hepg2-foxa2-imbalance-details.md

Details of imbalance analysis of HepG2-FOXA2 ChIP-seq

Here we'll consider studying allelic imbalance in five HepG2-FOXA2 ChIP-seq datasets.

Sample	Read Count (non-dup)	Duplication Rate	Peaks	FRiP
`ENCODE_HepG2_FOXA2_1`	7,387,781	57.81 %	15,646	6.91 %
`ENCODE_HepG2_FOXA2_2`	29,695,447	11.79 %	54,430	16.60 %
`2018_HepG2_FOXA2_1`	15,278,836	8.13 %	27,037	8.57 %
`2018_HepG2_FOXA2_2`	16,463,009	7.15 %	21,266	6.56 %
`2018_HepG2_FOXA2_3`	8,153,199	74.97 %	40,997	10.05 %

Effect sizes

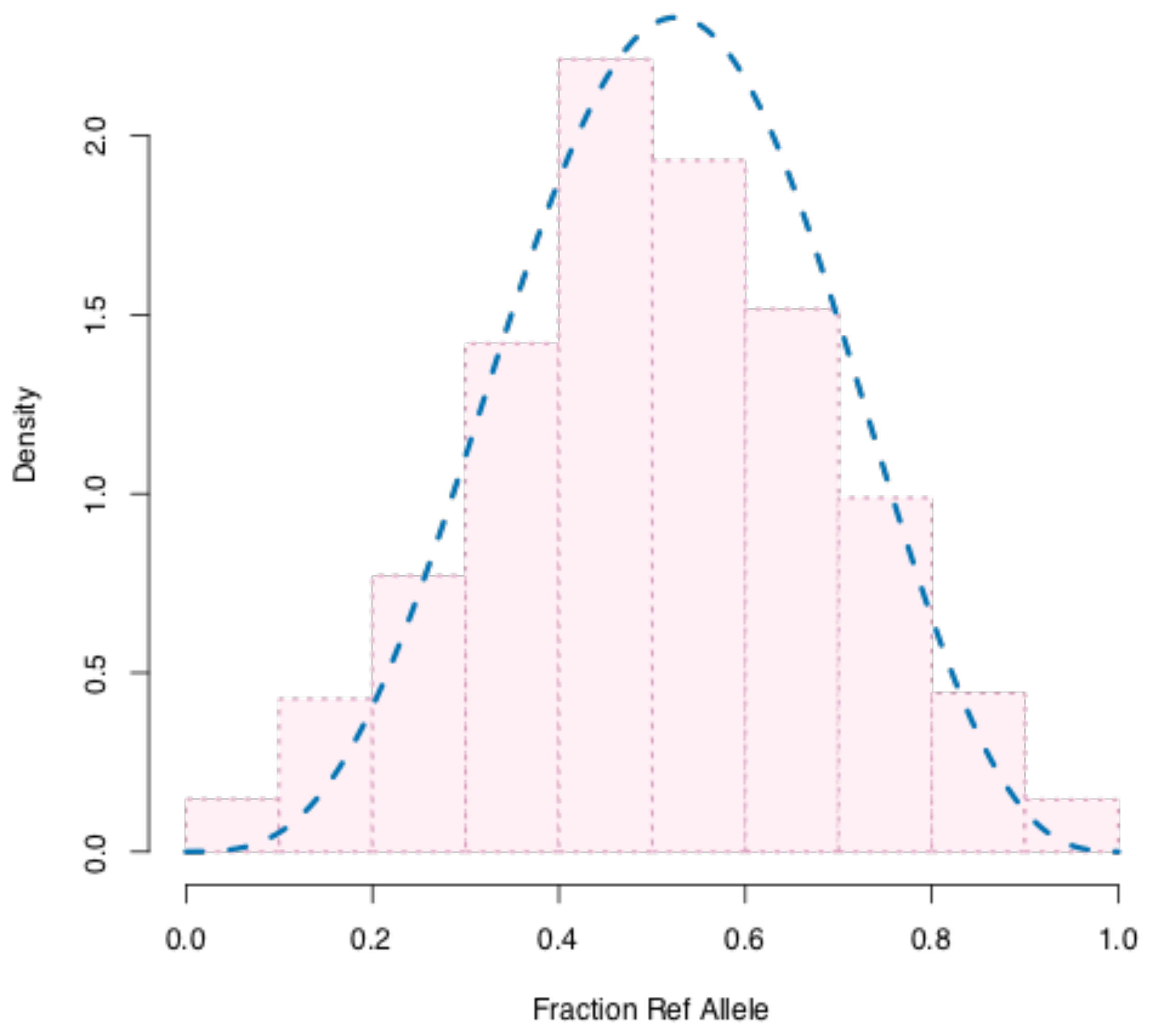
Beta-binomial fit

After aligning reads, identifying heterozygous SNPs with QuASAR, and correcting reference bias with WASP, we fit a beta-binomial distribution to the data using the method of Chen et al.. That method fits only the "overdispersion" parameter of the beta-binomial distribution. We extend it to also fit the "mean" parameter. This allows for a slightly improved fit that can account for a small residual reference bias that may persist after running WASP.

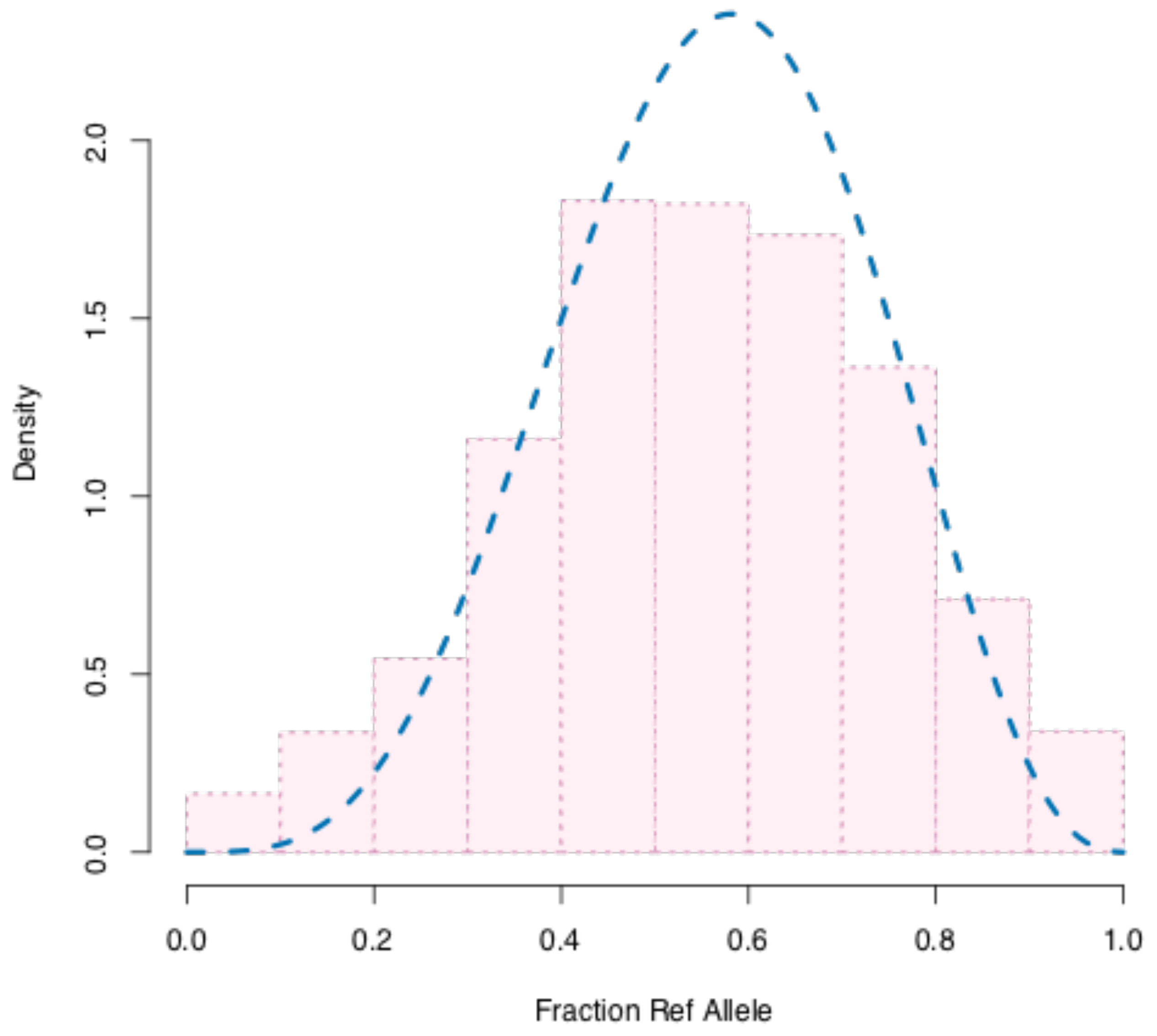
(While the authors of that paper used an overdispersion/mean parametrization of the beta distribution, we'll use the α/β shape parametrization from here on.)

The key component of the beta-binomial fit is the beta prior distribution, illustrated here:

> Histogram of observed ref fraction with estimated beta prior for the 2018 datasets (combined). The best-fit Beta parameters were α = 4.676, β = 4.324. Heterozygous SNPs with 10-fold coverage or greater were used for the fit.



Histogram of observed ref fraction with estimated beta prior for the ENCODE datasets (combined). The best-fit Beta parameters were α = 5.063, β = 3.938. Heterozygous SNPs with 10-fold coverage or greater were used for the fit.



Note that the distribution estimated for the ENCODE datasets is shifted to the right compared to the estimte for the 2018 datasets. This suggests that the underlying distributions of the two groups are different (possibly due to differences in experimental protocol, sequencing platform, etc.), so we treat them separately for the next step.

Bayes estimator of ref allele fraction

This beta distribution provides a prior model for the reference allele fraction at any given SNP. It will be the basis for a bayesian estimate of the imbalance effect size.

Specifically, we compute for each SNP a bayes estimator of the "true underlying" reference allele fraction, using the beta parameters and the observed allele counts:

$$\hat{p}_i = \frac{\alpha + x_i}{\alpha + \beta + n_i}$$

Where *x* and *n* are, respectively, the reference allele count and total coverage at the *i*'th SNP.

Log fold change

Following (Mohammadi e al., we convert our estimate of the ref allele fraction to log fold change. We also add a shift term which accounts for the small residual reference biases we have observed. The resulting formula is:

$$LPAFC_i = \log \frac{1 - \hat{p}_i}{\hat{p}_i} - \log \frac{\beta}{\alpha}$$

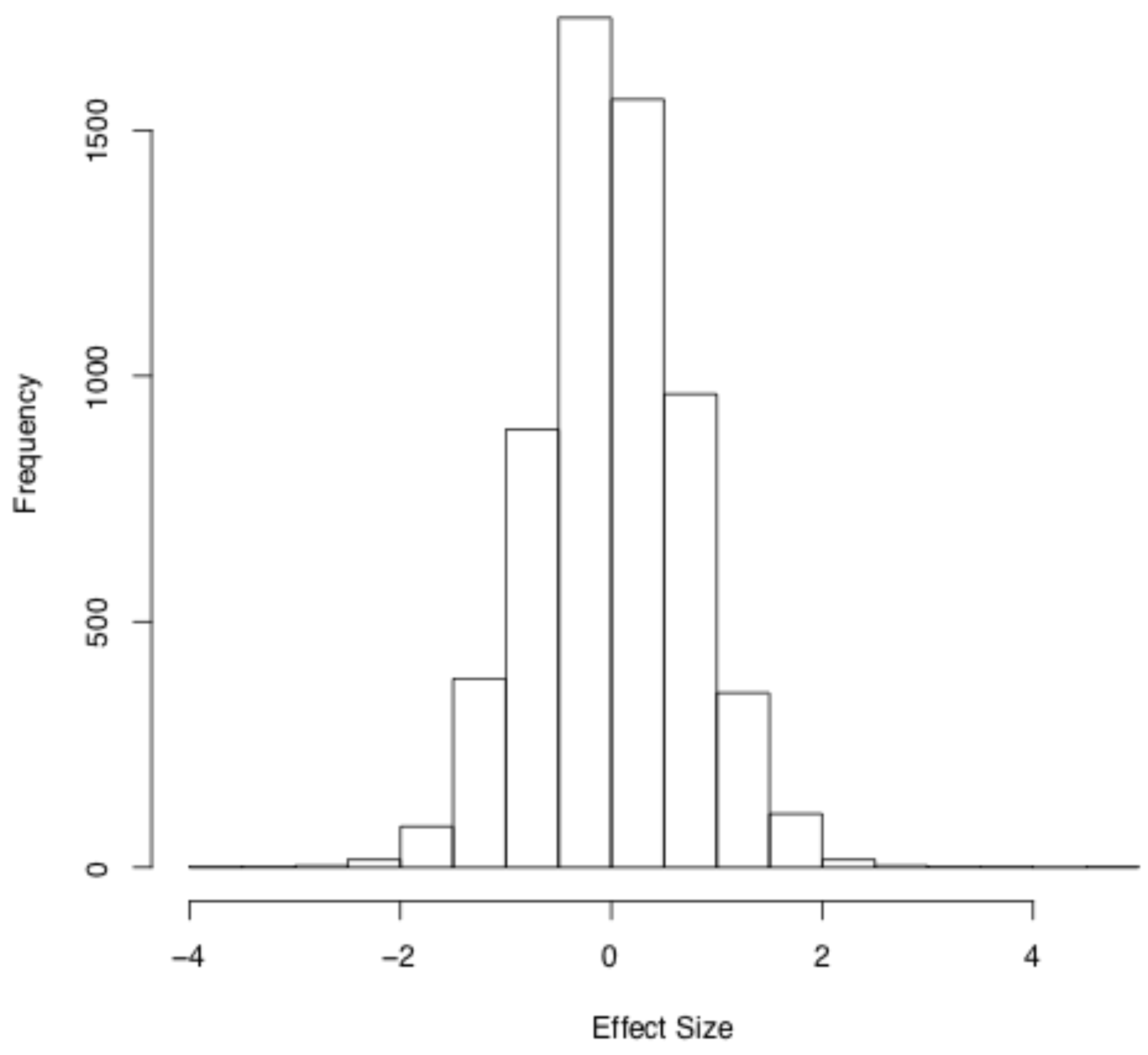
or, expanded:

$$LPAFC_i = \log \frac{\beta + n_i - x_i}{\alpha + x_i} - \log \frac{\beta}{\alpha}$$

We call this value "log posterior allelic fold change", since it is based on a bayesian posterior estimate of the ref allele fraction.

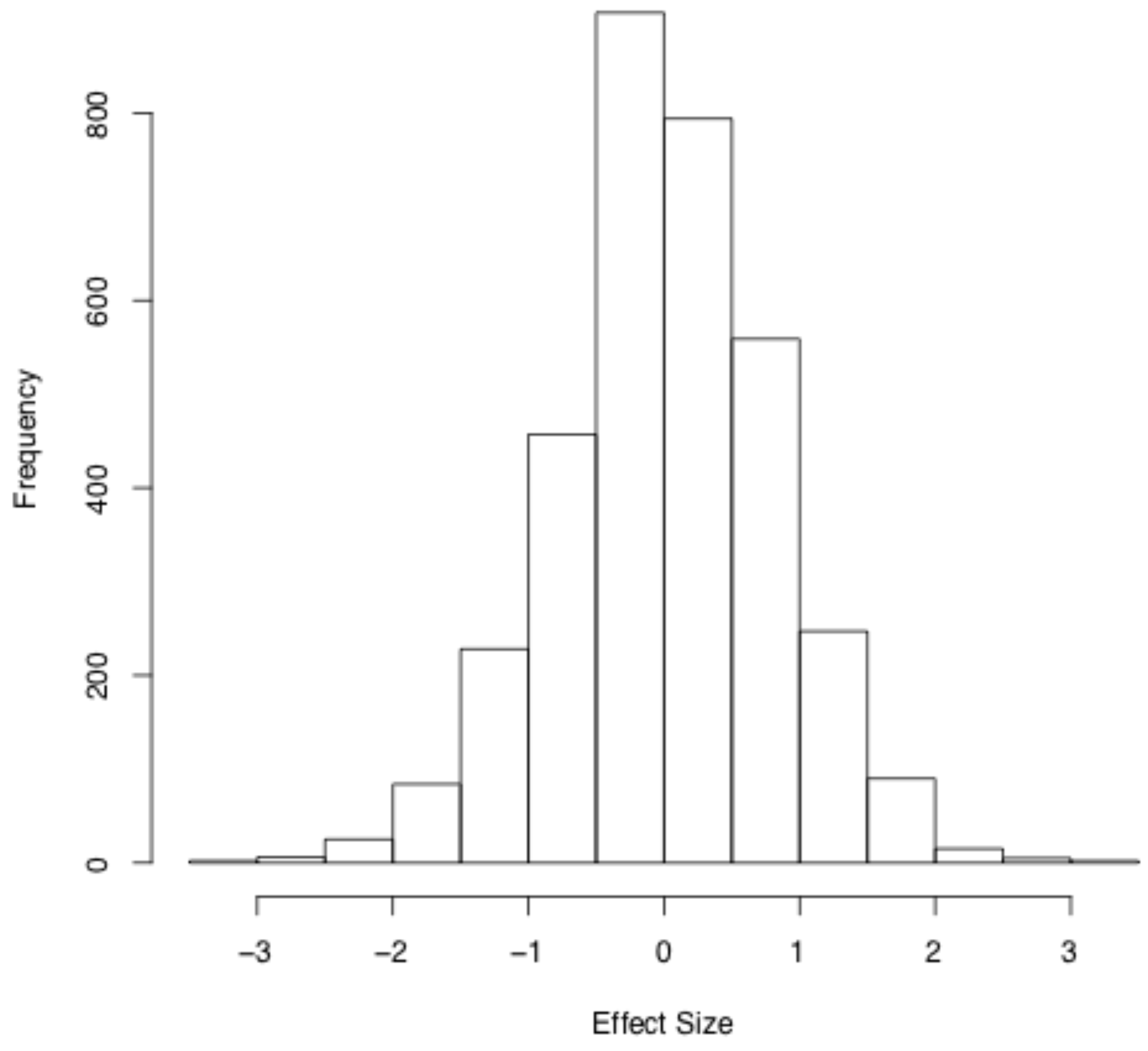
Once these effect sizes are computed, we can check on how they are distributed in the 2018 and ENCODE data. Note that in both cases the effects are centered on zero, thanks to the shift term.

> Histogram of estimated effect sizes in 2018 data (SNPs with > 10-fold coverage)
>
> x-axis: effect size (LPAFC), y-axis: number of SNPs



Histogram of estimated effect sizes in ENCODE data (SNPs with > 10-fold coverage)

x-axis: effect size (LPAFC), y-axis: number of SNPs



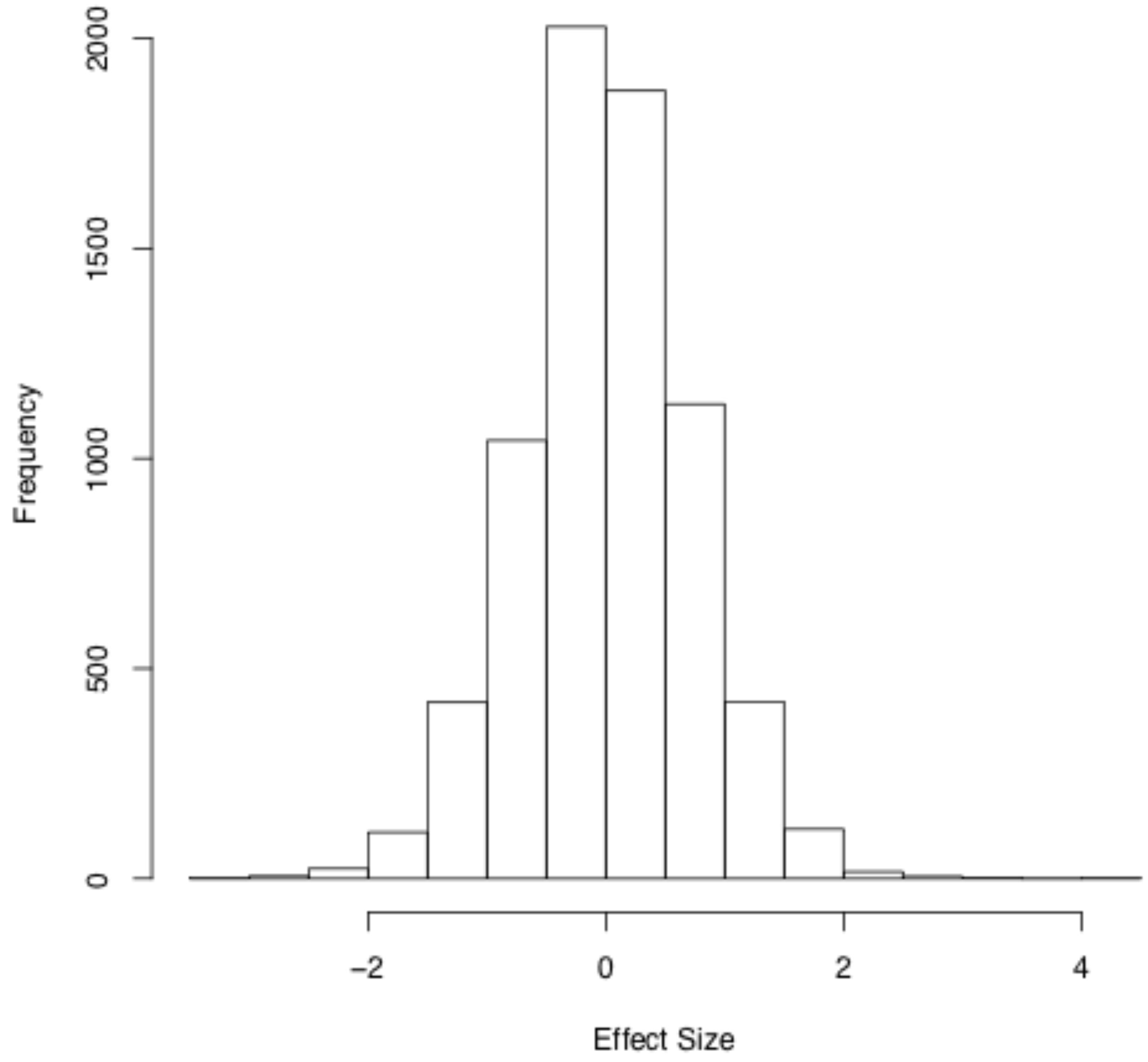
Meta-analysis

Where we have computed an effect size for a variant in both the 2018 and ENCODE datasets, we can meta-analyze them using the two coverage levels, according to:

$$E_{meta} = \frac{n_1 E_1 + n_2 E_2}{n_1 + n_2}$$

Where *E1* and *E2* are the two effect sizes, and *n1* and *n2* the two coverage levels.

> Histogram of estimated effect sizes after meta-analysis
>
> x-axis: effect size (LPAFC), y-axis: number of SNPs


Frequency
Effect Size