SUPPLEMENTARY DATA

Top-Down Crawl: A method for the ultra-rapid and motif-free alignment of sequences with associated binding metrics

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Supplementary Table S1: Pseudocode of entire alignment process performed by Top-Down Crawl (TDC).

TDC Algorithm
Input: Table containing standard DNA sequences (A,C,G,T) of equal length in column 1 and binding metrics in column 2
df ← dataframe containing binding metrics indexed by sequence
// Average reverse complements or copy value from partner if absent from input
df_rc ← Copy *df* and reverse complement all sequences
df ← Append *df_rc* to *df*, group by index, and save mean for each index
// Initialization
df ← Insert boolean column, *isAligned*, filled with False, to keep track of sequences which have already been added to the alignment
df ← Insert boolean column, *wasRef*, filled with False, to keep track of sequences which have already been used as a reference for adding other sequences to the alignment
df ← Insert integer column, *shift*, filled with N/A, to keep track of the shift assigned to each sequence
top ← Index of seqeunce with largest binding metric from *df*
k ← *length(top)*
df[top][shift] ← 0
df[top][isAligned] ← True
Delete *reverseComplement(top)* from *df*
// Continue iterating until all aligned sequences have been used as a reference or until all seqeunces are aligned
while (*isAligned* == True **and** *wasRef* == False for any row in *df*) **and** (*isAligned* == False for any row in *df*)
unchecked ← Subset of *df* including rows where *isAligned* == True **and** *wasRef* == False
ref ← Index with largest binding metric from unchecked
refshift ← *df[ref][shift]*
SNPs ← Indices within *df* that are 1 mismatch away from *ref* **and** where *isAligned* == False
df[SNPs][shift] ← *refshift*
df[SNPs][isAligned] ← True
Delete *reverseComplement(SNPs)* from *df*

olap-1 ← Indices within *df* that overlap with the first $k-1$ bases of *ref* **and** where *isAligned* == False
df[olap-1][shift] ← *refshift* – 1
df[olap-1][isAligned] ← True
Delete *reverseComplement(olap-1)* from *df*

olap-2 ← Indices within *df* that overlap with the first $k-2$ bases of *ref* **and** where *isAligned* == False
df[olap-2][shift] ← *refshift* – 2
df[olap-2][isAligned] ← True
Delete *reverseComplement(olap-2)* from *df*

olap+1 ← Indices within *df* that overlap with the last $k-1$ bases of *ref* **and** where *isAligned* == False
df[olap+1][shift] ← *refshift* + 1
df[olap+1][isAligned] ← True
Delete *reverseComplement(olap+1)* from *df*

olap+2 ← Indices within *df* that overlap with the last $k-2$ bases of *ref* **and** where *isAligned* == False
df[olap+2][shift] ← *refshift* + 2
df[olap+2][isAligned] ← True
Delete *reverseComplement(olap+2)* from *df*

d*f[ref][wasRef]* ← True

df ← Subset of df where *isAligned* == True
df ← Pad indices of *df* with "-" based on *shift*
Output Save *df* as a table including the padded sequence, averaged binding metric, and *shift*

Supplementary Table S2: Peak memory usage for calculation of enrichment (Riley et al., 2014; Slattery et al., 2011) and TDC, BEESEM (Ruan et al., 2017), SelexGLM (Zhang et al., 2018), or MEME (Bailey & Elkan, 1994) based alignments, evaluated for 12 SELEX-seq datasets (Abe et al., 2015; Dantas Machado et al., 2020; Zhang et al., 2018). We also report the memory requirements for *k*-mer level enrichment calculation, since this a necessary step preceding TDC or MEME based alignment as described in the text. Data is plotted in Supplementary Figure S1.

	Enrichment	TDC + Enrichment	BEESEM	SelexGLM	MEME + Enrichment
AR	19 GB	20 GB	63 GB	116 GB	20 GB
GR	19 GB	19 GB	55 GB	81 GB	19 GB
MEF2B	23 GB	23 GB	31 GB	228 GB	23 GB
Exd-AbdA	18 GB	18 GB	15 GB	42 GB	18 GB
Exd-AbdB	18 GB	18 GB	20 GB	58 GB	18 GB
Exd-Antp	18 GB	18 GB	17 GB	46 GB	18 GB
Exd-Dfd	18 GB	18 GB	14 GB	35 GB	18 GB
Exd-Lab	18 GB	18 GB	16 GB	47 GB	18 GB
Exd-PbFl	12 GB	12 GB	28 GB	110 GB	12 GB
Exd-Scr	16 GB	16 GB	15 GB	52 GB	16 GB
Exd-Ubxla	17 GB	17 GB	18 GB	68 GB	17 GB
Exd-UbxIVa	18 GB	18 GB	16 GB	47 GB	18 GB

Supplementary Table S3: Table of alignment agreements, indicating what fraction of sequences were assigned to the same shift according to TDC and a given method. Data is plotted in Supplementary Figure S1.

	BEESEM	SelexGLM	MEME
AR	63%	44%	43%
GR	68%	74%	56%
MEF2B	86%	85%	57%
Exd-AbdA	98%	97%	95%
Exd-AbdB	71%	69%	27%
Exd-Antp	73%	67%	34%
Exd-Dfd	97%	89%	65%
Exd-Lab	96%	90%	51%
Exd-PbFl	85%	83%	72%
Exd-Scr	96%	92%	87%
Exd-Ubxla	99%	97%	93%
Exd-UbxIVa	100%	98%	99%

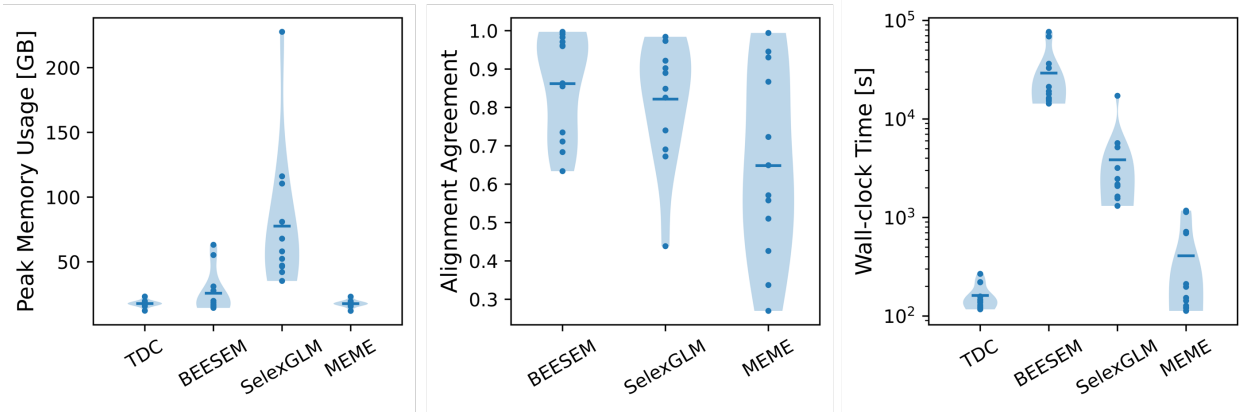
Supplementary Table S4: Multiple linear regression (MLR) models were trained using base sequence, minor groove width, and electro-static potential information along aligned 10-mers to predict the log enrichment of 10-mers with a Z-score larger than 2. Models were trained using 5-fold cross validation with elastic net regularization and the median performance across the tests is reported. Data is plotted in Figure 1.

	TDC	BEESEM	SelexGLM	MEME
AR	0.84	0.80	0.83	0.83
GR	0.76	0.75	0.74	0.78
MEF2B	0.56	0.60	0.63	0.40
Exd-AbdA	0.67	0.69	0.70	0.66
Exd-AbdB	0.37	0.33	0.33	0.22
Exd-Antp	0.42	0.36	0.34	0.20
Exd-Dfd	0.65	0.64	0.59	0.64
Exd-Lab	0.69	0.66	0.59	0.40
Exd-PbFl	0.75	0.76	0.75	0.73
Exd-Scr	0.82	0.76	0.78	0.65
Exd-Ubxla	0.81	0.77	0.73	0.59
Exd-UbxIVa	0.85	0.85	0.84	0.85

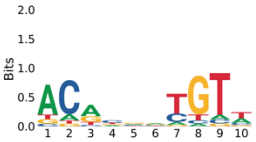
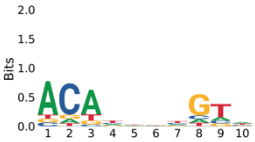
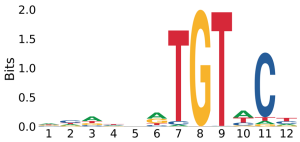
Supplementary Table S5: Wall-clock time required for each of the methods evaluated. We also report the time requirements for *k*-mer level enrichment calculation, since this a necessary step preceding TDC or MEME based alignment as described in the text. Data is plotted in Supplementary Figure S1.

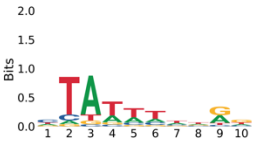
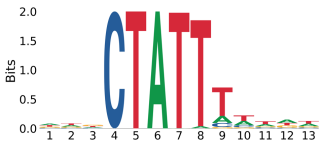
	Enrichment	TDC + Enrichment	BEESEM	SelexGLM	MEME + Enrichment
AR	0h 3m 9s	0h 3m 41s	21h 17m 59s	1h 26m 0s	0h 11m 32s
GR	0h 3m 5s	0h 3m 40s	19h 6m 31s	0h 41m 6s	0h 18m 55s
MEF2B	0h 4m 1s	0h 4m 28s	10h 6m 30s	4h 46m 34s	0h 11m 56s
Exd-AbdA	0h 1m 47s	0h 1m 57s	4h 24m 37s	0h 27m 10s	0h 1m 53s
Exd-AbdB	0h 2m 8s	0h 2m 38s	5h 53m 40s	0h 35m 27s	0h 3m 31s
Exd-Antp	0h 2m 2s	0h 2m 22s	4h 59m 24s	0h 34m 47s	0h 2m 23s
Exd-Dfd	0h 2m 2s	0h 2m 9s	3h 58m 42s	0h 21m 53s	0h 2m 7s
Exd-Lab	0h 2m 23s	0h 2m 37s	4h 28m 12s	0h 26m 6s	0h 2m 33s
Exd-PbFl	0h 1m 30s	0h 2m 17s	9h 8m 54s	1h 34m 51s	0h 19m 35s
Exd-Scr	0h 1m 46s	0h 2m 1s	4h 14m 29s	0h 36m 9s	0h 2m 0s
Exd-UbxIa	0h 2m 9s	0h 2m 27s	5h 18m 2s	0h 53m 4s	0h 3m 18s
Exd-UbxIVa	0h 1m 51s	0h 2m 4s	4h 31m 49s	0h 27m 14s	0h 2m 2s

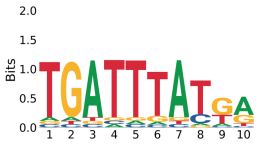
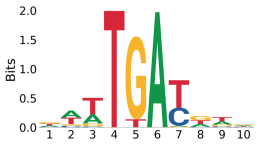
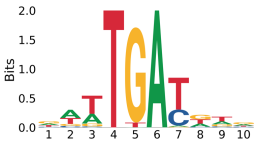
Supplementary Figure S1: Violin plots of data given in Supplementary Tables S2, S3, and S5. (Violin plots of data given in Supplementary Table S4 are shown in Figure 1.)

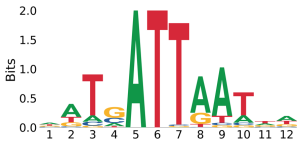
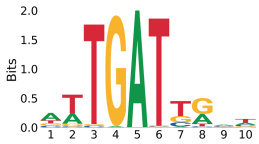


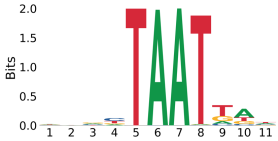
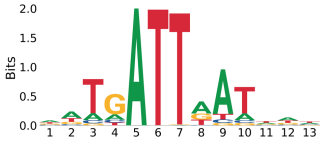
Supplementary Figure S2: Comparison of PWMs generated from each method. The TDC PWM is generated using all 10-mers aligned with a shift of ±5, weighting each sequence by its relative enrichment. The units of the SelexGLM method are provided in terms of $-\Delta\Delta G/RT$ as described in the original method. All others are shown in terms of bits.

	AR	GR
TDC		
BEESEM		
SelexGLM		
MEME		

	MEF2B	Exd-AbdA
TDC		
BEESEM		
SelexGLM		
MEME		

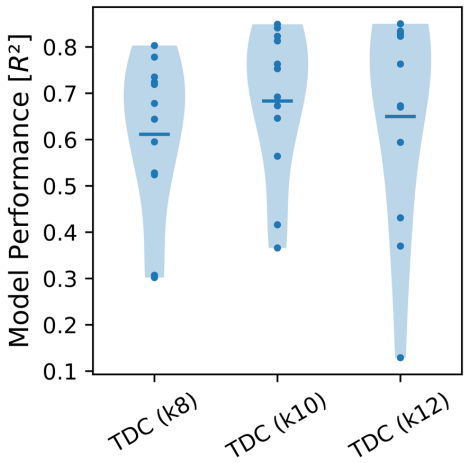
	Exd-AbdB	Exd-Antp
TDC		
BEESEM		
SelexGLM		
MEME		

	Exd-Dfd	Exd-Lab
TDC		
BEESEM		
SelexGLM		
MEME		

	Exd-PbFl	Exd-Scr
TDC		
BEESEM		
SelexGLM		
MEME		

	Exd-UbxIa	Exd-UbxIVa
TDC		
BEESEM		
SelexGLM		
MEME		

Supplementary Figure S3: Violin plots showing model performance across 12 SELEX-seq datasets, using various length *k*-mers as input to TDC. MLR models were trained using base sequence, minor groove width, and electrostatic potential information along aligned *k*-mers to predict the log enrichment of *k*-mers with a *Z*-score larger than 2. Models were trained using 5-fold cross validation with elastic net regularization and the median performance across the tests is reported.



Supplementary References

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Author Contributions

B.H.C. conceived the TDC method, independently executed the project, and wrote the manuscript. T.P.C. tested the method and provided advice on implementation. R.R. supervised the project. The authors thank Luigi Manna for help with server setup.