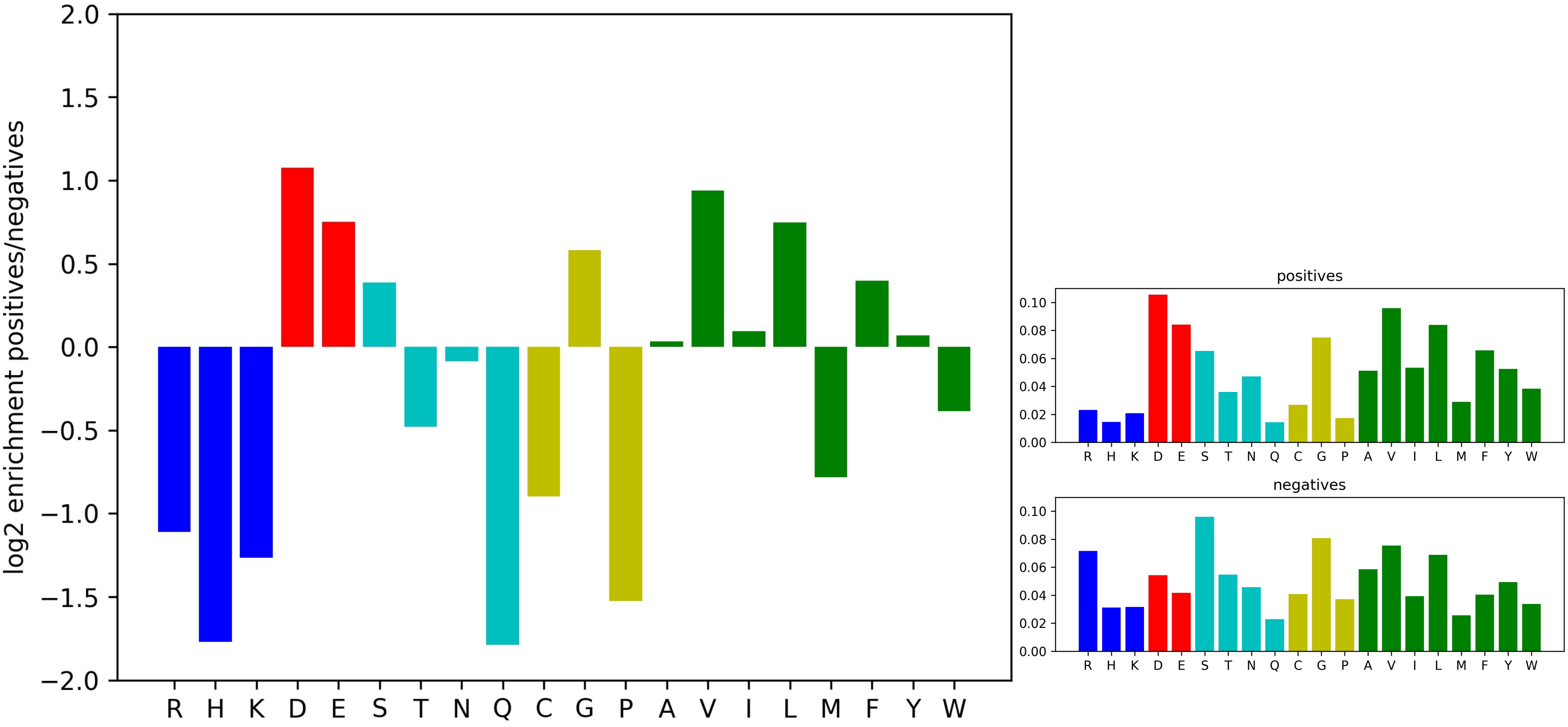
Based on 18 mutants experimentally validated and with known distribution of reads across Bin1-4 and Bkgd





$$\frac{\sum reads_i \times MeanFluo_i}{reads_0}$$

Enrichment in aa content

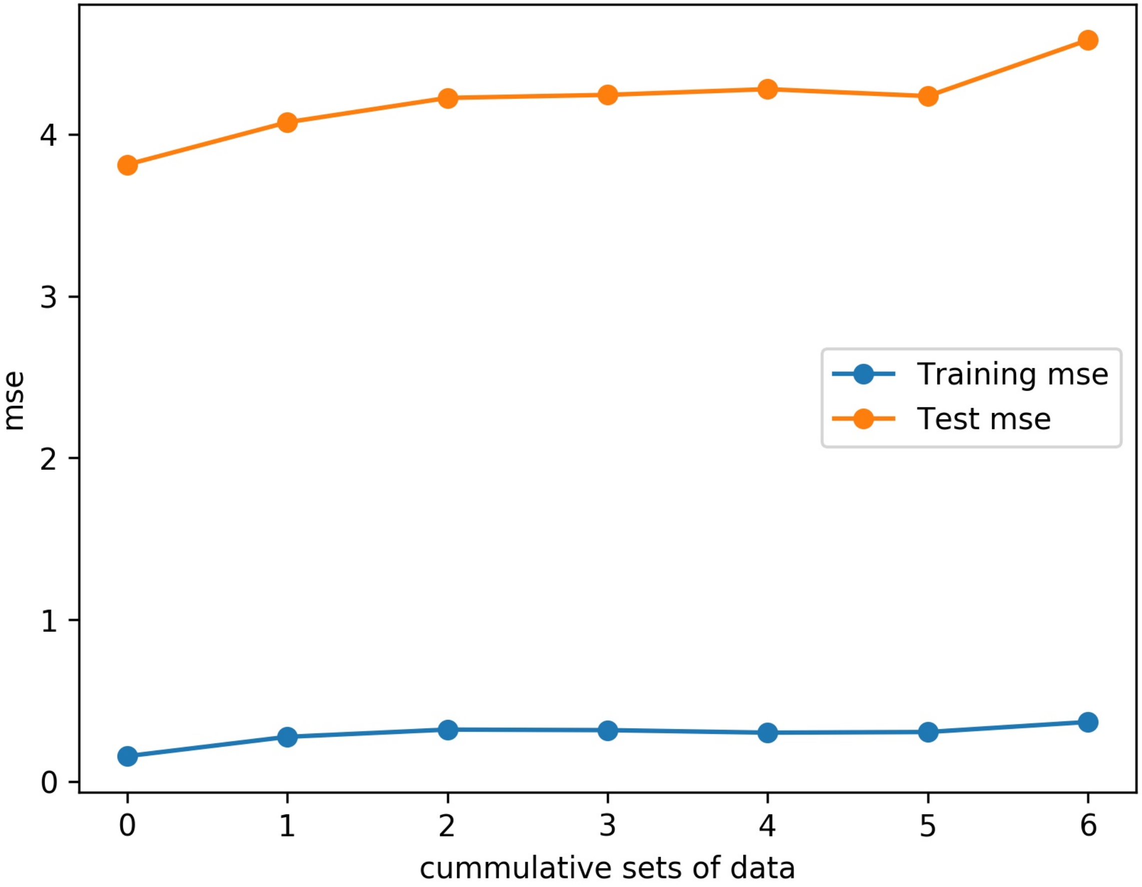


Features for ML

- AA seq
- AA hydrophobicity
- AA Charge
- AA Secondary Structure
- AA Disorder

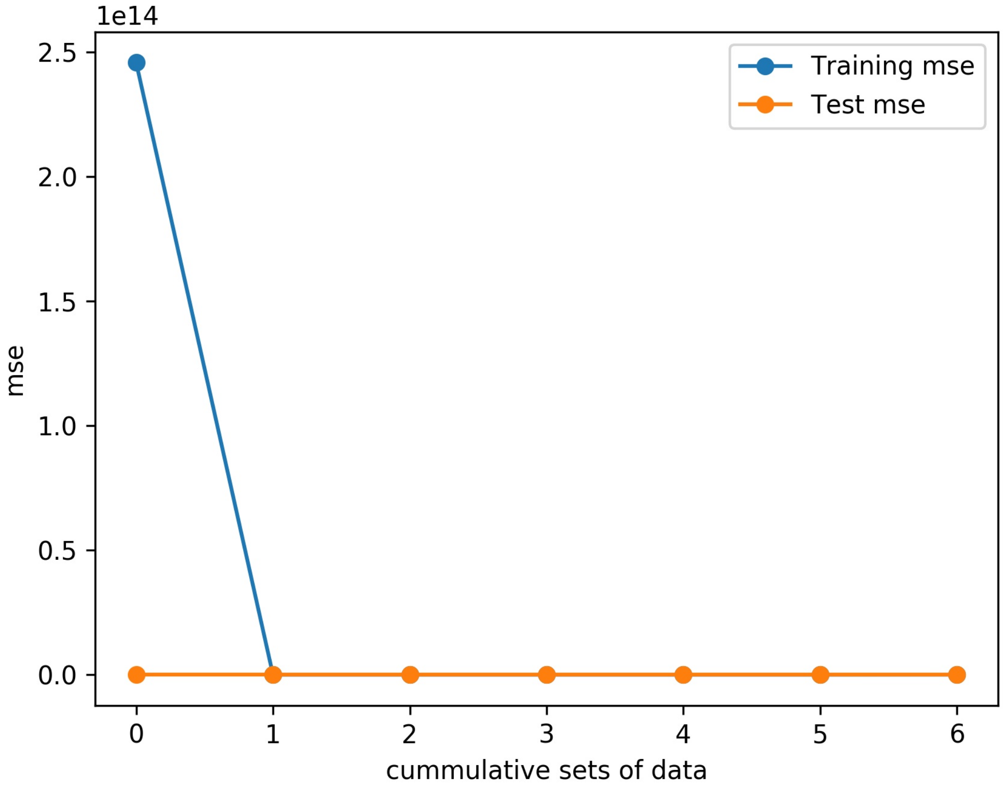
Regression with a Dense model without regularization

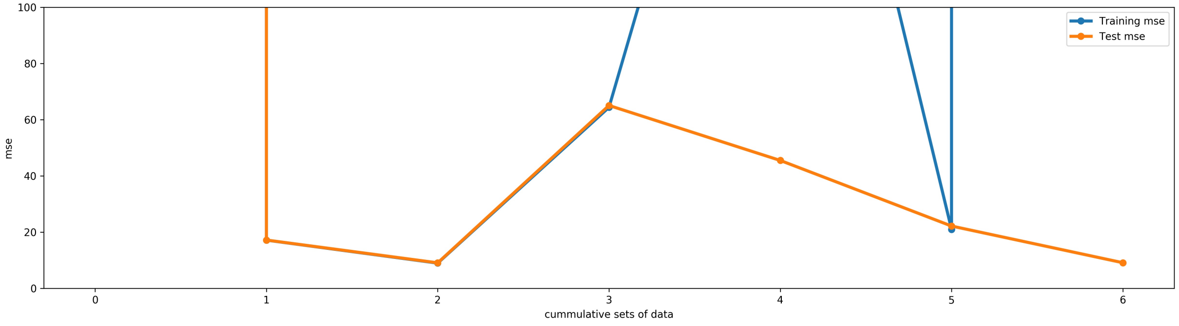
```
Layer (type)                 Output Shape              Param #
=================================================================
dense_1 (Dense)              (None, 500)               3500
_________________________________________________________________
dense_2 (Dense)              (None, 250)               125250
_________________________________________________________________
dense_3 (Dense)              (None, 60)                15060
_________________________________________________________________
dense_4 (Dense)              (None, 1)                 61
=================================================================
Total params: 143,871
Trainable params: 143,871
Non-trainable params: 0
```



Regression with Convolutional model

```
Layer (type)                 Output Shape              Param #
=================================================================
input_1 (InputLayer)         (None, 60, 1)             0
_________________________________________________________________
conv1d_1 (Conv1D)            (None, 60, 100)           1100
_________________________________________________________________
conv1d_2 (Conv1D)            (None, 60, 100)           100100
_________________________________________________________________
dropout_1 (Dropout)          (None, 60, 100)           0
_________________________________________________________________
max_pooling1d_1 (MaxPooling1 (None, 30, 100)           0
_________________________________________________________________
dropout_2 (Dropout)          (None, 30, 100)           0
_________________________________________________________________
flatten_1 (Flatten)          (None, 3000)              0
_________________________________________________________________
dense_1 (Dense)              (None, 500)               1500500
_________________________________________________________________
batch_normalization_1 (Batch (None, 500)               2000
_________________________________________________________________
dense_2 (Dense)              (None, 100)               50100
_________________________________________________________________
batch_normalization_2 (Batch (None, 100)               400
_________________________________________________________________
dense_3 (Dense)              (None, 50)                5050
_________________________________________________________________
batch_normalization_3 (Batch (None, 50)                200
_________________________________________________________________
dense_4 (Dense)              (None, 1)                 51
=================================================================
Total params: 1,659,501
Trainable params: 1,658,201
Non-trainable params: 1,300
```





Classification using convolutional or recurrent models

- Sigmoid activation in output layer
- Loss = binary crossentropy
- Best. accuracy = 0.68 ± 0.06 (benchmark ~50%)

Hyper-parameters tunning

- GridSearchCV(sklearn)
- batch_size = [64, 128, 256]
- epochs = [10]
- kernel_init = ['uniform', ‘normal’]
- pDropout = [0.3-0.5]
- Convolutions2D_shape1= [3,2]
- learning_rate = [0.01, 0.0001] #0.1, 0.01, 0.001]
- Optimizer = [‘RMSprop’, ‘Adam’]
- decay = [1e-4, 1e-6]

Questions

- Stacking ohe-AA and other features into a nD tensor?
- Keeping aa-Ids and other features separately?
- Working with 1D or nD tensors? This for convolutional models and RNN.
- Embedding layers?

Library design and construction

- ✔ **NNN** → 3 out of 64 (~5%) are stop codons... → short peptides rather than 30 residues long sequence
- ✔ **NNK** or **NNS** → > 3% stop codons
- ✔ **NNY** and RNN repeats (Y=primidines, R=purines) avoid Stop codon but do not encode for 2 amino-acids
- ✔ **SOLUTION**: Biasing the **ratios of nucleotides** at all three positions in the randomized codons.

- Codon Optimized Libraries

	A	C	G	T
0	0.26	0.26	0.24	0.21
1	0.38	0.19	0.17	0.22
2	0.00	0.46	0.34	0.16

	Ideal	Optimized
F	0,05	0,035
L	0,05	0,08
I	0,05	0,045
M	0,05	0,03
V	0,05	0,065
S	0,05	0,08
P	0,05	0,04
T	0,05	0,05
A	0,05	0,045
Y	0,05	0,04
H	0,05	0,04
Q	0,05	0,03
N	0,05	0,055
K	0,05	0,04
D	0,05	0,045
E	0,05	0,035
C	0,05	0,03
W	0,05	0,025
R	0,05	0,085
G	0,05	0,06
STOP	0	0,03

- Optimized for Equal Ratios
- Optimized for Disordered regions

Searching nucleotide composition space

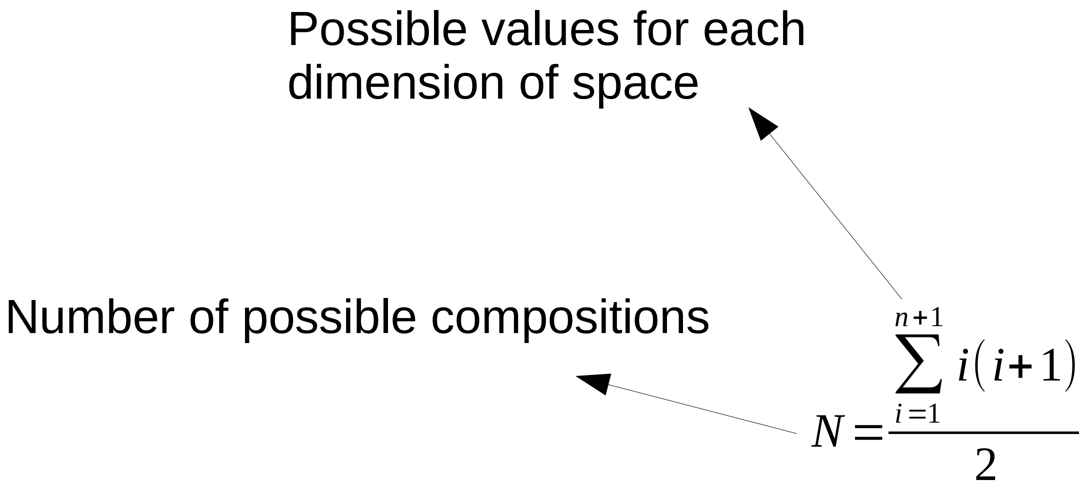
- Space of all possible sets of 3 nucleotide mixture $X_1X_2X_3$
- Each point in nucleotide space specifies a list of probabilities for the codons and therefore values for aminoacids and stop codons frequencies.
- Difference between ***target*** values and the ***encoded*** amino acid ratios correspond to a cost that we seek to minimize

SPACE

$$C=\sum_{i=1}^{21}\left(t_i-e_i\right)^2$$

Surface, where the deepest valley contains the nucleotide composition that most closely match the design target.

Complete enumeration of the space



Possible values for each dimension of space

Number of possible compositions

$$N=\frac{\sum_{i=1}^{n+1} i(i+1)}{2}$$

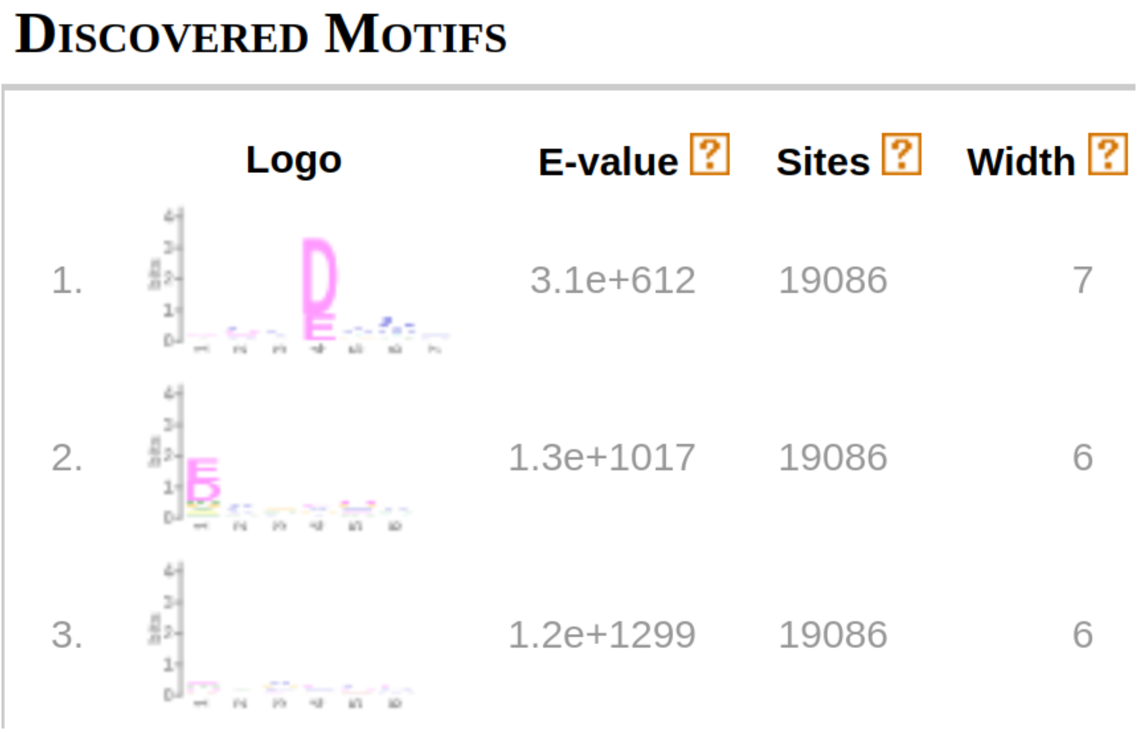
1% resolution = 100 possible values

~174000 compositions for one nucleotide
~10^{15} possible 3-based combinations

~30 years to test all possible combinations

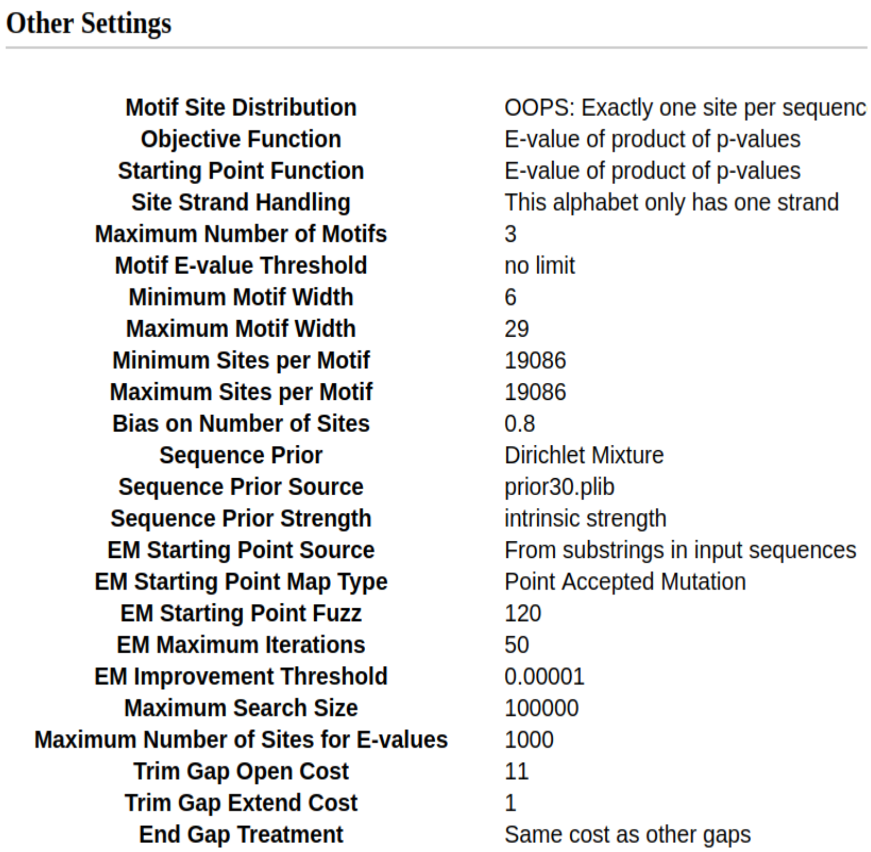
Scatter plot – design vs experimental

MEME



Discovered Motifs

	Logo	E-value ?	Sites ?	Width ?
1.		3.1e+612	19086	7
2.		1.3e+1017	19086	6
3.		1.2e+1299	19086	6

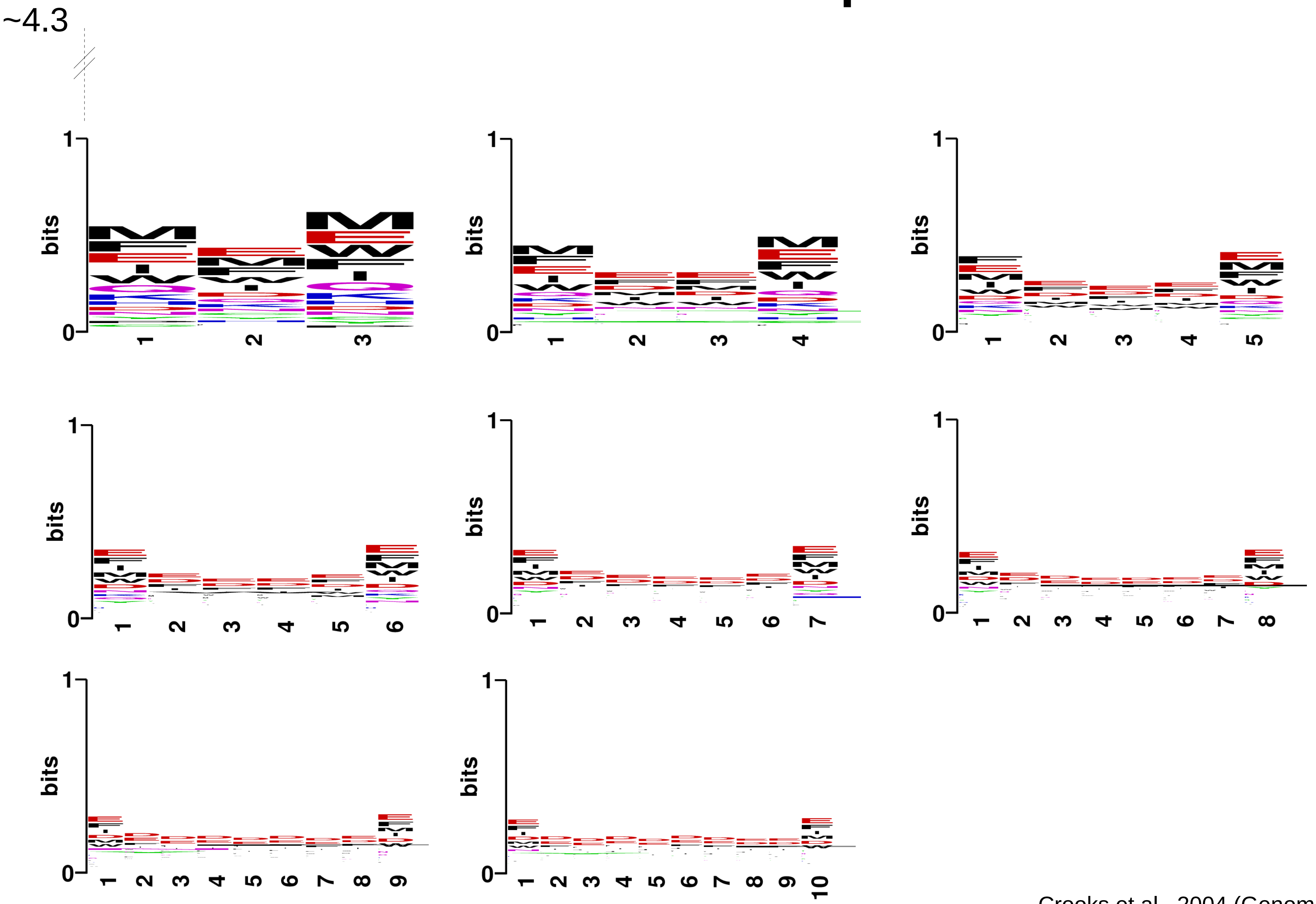


Other Settings

Motif Site Distribution	OOPS: Exactly one site per sequenc
Objective Function	E-value of product of p-values
Starting Point Function	E-value of product of p-values
Site Strand Handling	This alphabet only has one strand
Maximum Number of Motifs	3
Motif E-value Threshold	no limit
Minimum Motif Width	6
Maximum Motif Width	29
Minimum Sites per Motif	19086
Maximum Sites per Motif	19086
Bias on Number of Sites	0.8
Sequence Prior	Dirichlet Mixture
Sequence Prior Source	prior30.plib
Sequence Prior Strength	intrinsic strength
EM Starting Point Source	From substrings in input sequences
EM Starting Point Map Type	Point Accepted Mutation
EM Starting Point Fuzz	120
EM Maximum Iterations	50
EM Improvement Threshold	0.00001
Maximum Search Size	100000
Maximum Number of Sites for E-values	1000
Trim Gap Open Cost	11
Trim Gap Extend Cost	1
End Gap Treatment	Same cost as other gaps

Bailey & Elkan, 1994 (PSICISMB)

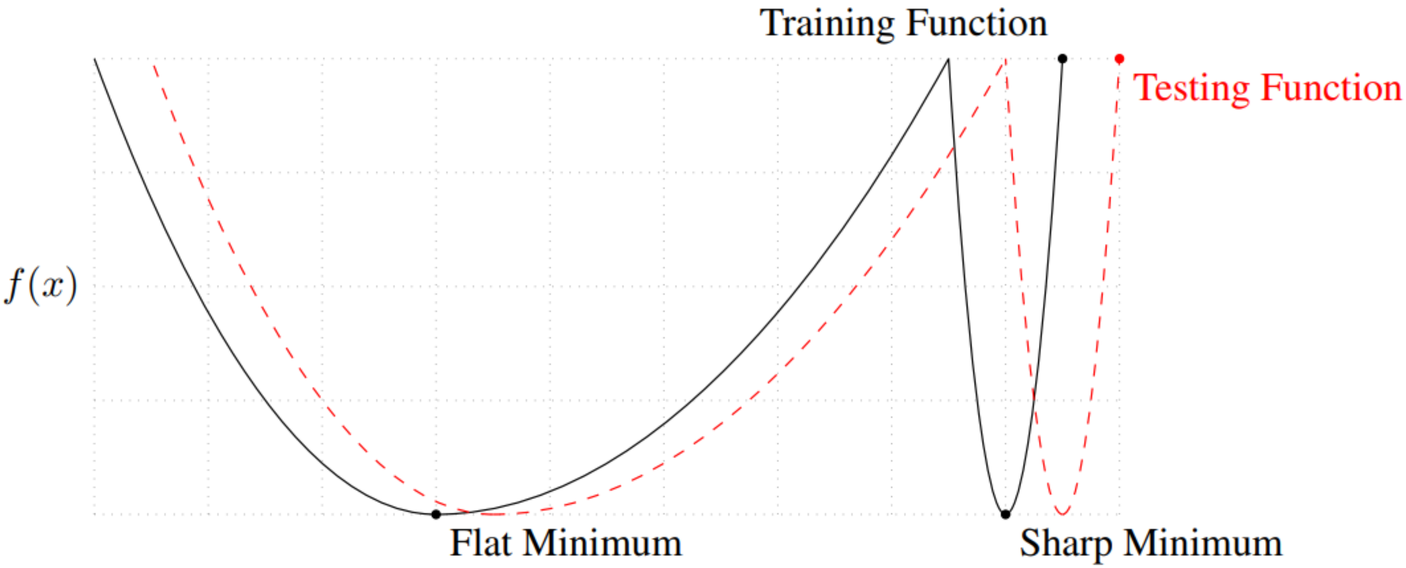
Gibbs sampler



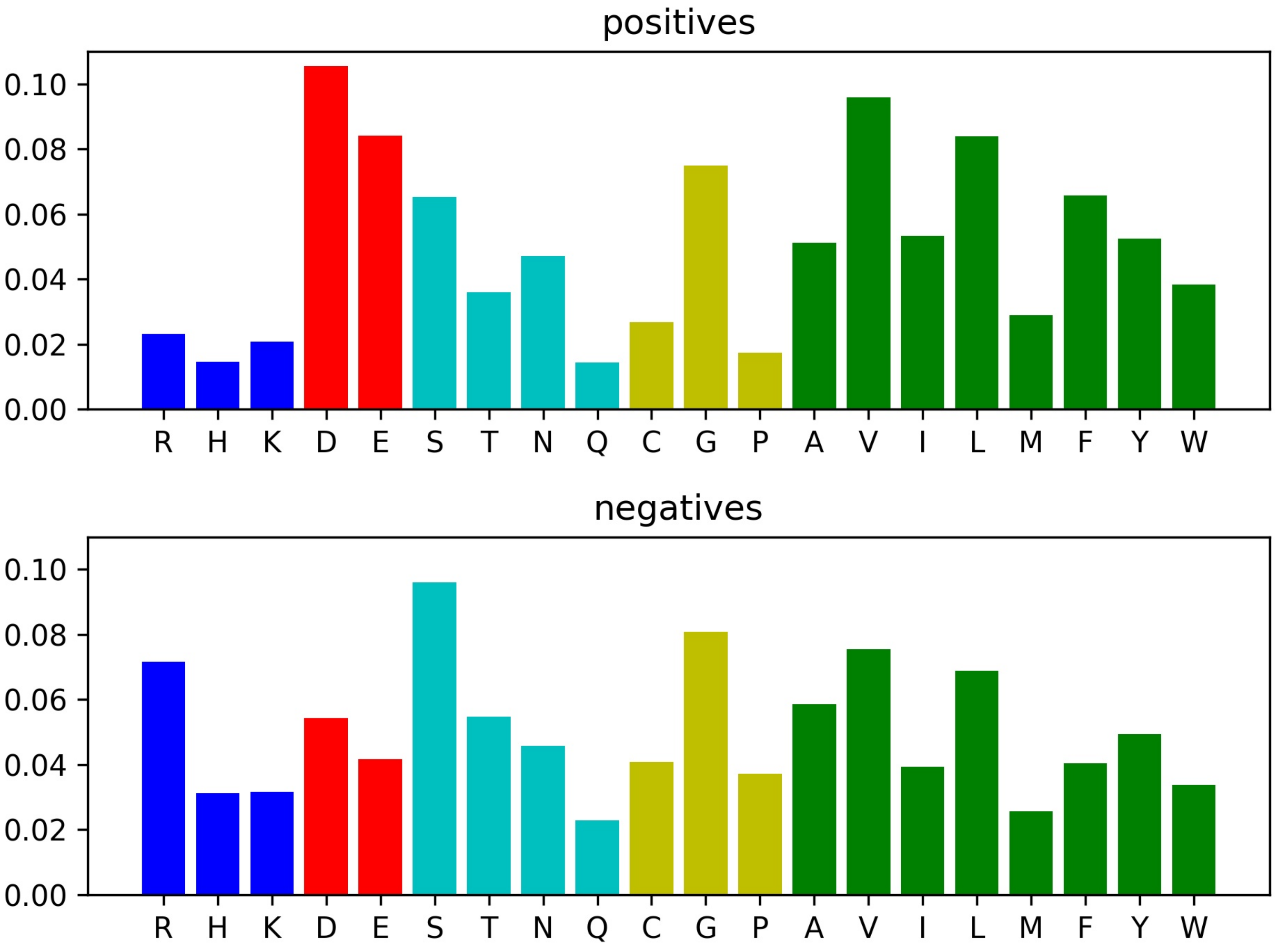
Crooks et al., 2004 (Genome research)

Batch size, not just a matter of learning speed?

0.41 ± 0.29	{'batch_size': **64**, 'decay': 1e-06, 'epochs': 10, 'init': 'uniform', 'k1': 3, 'lr': 0.001, 'pDrop': 0.4}
0.68 ± 0.07	{'batch_size': **128**, 'decay': 1e-06, 'epochs': 10, 'init': 'uniform', 'k1': 3, 'lr': 0.001, 'pDrop': 0.4}
0.55 ± 0.15	{'batch_size': **256**, 'decay': 1e-06, 'epochs': 10, 'init': 'uniform', 'k1': 3, 'lr': 0.001, 'pDrop': 0.4}

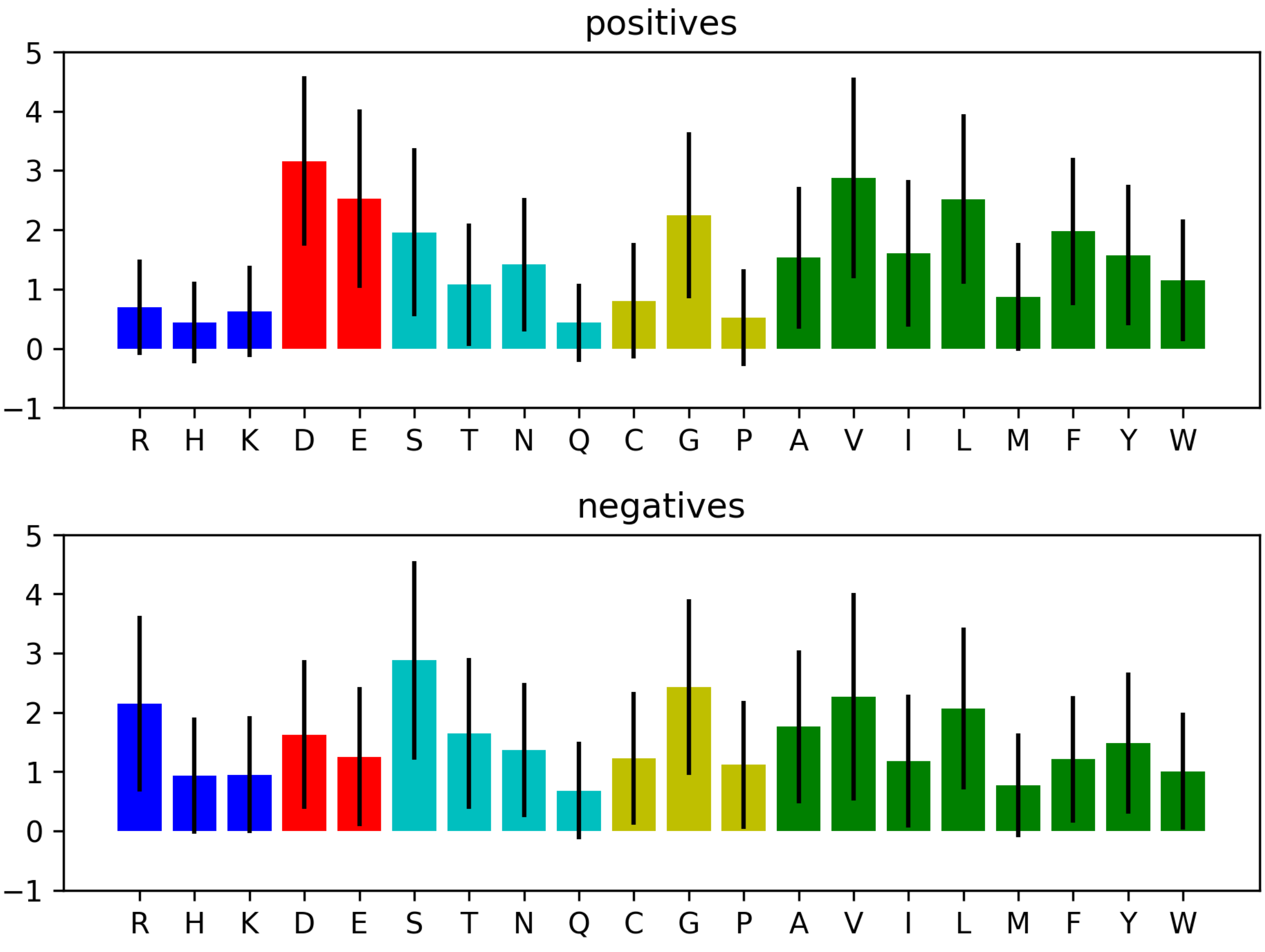


Amino-acid content of positivie and negative sets



Unique sequences

Average aa content per sequence



Unique sequences