ASE analysis in Geuvadis

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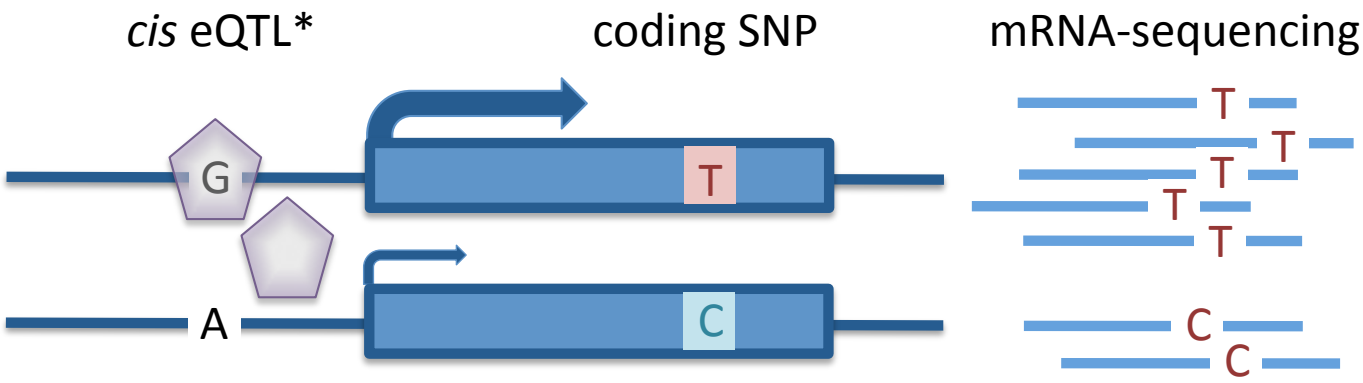
The allele specific expression (ASE) approach

- Read counts over all heterozygous sites of an individual partitioned by the allele
    - Binomial test to detect deviation from the expected ~50/50 ratio
        - the expected is calculated from the overall reference/total ratio across the genome, partitioned by mapping quality and SNP alleles
        - filtering of sites with poor mapability and simulated evidence of allele-specific mapping bias
        - robust to confounding factors between individuals
- Regulatory variation in cis (or an epigenetic effect shared by all the cells)



ASE pipeline @ UNIGE

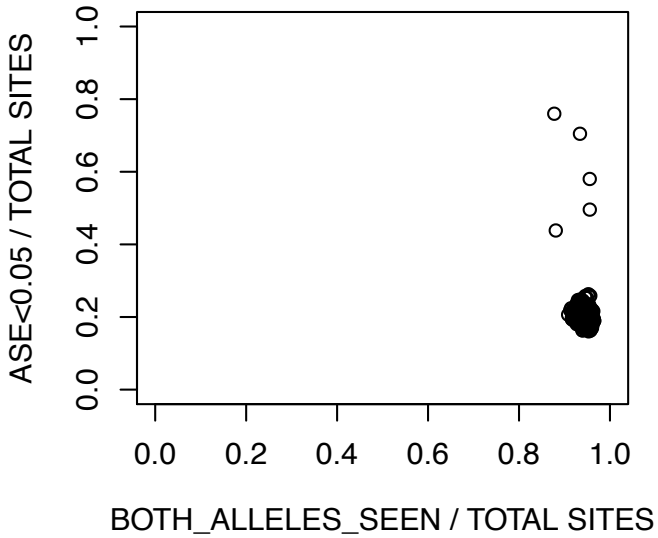
- Get the positions of all the variant sites in the entire study sample
  - Filter sites that are likely to have mapping error based on general mapability (UCSC track) and allelic mapping bias simulations
- For each individual, build pileups over these sites
- Parse the pileups to include only sites that are heterozygous in each individual and covered by >=16 reads, and count the alleles.
- Calculate a factor to correct for systematic bias in allelic ratios
  - calculate overall reference/total allele ratio for each individual for each SNP base combination
  - these ratios (generally only max 3% away from 0.5) are then used as the expected ratios.
- Calculate binomial probability for each covered site in each individual separately
- Build a results master file with annotations of the variants.

Caveats and concerns

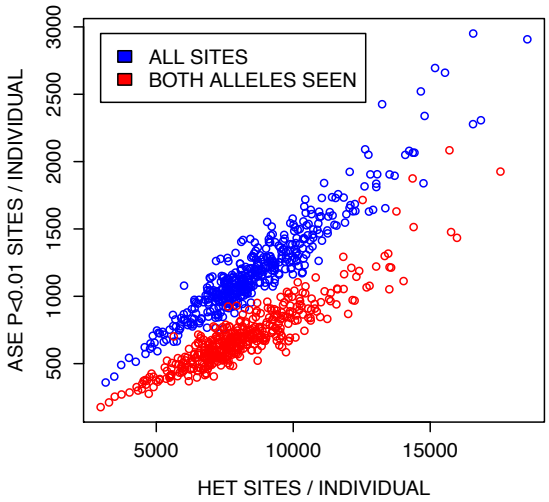
- Mapping bias = if your reference and alternative alleles don't map equally well (due to the variant itself or other flanking, linked variants)
  - I have developed our mapability filtering to relatively high sophistication
  - there isn't a filter that would get rid of all the bias
- Genotyping error
  - If you think that you're calculating ASE over a heterozygous site but the individual is actually homozygous, you'll have an extreme ASE signal that is completely false
  - We filter this by requiring to observe both alleles in RNAseq data (minor allele ratio >2%) by default. In some analysis this can be relaxed, but only with *a lot of caution*.
- P-value limit
  - <0.01 nominal p-value limit seems to work quite well, but it's not very stringent.
  - highly dependant on covegare and thus not really comparable between individuals or between sites
  - in many analyses I try to use continuous data of allelic ratios rather than the p-value.

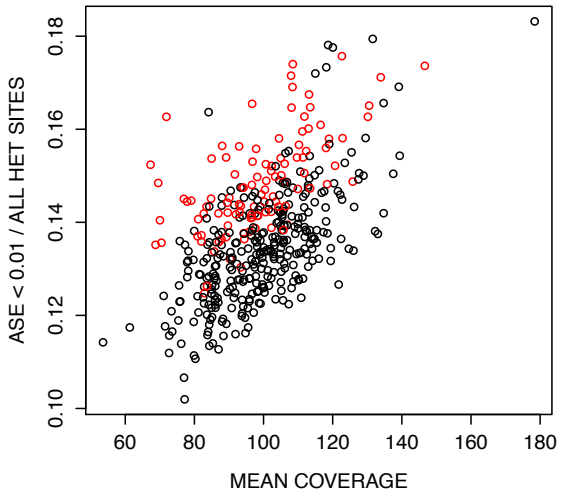
ASE as a QC tool

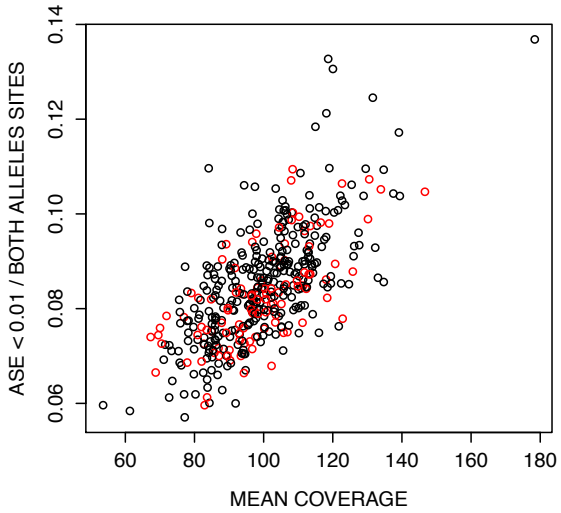
Cross-contamination in 5 samples



Differences in genotyping quality between individuals



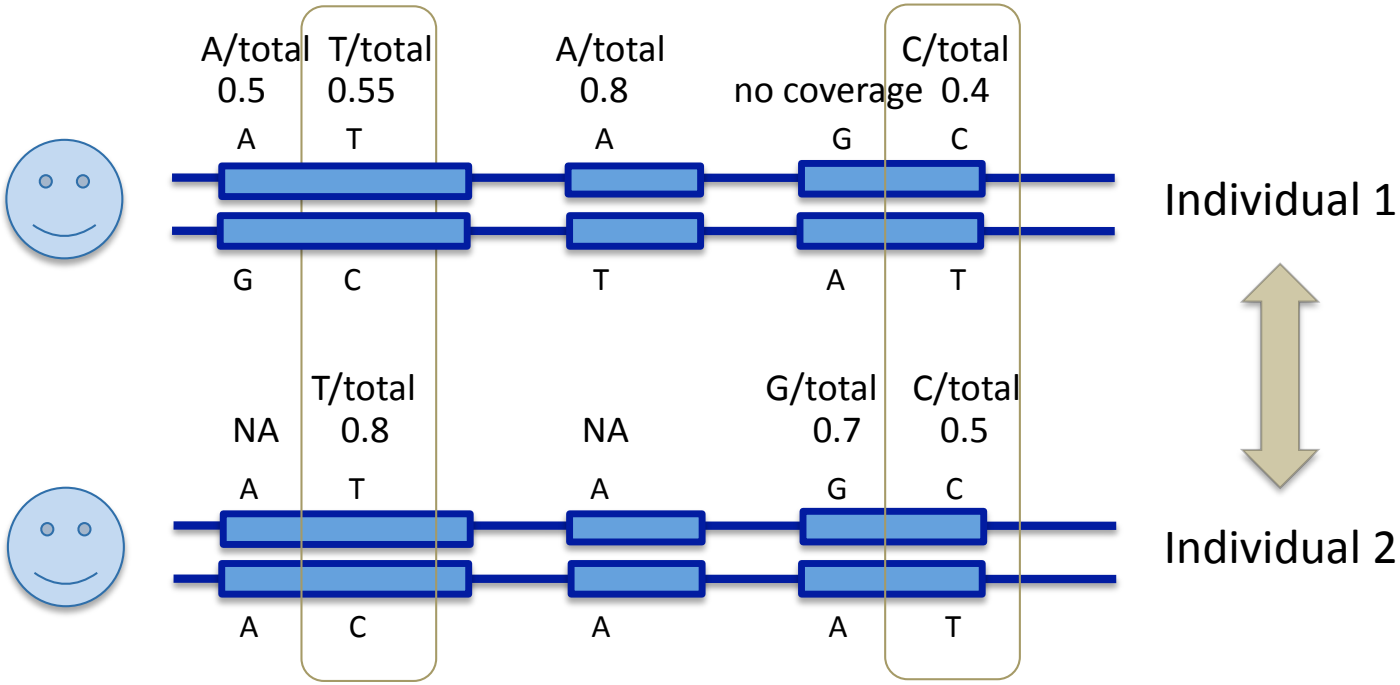




Geuvadis data: 462 full-coverage individuals

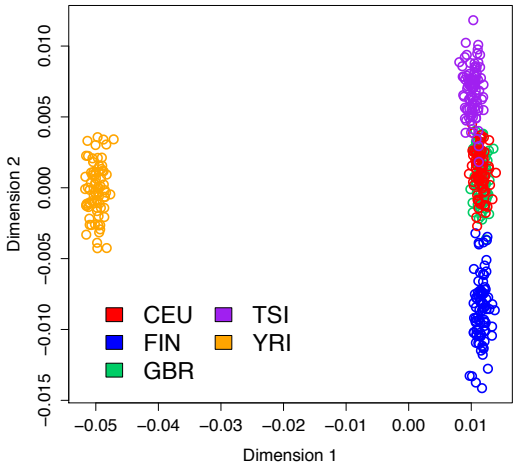
- Total number of heterozygous sites with enough coverage across individuals: 4,523,183
  - Unique SNPs: 200,133
  - Median per sample: 7,435
- Total number sites with ASE $p < 0.01$ across individuals 372,710
  - Unique SNPs: 59,022
  - Median per sample: 600 (7.79%)

Allelic expression distance between individuals

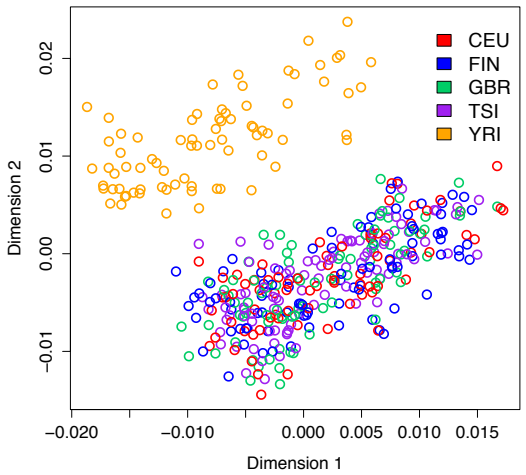


dist = median(c(abs(0.55-0.8), abs(0.4-0.5), …)

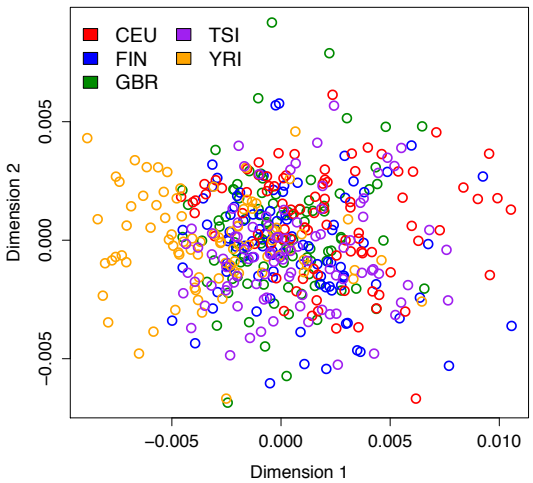
Genetic distance



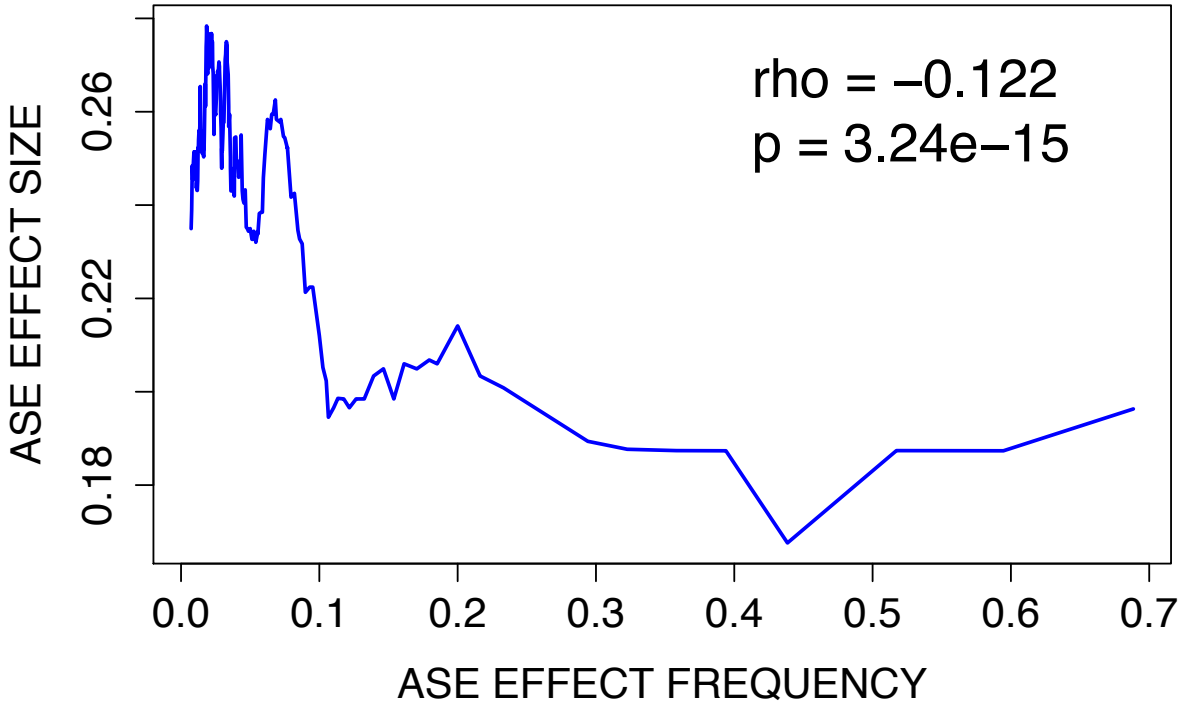
Allelic expression distance



Exon quantifications

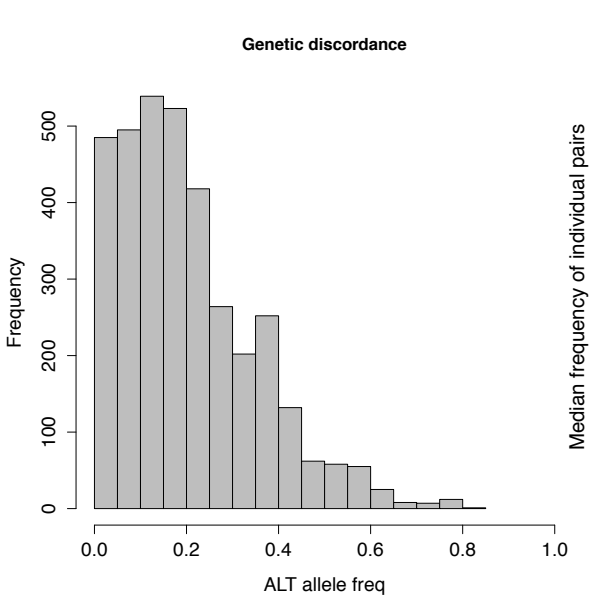


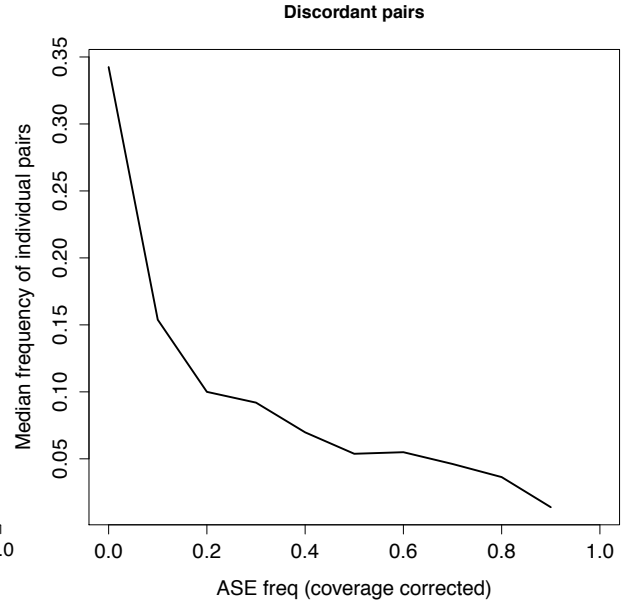
Effect sites of regulatory events

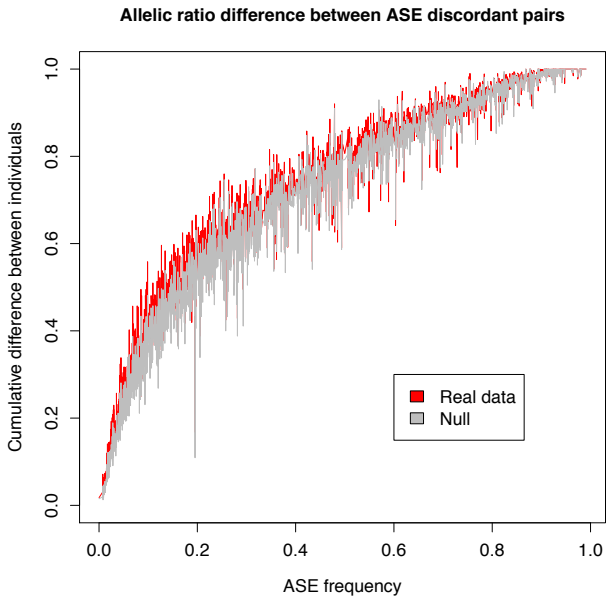


- for each SNP, what's the proportion of individuals having significant ASE in this SNP? -> x-axis
- for each individual with ASE, calculate how far the allelic ratio is it from 0.5 = magnitude of the cis regulatory effect -> y-axis
- rare effects have bigger effect sizes

What is the contribution of rare and common variants to phenotypic (allelic ratio) difference between two individuals?







For genetic variants, when looking at the allele frequency of sites that are genetically discordant between two individuals, much more are common (Fig 1). When two individuals are ASE-discordant, in the vast majority of cases the ASE effect is rare in the population (Fig 2).

Fig 3 is cumulative distance between individual pairs as a function of ASE frequency. Rare ASE effects contribute more mostly because there are so many of them, but their higher effect size (difference from the null) adds extra couple of percent. The difference is very significant for the rare ASE effects.