

Information theory based measures for quantification of  
private information leakage of functional genomics data  
and privacy-preserving file formats

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## Abstract

207 words

Functional genomics experiments provide important insight on genomic activities such as gene expression levels or transcription factor enrichment essential for personalized medicine. While publicly sharing of such data is extremely valuable for biomedical research, it invokes privacy concerns. Although functional genome are not necessarily tied to an individual's genotype, extracting information related to genomic activities from these experiments involves using raw sequencing reads that contain a large number of genetic variants. Therefore, reads from functional genomics experiments are considered to be an important set point, which public sharing is only possible in the form of summary representations such as signal profiles. There is, however, great desire to share as much data as possible to enhance research reproducibility and enable scientific discovery. Here, we study the quantification of sensitive information in functional genomics data by deriving information theory based measures. We show that functional genomics reads indeed leak a large amount of private information even at small sequencing depths and can be used to construct an individual's complete variant set when combined with imputation. We propose a privacy-enhancing file format enabling public sharing of reads, which constitutes the largest portion of functional genomics data production. Our file format allows accurate quantification of genomic activities with minimum utility loss.

# 1 Introduction

With the decreasing cost of DNA sequencing technologies, the number and the size of the available genomic data have exponentially increased and become available to a wider group of audiences such as hospitals, research institutions and individuals [?]. In turn, privacy of individuals has become an important aspect of biomedical data science [?, ?] as availability of genetic information gives rise to privacy concerns such that genetic predisposition to diseases may bias insurance companies [?] or create unlawful discrimination by employers.

Early genomic privacy studies focused on identification of individuals in a mixture by using phenotype-genotype association [?, ?]. These revealed that private information of an individual such as participation to a drug-abuse study [?, ?] can be revealed. With the increase of large-scale genomic projects such as Personal Genome Project (PGP) [?] or recreational/direct-to-consumer genomic databases, researchers showed that multiple datasets can be linked together to infer sensitive information such as participant's surnames [?] or addresses [?]. Such cross-referencing relies on quasi-identifiers, which are pieces of information that are not unique identifiers by themselves, but are well correlated with unique identifiers or can be unique identifiers when combined with other quasi-identifiers [?].

Functional genomics experiments provide a wealth of information on genomic activities related to developmental stages or diseases that are essential for personalized medicine. These are large-scale, high-throughput assays to quantify transcription (RNA-Seq) [?], epigenetic regulation (ChIP-Seq) [?] or 3D organization of genome (Hi-C) [?] in a genome-wide fashion under different conditions (e.g. samples from patients and healthy individuals). The biggest component in inferring biological information from functional genomics experiments is the data analysis step. It starts with the generation of DNA/RNA sequencing reads that are stored in special file formats called fastq [?]. These files are large in size ranging from 5 GB up to 60 GB depending on the

purpose of the experiment. They are then mapped to human reference genome and these mapped reads are stored in compressed binary file types called BAM [?]. While mapping sequences from fastq files to human reference genome is computationally expensive, BAM files provide 1 to 2 fold reduction in data size compared to fastq. The BAM files from functional genomics experiments are of particular interest due to several reasons. First, they contain raw data with the coordinate of each read in the human genome. Therefore, they constitute the most important input of majority of genomic annotation pipelines. Second, the raw reads contain sequence information of the individual that may leak sensitive data. Depending on the depth of the functional genomics experiment, raw reads can be used to identify the private SNPs, small indels, and structural variants. Consequently, BAM files might create a set point in the data production process in terms of privacy concern. However, current policies related to public sharing of the BAM files are somewhat ad-hoc. For example, the genome of HeLa cell line and the raw reads from Hi-C experiments require special access, while reads from ChIP-Seq and RNA-Seq experiments are publicly available [?]. That is, reads from the experiments that do not require substantial depth are sometimes considered to be safe to share without privacy concerns owing to partial and biased sequencing, although it is not clear if these reads indeed leak sensitive information. The lack of a systematic quantification of private data leakage from BAM files of functional genomics experiments makes it difficult for biomedical data sharing policy makers to protect individual's sensitive information in a consistent fashion. The progressive summarization of the raw data (such as signal profiles or gene expression quantification) still allows researchers to make accurate biological conclusions, while providing further data reduction of a  $\sim 20$  fold. Although overall aggregation and averaging reduces biological information, private information leakage also greatly decreases (Figure ??). On the other hand, recent studies on cross-referencing eQTLs with gene expression levels [?] as well as inferring structural variations from signal profiles [?] showed that there is no static set point in terms of private information leakage and the private information leakage in all levels of the data production process need to be quantified and further processed before making them publicly available.

On the flip side of the coin is the utility of the mapped reads (BAM files) and challenges related to dealing with private data. Accession to private data require use agreement that has an expiration date and a tremendous amount of bureaucracy connected to it. Moreover, any secondary data product becomes private and cannot be distributed. Problems associated with the distribution of secondary data products from private biomedical data is exacerbated due to large file sizes. For example, genome annotations that are derived from private functional genomics data require establishment of their own databases. However, since such annotations are derived from private data, establishment and distribution of these databases require extra levels of privacy related bureaucracy. Another example to the challenges associated with private data is that big consortia such as ENCODE [?], TCGA [?] or GTEx [?] fund multiple research institutions and enable a collaborative working environment through dedicated phone calls and meetings. In turn, all the participants need to go through required access procedures with their institutions. Otherwise communication based on private data is prohibited due to data use agreements. Moreover, when multiple institutions have required access to the same data, they still cannot exchange files with each other. These challenges create a bottleneck and hinder the progress of important biomedical findings. Open data helps the advancement of biomedical data science not only with the easy access to the data, but also helping with the speedy assesment of tools and methods and in turn reproducibility. Funding agencies and research organizations are increasingly supporting new means of data sharing and new requirements for making data publicly available while preserving the participant's privacy [?]. **In an attempt to consider both sides of the coin,** we ask the questions of how much information is enough information to identify individuals and how we can protect the sensitive information with minimum loss of utility in a publicly data sharing mode. This allows to push the set point further down the data analysis, which in turn helps with the solving the complexity associated with private biomedical data sharing. To this end, we derive novel information theory based measures and apply these measures to quantify the amount of leaked information in 24 functional genomic assays from ENCODE [?] at varying coverages. Based on our findings, we develop new file formats that

allow the public sharing of read alignments of functional genomics experiments while protecting the sensitive information as well as minimizing the amount of private data that requires special access and storage.

In this study, we use NA12878 as a case example and her 1000 genomes [?] genotypes as gold standard genotypes. We sample reads from the sequencing data of functional genomics experiments at increasing coverages and detect SNVs and indels using Genome Analysis Toolkit (GATK) best practices recommendations [?, ?]. We propose a new metric for quantifying the amount of information that can be obtained from sequencing data with respect to the gold standard. We next present a simple and practical instantiation of a linking attack with the assumption of adversaries accessing increasing amount of the sequencing data. We show that individuals are vulnerable to identifications even at small coverages of sequencing data. We further show that with summation of reads from functional genomics experiments and imputation through linkage disequilibrium, the leaked number of variants can reach the total number of variants in an individual's genome. We then provide a theoretical framework where the amount of leaked information can be estimated from depth and breadth of the coverage as well as the bias of the experiments. Finally, we focus on ways to publicly share alignment data without comprimizing individual's sensitive information. We propose privacy enhancing file formats that hide variant information, are compressed and have minimum amount of utility loss.

## **2 Results**

### **2.1 Information Theory to quantify private information in an individual's genome**

An individual's genome can be represented as a set of variants. Each variant is composed of the chromosome it belongs to, location on that chromosome, the alternative allele and its correspond-

ing genotype. Let  $S = \{s_1, s_2, \dots, s_i, \dots, s_N\}$  be the set of variants, then each variant can be represented as  $s_i = \{v_i, g_i\}$ , where  $v_i$  consists of the location and alternative allele information and  $g_i$  denotes the genotype of the variant as 1 for heterozygous variant and 2 for homozygous variant. We can then calculate the naive self-information of  $S$  in bits as

$$h(S) = - \sum_{i=1}^{i=N} \log_2(p(s_i)). \quad (1)$$

In eq. 1  $N$  is the total number of variants in an individual's genome,  $p(s_i) = n_i/n_T$  is the genotype frequency, in which  $n_i$  is the number of individuals with variant  $s_i = \{v_i, g_i\}$  and  $n_T$  is the total number of individuals in the panel. Note that we denote  $h(S)$  as "naive" information, because it is an estimate of the real information in a situation where the population that the individual belongs to is not known and the number of individuals are finite. Eq.1 holds only if variants are independent of each other, which is not the case due to the correlation between variants in linkage disequilibrium (LD). In theory, the population that the individual belongs to can easily be predicted by using a few variants. However, from an adversary's perspective, this will add one more layer of calculation, i.e computational and time cost to identification attack. Eq.1 also an estimate to the information when we consider all the individuals in the world (i.e  $\lim_{n_i \rightarrow \infty} h(S)$ ).

To be able to understand whether naive information is a good estimate, we first calculate the information with the consideration of LD scores taken from the European population of HapMap project [?]. LD scores are pairwise correlations between variants, which we consider as the prior information on the existence of a variant given other variants in the same LD block exist in a genome. Then the information with LD consideration is calculated as

$$h^{LD}(S) = - \sum_{i=1}^{i=N} (1 - mLD(s_i, s_j)) h(s_i) \quad (2)$$

$LD(s_i, s_j)$  is the maximum LD correlation of variant  $s_i$  such that  $mLD(s_i, s_j) = \max_{i \neq j, j \in (1, \dots, N)} LD(s_i, s_j)$ , where  $mLD(s_i, s_j) \neq mLD(s_j, s_i)$ .

Figure ??a shows a negligible difference between the naive information and information with LD consideration for NA12878 genome. To understand the lack of difference better, we calculate the self-information of each variant in an LD block with and without LD consideration. We show that highly informative variants do not exhibit any difference due to the low LD correlations (Figure ??b). We further show that the number of variants that have difference between information with and without LD consideration is small compared to highly informative variants having low LD correlations on average.

We then estimate the information when the population size is infinite [?]. We sample fractions in the order of 10%, 20%,..., 100% individuals from the 1000 genomes phase I panel (total of 2504 individuals) and calculate the information using the sampled distribution of genotypes. We repeat this calculation for 100 times and calculate the mean information for each sampled fraction. The relationship between the inverse of the sample fraction and the information fits best to a power function with two terms ( $y = ax^b + c$ ,  $R = 0.99$ ). The y-intercept ( $c$ ) of the curve is the extrapolation of information when the population size goes to infinity ( $1/\infty = 0$ , Figure ??c). We again found a negligible difference between the naive information and the information when the population size is infinite (Figure ??a). The information is also calculated by starting from a single individual and adding individuals one by one to the population (SI Figure 1). These individuals are simulated using the genotype frequencies in the 1000 genomes panel and the LD information from HapMap project (see SI methods). Both the information calculation and the  $KL$ -divergence between different size populations show that as the size of the population increases, the difference in the information decreases and eventually becomes negligible (SI Figure 1a-b)



In summary, calculations above show that the naive information can be an accurate approximate to the private information content of an individual’s genome when the individual’s population is not known and the population size is bound by the number of individuals in 1000 genomes panel due to the relationship of information at  $n \rightarrow \infty \geq \text{naive information} \geq \text{information with LD}$  (Figure ??a). That is, an adversary with no prior knowledge on the population of the sample and limited number of individuals in a known genotype panel can accurately approximate the private information in the sample.

## 2.2 Information Theory to quantify private information leakage in functional genomics data

In an effort to understand the relationship between the leaked information and the coverage as well as for a fair comparison,  $k$  amount of reads were sampled from the 24 different functional genomic experiments and from WGS and WES data of NA12878 (see SI Table 1). Genome Analysis Tool Kit (GATK) is used to call SNVs and indels with the parameters and filtering suggested in GATK best practices [?, ?]. The genotypes in 1000 genomes panel for NA1278 is used as the gold standard. We use “naive” pointwise mutual information (pmi) as a measure to quantify the association between the gold standard and the called variants. If  $S^{GS} = \{s_1^*, \dots, s_i^*, \dots, s_M^*\}$  is the set of variants from the gold standard and  $S^{FGE}(k) = \{s_1, \dots, s_i, \dots, s_M\}$  is the set of variants called from the  $k$  reads of a functional genomics experiment, then the set  $A = S^{GS} \cap S^{FGE}(k)$  contains the variants that are called and are in the gold standard set. If  $A = \{a_1, \dots, a_i, \dots, a_T\}$ , then

$$pmi(S^{GS}; S^{FGE}(k)) = - \sum_{i=1}^{i=T} \log_2(p(a_i)) \quad (3)$$

We then add  $k$  more reads to the sampled reads and repeat the calculation. This procedure is repeated till we deplete all the reads of a functional genomics experiment. Overall process is depicted in Figure ??e.

## 2.3 Private information leakage in 24 functional genomics experiment at different coverages

The pmi values for 24 functional genomics experiments are calculated at different coverages. These experiments involve whole genome approaches such as Hi-C, transcriptome-wide assays such as RNA-Seq and targeted assays such as ChIP-Seq of histone modifications and transcription factor binding. In addition, the pmi is also calculated for WGS, WES, and SNP-ChIP for comparison (Figure ??).

As expected Hi-C data contains almost as much information as WGS and more information than SNP ChIP arrays. WGS data contains more information than Hi-C in the beginning of the sampling process. As we sample nucleotides that are between around 1.1 and 10 billion bps, the information content of Hi-C surpasses the WGS data (Figure ??a). We speculate that this is due to better genotyping quality of the genomics regions that are in spatial proximity, as Hi-C has a bias of sequencing more reads from those regions. As expected, we cannot infer as much information from ChIP-Seq reads (Figure ??b). However, surprisingly many of the ChIP-Seq assays such as the ones targeting CTCF and RNAPII contain a great amount of information at low coverages. Furthermore, comparison between WES and different RNA-Seq experiments show that none of the RNA-Seq experiments contain as much information as WES, which is due to the fact that RNA-Seq captures reads only from expressed genes in a given cell (Figure ??c). The unexpected observation is that more information can be inferred from polyA RNA-Seq data at low coverages compared to WES and total RNA-Seq. To be able to make a fair comparison between all these assays, we calculate the pointwise mutual information per bp at the lowest coverages depicted in Figure ??a–c ( $pmi(S^{FGE}(k_{min}); S^{GS})/k_{max}$ ). We found that ChIP-Seq reads targeting CTCF contains even more information per basepair than WGS data at the lowest coverage we sample (Figure ??d).

## 2.4 Genotyping accuracy

In light of the above findings, in which genotyping can be done using low depth, biased functional genomics experiments, we assess the accuracy of genotyping by calculating the false discovery rate at different coverages. This also measures how much noise that each assay captures. The false discovery rate is defined as the ratio between the information obtained from the incorrectly called variants ( $h(S^{FGE} | S^{GS})$ ) and the information obtained from all the called variants ( $h(S^{FGE})$ ), namely

$$FDR(S^{FGE}(k)) = h(S^{FGE}(k) | S^{GS}) / h(S^{FGE}(k)) \quad (4)$$

Figure ??a shows that the false discovery rate for Hi-C data is lower compared to WGS data at lower coverages. We attribute it to the deeper sequencing of the genomics regions in close spatial proximity. Hence, sampling more reads from those regions at low coverages is more likely compared to uniform sampling of reads from WGS. ChIP-Seq data has comparable false discovery rate to WGS and Hi-C data, ChIP-Seq targeting CTCF having the lowest FDR (Figure ??b). We further find that assays targeting transcriptome such as WES and RNA-Seq produce the noisiest genotypes among all the assays, only around 10% of the called variants being the correctly called variants (Figure ??c).

## 2.5 Linking attack scenario

Linking attacks aim at re-identification of an individual by cross-referencing datasets (Figure ??a). For example, in an hypothetical scenario, the attacker aims at querying an individual's HIV status from his/her phenotype data. This phenotype data is released with the individuals' genotype information with an anonymized identifier for each individual. We assume that adversary obtains access to this dataset either lawful or unlawful means. Now let's assume that attacker has access to a biosample. This could be partial or complete mapped reads from functional genomics experiments or a saliva sample taken from a used glass. The idea is to do genotyping to

the biosample and find the matching genotype in the HIV status database. However, individuals share many common variants with each other. The number of shared variants between individuals is large within a racial population and even larger within a family. Then the question becomes how well an adversary has to sequence an individual's genome to be able to do successful linking. Specifically, adversary is interested in investigating whether noisy and incomplete reads from functional genomics experiments can be used as quasi-identifiers and how accurate the genotyping need to be in order to link individuals to databases.

For this, the attacker calls variants directly from the reads of anonymized functional genomic experiments. Then he/she compares the called noisy and incomplete genotypes to the genotype data panel and finds the entry with the highest pointwise mutual information. This reveals the sensitive information for the linked individual to the attacker. We then consider a scenario that the attacker has access partial or increasing amount of reads to find out when the data crosses the set point and becomes private.

Based on the pmi values of each experiment at different coverages, we define a metric for linking accuracy called  $gap_i$ . To calculate this metric, we first rank all the  $pmi(S^{FGE}(k); S^i)$  where  $S^{FGE}(k)$  is the set of called genotypes from the functional genomics experiment at total coverage  $k$  and  $S^i$  is the set of genotypes of individual  $i$  in the panel of genotypes.  $gap_i$  for each individual  $i$  at total coverage  $k$  is calculated as;

$$gap_i = \begin{cases} \frac{pmi(S^{FGE}(k); S^i)}{pmi(S^{FGE}(k); S^j)}, & \text{if } rank(pmi(S^{FGE}(k); S^i)) \leq 5 \text{ and } rank(pmi(S^{FGE}(k); S^j)) = 2 \\ 0, & \text{otherwise} \end{cases}$$

We then define that if  $gap_i$  is 0 for the individual  $i$ , whose functional genomics data is used, then the individual cannot be identified as there are other individuals in the panel that have the matching

genotypes. If  $0 < gap_i \leq 1$ , then the individual  $i$  might be vulnerable with auxiliary data such as gender or ethnicity, because he/she is in the top 5 matching individuals. If  $1 < gap_i \leq 2$ , then the individual  $i$  is vulnerable as we can identify him/her with 1 to 2 fold difference between him/her and the second best match. Lastly, if  $gap_i > 2$ , then the individual is extremely vulnerable with more than 2 fold difference between him/her and the second best match (Figure ??a).

We find that NA12878 is extremely vulnerable even at the lowest sampled coverages for Hi-C and RNA-Seq data (Figure ??b). More interestingly between around 1.1 and 10 billion basepairs, the Hi-C data exhibits higher linking accuracy than WGS data, consistent with the previous observation of pmi shown in Figure ??a. The total of coverage of ChIP-Seq data compared to Hi-C and RNA-Seq is quite low (SI Table I). However, the linking accuracy of ChIP-Seq is as good as Hi-C and WGS (Figure ??b), which shows extreme vulnerability of individuals with respect to release of such small amount of data. More strikingly, attacker can link NA12878 by using the reads of single-cell RNA-Seq data, which cover a small portion of the genome in a single cell (Figure ??d). We then added the variants of NA12878's parents to the 1000 genomes genotype panel and repeated the linking attack. We found that although NA12878 is still extremely vulnerable to re-identification in the presence of her parents in the database, the second best matching individuals are her parents (SI Figure 2). This shows that using the metric  $gap$ , an adversary can also identify individuals related to the target individual.

## 2.6 Individual's genome can be accurately approximated from publicly available data by imputation

To answer the question whether an attacker can correctly assemble an individual's variants by only using the reads from ChIP-Seq and RNA-Seq experiments, we impute variants by using IMPUTE2 [?, ?, ?] and the variants called from ChIP-Seq and RNA-Seq experiments. We then collected all the called and imputed variants in a set. Although imputed variants do not contribute

to the information due to high correlation with the called variants (SI Methods and SI Figure 3), total number of captured variants increases significantly (Figure ??a). By using shallow sequencing data of ChIP-Seq and RNA-Seq, we were able to call and impute variants almost as many as the gold standard variants.

We then ask the question if we can infer potentially sensitive phenotypes from these variants. Figure ??b shows a small set of example variants associated with physical traits such as eye color, hair color or freckles. Many of these variants are in the called set of Hi-C, ChIP-Seq and RNA-Seq data. Number of variants associated with traits further increases with imputation as expected.

## 2.7 Toy model for estimation of amount of leaked data without variant calling

Genotyping from DNA sequences is the process of comparing the DNA sequence of an individual to that of reference human genome. To be able to do successful genotyping, one needs substantial depth of sequencing reads for each base pair. According to the Lander-Waterman statistics for DNA sequencing, when random chunks of DNA is sequenced repeatedly, the depth per basepair follows Poisson distribution with a mean that can be estimated from the read length, number of reads and the length of the genome [?]. Since functional genomics experiments aim at finding highly expressed genes, TF binding enrichment or 3D interactions of the genome, it is expected that the sequencing depth per basepair does not follow the Poisson statistics. Thus, the genotyping using reads from functional genomics experiments is biased towards the variants that are in the functional regions of the cell types/lines of interest.

To this end, we hypothesized that the genotyping from the sequencing based functional genomics data depends on the average depth per base pair ( $\bar{d}$ ), the total fraction of the genome that is represented at least by one read, also called the breadth ( $b = \sum_{i=1}^N [d_i \geq 1]$ ),  $N$  is the total number of

nucleotides in the genome) and a parameter  $\beta$  that estimates the sequencing bias, i.e. how much the distribution of depth per basepair deviates from the Poisson distribution (Fig. ??c). The bias parameter  $\beta$  is composed of two terms: (1) the negative bias  $\beta-$  and (2) the positive bias  $\beta+$ . Negative bias estimates if there is an increase in the number of low depth basepairs relative to mean with respect to expected Poisson distribution and the positive bias estimates the increase in the number of high depth basepairs (see SI for more details).

To quantify the genotyping from the functional genomics data, we used “naive” normalized pointwise mutual information (npmi). It takes into account the information from the correctly identified genotypes ( $pmi(S^{FGE}; S^{GS})$ ), the information missed that is in the gold standard ( $h(S^{GS} | S^{FGE})$ ) and the information from the incorrectly identified genotypes, i.e FDR ( $h(S^{FGE} | S^{GS})$ ) as;

$$npmi(S^{FGE}; S^{GS}) = \frac{pmi(S^{FGE}; S^{GS})}{h(S^{FGE}, S^{GS})} = \frac{pmi(S^{FGE}; S^{GS})}{h(S^{GS} | S^{FGE}) + pmi(S^{FGE}; S^{GS}) + h(S^{FGE} | S^{GS})} \quad (5)$$

With the assumption of  $npmi(S^{FGE}; S^{GS}) = f(\overline{d_{FGE}}, b_{FGE}, \beta_{FGE})$ , we used Gaussian Process Regression (GPR) [?] to fit 40 training data points and achieved a root mean square error (RMSE) of 0.06 with the values ranging between [0,35] (Fig. ??d). 5 separate data points were used as test set and an RMSE of 0.07 was achieved (Fig. ??d), see SI for more details). The regression learning is performed using 10 fold cross-validation to protect against overfitting. This toy model represents a proof of concept suggesting a theoretical framework for the estimation of amount of leaked data from functional genomics experiments without the need of performing time-consuming genotyping calculations.

## 2.8 Unique combination of common variants contribute significantly to the information leakage and linking accuracy

We next analyze whether a linking attack can be prevented by removing rare variants from the datasets as their contribution to the information is the highest. We first speculated that the removal of the variants that are unique to NA12878 might be enough to fail at linking. A total of 11,472 variants along with their genotypes are only observed in NA12878, which we refer as set I (Fig. ??a). After the removal of set I variants from the NA12878 variant set, we calculated the  $gap_{NA12878}$  and surprisingly found that linking accuracy is affected minimally compared to using the all of NA12878 variants (set 0, Fig. ??b). We then created another set (set II, Fig. ??a), that includes the variants that are observed in NA12878's genome as well as one more individual in the 1000 genomes genotype panel (total of 16,305 genotypes). We again found that individual is extremely vulnerable to linking attacks ( $gap_{NA12878} > 2$ , Fig. ??b). We then relaxed our cut-off further to remove the variants that are observed in NA12878's genome as well as at most 1.5% of the population (set III, total of 124,093 genotypes, Fig. ??a). This also did not affect the overall linking ( $gap_{NA12878} > 2$ , Fig. ??b).

The genotypes in these 3 sets are rare in the 1k genome panel. They are observed in 64 or less individuals including NA12878. A practical solution to the re-identification problem using functional genomics data would be masking or removing such rare genotypes from the reads. However, as iteratively shown here that although rare variants are extremely informative and sufficient enough to do re-identification through linking attacks, their removal is not sufficient to fail at re-identification. That is, not only the rare genotypes but also the unique combination of common genotypes are identifiers of genetic make-up of an individual. To further support this calculation, we added the genotypes of the parents of NA12878 to the panel and found that linking is still successful with an extreme vulnerability ( $gap_{NA12878} > 2$ , SI Fig.2).



We then analyze the contribution of small indels to the naive information and whether accurate linking is possible when we remove all the single nucleotide mutations from the data and keep the indels. Fig. ??c shows the information contribution of the indels. Although naive pointwise mutual information from indels are much smaller compared to single nucleotide mutations, a high linking accuracy can be achieved by using only indels even at small coverages (Fig. ??d). This linking attack is done using the most noisy data set we have (total RNA-Seq) to make linking more difficult.

## 2.9 Privacy-enhancing file formats for functional genomics experiments

After discovering neither common variants nor indels can be publicly shared, we seek for ways to share the mapped reads of functional genomics data. The purpose is to share maximum amount of information with minimum utility lost while maintaining the individual’s privacy. As a privacy metric, we aim to prevent leakage of any variants as well as any quasi-identifier that can lead to identification of position of variants in the genome. For utility measure, we used the following equation:

$$U = \frac{\bar{d} - RMSE}{\bar{d}} \quad (6)$$

In Eq. 7,  $\bar{d}$  is the mean number of reads that overlap with a basepair or with a functional unit such as exons.  $RMSE$  is the root mean square error between the real depth and the depth after distorting the file to make it private. For a genome with  $N$  number of basepairs or exons, it can be calculated as:

$$RMSE = \frac{\sum_{i=1}^N |d_i - d_i^p|}{N} \quad (7)$$

$d_i$  is the real number of reads for  $i$ th basepair, whereas  $d_i^p$  is the number of reads obtained from the distorted file.  $U$  measures the percentage of the depth per bp that are correctly reported on the privatized file format, while  $RMSE$  is the mean difference between the depth of a nucleotide between privatized and original files. According to  $U$  and  $RMSE$ , the high depth regions of genome,

i.e. the functional regions, will be penalized more if the new file format reports the depth different than the original depth, while the low depth regions such as low expression genes will be penalized less. As the purpose of functional genomics experiments is to annotate functional genome, the utility metric measures the quality of the annotation when the analysis tools are performed using privatized file formats.

The reads from the SAM/BAM/CRAM files are categorized as perfectly mapped reads that includes also intronic reads and reads with mismatches, insertions, deletions, soft- and hard-clipping. We remove the sequence of the reads and keep mapping quality, start coordinates, fragment lengths and flags related to mappability of the reads while adjusting the cigar and alignment scores such that leakage of variants are masked (Fig. ??). The details of how new file format deals with reads are reported in detail in SI Methods with a detailed figure (SI Fig. 3).

Such treatment of **reads** introduce noise to the signal profiles especially with deletions since the start coordinates and total length of the fragments are unchanged (Fig. ??a-b, see SI Methods and SI Figure 4). However, our utility analysis showed > 99.9% accordance with the original depth of the nucleotides. As can be seen from the scatter plots, noise is mostly introduced to basepairs with low depth (Fig. ??c-d). We call this file format pSAM/BAM/CRAM. The pBAM file format contains the necessary information to be used in functional genomics pipelines such as gene expression quantification and transcription factor binding peak calling. We then create a “.diff” file that contains the information that are distorted in the pBAM files, except the sequence of the reads. Instead of reporting the entire sequence of a fragment, we reported the nucleotides that are different than the reference sequence (see SI). “.diff” files are private files that require special permission for access. The advantage of locking up the “.diff” files instead of the entire BAM files is that they are smaller in size, hence it is easier to store and move these files. A user is able to reach the original BAM file when they have access to the .diff file and a script can convert pBAM

+ .diff + reference genome into the original BAM file (Fig ??b).

### 3 Discussion

Functional genomics experiments provide large amount of biological data. These are large-scale, high-throughput assays based on sequencing. Although they aim at answering questions related to genomic activities such as gene expression, TF binding or 3D organization of genome, public sharing of sequencing data from these experiment can lead to recovery of genotype information and in turn raise privacy concerns. However, the systematic quantification of private information content of the functional genomics BAM files and open access to such data without compromising individuals' identity have not been well studied. Current policies regarding to public sharing of functional genomics BAM files are ad-hoc. The experiments that require high depth of sequencing such as Hi-C and sometimes RNA-Seq are considered to be private, while relatively low depth BAM files such as those from ChIP-Seq are often shared publicly. In this study, we derived information theory based measures to systematically quantify the sensitive information leakage in the BAM files of functional genomics experiments in low and high depth experiments.

Instantiating of linking attacks by doing genotyping to partial or complete functional genomics data showed that even at low coverages of low depth experiments such as ChIP-Seq, linking individuals to the databases can be done without error. When we compare the linking accuracy to the false discovery rate, we found that it is easier to link individuals to the databases than genotyping them accurately using functional genomics experiments. The implication is that noisy quasi-identifiers, i.e bad quality SNP calling, can be used to link the data to the high quality genotypes. For example, according to our calculations, reads from single-cell RNA-Seq data carry the most amount of noise. This is likely due to the bias towards expressed genes in such small amount of cells, mapping issues of splice sites, false positives from RNA editing sites and amplification bias.

However, the noisy genotypes called from small amount of cells, even when the number of reads are only a million, are quasi-identifiers that result in very high linking accuracy. This is worrisome in terms of biomedical data sharing as the number of individuals in genotype databases is increasing exponentially with the decreasing cost of sequencing. Furthermore, rich information about an individual's identity and his/her sensitive phenotypes can also be inferred by combining the reads from low depth functional genomics experiments and through genotype imputation.

Analysis and interpretation of functional genomics data start with the alignment of sequencing products obtained from functional regions of genome to the reference human genome. In this study, we showed that even a small portion of these reads leaks great amount of sensitive information. SNPs, small indels and structural variants can be identified using these reads. This marks a set point in the data processing chain, where below the set point data contains sensitive information and cannot be shared, whereas above set point the data is freely available (Fig. ??). Although the idea of set point is effective in sharing data without compromising individual's identity, in practice it does not work due to couple of reasons. Firstly, summary data such as signal profiles or gene expression levels are also shown to be strong quasi-identifiers that leak sensitive information. Secondly, functional genomics experiments advanced our understanding of health and disease by revealing function of the genome under different conditions. The quantification, analysis and the interpretation of functional genomics data are still an evolving field, hence extensive public sharing of functional genomics data accelerate collaborative research and reproducibility by removing the complexities associated with data accession procedures.

In order to overcome bottlenecks related to data sharing and answer privacy-concerns, researchers proposed solutions such as differential privacy [?, ?, ?]. Differential privacy solutions often suppress and add noise to the data, so that the dataset retrieves the same result with and without the individual's information in it [?]. Although, such solutions can be applied to the genotype databases

by removing the rare variants in order to prevent linking, we also showed that the unique combination of common variants can be used as strong quasi-identifiers. However, solution becomes less complex for sharing raw reads from functional genomics experiments as the main purpose of functional genomics data is not related to genotypes, but rather annotation of functional regions of the genome under different conditions such as cancer. Therefore, privacy-preserving solutions for sharing raw reads of an individual's functional genomics data can dismiss the issue of accurate sharing of genotypes and focus on the utility of the data in terms of annotation of functional genome.

To this end, inspired by the essence of differentially private solutions, we propose means to share raw reads from functional genomics data without comprising individual's sensitive information. We created privatized data formats, in which the functional annotation of genome is as accurate as possible with and without the genotype leakage from the reads. We showed that we can accurately compute the signal profiles and gene quantifications with more than 99% recovery. This new file format called pBAMs enable researchers to share the mapped reads, which are largest data product of functional genomics experiments. To easen the challenges associated with moving and storing of large special access files, we created light-weight .diff file format that consists of the differences between pBAM and BAM files in a compact format. This allows us not to repeat the sequence information in the human reference genome files in .diff files and reduces the size of the private files significantly. [\[\[GG2MG: I will add "ENCODE uses these files" after we have the call with them\]\]](#)

Presented framework can be used to quantification of sensitive information from the raw reads of functional genomics experiments and conversion of raw files to privacy-preserving file formats. We address the most obvious leakage and provide solutions for quick quantification and safe data sharing. However, it is useful to review all the sources of information leakage from func-

tional genomics experiments. For example, the next source of leakage is from the signal profiles in RNA-Seq, which was addressed elsewhere [?]. There is also leakage from gene expression quantifications, which was shown to be connected with variants through the eQTLs [?]. We also anticipate more leakages to be discovered as new functional genomics experiments are developed. Combined with the increasing attention to genomic privacy, we expect future studies will lead to novel privacy-preserving solutions in an open data sharing mode.

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## List of Figures

1	stack . . . . .	28
2	comparison and procedure . . . . .	29
3	pmi for all . . . . .	30
4	FDR . . . . .	31
5	linking and gap . . . . .	32
6	imputation, phenotype inference and theoretical framework . . . . .	33
7	differential privacy . . . . .	34
8	pBAM . . . . .	35

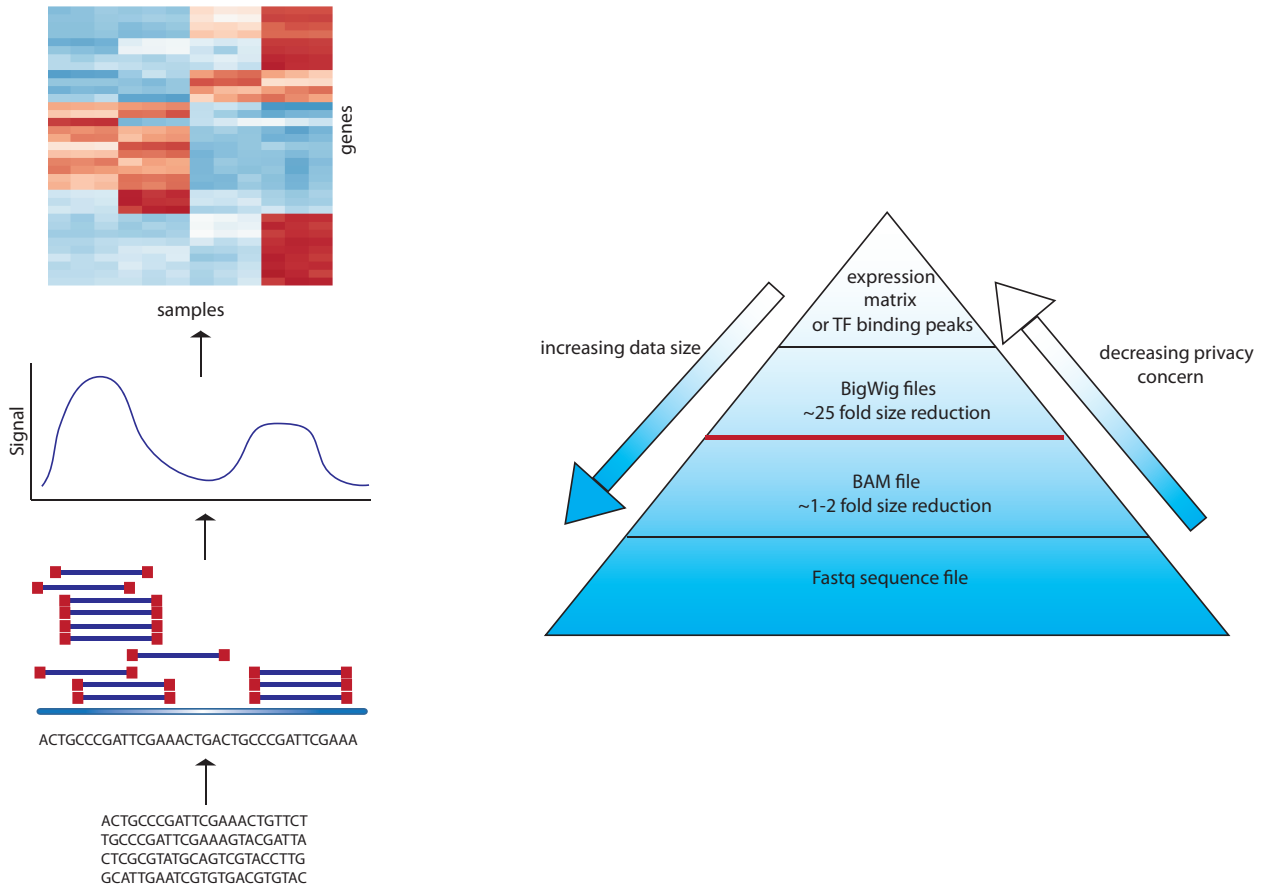
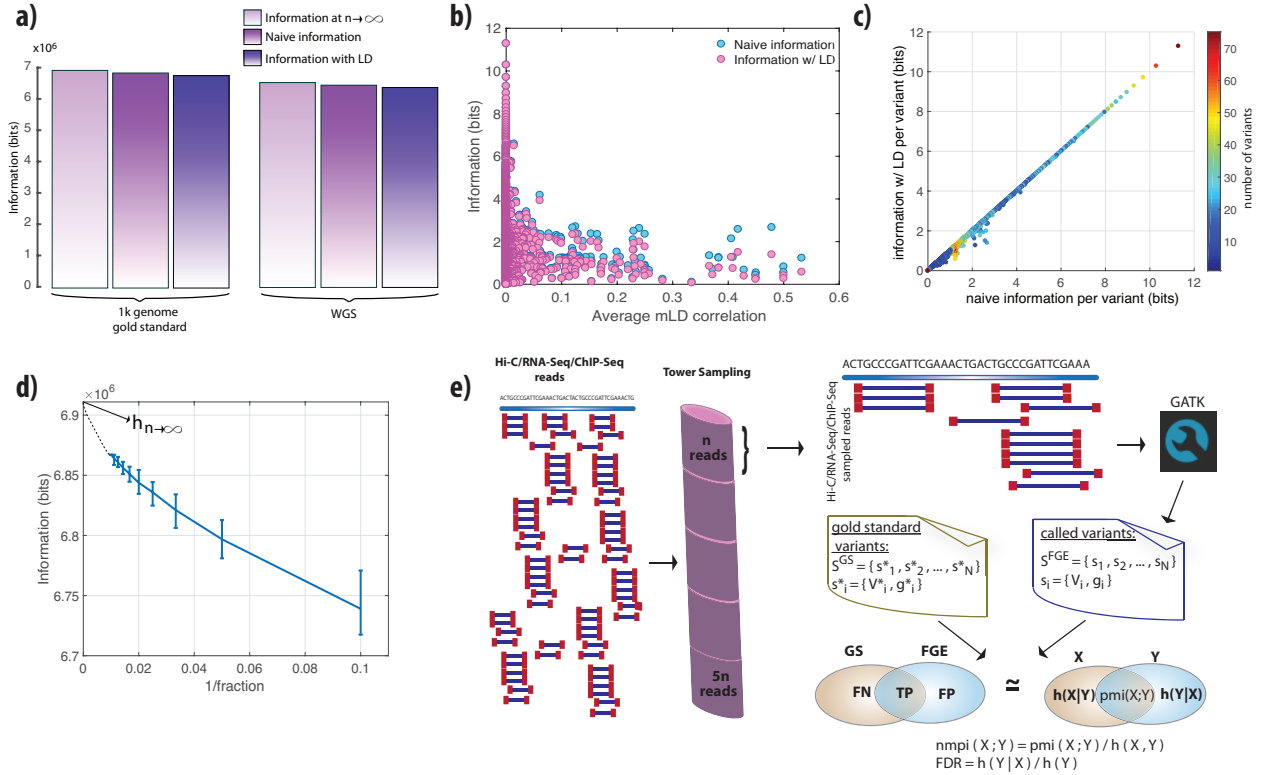
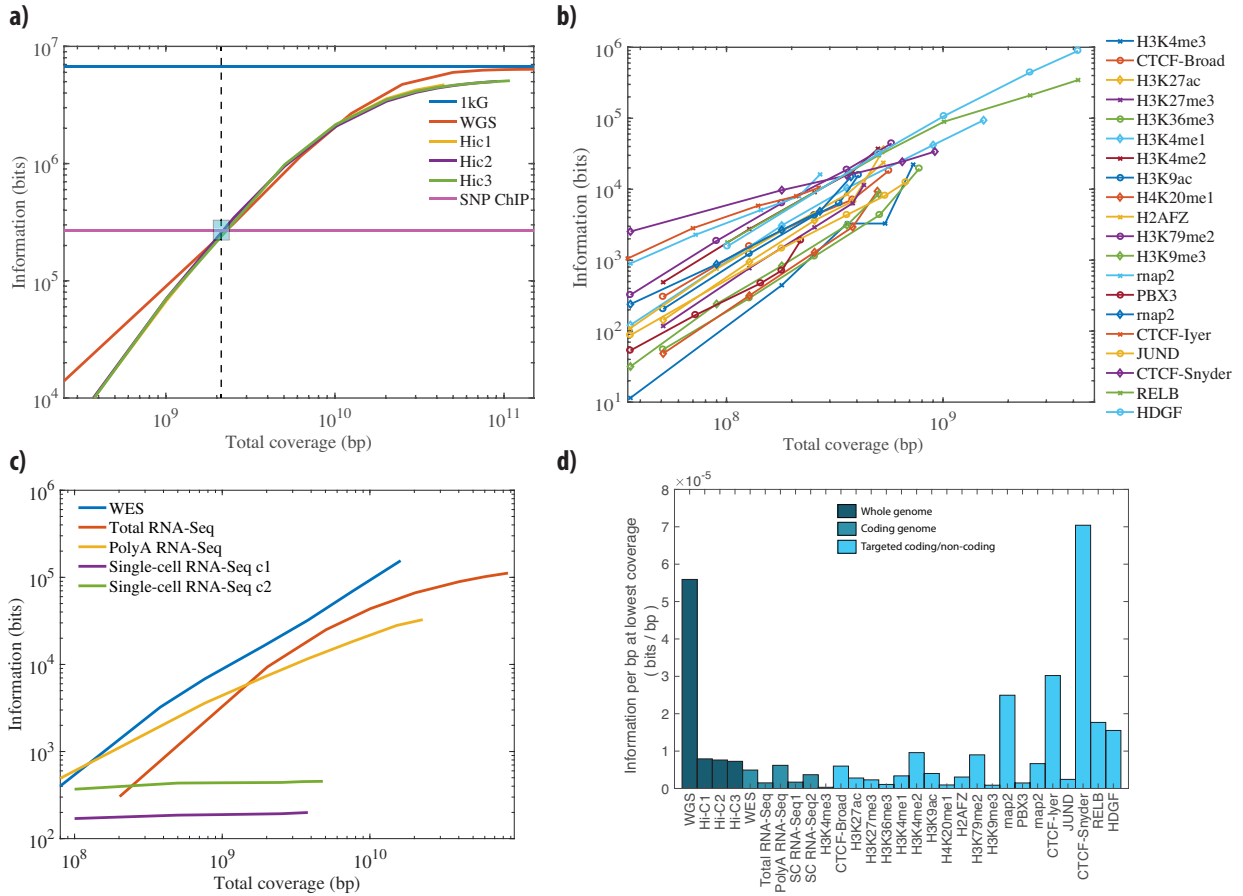


Figure 1: **Schematic of data types from functional genomics experiments.** (a) The flow for RNA-Seq data processing from mapped reads to the gene quantifications. (b) Different layers of produced data from RNA-Seq pipeline. Red line denotes the set point, where privacy concern vanishes afterwards.



**Figure 2: Comparison of naive information measure with information with LD consideration and sample size correction.** (a) Difference between the naive information, information with LD consideration and extrapolated information when population size is infinite. (b) The maximum LD score for each variant are averaged over per information and plotted against information. Highly informative variants do not exhibit difference when information is calculated using naive approach vs. with LD consideration. (c) Naive information vs. information with LD consideration per each variant in an LD block. Only low information variants show slight difference between two approaches. (d) Naive information vs. inverse fraction of the data sampled from the 1000 genomes population. y-intercept is extrapolated from the fitted curve and denotes the information when the population size is infinite. Error bars are calculated using  $100\times$  bootstrapping. (e) The process of sampling reads from functional genomics experiments for the calculation of pointwise mutual information between 1000 genomes gold standard variants for NA12878 in different coverages.



**Figure 3: The pointwise mutual information calculated for 24 different functional genomics assays and WGS, WES and SNP ChIP data using NA12878 1000 genomes variants as gold standard.** (a) The pmi values for WGS and three different primary Hi-C experiments plotted at different coverages. The information contents of the gold standard (1kG in blue) and SNP ChIP (in pink) are added for comparison. (b) The pmi values for 20 different ChIP-Seq experiments targeting histone modifications and transcription factor binding plotted at different coverages. (c) The pmi values for WES, total RNA-Seq, polyA RNA-Seq and single-cell RNA-Seq from two different cells plotted at different coverages. (d) The pmi values per basepair plotted using the lowest total coverage for all the assays.

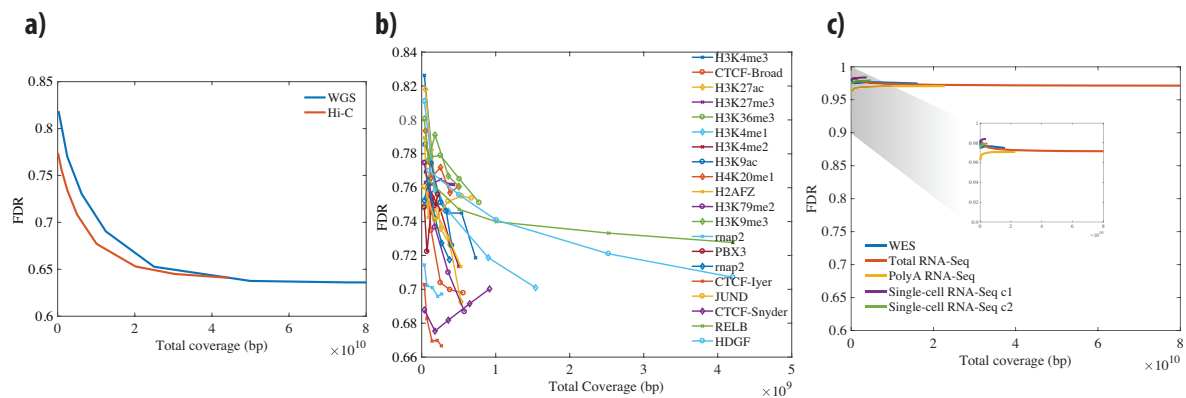


Figure 4: **False discovery rate of functional genomics experiments at different coverages (a)** FDR comparison for Hi-C and WGS data at different sampled coverages. **(b)** FDR comparison for different ChIP-Seq experiments at different coverages. **(c)** FDR comparison for WES and different RNA-Seq experiments.

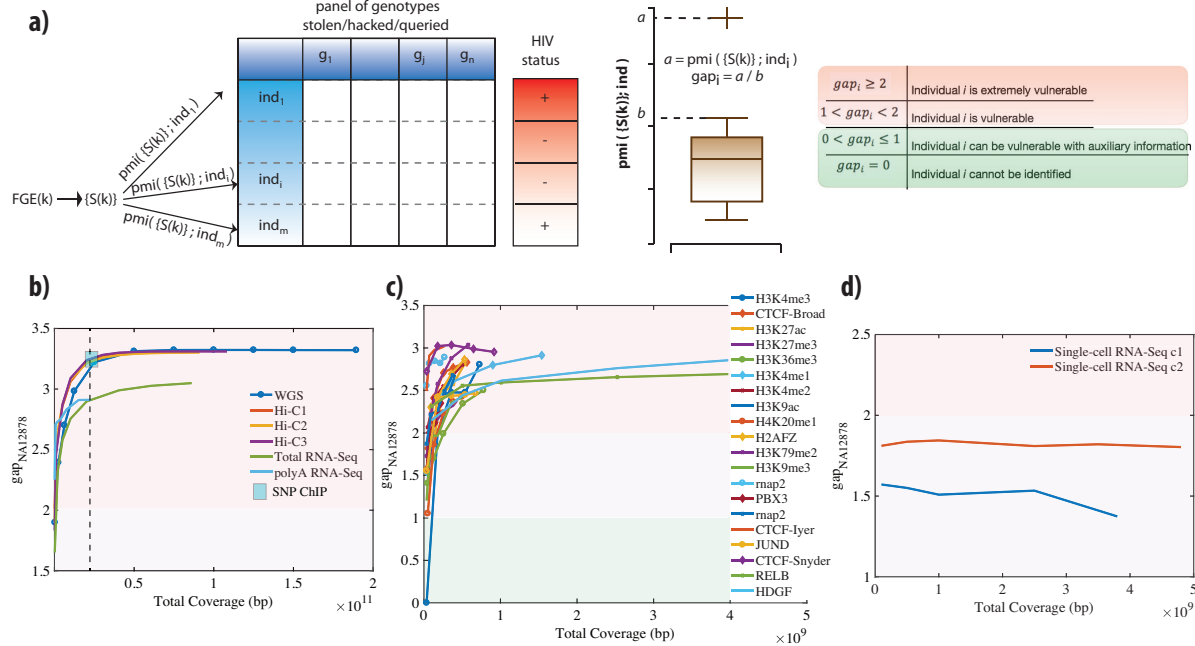
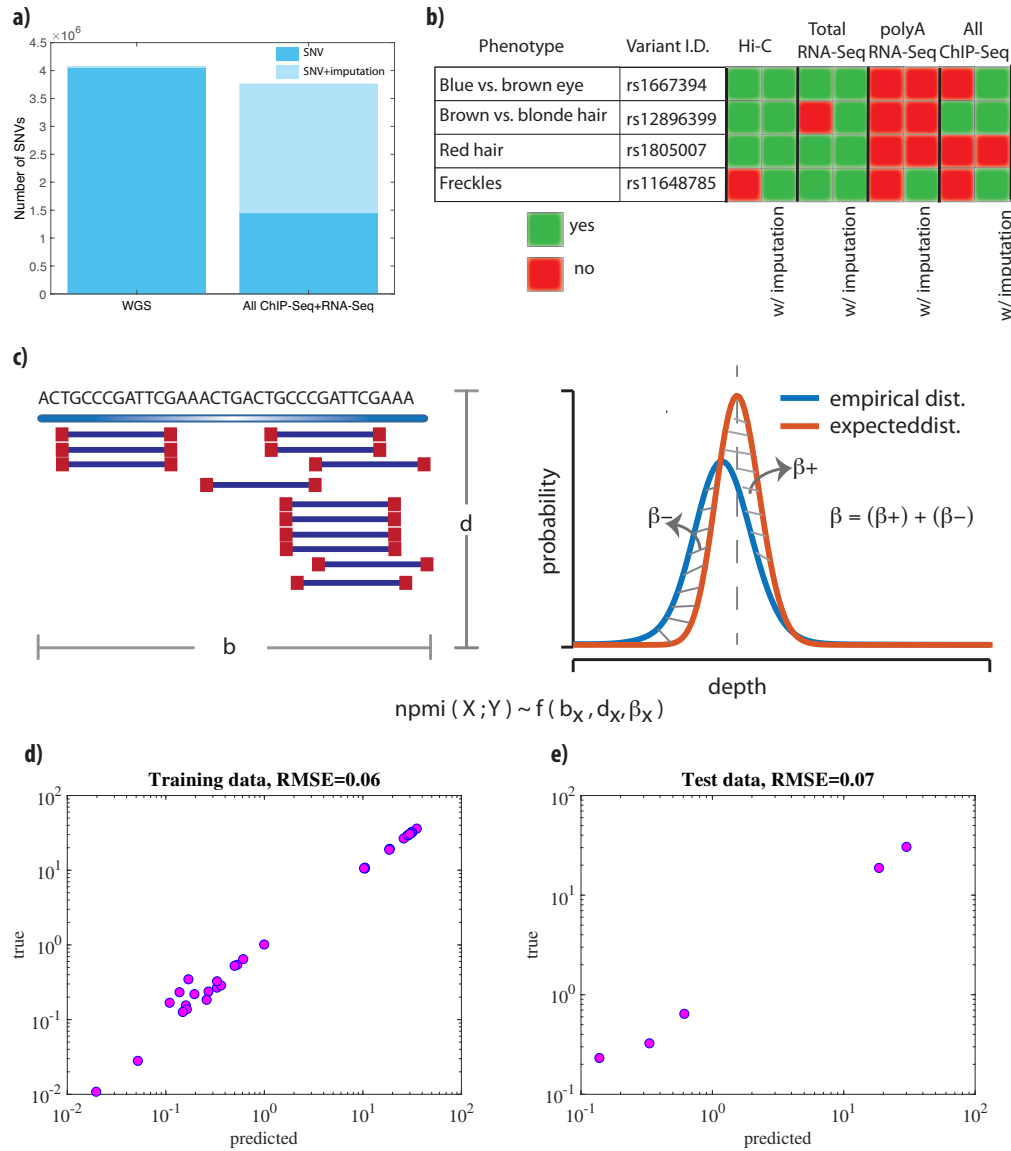


Figure 5: **Illustration of a linking attack and the accuracy of linking.** (a) The publicly available anonymized reads from functional genomics experiments contains a set of variants and HIV status for the sample that the functional genomics experiment was performed at increasing coverages. The panel of genotypes contains the variants and associated genotypes for  $m$  individuals. The attacker links the inferred variants and genotypes to the panel of genotypes by using the best matched pointwise mutual information. The linking potentially reveals the HIV status for the linked individual. (b) Comparison of  $gap$  for NA12878 at different coverages for Hi-C and Total/PolyA RNA-Seq reads. WGS and SNP-ChIP are also added for comparison. (c) Comparison of  $gap$  for NA12878 at different coverages for 20 different ChIP-Seq experiments. (d) Comparison of  $gap$  for NA12878 at different coverages for single-cell RNA-Seq experiments.





**Figure 6: Individual's genome can be approximated and sensitive phenotypes can be inferred from publicly available data by imputation and a theoretical framework for prediction of amount of leaked data (a) Number SNVs called from WGS data and all of the ChIP-Seq and RNA-Seq data together with and without imputation. (b) Variants associated with physical traits and if they present in the called variants from different functional genomics experiments before and after imputation. (c) Features of the theoretical framework - write more. (d) Accuracy of fitted model on training set- write more (e) Accuracy of fitted model on test set - write more**

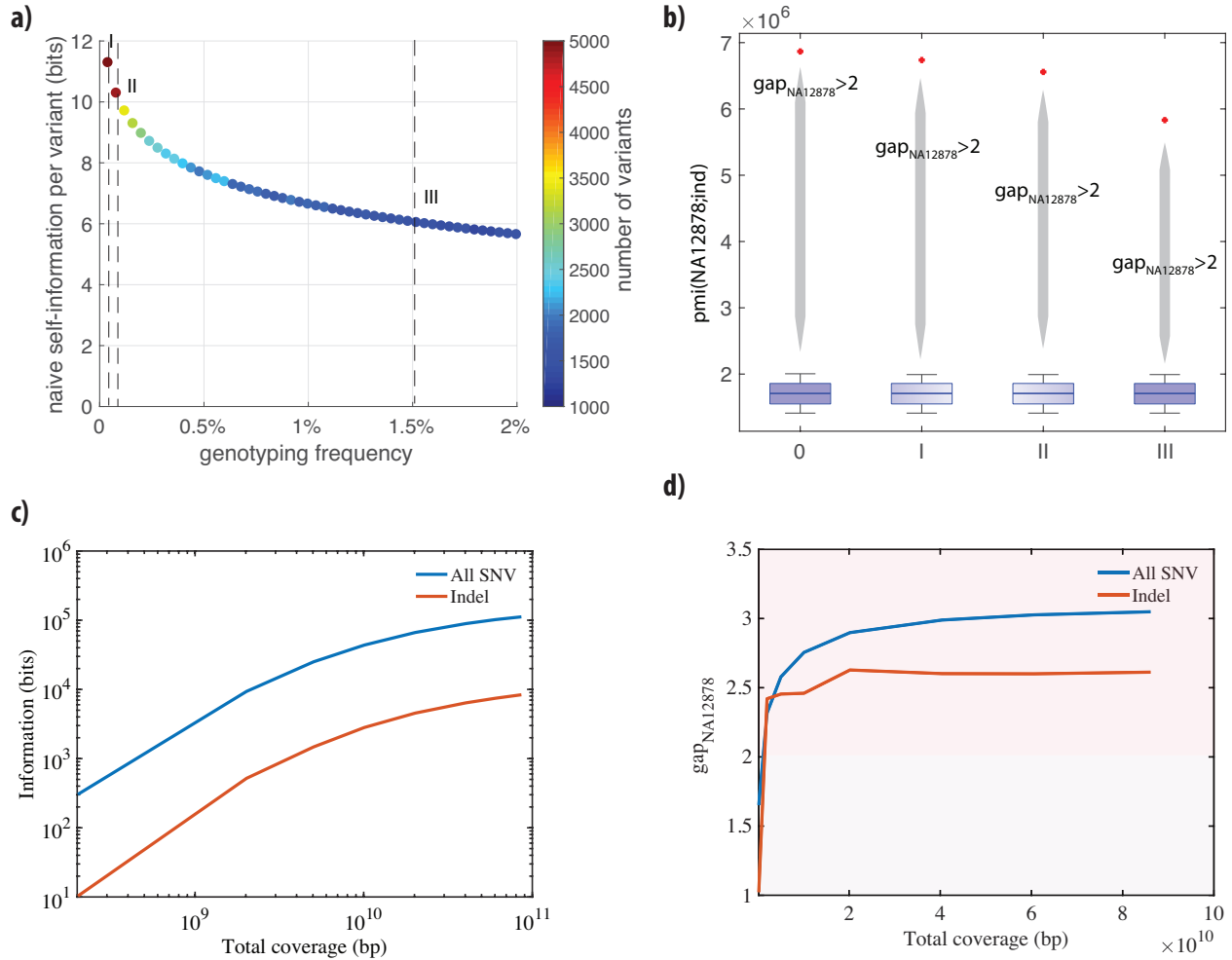
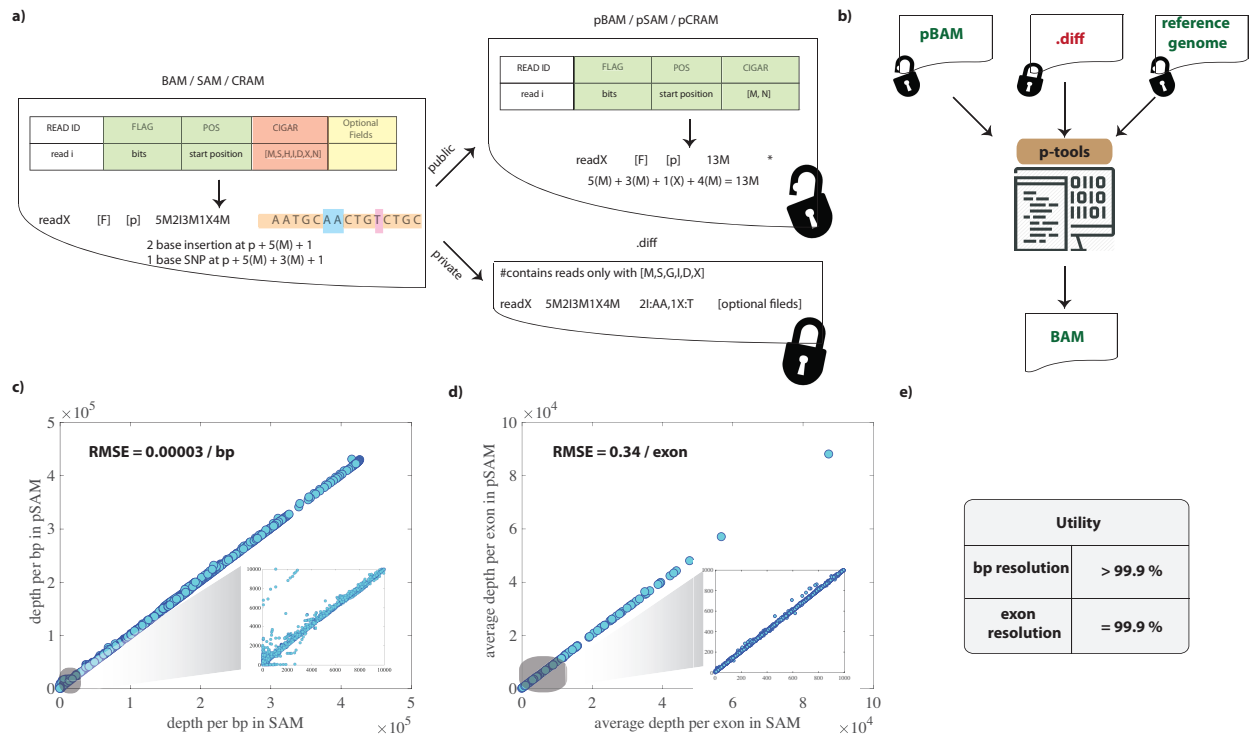


Figure 7: **Removal of rare variants and linking** (a) Information of the variant before and after addition of NA12878 to the population. We iteratively removed variants from the set as (I) only the variants that is only NA12878 specific, (II) the variants that have an information of 11 or higher bits after removal of NA12878 from the population, (III) the variants that have an information of 6 or higher bits after removal of NA12878 (b) Linking accuracy for every iteration of removal of NA12878 variants from the set. (c) Information of all the variants that are called from Total RNA-Seq reads vs. the information of the indels that are called from Total RNA-Seq reads. (d) Linking accuracy when we consider all the variants that are called from Total RNA-Seq reads vs. the linking accuracy when we consider only indels called from Total RNA-Seq reads.



**Figure 8: Privacy-preserving file formats for mapped reads** (a) The generation of public pSAM and private .diff files. (b) Schematic of how to go between pBAM and BAM formats by utilizing the human reference (c) Comparison of number of reads for each basepair in the original SAM file and the distorted pSAM file. Noise is mostly introduced to basepairs with low depth. (d) Comparison of number of reads for each exon in the original SAM file and the distorted pSAM file. Noise is mostly introduced to exons with low expression.