# Clipper: p-value-free FDR control on high-throughput data from two conditions

Xinzhou Ge[1,†], Yiling Elaine Chen[1,†], Dongyuan Song[2], MeiLu McDermott[3,4], Kyla Woyshner[3], Antigoni Manousopoulou[3], Wei Li[5], Leo D. Wang[3], and Jingyi Jessica Li[1,2,6,7,8,*]

[1]Department of Statistics, University of California, Los Angeles, CA 90095
[2]Interdepartmental Program in Bioinformatics, University of California, Los Angeles, CA 90095
[3]Beckman Research Institute, City of Hope National Medical Center, Duarte, CA 91010
[4]The Quantitative and Computational Biology section, University of Southern California, Los Angeles, CA 90089
[5]Division of Computational Biomedicine, Department of Biological Chemistry, School of Medicine, University of California, Irvine, CA 92697
[6]Department of Human Genetics, University of California, Los Angeles, CA 90095
[7]Department of Computational Medicine, University of California, Los Angeles, CA 90095
[8]Department of Biostatistics, University of California, Los Angeles, CA 90095
[†]These authors contributed equally to this work.
[*]To whom correspondence should be addressed. Email: jli@stat.ucla.edu

**Abstract**

High-throughput biological data analysis commonly involves identifying "interesting" features (e.g., genes, genomic regions, and proteins), whose values differ between two conditions, from numerous features measured simultaneously. The most widely-used criterion to ensure the analysis reliability is the false discovery rate (FDR), the expected proportion of uninteresting features among the identified ones. Existing bioinformatics tools primarily control the FDR based on p-values. However, obtaining valid p-values relies on either reasonable assumptions of data distribution or large numbers of replicates under both conditions, two requirements that are often unmet in biological studies. To address this issue, we propose Clipper, a general statistical framework for FDR control without relying on p-values or specific data distributions. Clipper is applicable to identifying both enriched and differential features from high-throughput biological data of diverse types. In comprehensive simulation and real-data benchmarking, Clipper outperforms existing generic FDR control methods and specific bioinformatics tools designed for various tasks, including peak calling from ChIP-seq data, differentially expressed gene identification from RNA-seq data, differentially interacting chromatin region identification from Hi-C data, and peptide identification from mass spectrometry data. Notably, our benchmarking results for peptide identification are based on the first mass spectrometry data standard with a realistic dynamic range. Our results demonstrate Clipper's flexibility and reliability for FDR control, as well as its broad applications in high-throughput data analysis.

## Introduction

High-throughput technologies are widely used to measure system-wide biological features, such as genes, genomic regions, and proteins ("high-throughput" means the number of features is large, at least in thousands). The most common goal of analyzing high-throughput data is to contrast two conditions so as to reliably screen "interesting features," where "interesting" means "enriched" or "differential." "Enriched features" are defined to have higher expected measurements (without measurement errors)

under the experimental (i.e., treatment) condition than the background (i.e., the negative control) condition. The detection of enriched features is called "enrichment analysis." For example, typical enrichment analyses include calling protein-binding sites in a genome from chromatin immunoprecipitation sequencing (ChIP-seq) data [1, 2] and identifying peptides from mass spectrometry (MS) data [3]. In contrast, "differential features" are defined to have different expected measurements between two conditions, and their detection is called "differential analysis." For example, popular differential analyses include the identification of differentially expressed genes (DEGs) from genome-wide gene expression data (e.g., microarray and RNA sequencing (RNA-seq) data [4–10]) and differentially interacting chromatin regions (DIRs) from Hi-C data [11–13] (Fig. 1a). In most scientific research, the interesting features only constitute a small proportion of all features, and the remaining majority is referred to as "uninteresting features."

The identified features, also called the "discoveries" from enrichment or differential analysis, are subject to further investigation and validation. Hence, to reduce experimental validation that is often laborious or expensive, researchers demand reliable discoveries that contain few false discoveries. Accordingly, the false discovery rate (FDR) [14] has been developed as a statistical criterion for ensuring discoveries' reliability. The FDR technically is defined as the expected proportion of uninteresting features among the discoveries under the frequentist statistical paradigm. In parallel, under the Bayesian paradigm, other criteria have been developed, including the Bayesian false discovery rate [15], the local false discovery rate (local fdr) [16], and the local false sign rate [17]. Among all these frequentist and Bayesian criteria, the FDR is the dominant criterion for setting thresholds in biological data analysis [1, 10, 18–24] and is thus the focus of this paper.

FDR control refers to the goal of finding discoveries such that the FDR is under a pre-specified threshold (e.g., $0.05$). Existing computational methods for FDR control primarily rely on p-values, one per feature. Among the p-value-based methods, the most classic and popular ones are the Benjamini-Hochberg (BH) procedure [14] and the Storey's q-value [25]; later development introduced methods that incorporate feature weights [26] or covariates (e.g., independent hypothesis weighting (IHW) [27], adaptive p-value thresholding [28], and Boca and Leek's FDR regression [29]) to boost the detection power. All these methods set a p-value cutoff based on the pre-specified FDR threshold. However, the calculation of p-values requires either distributional assumptions, which are often questionable, or large numbers of replicates, which are often unachievable in biological studies (see Results). Due to these limitations of p-value-based methods in high-throughput biological data analysis, bioinformatics tools often output ill-posed p-values. This issue is evidenced by serious concerns about the widespread miscalculation and misuse of p-values in the scientific community [30]. As a result, bioinformatics tools using questionable p-values either cannot reliably control the FDR to a target level [23] or lack power to make discoveries [31]; see Results. Therefore, p-value-free control of FDR is desirable, as it would make data analysis more transparent and thus improve the reproducibility of scientific research.

Although p-value-free FDR control has been implemented in the MACS2 method for ChIP-seq peak calling [1] and the SAM method for microarray DEG identification [32], these two methods are restricted to specific applications and lack theoretical guarantee for FDR control[1]. More recently, the Barber-Candès (BC) procedure has been proposed to achieve theoretical FDR control without using p-values [35], and it has been shown to perform comparably to the BH procedure with well-calibrated p-values [36]. The BC procedure is advantageous because it does not require well-calibrated p-values, so it holds tremendous potential in various high-throughput data analyses where p-value calibration is challenging [37]. For example, a recent paper has implemented the BC procedure to control the FDR in peptide identification from MS data [38].

[1] Although later works have studied some theoretical properties of SAM, they are not about the exact control of the FDR [33, 34].

Inspired by the BC procedure, we propose a general statistical framework Clipper to provide reliable FDR control for high-throughput biological data analysis, without using p-values or relying on specific data distributions. Clipper is a robust and flexible framework that applies to both enrichment and differential analyses and that works for high-throughput data with various characteristics, including data distributions, replicate numbers (from one to multiple), and outlier existence.

# Results

Clipper consists of two main steps: construction and thresholding of contrast scores. First, Clipper defines a contrast score for each feature, as a replacement of a p-value, to summarize that feature's measurements between two conditions and to describe the degree of interestingness of that feature. Second, as its name suggests, Clipper establishes a cutoff on features' contrast scores and calls as discoveries the features whose contrast scores exceed the cutoff (see Online Methods and Supplementary). Clipper is a flexible framework that only requires a minimal input: all features' measurements under two conditions and a target FDR threshold (e.g., $5\%$) (Fig. 1b).

Clipper only relies on two fundamental statistical assumptions of biological data analysis: (1) measurement errors (i.e., differences between measurements and their expectations, with the expectations including both biological signals and batch effects) are independent across all features and replicates; (2) every uninteresting feature has measurement errors identically distributed across all replicates under both conditions. These two assumptions are used in almost all bioinformatics tools and are commonly referred to as the "measurement model" in statistical genomics [39]. With these two assumptions, Clipper has a theoretical guarantee for FDR control under both enrichment and differential analyses with any number of replicates (see Online Methods and Supp. Section S2).

To verify Clipper's performance, we designed comprehensive simulation studies to benchmark Clipper against existing generic FDR control methods (Supp. Section S1). We also benchmarked Clipper against bioinformatics tools in studies including peak calling from ChIP-seq data, peptide identification from mass spectrometry data, DEG identification from RNA-seq data, and DIR identification from Hi-C data. Notably, our benchmarking results for peptide identification are based on the first MS data standard with a realistic dynamic range.

## Clipper has verified FDR control and power advantage in simulation

Simulation is essential because we can generate numerous datasets from the same distribution with known truths to calculate the FDR, which is not observable from real data. Our simulation covers both enrichment and differential analyses. In enrichment analysis, we consider four "experimental designs": 1vs1 design (one replicate per condition), 2vs1 design (two and one replicates under the experimental and background conditions, respectively), 3vs3 design (three replicates per condition), and 10vs10 design (ten replicates per condition). In differential analysis, since Clipper requires that at least one condition has two replicates, we only consider the 2vs1 and 3vs3 designs. For each analysis and design, we simulated data from three "distributional families"—Gaussian, Poisson, and negative binomial—for individual features under two "background scenarios" (i.e., scenarios of the background condition): homogeneous and heterogeneous. Under the homogeneous scenario, all features' measurements follow the same distribution under the background condition; otherwise, we are under the heterogeneous scenario, which is ubiquitous in applications, e.g., identifying DEGs from RNA-seq data and calling protein-binding sites from ChIP-seq data. The detailed simulation settings, each of which is a combination of an experimental design, a distributional family, and a background scenario, are described in

Supp. Section S4.

For both enrichment and differential analyses and each simulation setting, we compared Clipper against generic FDR control methods, including p-value-based methods and local-fdr-based methods. The p-value-based methods include BH-pair, BH-pool, qvalue-pair, and qvalue-pool, where "BH" and "qvalue" stand for p-value thresholding procedures, and "pair" and "pool" represent the paired and pooled p-value calculation approaches, respectively. The local-fdr-based methods include locfdr-emp and locfdr-swap, where "emp" and "swap" represent the empirical null and swapping null local-fdr calculation approaches, respectively. See Online Methods for detail.

The comparison results in Fig. 2 and Supp. Figs. S1–S11 show that Clipper controls the FDR and is overall more powerful than other methods, excluding those that fail to control the FDR, under all settings. Clipper is also shown to be more robust to the number of features and the existence of outliers than other methods. In detail, in both enrichment analyses (1vs1, 2vs1, 3vs3, and 10vs10 designs) and differential analyses (2vs1 and 3vs3 designs), Clipper consistently controls the FDR, and it is more powerful than the generic methods in most cases under the realistic, heterogeneous background, where features do not follow the same distribution under the background condition. Under the idealistic, homogeneous background, Clipper is still powerful and only second to BH-pool and qvalue-pool, which, however, cannot control the FDR under the heterogeneous background.

Here we summarize the performance of the generic FDR control methods. First, the two p-value-based methods using the pooled approach, BH-pool and qvalue-pool, are the most powerful under the idealistic, homogeneous background, which is their inherent assumption; however, they cannot control the FDR under the heterogeneous background (Fig. 2b). Besides, they cannot control the FDR when the number of features is small (Fig. 2a and Supp. Fig. S1). These results show that the validity of BH-pool and qvalue-pool requires a large number of features and the homogeneous background assumption, two requirements that rarely hold in biological applications. Second, the four p-value-based methods using the paired approach with misspecified models or misformulated tests (BH-pair-mis, qvalue-pair-mis, BH-pair-2as1, and qvalue-pair-2as1; see Online Methods) fail to control the FDR by a large margin in most cases, and rarely when they control the FDR, they lack power (Fig. 2c–d and Supp. Figs. S1–S8). These results confirm that the BH-pair and qvalue-pair rely on the correct model specification to control the FDR; however, the correct model specification is hardly achievable with no more than three replicates per condition. Third, even when models are correctly specified (an idealistic scenario), the p-value-based methods that use the paired approach—BH-pair-correct and qvalue-pair-correct (see Online Methods)—fail to control the FDR in the existence of outliers (Fig. 2e and Supp. Figs. S3 and S7) or for the negative binomial distribution with unknown dispersion (Fig. 2f and Supp. Fig. S9). It is worth noting that even when they control the FDR, they are less powerful than Clipper in most cases except for the 3vs3 differential analysis with the Poisson distribution (Fig. 2d and Supp. Figs. S4 and S8). Fourth, the two local-fdr-based methods—locfdr-emp and locfdr-swap—achieve the FDR control under all designs and analyses; however, they are less powerful than Clipper in most cases (Supp. Figs. S1–S4). Fifth, when the numbers of replicates are large (10vs10 design), non-parametric tests become applicable. We compared Clipper with three BH-pair methods that use different statistical tests: BH-pair-Wilcoxon (the non-parametric Wilcoxon rank-sum test), BH-pair-permutation (the non-parametric permutation test), and BH-pair-parametric (the parametric test based on the correct model specification, equivalent to BH-pair-correct). Although all the three methods control the FDR, they are less powerful than Clipper (Supp. Fig. S10).

The above results are all based on simulations with independent features. To examine the robustness of Clipper, we introduced feature correlations to our simulated data, on which we compared Clipper with other generic FDR control methods. The comparison results in Supp. Fig. S11 show that even when the feature independence assumption is violated, Clipper still demonstrates strong performance in both

FDR control and power.

## Clipper has broad applications in omics data analyses

We then demonstrate the use of Clipper in four omics data applications: peak calling from ChIP-seq data, peptide identification from MS data, DEG identification from RNA-seq data, and DIR identification from Hi-C data. The first two applications are enrichment analyses, and the last two are differential analyses. In each application, we compared Clipper with mainstream bioinformatics methods to demonstrate Clipper's superiority in FDR control and detection power.

### Peak calling from ChIP-seq data (enrichment analysis I)

ChIP-seq is a genome-wide experimental assay for measuring binding intensities of a DNA-associated protein [40], often a transcription factor that activates or represses gene expression [41, 42]. ChIP-seq data are crucial for studying gene expression regulation, and the indispensable analysis is to identify genomic regions with enriched sequence reads in ChIP-seq data. These regions are likely to be bound by the target protein and thus of biological interest. The identification of these regions is termed "peak calling" in ChIP-seq data analysis.

As the identified peaks are subject to experimental validation that is often expensive [43], it is essential to control the FDR of peak identification to reduce unnecessary costs. The two most highly-cited peak-calling methods are MACS2 [1] and [2], both of which claim to control the FDR for their identified peaks. Specifically, both MACS2 and HOMER assume that the read counts for each putative peak (one count per sample/replicate) follow the Poisson distribution, and they use modified paired approaches to assign each putative peak a p-value and a corresponding Storey's q-value. Then given a target FDR threshold $0 < q < 1$, they call the putative peaks with q-values $\leq q$ as identified peaks. Despite being popular, MACS2 and HOMER have not been verified for their FDR control, to our knowledge.

To verify the FDR control of MACS2 and HOMER (Supp. Section S5.1), we used ENCODE ChIP-seq data of cell line GM12878 [44] and generated semi-synthetic data with spiked-in peaks (Supp. Section S6.1). We examined the actual FDR and power of MACS2 and HOMER in a range of target FDR thresholds: $q = 1\%, 2\%, \ldots, 10\%$. Fig. 3a shows that MACS2 and HOMER cannot control the FDR as standalone peak-calling methods. However, with Clipper as an add-on (Supp. Section S7.1), both MACS2 and HOMER can guarantee the FDR control. This result demonstrates the flexibility and usability of Clipper for reducing false discoveries in peak calling analysis.

Technically, the failed FDR control by MACS2 and HOMER is attributable to the likely model misspecification and test misformulation in their use of the paired approach. Both MACS2 and HOMER assume the Poisson distribution for read counts in a putative peak; however, it has been widely acknowledged that read counts are over-dispersed and thus better modeled by the negative binomial distribution [45]. Besides, MACS2 uses one-sample tests to compute p-values when two-sample tests should have been performed. As a result, the p-values of MACS2 and HOMER are questionable, so using their p-values for FDR control would not lead to success. (Note that MACS2 does not use p-values to control the FDR but instead swaps experimental and background samples to calculate the empirical FDR; yet, we emphasize that controlling the empirical FDR does not guarantee the FDR control.) As a remedy, Clipper strengthens both methods to control the FDR while maintaining high power.

### Peptide identification from MS data (enrichment analysis II)

The state-of-the-art proteomics studies use MS experiments and database search algorithms to identify and quantify proteins in biological samples. In a typical proteomics experiment, a protein mixture

sample is first digested into peptides and then measured by tandem MS technology as mass spectra, which encode peptide sequence information. "Peptide identification" is the process that decodes mass spectra and converts mass spectra into peptide sequences in a protein sequence database via search algorithms. The search process matches each mass spectrum to peptide sequences in the database and outputs the best match, called a "peptide-spectrum match" (PSM). The identified PSMs are used to infer and quantify proteins in a high-throughput manner.

False PSMs could occur when mass spectra are matched to wrong peptide sequences due to issues such as low-quality spectra, data-processing errors, and incomplete protein databases, causing problems in the downstream protein identification and quantification [46]. Therefore, a common goal of database search algorithms is to simultaneously control the FDR and maximize the number of identified PSMs, so as to maximize the number of proteins identified in a proteomics study [3, 47, 48]. A widely used FDR control strategy is the target-decoy search, where mass spectra of interest are matched to peptide sequences in both the original (target) database and a decoy database that contains artificial false protein sequences. The resulting PSMs are called the target PSMs and decoy PSMs, respectively. The decoy PSMs, i.e., matched mass spectrum and decoy peptide pairs, are known to be false and thus used by database search algorithms to control the FDR. Mainstream database search algorithms output a q-value for each PSM, target or decoy. Discoveries are the target PSMs whose q-values are no greater than the target FDR threshold $q$.

We used the first comprehensive benchmark dataset from an archaea species *Pyrococcus furiosus* to examine the FDR control and power of a popular database search algorithm Mascot [3] (Supp. Section S5.2). Using this benchmark dataset (Supp. Section S6.2), we demonstrate that, as an add-on, Clipper improves the power of Mascot. Specifically, Clipper treats mass spectra as features. For each mass spectrum, Clipper considers its measurement under the experimental condition as the $-\log_{10}$-transformed q-value of the target PSM that includes it, and its measurement under the background condition as the $-\log_{10}$-transformed q-value of the decoy PSM that includes it. Then Clipper decides which mass spectra and their corresponding target PSMs are discoveries (Supp. Section S7.2). Based on the benchmark dataset, we examined the empirical FDR, i.e., the FDP calculated based on the true positives and negatives, and the power of Mascot with or without Clipper as an add-on, for a range of target FDR thresholds: $q = 1\%, 2\%, \ldots, 10\%$. Fig. 3b shows that although Mascot and Mascot+Clipper both control the FDR, Mascot+Clipper consistently improves the power, thus enhancing the peptide identification efficiency of proteomics experiments.

While preparing this manuscript, we found a recent work [38] that used a similar idea to identify PSMs without using p-values. Clipper differs from this work in two aspects: (1) Clipper is directly applicable as an add-on to any existing database search algorithms that output q-values; (2) Clipper is not restricted to the peptide identification application.

### DEG identification from RNA-seq data (differential analysis I)

RNA-seq data measure genome-wide gene expression levels in biological samples. An important use of RNA-seq data is the DEG analysis, which aims to discover genes whose expression levels change between two conditions. The FDR is a widely used criterion in DEG analysis [4–9].

We compared Clipper with two popular DEG identification methods DESeq2 [5] and edgeR [4] (Supp. Section S5.3). Specifically, we applied Clipper to two gene expression matrices, one per condition, to identify DEGs (Supp. Section S7.3). To verify the FDR control of Clipper, DESeq2, and edgeR, we generated realistic semi-synthetic data from real RNA-seq data of classical and non-classical human monocytes [49], i.e., two conditions (Supp. Section S6.3). With ground truths (true DEGs and non-DEGs), the semi-synthetic data allow evaluating each method's FDR and power for a range of target

FDR thresholds: $q = 1\%, 2\%, \ldots, 10\%$. Fig. 3c shows that Clipper consistently controls the FDR and achieves high power. In contrast, edgeR controls the FDR but has lower power than Clipper does, while DESeq2 fails to control the FDR. To explain this DESeq2 result, we examined the p-value distributions of $16$ non-DEGs that were most frequently identified from $200$ synthetic datasets by DESeq2 at the target FDR threshold $q = 0.05$. Our results in Fig. 3e show that the $16$ non-DEGs' p-values are non-uniformly distributed with a mode close to $0$. Such unusual enrichment of overly small p-values makes these non-DEGs mistakenly called discoveries by DESeq2.

IHW is a popular procedure for boosting the power of p-value-based FDR control methods by incorporating feature covariates [27]. We used IHW as an add-on for DESeq2 and edgeR by adding every gene's mean expression across replicates and conditions as that gene's covariate, as suggested in [50]. The result in Fig. 3c shows that DESeq2+IHW and edgeR+IHW are similar to DESeq2 and edgeR, respectively, in FDR control and power.

We further compared Clipper with DESeq2 and edgeR on the real RNA-seq data of classical and non-classical human monocytes [49]. In this dataset, gene expression changes are expected to be associated with the immune response process. We input three classical and three non-classical samples into Clipper, DESeq2, and edgeR for DEG identification. Fig. 4a shows that edgeR identifies the fewest DEGs, while DESeq2 identifies the most DEGs, followed by Clipper. Notably, most DEGs identified by DESeq2 are not identified by Clipper or edgeR. To investigate whether DESeq2 makes too many false discoveries and whether the DEGs found by Clipper but missed by DESeq2 or edgeR are biologically meaningful, we performed functional analysis on the set of DEGs identified by each method. We first performed the gene ontology (GO) analysis on the three sets of identified DEGs using the R package `clusterProfiler` [51]. Fig. 4b ("Total") shows that more GO terms are enriched (with enrichment q-values $\leq 0.01$) in the DEGs identified by Clipper than in the DEGs identified by DESeq2 or edgeR. For the GO terms enriched in all three sets of identified DEGs, Fig. 4c shows that they are all related to the immune response and thus biologically meaningful. Notably, these biologically meaningful GO terms have more significant enrichment in the DEGs identified by Clipper than in those identified by edgeR and DESeq2. We further performed GO analysis on the DEGs uniquely identified by one method in pairwise comparisons of Clipper vs. DESeq2 and Clipper vs. edgeR. Fig. 4b and Supp. Fig. S12 show that multiple immune-related GO terms are enriched in Clipper-specific DEGs, while no GO terms are enriched in edgeR-specific or DESeq2-specific DEGs. Altogether, these results confirm the capacity of Clipper in real-data DEG analysis, and they are consistent with our simulation results that edgeR lacks power, while DESeq2 fails to control the FDR.

### DIR analysis of Hi-C data (differential analysis II)

Hi-C experiments are widely used to investigate spatial organizations of chromosomes and map chromatin interactions across the genome. A Hi-C dataset is often processed and summarized into an interaction matrix, whose rows and columns represent manually binned chromosomal regions and whose $(i, j)$-th entry represents the measured contact intensity between the $i$-th and $j$-th binned regions. The DIR analysis aims to identify pairs of genomic regions whose interaction intensities differ between conditions. Same as DEG analysis, DIR analysis also uses the FDR as a decision criterion [11–13].

We compared Clipper with three popular DIR identification methods: diffHic [13], FIND [12], and multiHiCcompare [11] (Supp. Section S5.4). Specifically, we applied Clipper to DIR identification by treating pairs of genomic regions as features and interaction intensities as measurements. To verify the FDR control of Clipper (Supp. Section S7.4), diffHiC, FIND, and multiHiCcompare, we generated realistic synthetic data from real interaction matrices of ENCODE cell line GM12878 [44] with true spiked-in DIRs to evaluate the FDR and power (Supp. Section S6.4). We examined the actual FDR

and power in a range of target FDR thresholds: $q = 1\%, 2\%, \ldots, 10\%$. Fig. 3d shows that Clipper and diffHic are the only two methods that consistently control the FDR, while multiHiCcompare and FIND fail by a large margin. In terms of power, Clipper outperforms diffHic except for $q = 0.01$ and $0.02$, even though Clipper has not been optimized for Hi-C data analysis. This result demonstrates Clipper's general applicability and strong potential for DIR analysis.

## Discussion

In this paper, we proposed a new statistical framework, Clipper, for identifying interesting features with FDR control from high-throughput data. Clipper avoids the use of p-values and makes FDR control more reliable and flexible. We used comprehensive simulation studies to verify the FDR control by Clipper under various settings. We demonstrate that Clipper outperforms existing generic FDR control methods by having higher power and greater robustness to model misspecification. We further applied Clipper to four popular bioinformatics analyses: peak calling from ChIP-seq data, peptide identification from MS data, DEG identification from RNA-seq data, and DIR identification from Hi-C data. Our results indicate that Clipper can provide a powerful add-on to existing bioinformatics tools to improve the reliability of FDR control and thus the reproducibility of scientific discoveries.

We validated the FDR control by Clipper using extensive and concrete simulations, including both model-based and real-data-based data generation with ground truths. In contrast, in most bioinformatics method papers, the FDR control was merely mentioned but rarely validated. Many of them assumed that using the BH procedure on p-values would lead to valid FDR control; however, the reality is often otherwise because p-values would be invalid when model assumptions were violated or the p-value calculation was problematic. Here we voice the importance of validating the FDR control in bioinformatics method development, and we use this work as a demonstration. We believe that Clipper provides a powerful booster to this movement. As a p-value-free alternative to the classic p-value-based BH procedure, Clipper relies less on model assumptions and is thus more robust to model misspecifications, making it an appealing choice for FDR control in diverse high-throughput biomedical data analyses.

Clipper is a flexible framework that is easily generalizable to identify a variety of interesting features. The core component of Clipper summarizes each feature's measurements under each condition into an informative statistic (e.g., the sample mean); then Clipper combines each feature's informative statistics under two conditions into a contrast score to enable FDR control. The current implementation of Clipper only uses the sample mean as the informative statistic to identify the interesting features that have distinct expected values under two conditions. However, by modifying the informative statistic, we can generalize Clipper to identify the features that are interesting in other aspects, e.g., having different variances between two conditions. Regarding the contrast score, Clipper makes careful choices between two contrast scores, difference and maximum, based on the number of replicates and the analysis task (enrichment vs. differential). Future studies are needed to explore other contrast scores and their power with respect to data characteristics and analysis tasks.

We have demonstrated the broad application potential of Clipper in various bioinformatics data analyses. Specifically, when used as an add-on to established, popular bioinformatics methods such as MACS2 for peak calling and Mascot for peptide identification, Clipper guaranteed the desired FDR control and in some cases boosted the power. However, many more careful thoughts are needed to escalate Clipper into standalone bioinformatics methods for specific data analyses, for which data processing and characteristics (e.g., peak lengths, GC contents, proportions of zeros, and batch effects) must be appropriately accounted for before Clipper is used for the FDR control [52, 53]. We expect that the Clipper framework will propel future development of bioinformatics methods by providing a flexible

p-value-free approach to control the FDR, thus improving the reliability of scientific discoveries.

# Online Methods

## Notations and assumptions

We first introduce notations and assumptions used in Clipper. While the differential analysis treats the two conditions symmetric, the enrichment analysis requires one condition to be the experimental condition (i.e., the condition of interest) and the other condition to be the background condition (i.e., the negative control). For simplicity, we use the same set of notations for both analyses. For two random vectors $\boldsymbol{X} = (X_1, \ldots, X_m)^\top$ and $\boldsymbol{Y} = (Y_1, \ldots, Y_n)^\top$, we write $\boldsymbol{X} \perp \boldsymbol{Y}$ if $X_i$ is independent of $Y_j$ for all $i = 1, \ldots, m$ and $j = 1, \ldots, n$. To avoid confusion, we use $\mathrm{card}(A)$ to denote the cardinality of a set $A$ and $|c|$ to denote the absolute value of a scalar $c$. We define $a \vee b := \max(a, b)$.

Clipper only requires two inputs: the target FDR threshold $q \in (0, 1)$ and the input data. Regarding the input data, we use $d$ to denote the number of features with measurements under two conditions, and we use $m$ and $n$ to denote the numbers of replicates under the two conditions. For each feature $j = 1, \ldots, d$, we use $\boldsymbol{X}_j = (X_{j1}, \ldots, X_{jm})^\top \in \mathbb{R}^m_{\geq 0}$ and $\boldsymbol{Y}_j = (Y_{j1}, \ldots, Y_{jn})^\top \in \mathbb{R}^n_{\geq 0}$ to denote its measurements under the two conditions, where $\mathbb{R}_{\geq 0}$ denotes the set of non-negative real numbers. We assume that all measurements are non-negative, as in the case of most high-throughput experiments. (If this assumption does not hold, transformations can be applied to make data satisfy this assumption.)

Clipper has the following assumptions on the joint distribution of $\boldsymbol{X}_1, \ldots, \boldsymbol{X}_d, \boldsymbol{Y}_1, \ldots, \boldsymbol{Y}_d$. For $j = 1, \ldots, d$, Clipper assumes that $X_{j1}, \ldots, X_{jm}$ are identically distributed, so are $Y_{j1}, \ldots, Y_{jn}$. Let $\mu_{Xj} = \mathbb{E}[X_{j1}]$ and $\mu_{Yj} = \mathbb{E}[Y_{j1}]$ denote the expected measurement of feature $j$ under the two conditions, respectively. Then conditioning on $\{\mu_{Xj}\}_{j=1}^d$ and $\{\mu_{Yj}\}_{j=1}^d$,

$$\begin{aligned} &X_{j1}, \cdots, X_{jm}, Y_{j1}, \cdots, Y_{jn} \text{ are mutually independent}\,; \\ &\boldsymbol{X}_j \perp \boldsymbol{X}_k, \boldsymbol{Y}_j \perp \boldsymbol{Y}_k \text{ and } \boldsymbol{X}_j \perp \boldsymbol{Y}_k\,,\ \forall j, k = 1, \ldots, d. \end{aligned} \tag{1}$$

An enrichment analysis aims to identify interesting features with $\mu_{Xj} > \mu_{Yj}$ (with $\boldsymbol{X}_j$ and $\boldsymbol{Y}_j$ defined as the measurements under the experimental and background conditions, respectively), while a differential analysis aims to call interesting features with $\mu_{Xj} \neq \mu_{Yj}$. We define $\mathcal{N} := \{j : \mu_{Xj} = \mu_{Yj}\}$ as the set of uninteresting features and denote $N := \mathrm{card}(\mathcal{N})$. In both analyses, Clipper further assumes that an uninteresting feature $j$ satisfies

$$X_{j1}, \cdots, X_{jm}, Y_{j1}, \cdots, Y_{jn} \text{ are identically distributed}\,, \forall j \in \mathcal{N}\,. \tag{2}$$

Clipper consists of two main steps: construction and thresholding of contrast scores. First, Clipper computes contrast scores, one per feature, as summary statistics that reflect the extent to which features are interesting. Second, Clipper establishes a contrast-score cutoff and calls as discoveries the features whose contrast scores exceed the cutoff.

To construct contrast scores, Clipper uses two summary statistics $t(\cdot, \cdot) : \mathbb{R}^m_{\geq 0} \times \mathbb{R}^n_{\geq 0} \to \mathbb{R}$ to extract data information regarding whether a feature is interesting or not:

$$t^{\mathrm{diff}}(\boldsymbol{x}, \boldsymbol{y}) := \bar{x} - \bar{y}\,; \tag{3}$$

$$t^{\max}(\boldsymbol{x}, \boldsymbol{y}) := \max(\bar{x}, \bar{y}) \cdot \mathrm{sign}(\bar{x} - \bar{y})\,, \tag{4}$$

where $\boldsymbol{x} = (x_1, \ldots, x_m)^\top \in \mathbb{R}^m_{\geq 0}$, $\boldsymbol{y} = (y_1, \ldots, y_n)^\top \in \mathbb{R}^n_{\geq 0}$, $\bar{x} = \sum_{i=1}^m x_i/m$, $\bar{y} = \sum_{i=1}^n y_i/n$, and

$\mathrm{sign}(\cdot) : \mathbb{R} \to \{-1, 0, 1\}$ with $\mathrm{sign}(x) = 1$ if $x > 0$, $\mathrm{sign}(x) = -1$ if $x < 0$, and $\mathrm{sign}(x) = 0$ otherwise.

Then we introduce how Clipper works in three analysis tasks: the enrichment analysis with equal numbers of replicates under two conditions ($m = n$), the enrichment analysis with different numbers of replicates under two conditions ($m \neq n$), and the differential analysis (when $m + n > 2$).

## Enrichment analysis with equal numbers of replicates ($m = n$)

Under the enrichment analysis, we assume that $\boldsymbol{X}_j \in \mathbb{R}^m_{\geq 0}$ and $\boldsymbol{Y}_j \in \mathbb{R}^n_{\geq 0}$ are the measurements of feature $j$, $j = 1, \ldots, d$, under the experimental and background conditions with $m$ and $n$ replicates, respectively. We start with the simple case when $m = n$. Clipper defines a contrast score $C_j$ of feature $j$ in one of two ways:

$$C_j := t^{\mathrm{diff}}(\boldsymbol{X}_j, \boldsymbol{Y}_j) \qquad \textbf{difference contrast score}\,, \tag{5}$$

or

$$C_j := t^{\max}(\boldsymbol{X}_j, \boldsymbol{Y}_j) \qquad \textbf{maximum contrast score}\,. \tag{6}$$

Fig. 5a shows a cartoon illustration of contrast scores when $m = n = 1$. Accordingly, a large positive value of $C_j$ bears evidence that $\mu_{Xj} > \mu_{Yj}$. Motivated by Barber and Candès [35], Clipper uses the following procedure to control the FDR under the target level $q \in (0, 1)$.

**Definition 1 (Barber-Candès (BC) procedure for thresholding contrast scores [35])** *Given contrast scores $\{C_j\}_{j=1}^d$, $\mathcal{C} = \{|C_j| : C_j \neq 0\,;\ j = 1, \ldots, d\}$ is defined as the set of non-zero absolute values of $C_j$'s. The BC procedure finds a contrast-score cutoff $T^{\mathrm{BC}}$ based on the target FDR threshold $q \in (0, 1)$ as*

$$T^{\mathrm{BC}} := \min\left\{t \in \mathcal{C} : \frac{\mathrm{card}(\{j : C_j \leq -t\}) + 1}{\mathrm{card}(\{j : C_j \geq t\}) \vee 1} \leq q\right\} \tag{7}$$

*and outputs $\{j : C_j \geq T^{\mathrm{BC}}\}$ as discoveries.*

## Enrichment analysis with any numbers of replicates $m$ and $n$

When $m \neq n$, Clipper constructs contrast scores via permutation of replicates across conditions. The idea is that, after permutation, every feature becomes uninteresting and can serve as its own negative control.

**Definition 2 (Permutation)** *We define $\sigma$ as permutation, i.e., a bijection from the set $\{1, \cdots, m + n\}$ onto itself, and we rewrite the data $\boldsymbol{X}_1, \ldots, \boldsymbol{X}_d, \boldsymbol{Y}_1, \ldots, \boldsymbol{Y}_d$ into a matrix $\mathbf{W}$:*

$$\mathbf{W} = \begin{bmatrix} W_{11} & \cdots & W_{1m} & W_{1(m+1)} & \cdots & W_{1(m+n)} \\ & \vdots & & & \vdots & \\ W_{d1} & \cdots & W_{dm} & W_{d(m+1)} & \cdots & W_{d(m+n)} \end{bmatrix} := \begin{bmatrix} X_{11} & \cdots & X_{1m} & Y_{11} & \cdots & Y_{1n} \\ & \vdots & & & \vdots & \\ X_{d1} & \cdots & X_{dm} & Y_{d1} & \cdots & Y_{dn} \end{bmatrix}.$$

*We then apply $\sigma$ to permute the columns of $\mathbf{W}$ and obtain*

$$\mathbf{W}_\sigma := \begin{bmatrix} W_{1\sigma(1)} & \cdots & W_{1\sigma(m)} & W_{1\sigma(m+1)} & \cdots & W_{1\sigma(m+n)} \\ & \vdots & & & \vdots & \\ W_{d\sigma(1)} & \cdots & W_{d\sigma(m)} & W_{d\sigma(m+1)} & \cdots & W_{d\sigma(m+n)} \end{bmatrix},$$

*from which we obtain the permuted measurements* $\{(\boldsymbol{X}_j^\sigma, \boldsymbol{Y}_j^\sigma)\}_{j=1}^d$*, where*

$$\begin{aligned} \boldsymbol{X}_j^\sigma &:= \left(W_{j\sigma(1)}, \ldots, W_{j\sigma(m)}\right)^\top, \\ \boldsymbol{Y}_j^\sigma &:= \left(W_{j\sigma(m+1)}, \ldots, W_{j\sigma(m+n)}\right)^\top. \end{aligned} \tag{8}$$

In the enrichment analysis, if two permutations $\sigma$ and $\sigma'$ satisfy that

$$\{\sigma(1), \cdots, \sigma(m)\} = \{\sigma'(1), \cdots, \sigma'(m)\},$$

then we define $\sigma$ and $\sigma'$ to be in one equivalence class. That is, permutations in the same equivalence class lead to the same division of $m+n$ replicates (from the two conditions) into two groups with sizes $m$ and $n$. In total, there are $\binom{m+n}{m}$ equivalence classes of permutations.

We define $\sigma_0$ as the identity permutation such that $\sigma_0(i) = i$ for all $i \in \{1, \cdots, m+n\}$. In addition, Clipper randomly samples $h$ equivalence classes $\sigma_1, \ldots, \sigma_h$ with equal probabilities without replacement from the other $h_{\max} := \binom{m+n}{m} - 1$ equivalence classes (after excluding the equivalence class containing $\sigma_0$). Note that $h_{\max}$ is the maximum value $h$ can take.

Clipper then obtains $\{(\boldsymbol{X}_j^{\sigma_0}, \boldsymbol{Y}_j^{\sigma_0}), (\boldsymbol{X}_j^{\sigma_1}, \boldsymbol{Y}_j^{\sigma_1}), \cdots, (\boldsymbol{X}_j^{\sigma_h}, \boldsymbol{Y}_j^{\sigma_h})\}_{j=1}^d$, where $(\boldsymbol{X}_j^{\sigma_\ell}, \boldsymbol{Y}_j^{\sigma_\ell})$ are the permuted measurements based on $\sigma_\ell$, $\ell = 0, 1, \ldots, h$. Then Clipper computes $T_j^{\sigma_\ell} := t^{\text{diff}}(\boldsymbol{X}_j^{\sigma_\ell}, \boldsymbol{Y}_j^{\sigma_\ell})$ to indicate the degree of "interestingness" of feature $j$ reflected by $(\boldsymbol{X}_j^{\sigma_\ell}, \boldsymbol{Y}_j^{\sigma_\ell})$. Note that Clipper chooses $t^{\text{diff}}$ instead of $t^{\max}$ because empirical evidence shows that $t^{\text{diff}}$ leads to better power. Sorting $\{T_j^{\sigma_\ell}\}_{\ell=0}^h$ gives

$$T_j^{(0)} \geq T_j^{(1)} \geq \cdots \geq T_j^{(h)}.$$

Then Clipper defines the contrast score of feature $j$, $j = 1, \ldots, d$, in one of two ways:

$$C_j := \begin{cases} T_j^{(0)} - T_j^{(1)} & \text{if } T_j^{(0)} = T_j^{\sigma_0} \\ T_j^{(1)} - T_j^{(0)} & \text{otherwise} \end{cases} \qquad \textbf{difference contrast score}, \tag{9}$$

or

$$C_j := \begin{cases} \left|T_j^{(0)}\right| & \text{if } T_j^{(0)} = T_j^{\sigma_0} > T_j^{(1)} \\ 0 & \text{if } T_j^{(0)} = T_j^{(1)} \\ -\left|T_j^{(0)}\right| & \text{otherwise} \end{cases} \qquad \textbf{maximum contrast score}. \tag{10}$$

The intuition behind the contrast scores is that, if $C_j < 0$, then $T_j^{(0)} \neq T_j^{\sigma_0}$, which means that at least one of $T_j^{\sigma_1}, \ldots, T_j^{\sigma_h}$ (after random permutation) is greater than $T_j^{\sigma_0}$ calculated from the original data (identity permutation), suggesting that feature $j$ is likely an uninteresting feature in enrichment analysis. Fig. 5b (right) shows a cartoon illustration of contrast scores when $m = 2$ and $n = 1$. Motivated by Gimenez and Zou [54], we propose the following procedure for Clipper to control the FDR under the target level $q \in (0, 1)$.

**Definition 3 (Gimenez-Zou (GZ) procedure for thresholding contrast scores [54])** *Given* $h \in \{1, \cdots, h_{\max}\}$ *and contrast scores* $\{C_j\}_{j=1}^d$*,* $\mathcal{C} = \{|C_j| : C_j \neq 0 \,;\, j = 1, \ldots, d\}$ *is defined as the set of non-zero absolute values of* $C_j$*'s. The GZ procedure finds a contrast-score cutoff* $T^{\text{GZ}}$ *based on the target FDR threshold* $q \in (0, 1)$ *as:*

$$T^{\text{GZ}} := \min\left\{t \in \mathcal{C} : \frac{\frac{1}{h} + \frac{1}{h}\text{card}\left(\{j : C_j \leq -t\}\right)}{\text{card}\left(\{j : C_j \geq t\}\right) \vee 1} \leq q\right\} \tag{11}$$

*and outputs* $\left\{j : C_j \geq T^{\text{GZ}}\right\}$ *as discoveries.*

## Differential analysis with $m + n > 2$

For differential analysis, Clipper also uses permutation to construct contrast scores. When $m \neq n$, the equivalence classes of permutations are defined the same as for the enrichment analysis with $m \neq n$. When $m = n$, there is a slight change in the definition of equivalence classes of permutations: if $\sigma$ and $\sigma'$ satisfy that

$$\{\sigma(1), \cdots, \sigma(m)\} = \{\sigma'(1), \cdots, \sigma'(m)\} \text{ or } \{\sigma'(m+1), \cdots, \sigma'(2m)\},$$

then we say that $\sigma$ and $\sigma'$ are in one equivalence class. In total, there are $h_{\text{total}} := \binom{m+n}{m}$ (when $m \neq n$) or $\binom{2m}{m}/2$ (when $m = n$) equivalence classes of permutations. Hence, to have more than one equivalence class, we cannot perform differential analysis with $m = n = 1$; in other words, the total number of replicates $m + n$ must be at least $3$.

Then Clipper randomly samples $\sigma_1, \ldots, \sigma_h$ with equal probabilities without replacement from the $h_{\max} := h_{\text{total}} - 1$ equivalence classes that exclude the class containing $\sigma_0$, i.e., the identity permutation. Note that $h_{\max}$ is the maximum value $h$ can take. Next, Clipper computes $T_j^{\sigma_\ell} := \left|t^{\text{diff}}(\boldsymbol{X}_j^{\sigma_\ell}, \boldsymbol{Y}_j^{\sigma_\ell})\right|$, where $\boldsymbol{X}_j^{\sigma_\ell}$ and $\boldsymbol{Y}_j^{\sigma_\ell}$ are the permuted data defined in (8), and it defines $C_j$ as the contrast score of feature $j$, $j = 1, \ldots, d$, in the same ways as in (9) or (10). Fig. 5b (left) shows a cartoon illustration of contrast scores when $m = 2$ and $n = 1$.

Same as in the enrichment analysis with $m \neq n$, Clipper also uses the GZ procedure [54] to set a cutoff on contrast scores to control the FDR under the target level $q \in (0, 1)$.

Granted, when we use permutations to construct contrast scores in the GZ procedure, we can convert contrast scores into permutation-based p-values (see Supp. S1.1.2). However, when the numbers of replicates are small, the number of possible permutations is small, so permutation-based p-values would have a low resolution (e.g., when $m = 2$ and $n = 1$, the number of non-identity permutations is only $2$). Hence, applying the BH procedure to the permutation-based p-values would result in almost no power. Although Yekutieli and Benjamini proposed another thresholding procedure for permutation-based p-values [55], it still requires the number of permutations to be large to obtain a reliable FDR control. Furthermore, if we apply the SeqStep+ procedure by Barber and Candés [35] to permutation-based p-values, it would be equivalent to our application of the GZ procedure to contrast scores (Supp. Section S1.1.2).

## Generic FDR control methods

In our simulation analysis, we compared Clipper against generic FDR control methods including p-value-based methods and local-fdr-based methods. Briefly, each p-value-based method is a combination of a p-value calculation approach and a p-value thresholding procedure. We use either the "paired" or "pooled" approach (see next paragraph) to calculate p-values of features and then threshold the p-values using the BH procedure (Supp. Definition S1) or Storey's qvalue procedure (Supp. Definition S2) to make discoveries (Supp. Section S1.1). As a result, we have four p-value-based methods: BH-pair, BH-pool, qvalue-pair, and qvalue-pool (Fig. 1b).

Regarding the existing p-value calculation approaches in bioinformatics tools, we categorize them as "paired" or "pooled." The paired approach has been widely used to detect DEGs and protein-binding sites [1, 2, 4, 5]. It examines one feature at a time and compares the feature's measurements between two conditions using a statistical test. In contrast, the pooled approach is popular in proteomics for identifying peptide sequences from MS data [56]. For every feature, it defines a test statistic and estimates a null distribution by pooling all features' observed test statistic values under the background condition.

Finally, it calculates a p-value for every feature under the experimental condition based on the feature's observed test statistic and the null distribution.

In parallel to p-value-based methods, local-fdr-based methods estimate local fdrs of features and then threshold the local fdrs using the locfdr procedure (Supp. Definition S5) to make discoveries. The estimation of local fdrs takes one of two approaches: (1) empirical null, which is estimated parametrically from the test statistic values that are likely drawn from the null distribution, and (2) swapping null, which is constructed by swapping measurements between experimental and background conditions. The resulting two local-fdr-based-methods are referred to as locfdr-emp and locfdr-swap (Figs. 1b and 2). Supp. Section S1 provides a detailed explanation of these generic methods and how we implemented them in this work.

Specific to the p-value-based methods, for the paired approach, besides the ideal implementation that uses the correct model to calculate p-values (BH-pair-correct and qvalue-pair-correct), we also consider common mis-implementations. The first mis-implementations is misspecification of the distribution (BH-pair-mis and qvalue-pair-mis). An example is the detection of protein-binding sites from ChIP-seq data. A common assumption is that ChIP-seq read counts in a genomic region (i.e., a feature) follow the Poisson distribution [1, 2], which implies that the counts have the variance equal to the mean. However, if only two replicates are available, it is impossible to check whether this Poisson distribution is reasonably specified. The second mis-implementation is the misspecification of a two-sample test as a one-sample test (BH-pair-2as1 and qvalue-pair-2as1), which ignores the sampling randomness of replicates under one condition. This issue is implicit but widespread in bioinformatics methods [1, 57].

To summarize, we compared Clipper against the following implementations of generic FDR control methods:

- **BH-pool** or **qvalue-pool**: p-values calculated by the pooled approach and thresholded by the BH or qvalue procedure.
- **BH-pair-correct** or **qvalue-pair-correct**: p-values calculated by the paired approach with the correct model specification and thresholded by the BH or qvalue procedure.
- **BH-pair-mis** or **qvalue-pair-mis**: p-values calculated by the paired approach with a misspecified model and thresholded by the BH or qvalue procedure.
- **BH-pair-2as1** or **qvalue-pair-2as1**: p-values calculated by the paired approach that misformulates a two-sample test as a one-sample test (2as1) and thresholded by the BH or qvalue procedure.
- **locfdr-emp**: local fdrs calculated by the empirical null approach and thresholded by the locfdr procedure.
- **locfdr-swap**: local fdrs calculated by the swapping approach and thresholded by the locfdr procedure.

## Real datasets

- The H3K4me3 ChIP-seq dataset with one experimental sample (GEO accession number GSM733708) and two control samples (GEO accession number GSM733742) from the cell line GM12878 is available at `ftp://hgdownload.cse.ucsc.edu/goldenPath/hg19/encodeDCC/wgEncodeBroadHistone/`, with the experimental sample under the filename `wgEncodeBroadHistoneGm12878H3k4me3StdAlnRep1.bam` and the two control samples under the filenames `wgEncodeBroadHistoneGm12878ControlStdAlnRep1.bam`

and wgEncodeBroadHistoneGm12878ControlStdAlnRep2.bam. The processed dataset is available at https://zenodo.org/record/4404882.

- The MS benchmark dataset will be published in a future manuscript. Interested readers should contact Dr. Leo Wang at lewang@coh.org.
- The human monocyte RNA-Seq dataset is available at https://www.ncbi.nlm.nih.gov/Traces/study/?acc=srp082682. The dataset includes 17 samples of classical monocytes and 17 samples of nonclassical monocytes, and it is converted to a sample-by-gene count matrix by R package GenomicFeatures (v 1.40.1). The processed count matrix is available at https://zenodo.org/record/4404882.
- The Hi-C dataset from the cell line GM12878 is available at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE63525. The count matrix is under the filename GSE63525_GM12878_primary_intrachromosomal_contact_matrices.tar.gz, and the matrix corresponding to Chromosome 1 and bin width 1MB is used. The processed dataset is available at https://zenodo.org/record/4404882.

## Software packages used in this study

- **p.adjust** R function (in R package stats v 4.0.2 with default arguments) [14]: used for BH-pool, BH-pair-correct, BH-pair-mis, and BH-pair-2as1.
- **qvalue** R package (v 2.20.0 with default arguments) [58]: used for qvalue-pool, qvalue-pair-correct, qvalue-pair-mis, and qvalue-pair-2as1.
- **locfdr** R package (v 1.1-8 with default arguments) [59]: used for locfdr-emp.
- **MACS2** software package (v 2.2.6 with default settings) [1]: available at https://github.com/macs3-project/MACS/releases/tag/v2.2.6.
- **HOMER** software package (findPeaks v 3.1.9.2 with default settings) [2]: available at https://www.bcgsc.ca/platform/bioinfo/software/findpeaks/releases/3.1.9.2/findpeaks3-1-9-2-tar.gz.
- **Mascot** in Proteome Discoverer (v 2.3.0.523 with default settings) [3]: commercial software by ThermoScientific.
- **edgeR** R package (v 3.30.0 with default arguments) [4]: available at https://www.bioconductor.org/packages/release/bioc/html/edgeR.html.
- **DESeq2** R package (v 1.28.1 with default arguments) [5]: available at http://bioconductor.org/packages/release/bioc/html/DESeq2.html.
- **MultiHiCcompare** R package (v 1.6.0 with default arguments) [11]: available at https://bioconductor.org/packages/release/bioc/html/multiHiCcompare.html.
- **diffHic** R package (v 1.20.0 with default arguments) [13]: available at https://www.bioconductor.org/packages/release/bioc/html/diffHic.html.
- **FIND** R package (v 0.99 with default arguments) [12]: available at https://bitbucket.org/nadhir/find/src/master/.

## Software, code, and video tutorial

- The **Clipper** `R` package is available at https://github.com/JSB-UCLA/Clipper/.
- The code and processed data for reproducing the figures are available at https://zenodo.org/record/4404882.
- A video introduction of Clipper is available at https://youtu.be/-GXyHiJMpLo.

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## Conflicts of interests

L.D.W. holds equity in Magenta Therapeutics.

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## Figures

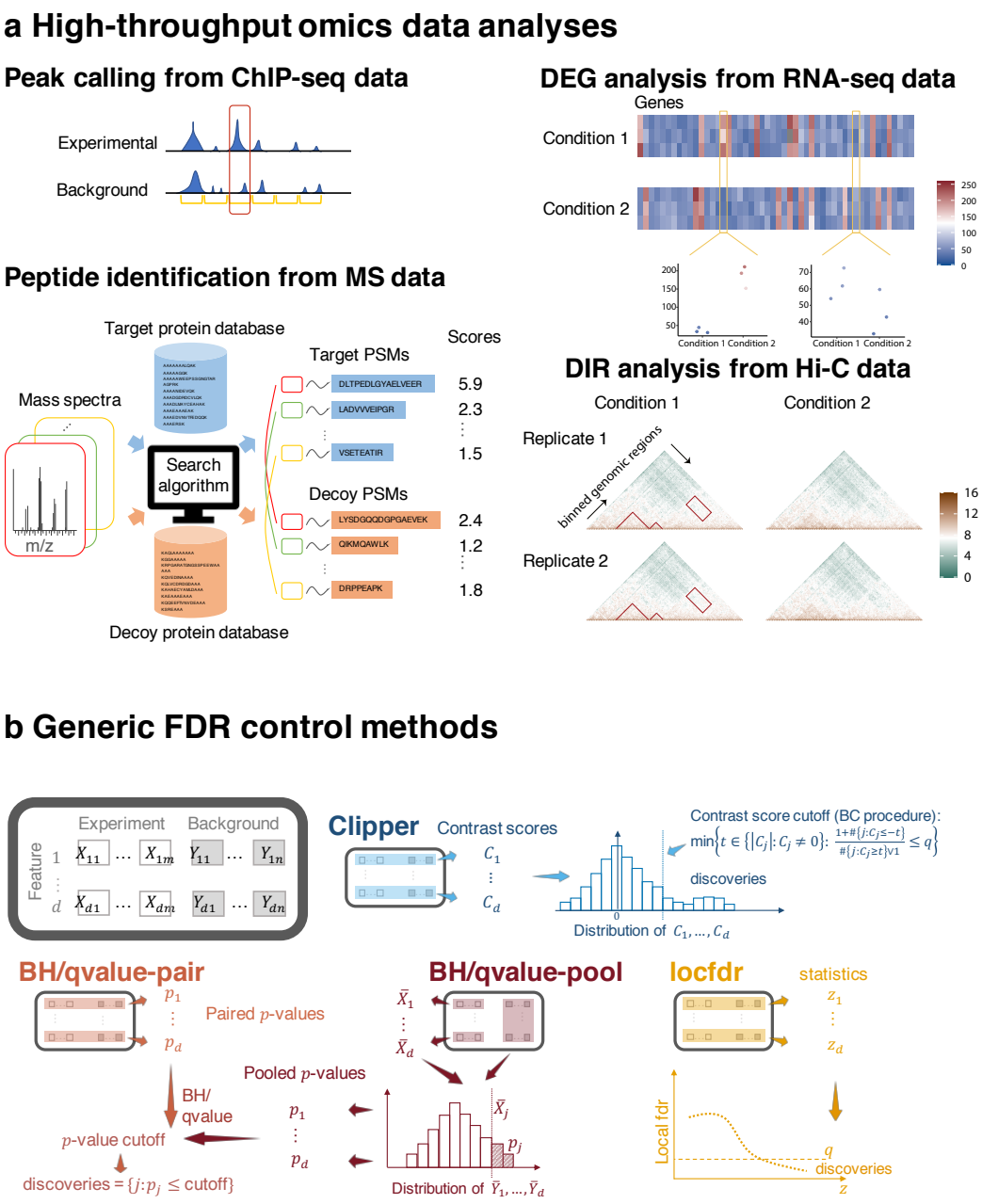



**Figure 1: High-throughput omics data analyses and generic FDR control methods. (a)** Illustration of four common high-throughput omics data analyses: peak calling from ChIP-seq data, peptide identification from MS data, DEG analysis from RNA-seq data, and DIR analysis from Hi-C data. In these four analyses, the corresponding features are genomic regions (yellow intervals), peptide-spectrum matches (PSMs; a pair of a mass spectrum and a peptide sequence), genes (columns in the heatmaps), and chromatin interacting regions (entries in the heatmaps). **(b)** Illustration of Clipper and five generic FDR control methods: BH-pair (and qvalue-pair), BH-pool (and qvalue-pool), and locfdr. The input data are $d$ features with $m$ and $n$ repeated measurements under the experimental and background conditions, respectively. Clipper computes a contrast score for each feature based on the feature's $m$ and $n$ measurements, decides a contrast-score cutoff, and calls the features with contrast scores above the cutoff as discoveries. (This illustration is Clipper for enrichment analysis with $m = n$.) BH-pair or qvalue-pair computes a p-value for each feature based on the feature's $m$ and $n$ measurements, sets a p-value cutoff, and calls the features with p-values below the cutoff as discoveries. BH-pool or qvalue-pool constructs a null distribution from the $d$ features' average (across the $n$ replicates) measurements under the background condition, calculates a p-value for each feature based on the null distribution and the feature's average (across the $m$ replicates) measurements under the experimental condition, sets a p-value cutoff, and calls the features with p-values below the cutoff as discoveries. The locfdr method computes a summary statistic for each feature based on the feature's $m$ and $n$ measurements, estimates the empirical null distribution and the empirical distribution of the statistic across features, computes a local fdr for each feature, sets a local fdr cutoff, and calls the features with local fdr below the cutoff as discoveries.

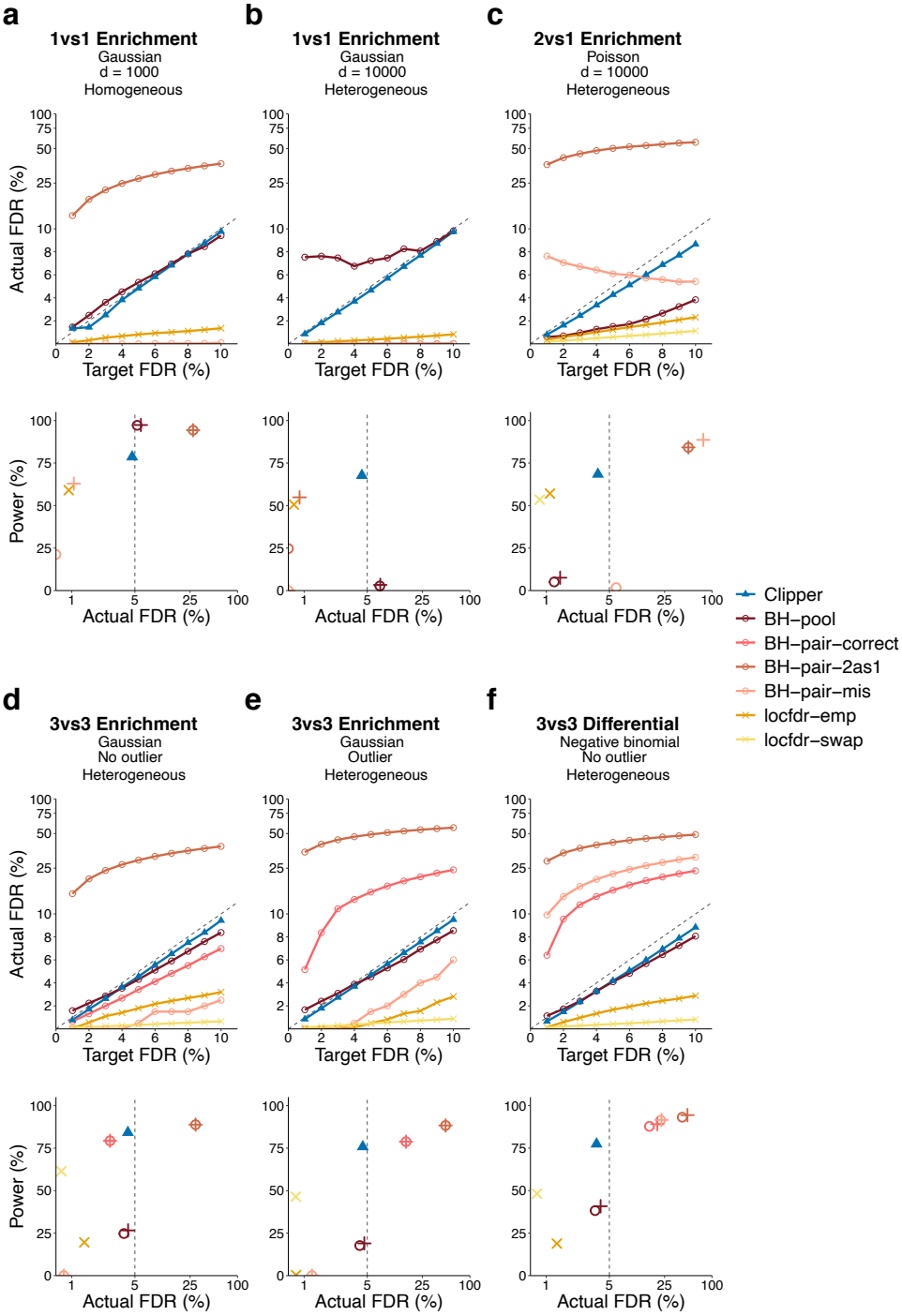



**Figure 2: Comparison of Clipper with generic FDR control methods in terms of their FDR control and power in six example simulation studies. (a)** 1vs1 enrichment analysis with $1000$ features generated from the Gaussian distribution with a homogeneous background; **(b)** 1vs1 enrichment analysis with $10{,}000$ features generated from the Gaussian distribution with a heterogeneous background; **(c)** 2vs1 enrichment analysis with $10{,}000$ features generated from the Poisson distribution with a heterogeneous background; **(d)** 3vs3 enrichment analysis with $10{,}000$ features generated from the Gaussian distribution without outliers and with a heterogeneous background; **(e)** 3vs3 enrichment analysis with $10{,}000$ features generated from the Gaussian distribution without outliers and with a heterogeneous background; **(f)** 3vs3 differential analysis with $10{,}000$ features generated from the negative binomial distribution with a heterogeneous background. At target FDR thresholds $q \in \{1\%, 2\%, \cdots, 10\%\}$, each method's actual FDRs and power are approximated by the averages of false discovery proportions (see Eq. (S14) in the Supplementary) and power evaluated on $200$ simulated datasets. In each panel, the top row shows each method's actual FDRs at target FDR thresholds: whenever the actual FDR is larger than the target FDR (the solid line is higher than the dashed line), FDR control is failed; the bottom row shows each method's actual FDRs and power at the target FDR threshold $q = 5\%$: whenever the actual FDR is greater than $q$ (on the right of the vertical dashed line), FDR control is failed. Under the FDR control, the larger the power, the better. Note that BH-pair-correct is not included in (a)–(c) because it is impossible to correctly specify the model with only one replicate per condition; locfdr-swap is not included in (a)–(b) because it is inapplicable to the 1vs1 design.

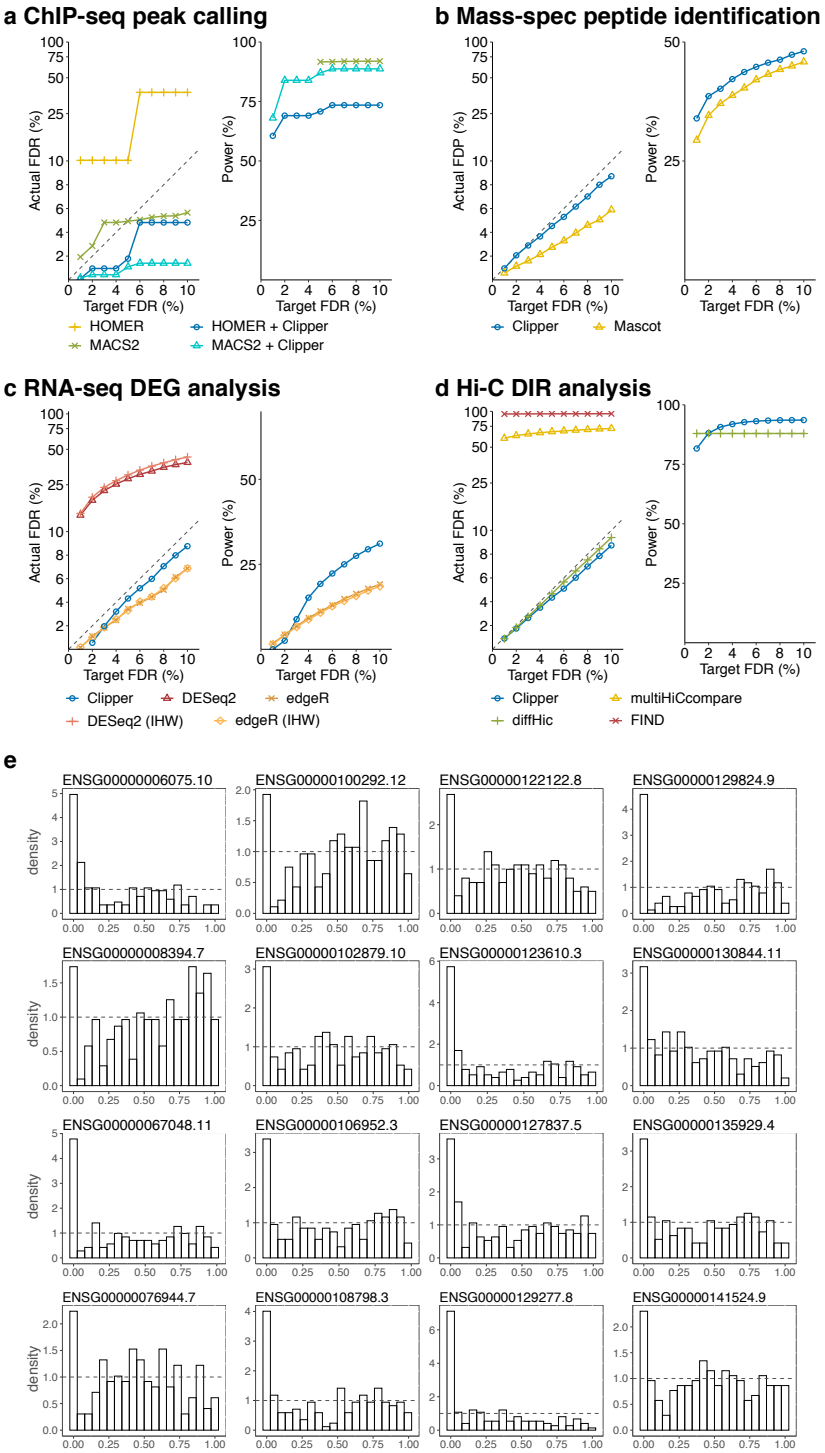
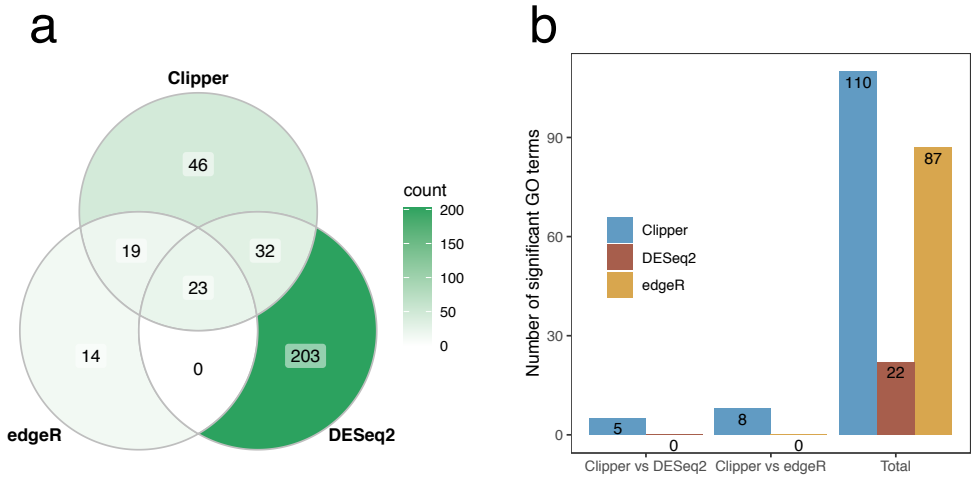



**Figure 3: Comparison of Clipper and popular bioinformatics methods in terms of FDR control and power.** **(a)** peaking calling analysis on synthetic ChIP-seq data; **(b)** peptide identification on real proteomics data; **(c)** DEG analysis on synthetic bulk RNA-seq data; **(d)** DIR analysis on synthetic Hi-C data. In all four panels, the target FDR level $q$ ranges from $1\%$ to $10\%$. Points above the dashed line indicate failed FDR control; when this happens, the power of the corresponding methods is not shown, including HOMER in (a), MACS2 for target FDR less than $5\%$ in (a), DESeq2 and DESeq2 (IHW) in (c), and multiHICcompare and FIND in (d). In all four applications, Clipper controls the FDR while maintaining high power, demonstrating Clipper's broad applicability in high-throughput data analyses. **(e)** The p-value distributions of $16$ non-DEGs that are most frequently identified by DESeq2 at $q = 5\%$ from $200$ synthetic datasets. The p-values of these $16$ genes tend to be overly small, and their distributions are non-uniform with a mode close to $0$.




c

Top GO terms enriched in all three sets of identified DEGs

| GO term (ID) | qvalue (DESeq2) | qvalue (edgeR) | qvalue (Clipper) |
|---|---|---|---|
| leukocyte chemotaxis (GO:0030595) | 9.930044e−06 | 9.594885e−09 | 3.104557e−10 |
| myeloid leukocyte migration (GO:0097529) | 1.107612e−05 | 2.921486e−08 | 5.740217e−10 |
| granulocyte chemotaxis (GO:0071621) | 2.698853e−05 | 1.008808e−08 | 1.167108e−09 |
| neutrophil chemotaxis (GO:0030593) | 2.698853e−05 | 2.921486e−08 | 2.691033e−09 |

**Figure 4: Application of Clipper, DESeq2, and edgeR to identifying DEGs from the classical and non-classical human monocyte dataset. (a)** A Venn diagram showing the overlaps of the identified DEGs (at the FDR threshold $q = 5\%$) by the three DE methods. **(b)** Numbers of GO terms enriched (with enrichment q-values $< 0.01$) in the DEGs found by Clipper, DESeq2 and edgeR (column 3), or in the DEGs specifically identified by Clipper or DESeq2/edgeR in the pairwise comparison between Clipper and DESeq2 (column 1) or between Clipper and edgeR (column 2). More GO terms are enriched in the DEGs identified by Clipper than in those identified by edgeR or DESeq2. **(c)** Enrichment q-values of four GO terms that are found enriched (with enrichment q-values $< 0.01$) in all three sets of identified DEGs, one set per method. All the four terms are most enriched in the DEGs identified by Clipper.

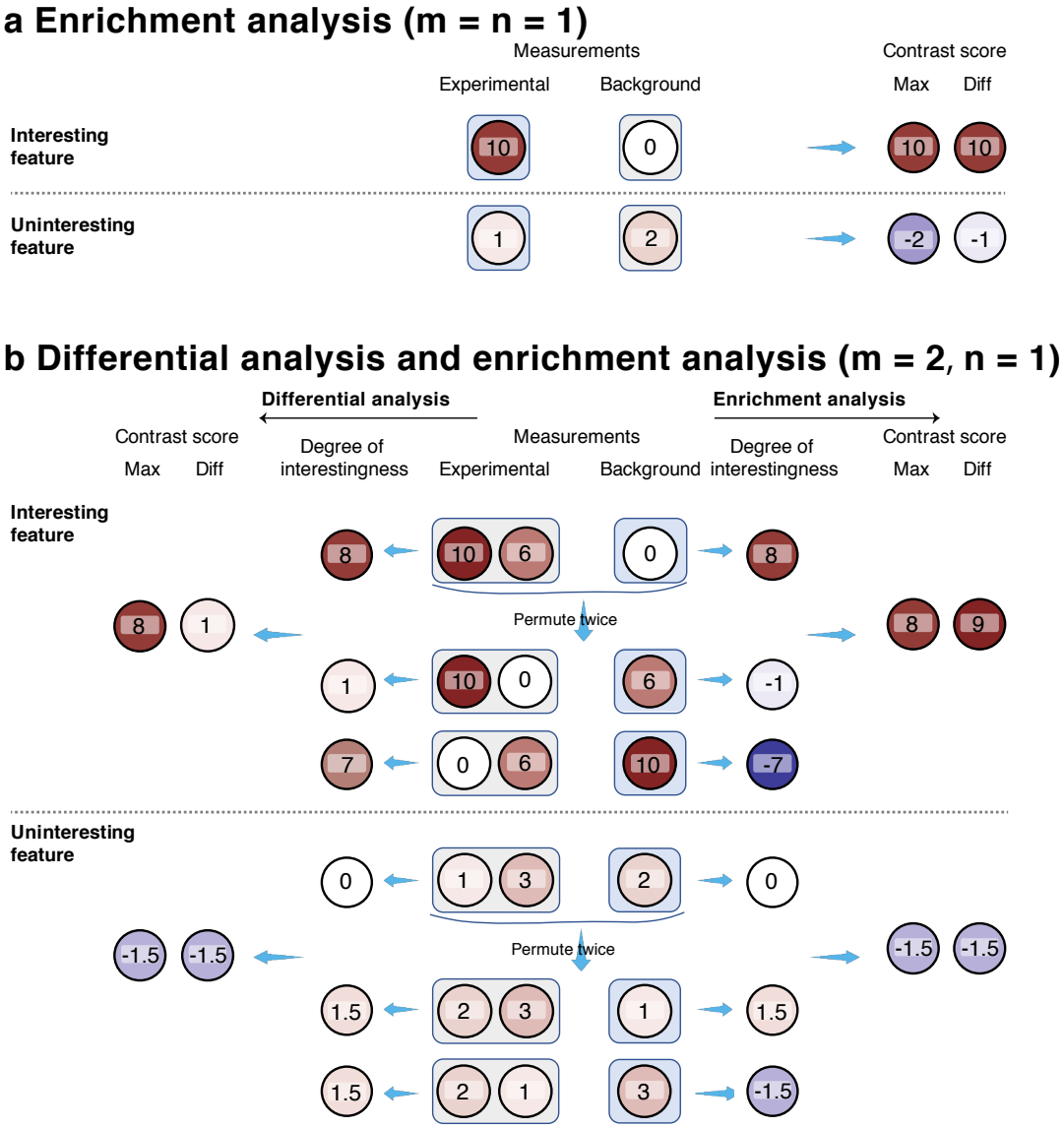



**Figure 5: Illustration of the construction of contrast scores. (a)** 1vs1 enrichment analysis; **(b)** 2vs1 differential analysis (left) or enrichment analysis (right). In each panel, an interesting feature (top) and an uninteresting feature (bottom) are plotted for contrast; both features have measurements under the experimental and background conditions. In (a), each feature's measurements are summarized into a maximum (max) contrast score or a difference (diff) contrast score. In (b), each feature's measurements are permuted across the two conditions, resulting in two sets of permuted measurements. Then for each feature, we calculate its degrees of interestingness (as the difference that equals the average of experimental measurements minus the average of background measurements (in enrichment analysis; right), or the absolute value of the difference (in differential analysis; left)) from its original measurements and permuted measurements, respectively. Finally, we summarize each feature's degrees of interestingness into a maximum (max) contrast score or a difference (diff) contrast score.