Supplementary Information for

Important biological information uncovered in previously unaligned reads from chromatin immunoprecipitation experiments (ChIP-Seq)

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This supplementary information document includes:

1. A description of Supplementary Files
2. Supplementary Figure S1 to S9
3. Supplementary Table S1 to S3
4. Supplementary Sections 3, 4, 5 and 6

Description of Supplementary Files

Supplementary File 1 as XLS Sheet 1 — Short Read Archive (SRA) accession numbers and dataset description for *A. thaliana*

Supplementary File 1 as XLS Sheet 2 — Short Read Archive (SRA) accession numbers and dataset description for *H. sapiens*

Supplementary File 1 as XLS Sheet 3 — Short Read Archive (SRA) accession numbers and dataset description for *D. melanogaster*

Supplementary File 1 as XLS Sheet 4 — Short Read Archive (SRA) accession numbers and dataset description for *C. elegans*

Supplementary File 2 as XLS file — Genomic locations of TAL1 recovered peaks (Sheet 1); Genomic locations and target genes of validated TAL1 peaks (Sheet 2)

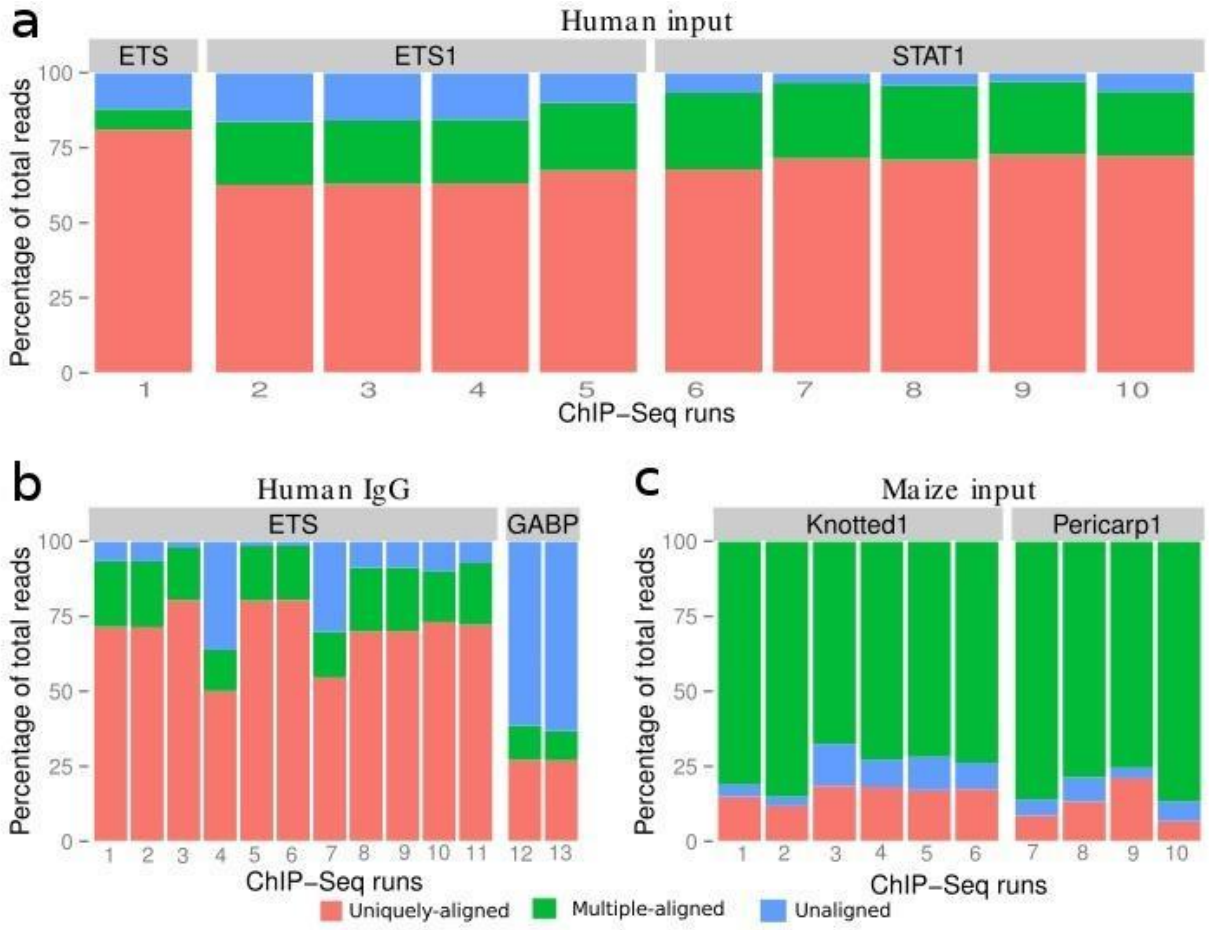
Supplementary Section 3 — Commands and description of parameters for running Bowtie, SHRiMP & MACS programs; versions of reference genomes used in analysis

Supplementary Section 4 — Published peaks repeat elements

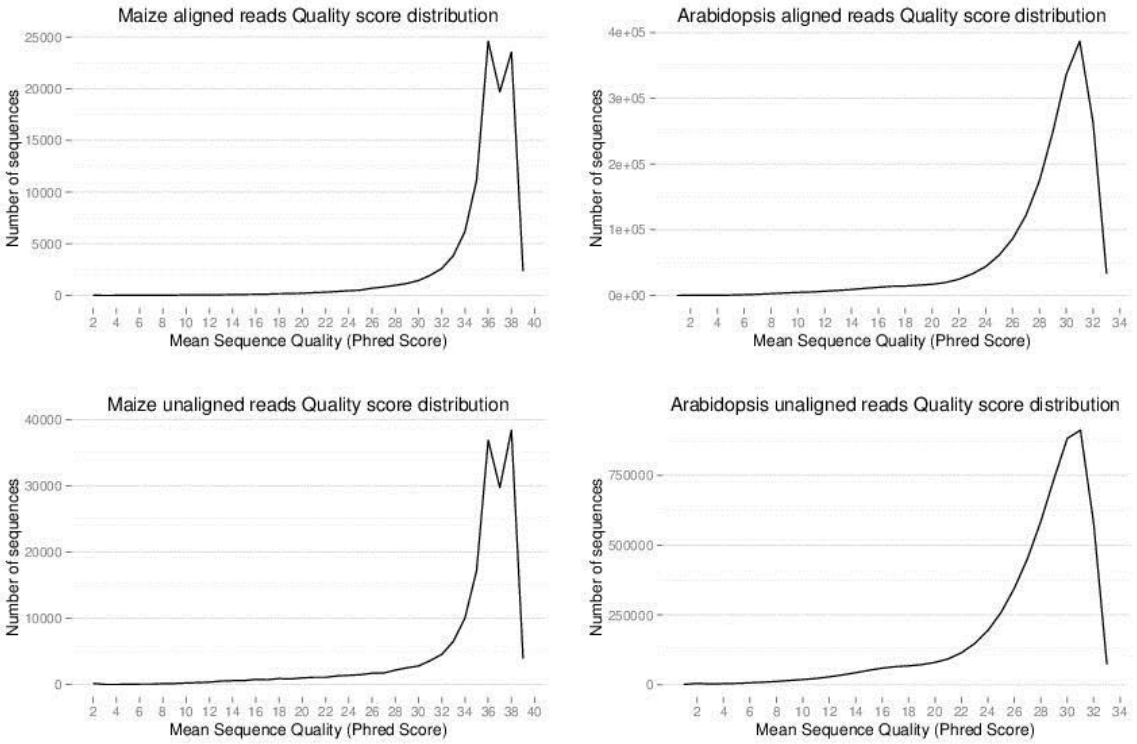
Supplementary Section 5 — Reanalyzed peaks repeat elements

Supplementary Section 6 — TAL1 recovered peaks repeat elements

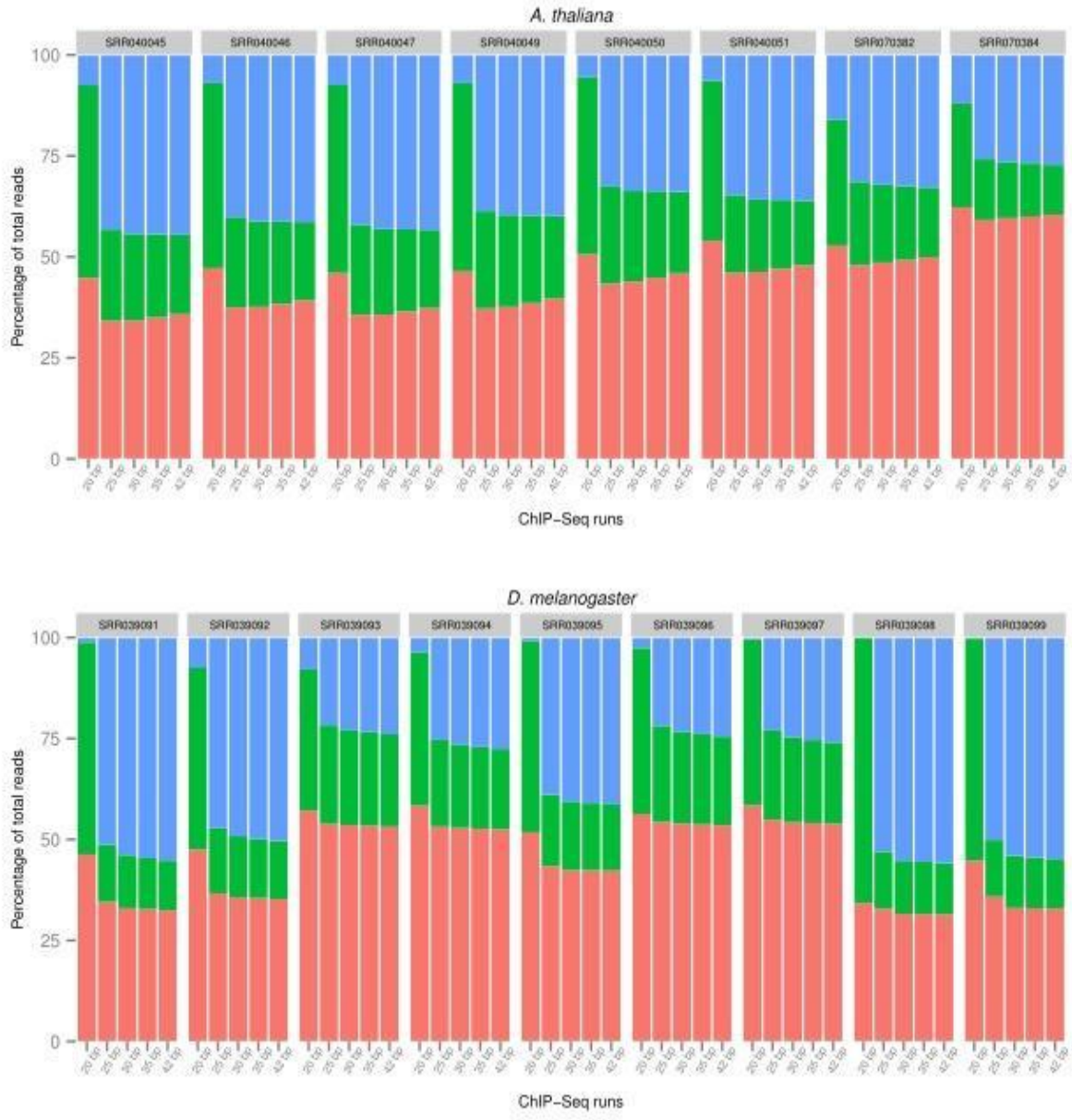
Supplementary Figures



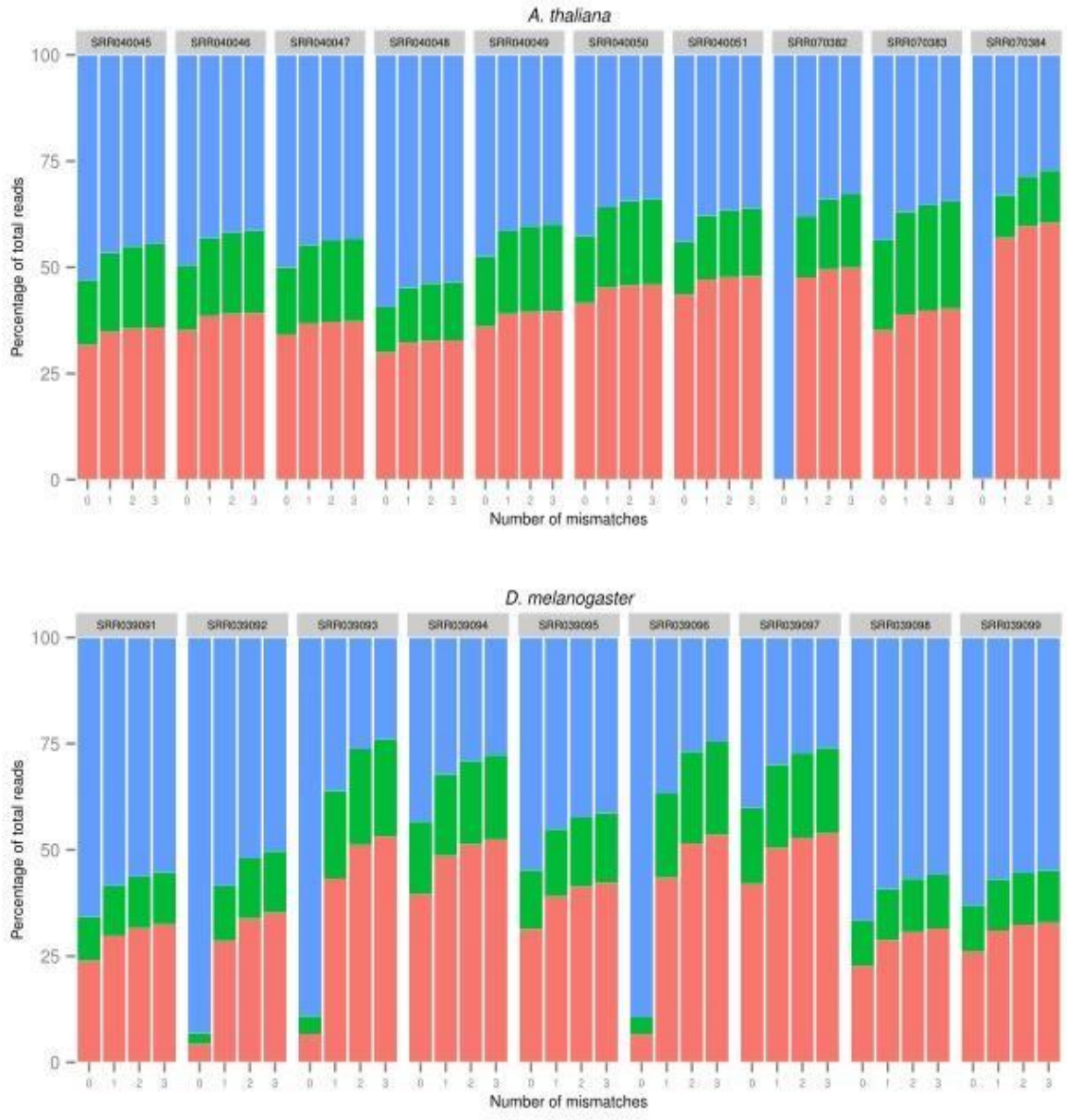
Supplementary Figure S1: Alignment proportions of control ChIP-Seq datasets. Each bar represents a ChIP-Seq run; several runs constitute an experiment for determining binding patterns of one transcription factor. Runs have been grouped into their respective TFs



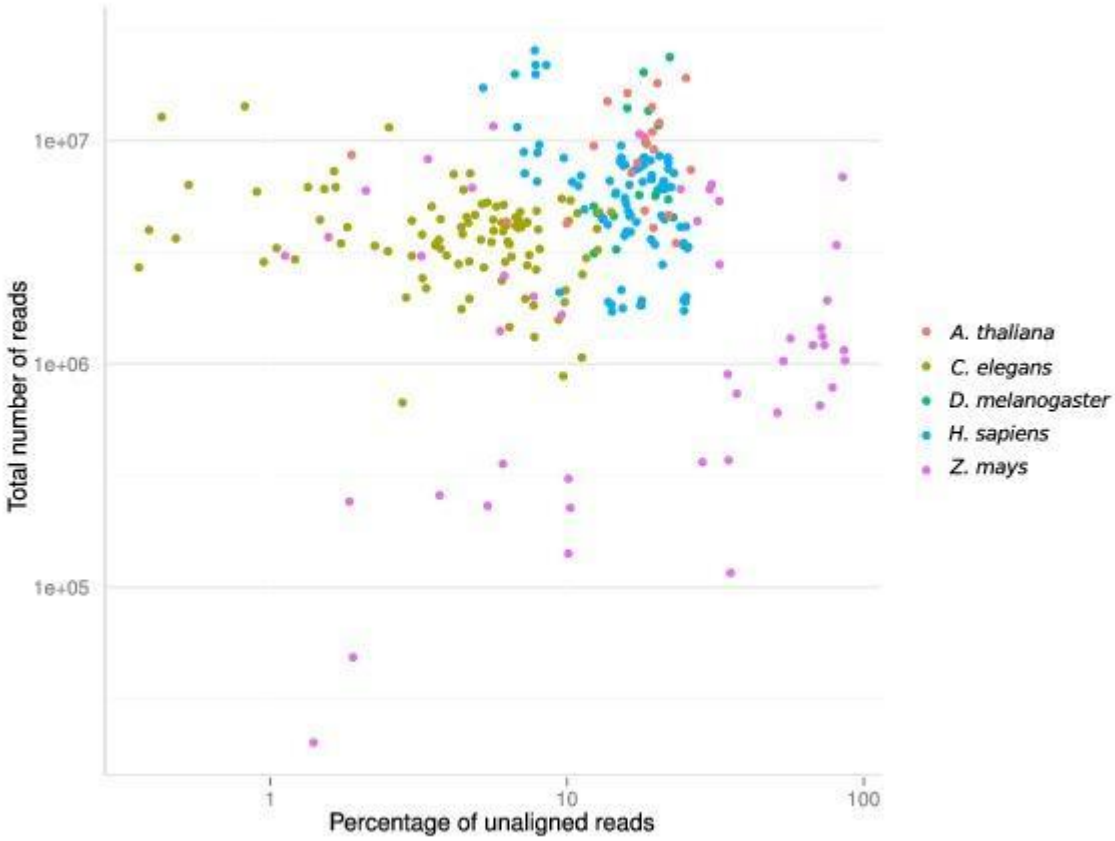
Supplementary Figure S2: Quality scores for maize and Arabidopsis ChIp-Seq reads. Both aligned and unaligned reads exhibit similar quality scores.



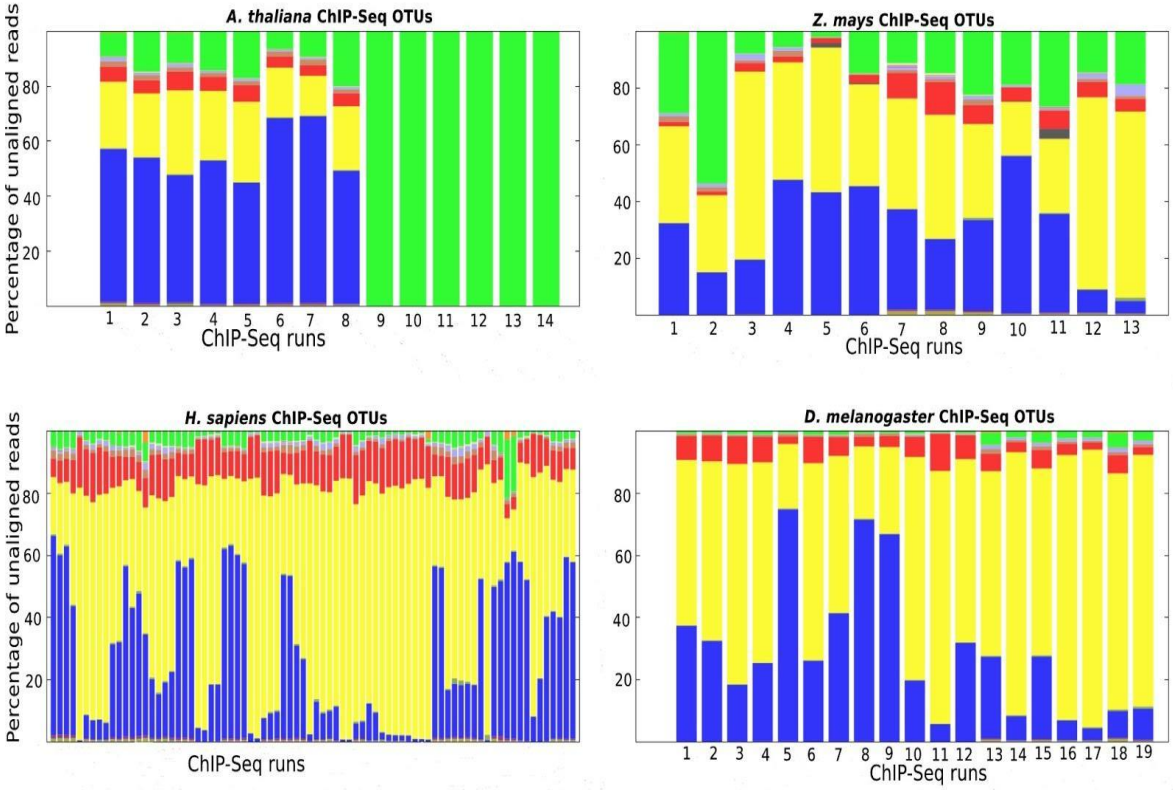
Supplementary Figure S3: Proportions of uniquely aligned, unaligned and multiple-aligned reads for *A. thaliana* and *D. melanogaster* trimmed datasets. Each facet is a ChIP-Seq run in which each bar represents a run whose reads have been trimmed to a particular read length shown on the x-axis.



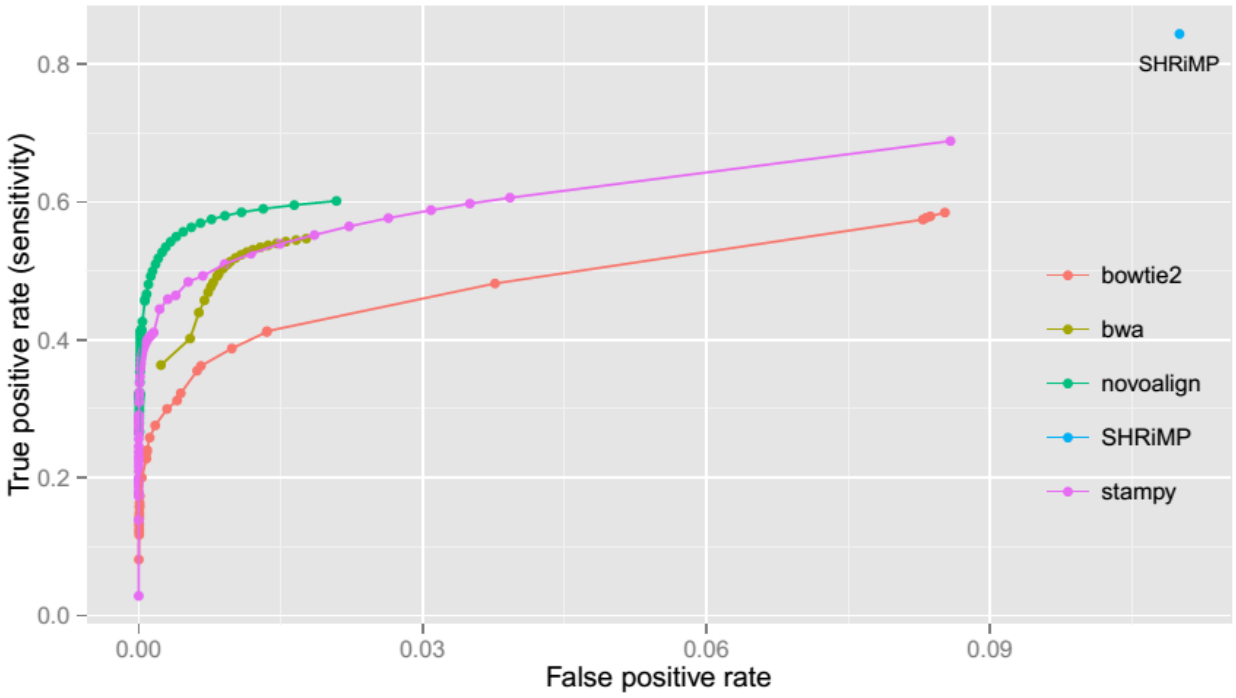
Supplementary Figure S4: Proportions of aligned, unaligned and multiple-aligned reads for different mismatches allowed in alignment process. Each facet is a ChIP-Seq run in which each bar represents a run whose reads have been aligned using the number of allowed mismatches showed on the x-axis.



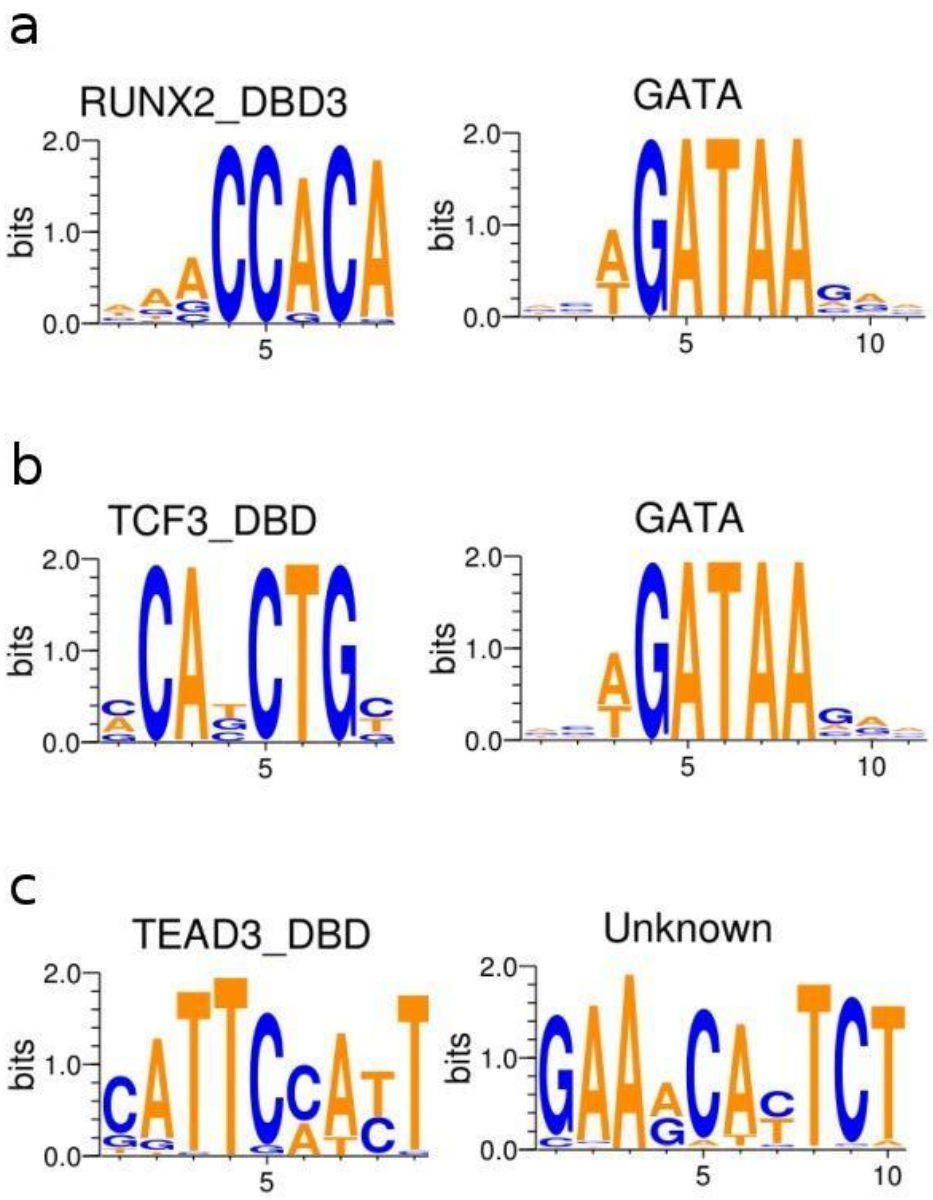
Supplementary Figure S5: Number of reads in a ChIP-Seq dataset as a function of proportion of unaligned reads from the same dataset. Lack of correlation suggests that sequencing more reads does not guarantee increased read mappability.



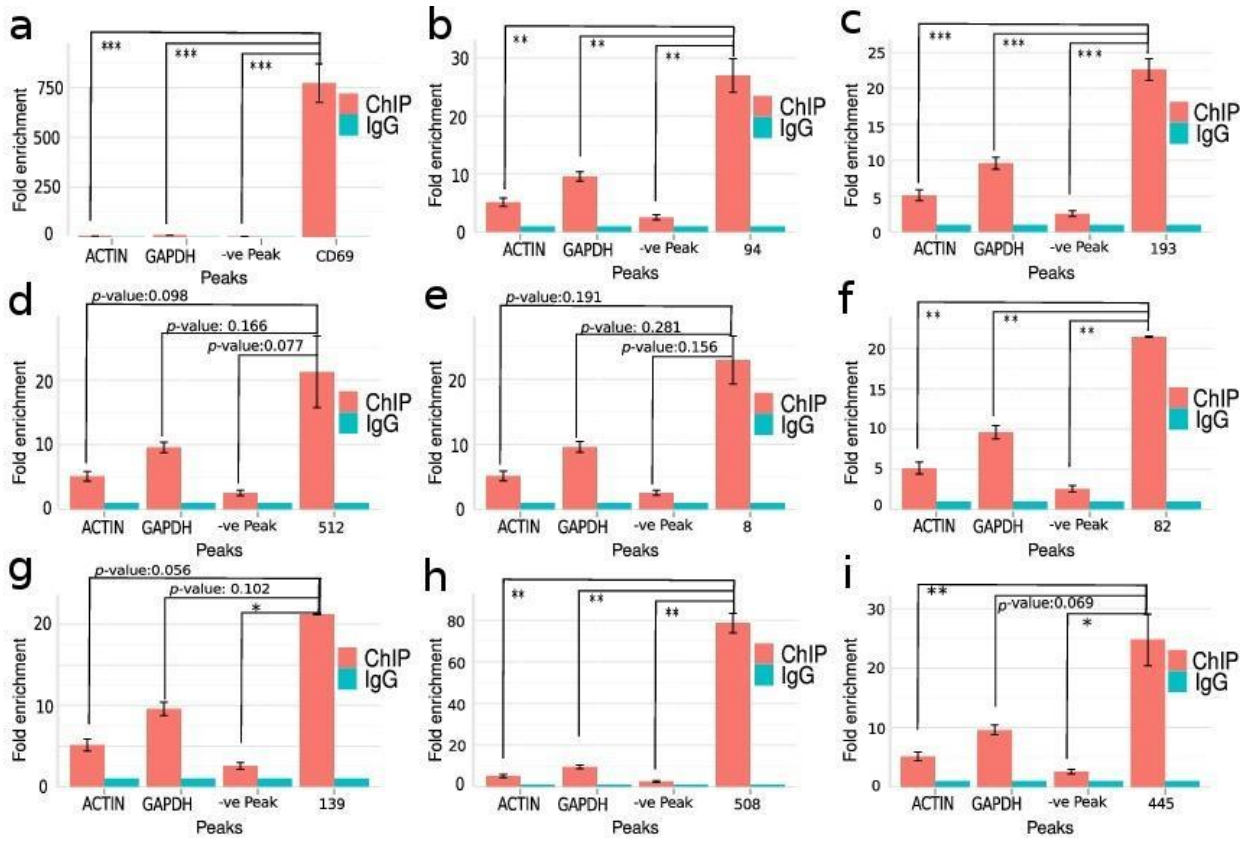
Supplementary Figure S6: Taxonomic classification of previously unaligned reads from *A. thaliana*, *Z. mays*, *H. sapiens*, and *D. melanogaster* datasets. Taxonomic classification reveals presence of bacterial (blue), metazoan (yellow) and plant derived (green) sequences. The Arabidopsis runs 9-14 correspond to Arabidopsis genome-simulated and aligned reads reads subjected taxonomic classification as a positive control (See Supplementary File 1 Sheets 5, 6 & 7 for the accession numbers corresponding to each number in the figure).



Supplementary Figure S7: A Receiver Operating Characteristic Curve illustrating the trade-off between true positive and false positive rates of selected next generation sequencing alignment programs



Supplementary Figure S8: Motifs enriched in published (a), reanalyzed (b) and recovered (c) peaks. Statistically over-represented motifs were identified using the *de novo* motif discovery tool MEME.



Supplementary Figure S9: ChIP-qPCR validation of selected recovered TAL1 peaks. Each bar represents fold enrichment of peaks relative to IgG. Error bars represent standard error. Statistical significance is as a result of t-test between the validated peak enrichment and the negative controls of ACTIN, GAPDH, and negative peak; n=3. Actual p-value is indicated when > 0.05, otherwise p-value < 0.05 indicated by *; < 0.01 by **; and < 0.001 by ***;- ve: negative.

Supplementary Tables

Supplementary Table S1: Average GC content for the five genomes analyzed.

Genome	GC content, %
H. sapiens	40.38
C. elegans	35.44
D. melanogaster	41.51
Z. mays	46.81
A. thaliana	36.05

Supplementary Table S2: GO term analysis of genes closest to TAL1 published, reanalyzed and recovered peaks

Published peaks GO terms:	**-log$_{10}$(*p*-value)**
T- cell differentiation	16.36
T-cell activation	16.09
T-cell receptor signaling pathway	13.41
Reanalyzed peaks GO terms:	
T-cell activation	18.74
T-cell differentiation	18.49
T-cell receptor signaling pathway	14.82
Recovered peaks GO terms:	
Pentose-phospahate shunt pathway	27.47
Sensory perception	17.12
Olfactory transduction	13.39
Apoptosis	2.38
T-cell activation	1.42

Supplementary Table S3: Repeat elements identified published (A), reanalyzed (B) and recovered peaks (C)

A: Published peaks

	Number of elements	Length occupied, bp	Percentage of sequence, %
Satellites	0	0	0.00
Simple repeats	93	3888	0.50
Low complexity	22	941	0.12

B: Reanalyzed peaks

	Number of elements	Length occupied, bp	Percentage of sequence, %
Satellites	2	1144	0.17
Simple repeats	65	4476	0.67
Low complexity	9	401	0.06

C: Recovered peaks

	Number of elements	Length occupied, bp	Percentage of sequence, %
Satellites	121	61773	18.56
Simple repeats	188	31934	9.59
Low complexity	55	7685	2.31

Section 3

Bowtie alignment command and parameters:

/nfs/15/osu4902/bowtie -p 10 -q -v 3 -m 1 -S --best --strata --chunkmbs 128 -t /nfs/15/osu4902/bowtie_db/a_thaliana SRR016811.fastq ../sam/SRR016811.sam --un ../unaligned/SRR016811_unaligned.fq --al ../aligned/SRR016811_aligned.fq --max ../aligned/SRR016811_multiple_align.fq

Parameters:
-p <integer>: number of processor core threads used in alignment
-q: input files are in FASTQ format
-v <integer>: report end-to-end hits with less than or equal v mismatches
-m <integer>: maximum number of multiple alignments allowed
-S: write hits in SAM format
--best --strata: only hits in best stratum are reported
--chunkmbs <integer>: maximum megabytes for RAM for best first search frames
-t: print wall-clock time taken by search phases
--un <filename>: write unaligned reads to <filename>
--al <filename>: write aligned reads to <filename>
--max <filename>: write multiple-aligned reads to to <filename>

SHRiMP alignment command and parameters:

/nfs/15/osu4902/applications/SHRiMP_2_2_0/bin/gmapper-ls --qv-offset 33 -L /nfs/proj14/PAS0107/reads_recovery/hg18-20gb-12_12_12_12seeds-"$i"of3-ls --strata --max-alignments 1 -N 12 --un SRR054882_homo_sapiens_${i}of3_unaligned.fq -E -Q SRR054882_homo_sapiens_sequence_reads.fq >SRR054882_homo_sapiens_${i}of3.sam

Parameters:
--qv-offset <integer>: interpret quality values in fastq input as PHRED+<integer>
-L: reference genome idex
--strata: print only best scoring hits
--max-alignments <integer>: maximum alignment per read
-N <integer>: number of processor core threads
--un <filename>: write unaligned reads to <filename>
-E: output SAM format
-Q: input reads are in FASTQ format

MACS peak calling command and parameters:

macs14 -t ChIP.bam -c Control.bam -f BAM -g h -n test -w -p 1e-5 -B

Parameters:
-t: ChIP-seq treatment files
-c: control files
-f: format of alignment file
-g: effective genome size
-n: experiment name
-w: Whether or not to save extended fragment pileup into a wiggle file
-p: Pvalue cutoff for peak detection
-B: Whether or not to save extended fragment pileup at every bp into a bedGraph file

Genome versions:
A. thaliana: TAIR9 genome release
Z. mays: B73 version 2 (ZmB73_RefGen_v2)
H. sapiens: version 19 (GRCh37/hg19)
C. elegans: version WS200
D. melanogaster: release 5.22

Section 4

```
==================================================
file name: GSM614003_jurkat.tal1_tab_hg19.bed.fasta
sequences:          2238
total length:     771448 bp  (771448 bp excl N/X-runs)
GC level:         48.05 %
bases masked:       4829 bp ( 0.63 %)
==================================================
               number of      length   percentage
               elements*    occupied  of sequence
--------------------------------------------------
SINEs:                0            0 bp    0.00 %
      ALUs            0            0 bp    0.00 %
      MIRs            0            0 bp    0.00 %

LINEs:                0            0 bp    0.00 %
      LINE1           0            0 bp    0.00 %
      LINE2           0            0 bp    0.00 %
      L3/CR1          0            0 bp    0.00 %

LTR elements:         0            0 bp    0.00 %
      ERVL            0            0 bp    0.00 %
      ERVL-MaLRs      0            0 bp    0.00 %
      ERV_classI      0            0 bp    0.00 %
      ERV_classII     0            0 bp    0.00 %

DNA elements:         0            0 bp    0.00 %
     hAT-Charlie      0            0 bp    0.00 %
     TcMar-Tigger     0            0 bp    0.00 %

Unclassified:         0            0 bp    0.00 %

Total interspersed repeats:        0 bp    0.00 %


Small RNA:            0            0 bp    0.00 %

Satellites:           0            0 bp    0.00 %
Simple repeats:      93         3888 bp    0.50 %
Low complexity:      22          941 bp    0.12 %
==================================================

* most repeats fragmented by insertions or deletions
  have been counted as one element


The query species was assumed to be homo
RepeatMasker version open-3.3.0 , default mode

run with rmblastn version : 2.2.27+
The query was compared to classified sequences in ".../RepeatMasker.lib"
RepBase Update 20110419-min, RM database version 20110419-min
```

Section 5

```
==================================================
file name: SRR070589-original_peaks.bed.fasta
sequences:          3344
total length:     666435 bp  (666435 bp excl N/X-runs)
GC level:         48.99 %
bases masked:       5264 bp ( 0.79 %)
==================================================
               number of      length   percentage
               elements*    occupied  of sequence
--------------------------------------------------
SINEs:                0            0 bp    0.00 %
      ALUs            0            0 bp    0.00 %
      MIRs            0            0 bp    0.00 %

LINEs:                0            0 bp    0.00 %
      LINE1           0            0 bp    0.00 %
      LINE2           0            0 bp    0.00 %
      L3/CR1          0            0 bp    0.00 %

LTR elements:         0            0 bp    0.00 %
      ERVL            0            0 bp    0.00 %
      ERVL-MaLRs      0            0 bp    0.00 %
      ERV_classI      0            0 bp    0.00 %
      ERV_classII     0            0 bp    0.00 %

DNA elements:         0            0 bp    0.00 %
     hAT-Charlie      0            0 bp    0.00 %
     TcMar-Tigger     0            0 bp    0.00 %

Unclassified:         0            0 bp    0.00 %

Total interspersed repeats:        0 bp    0.00 %


Small RNA:            0            0 bp    0.00 %

Satellites:           2         1144 bp    0.17 %
Simple repeats:      65         4476 bp    0.67 %
Low complexity:       9          401 bp    0.06 %
==================================================

* most repeats fragmented by insertions or deletions
  have been counted as one element


The query species was assumed to be homo
RepeatMasker version open-3.3.0 , default mode

run with rmblastn version : 2.2.27+
The query was compared to classified sequences in ".../RepeatMasker.lib"
RepBase Update 20110419-min, RM database version 20110419-min
```

Section 6

```
==================================================
file name: SRR070589_recovered_peaks.xls.bed.fasta
sequences:           594
total length:     332838 bp  (332697 bp excl N/X-runs)
GC level:         43.97 %
bases masked:      89271 bp ( 26.82 %)
==================================================
               number of      length   percentage
               elements*    occupied  of sequence
--------------------------------------------------
SINEs:                0            0 bp    0.00 %
      ALUs            0            0 bp    0.00 %
      MIRs            0            0 bp    0.00 %

LINEs:                0            0 bp    0.00 %
      LINE1           0            0 bp    0.00 %
      LINE2           0            0 bp    0.00 %
      L3/CR1          0            0 bp    0.00 %

LTR elements:         0            0 bp    0.00 %
      ERVL            0            0 bp    0.00 %
      ERVL-MaLRs      0            0 bp    0.00 %
      ERV_classI      0            0 bp    0.00 %
      ERV_classII     0            0 bp    0.00 %

DNA elements:         0            0 bp    0.00 %
     hAT-Charlie      0            0 bp    0.00 %
     TcMar-Tigger     0            0 bp    0.00 %

Unclassified:         0            0 bp    0.00 %

Total interspersed repeats:        0 bp    0.00 %


Small RNA:            0            0 bp    0.00 %

Satellites:         121        61773 bp   18.56 %
Simple repeats:     188        31934 bp    9.59 %
Low complexity:      55         7685 bp    2.31 %
==================================================

* most repeats fragmented by insertions or deletions
  have been counted as one element


The query species was assumed to be homo
RepeatMasker version open-3.3.0 , default mode

run with rmblastn version : 2.2.27+
The query was compared to classified sequences in ".../RepeatMasker.lib"
RepBase Update 20110419-min, RM database version 20110419-min
```