**Supporting Information for: Next-generation data filtering in the genomics era**

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**Supplementary Methods:**

**Empirical data preparation**

We acquired empirical data for ten taxa from published studies with taxonomic coverage including mammals (killer whales [*Orcinus orca*]1, deer mice [*Peromyscus maniculatus*]2, humans [*Homo sapiens*]3, mountain goats [*Oreamnos americanus*]4, and white-tailed deer [*Odocoileus virginianus*]5), arthropods (water fleas [*Daphnia pulex*]6, stoneflies [*Sweltsa coloradensis*]7, and monarch butterflies [*Danaus plexippus*]8), fish (yellow perch [*Perca flavescens*]9), and plants (*Arabidopsis thaliana*)10 (Table S2). The data types included whole-genome sequencing, low-coverage whole-genome sequencing, exome capture, and restriction site-associated DNA (RAD) sequencing. When more than two populations were sampled, we randomly selected two with 30 individuals per population for filtering. Populations with fewer than 30 individuals were not sub-sampled. For datasets where it was possible, we also applied GATK’s suggested hard-filters11 (QD > 2, FS < 60, SOR > 3, MQ > 40, MQRankSum > -12.5, ReadPosRankSum < -8) and a genotype quality (GQ) cutoff of 13 prior to filtering.

**Simulated data preparation**

We used the *scrm* coalescent simulator12 via the *coala* R package13 to simulate three genomic datasets under three different demographic histories: a neutral (static) scenario, a recent population bottleneck, and a recent population expansion (Table S3). For each model, we simulated three populations, all of which descended from a common ancestral population which split 1,000 generations before present to form populations A and (B + C). Populations B and C then split from each other 500 generations later. Population C then remained static for 450 generations, after which it either continued without change (neutral/static model), declined exponentially over five generations to 1/20th its original size (bottleneck model), or exponentially expanded ten-fold over the same time-frame (expansion model). Prior to demographic changes, all populations were held at a constant effective population size of 10,000. Gene flow between populations B and C was allowed following the population split at a rate of 0.1 migrants per generation. For each model, we sampled 30 individuals from populations B and C at the end of the simulation for 10 chromosomes, each with a length of 10mb and a recombination rate averaging at one per chromosome per cross per generation. Population C was used for all further analyses except *F*ST, for which both B and C were used. An R markdown document with the code used to perform these simulations is available in the Supplemental Materials.

To simulate selection for a range of recombination rates (Table S3), we used the *msms* simulator14, also via *coala*13. We used the same parameters as the neutral/static model, but each chromosome had a different recombination rate with selection on a single new mutation beginning 50 generations in the past. We varied recombination rates between 0.1 and 10 (results are reported for recombination rates of 0.1 and 1 in Box 1 of the main text). In all cases, we used a selection coefficient of 0.2 against the ancestral allele during selection. An R script with the code used to perform these simulations is available in the Supplemental Materials (Supplementary Notebook 3).

**Filtering**

We filtered each empirical and simulated dataset with a range of different filters and thresholds using the *filter\_snps* function in the *snpR* R package15. Specifically, we used the following filters and thresholds (function arguments listed in italics in parentheses):

* MAF (*maf*): 0.02–0.01 in 0.01 increments using within-group filtering (*maf\_facets = “pop”*) such that any locus with a MAF less than the threshold in *all* groups was removed. We did not filter at 0.01 because only 30 diploid individuals were included from each population, resulting in a minimum observable MAF of ~0.017 for polymorphic loci.
* HWP (*hwe*): 1x10-6–1x10-2 and 0.05 in increments of factors of ten (1x10-6, 1x10-5, and so on) using within-group filtering (*hwe\_facets = “pop”*) such that loci were removed only if they were significantly out of HWP in any individual sample group. HWP was assessed using an exact test16. No corrections for multiple testing were conducted to ensure that identical filtering thresholds were used for all loci and to ensure conservative removal of loci out of HWP.
* Required % individuals genotyped (*min\_ind*): 10–90% in increments of 10% such that loci were removed if they were not genotyped in at least the given percentage of individuals.
* Required % loci genotyped (*min\_loci*): 10–90% in increments of 10% such that individuals were removed if they were not genotyped in at least the given percentage of individuals.

When testing different thresholds for a given parameter value, we generally held all other parameter values constant at these values:

* MAF = 0, MGC = 1. Note: MGC = 1 (*mgc = 1*) removes any loci sequenced in only one individual, regardless of the genotypic state of that individual such that loci observed in a single homozygous individual were still removed.
* HWP = 1x10-6
* Required % individuals genotyped: 70%
* Required % loci genotyped: 70%

In addition to the solitary filter variation iterations, we also varied required % individuals and loci genotyped together for their ranges (both values 10-90%) for all datasets and performed a full factorial comparison of our filter thresholds for HWP and MAF for the mountain goat (RAD) and stonefly datasets specifically.

Following filtering, we computed expected heterozygosity (HE), observed heterozygosity (HO), nucleotide diversity (π), *F*IS and pairwise *F*ST according to Weir and Cockerham17, Tajima’s D18, Watterson’s 𝜃19, Tajima’s 𝜃18, a rarefaction-corrected measure of the number of segregating sites (Hemstrom and Christie, *in prep*), and a rarefaction-corrected estimate of the number of private alleles20 for each population or pair of populations (where applicable). We also conducted Principal Component Analysis using the smartPCA approach21 for all datasets and calculated site frequency spectra22 for each simulated dataset following filtering. Lastly, we also estimated effective population sizes (Ne) for each population in each dataset using the LD method in the NeEstimator software23 using only loci pairs on different chromosomes or scaffolds. All analyses were performed using the *snpR* R package15. Filtering R scripts, bash (shell) handling scripts, and parameter files are available at (https://github.com/ChristieLab/filtering\_simulation\_paper).

**Supplementary References:**

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**Supplementary Tables:**

**Supplementary Table 1:** Different types of filters available for genomic sequencing data.

|  |  |  |
| --- | --- | --- |
| **Filter** | **Stage** | **Description** |
| Base quality scores | *i* | Removal of reads with many poor-quality (likely mis-read) bases. |
| Poly-G tails | *i* | Removal of guanines (“G”s) erroneously called at the ends of reads on certain sequencing platforms. |
| Adapter/Barcode/Cut-site trimming | *i* | Removal of adapter, barcode, or cut-site sequences from the reads. |
| Adapter/Barcode/Cut-site mismatches | *i* | Removal of reads with sequences that do not match known adapter, barcode, or cut-site sequences. |
| Read K-mer distribution | *i, ii* | Removal of reads with too many very common or rare runs of base-pairs (K-mers). |
| Technical/PCR duplicates | *i, ii* | Thinning of technical or PCR duplicates down to a single representative read. |
| Alignment/Mapping scores | *ii* | Removal of reads that have mapping scores below a user-defined threshold. |
| Improperly paired reads (orientation and distance) | *ii* | Removal of paired-reads that are improperly paired (unexpectedly far apart or incorrectly oriented) |
| Stack depth of coverage | *ii* | Removal of loci "stacks" that have too low of a sequencing depth across samples; usually for reduced-representation sequencing. |
| Stack mismatches | *ii* | Removal of loci "stacks" that have too many mismatched base-pairs across samples; usually for reduced-representation sequencing. |
| Number of Alleles | *ii, iii* | Removal of genotypes, haplotypes, or "stacks" with too many possible alleles (usually > 2 for SNPs). Usually for computational efficiency, but also to remove potential errors. |
| Low coverage/Quality-by-depth | *iii* | Removal of individual called genotypes with coverage below a user-defined threshold. Joint "Quality-by-depth" often alternatively used. |
| Genotype Quality/Confidence | *iii* | Removal of individually called genotypes with genotyping confidence below a user-defined threshold. Joint "Quality-by-depth" often alternatively or additionally used. |
| High coverage | *iii* | Removal of individual called genotypes with coverage above a user-defined threshold (usually indicating errors in the reference, paralogs, or copy-number variants, all of which require additional investigation). |
| Insertion-deletions (Indels) | *iii* | Removal of insertions or deletions (indels), often required by many down-stream applications |
| Non-biallelic loci | *iii* | Removal of non-biallelic loci (for example, monomorphic or tri-allelic SNPs); required by many down-stream applications. |
| Allow/deny-listed variants | *iii* | Removal or inclusion of a set of user-defined loci. Common for methods that target specific loci or where specific variants are known to be problematic. |
| Variant Read Position | *iii* | Removal of variants that tend to occur in biased positions on shotgun-sequenced reads. |
| Missing data - per individual | *iii, iv* | Removal of individuals with called genotypes at fewer than a user-defined number of loci. |
| Missing data - per locus | *iii, iv* | Removal of loci with called genotypes at fewer than a user-defined number of individuals. |
| Minor allele frequency | *iii, iv* | Removal of loci with minor allele frequencies below a user-defined threshold. |
| Minor allele count | *iii, iv* | Removal of loci with a count of the minor allele below a user-defined threshold across samples. |
| Hardy-Weinberg proportions | *iii, iv* | Removal of loci out of Hardy-Weinberg proportions, typically below a user-defined p-value. |
| Strand Bias | *iii, iv* | Removal of loci where specific alleles are detected primarily on only the forward or reverse DNA strand. |
| Copy number variation | *iii, iv* | Removal of copy number variants. Often remain undiscovered. |
| Structural variants | *iii, iv* | Removal of structural variants, such as inversions. Often remain undiscovered. |
| Sex-linked loci | *iii, iv* | Removal of sex-linked loci, which may behave in unexpected ways or have biased statistical outcomes due to sex-specific sampling. |
| Paralogs - allelic imbalance/depth/heterozygosity | *ii, iii, iv* | Removal of reads aligned to paralogous genomic regions, where for recently diverged paralogs it can be unclear from which of the gene copies the read was sequenced. Additional analyses are required. |
| Mislabeling/Contamination | *iv* | Removal of individuals or loci that are likely mislabled, contaminated, or have similar issues. Can often be identified via PCA and other comparative analyses. |
| Transition-transversion bias | *iv* | Removal of loci from genomic regions with unexpected transition:transversion ratios. |
| *F*ST/Selection Outliers | *iv* | Removal of outlier loci likely to be under selection. Useful for cases where putatively neutral processes specifically are of interest (for example, gene flow). |

*i* = sequence QC (Quality control), *ii* = alignment to a reference, *iii* = variant discovery, and *iv* = data analysis. Note that stages *i* and *ii* constitute pre-variant filtering and stages *iii* and *iv* constitute post-variant filtering.

**Supplementary Table 2**: Empirical datasets used for filtering simulations.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Organism** | **Dataset Type** | **Number of SNPs** | **Number of Individuals** | **Reference** |
| *Arabidopsis* | lcWGS | 3,135,226 | 60 | 10 |
| Monarch butterflies | RAD | 238,368 | 54 | 8 |
| *Daphnia* | WGS |  |  | 6 |
| Stoneflies | RAD | 279,496 | 60 | 7 |
| Yellow perch | WGS | 6,586,547 | 57 | 9 |
| Humans | WGS | 17,458,468 | 60 | 3 |
| Deer mice | Exome capture | 5,373,633 | 55 | 2 |
| Mountain goats | RADseq/ WGS | 48,192/8,113,114 | 60/20 | 4 |
| White-tailed deer | WGS | 48,441,150 | 20 | 5 |
| Killer whales | WGS | 3,015,993 | 54 | 1 |

**Supplementary Table 3**: Simulated datasets used for filtering simulations.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Selection** | **Demographic History** | **Relevant Parameters** | **Number of SNPs** | **Number of Individuals** |
| No Selection | Neutral/Static | Ncurr = Nanc | 237,698 | 60 |
| No Selection | Large expansion in one population | Ncurr = 10Nanc | 247,922 | 60 |
| No Selection | Large bottleneck in one population | Ncurr = 0.05Nanc | 198,783 | 60 |
| Hard sweep at one locus in one population | Static | *s* = 0.2  *fs* = 1/2N  *r* = 0.1 or 1  *t­s* = 50 | 21228/21744 | 60/60 |

*N­­curr*: sample size at present

*Nanc*: ancestral sample size

*s*: selection coefficient against ancestral allele

*fs*: frequency of the new mutation

*r*: recombination rate, average number per chromosome per generation

*ts*: time (in generations) before present at which selection began

**Supplementary Table 4**: Threshold recommendations for low and high stringency datasets used to assess the impact of filtering decisions for different questions and objectives. Please note that these values are *suggestions* and should not necessarily supplant any existing knowledge of the study system or details of the base dataset. These values represent suggested extreme values to examine effects; moderate values may be preferable for final analysis. Note that suggested MAC values may actually be more strict than the suggested MAF values for datasets with low sample sizes and should be adjusted accordingly. Furthermore, some methods for questions may require different filter values that differ from these suggestions. See Supplementary Table 5 for brief justifications for many of these suggestions.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Question/Objective** | **MAF/MAC** | **Individuals missing data: % genotypes Required** | **Loci missing data: % genotypes Required** | **HWP** | **LD** |
| Population Structure | MAC > 1;  MAF > 0.05 | 50; 95% | 50; 95% | 1e-6; 0.01 | No; Yes |
| Demography | MAC > 0;  MAC = 1 | 75; 95% | 50; 95% | 1e-6; 0.01 | Yes |
| Relatedness/  Pedigree Construction | MAC > 2;  MAF > 0.05 | >5% | 80%; 95% | 0.001; 0.01 | No, although CI’s can be affected |
| Selection | MAC > 1;  MAF > 0.05 | 25; 75% | 25; 75% | 1e-6; 0.001 | No |
| Genetic Diversity | MAC > 1;  MAF > 0.05 | 50; 75% | 50; 75% | 1e-6; 0.01 | No, usually. |
| Phylogenetics | MAC > 1;  MAF > 0.05 | 50; 80% | 50; 80% | 0.001; 0.01 | Yes |
| GWAS | MAF > 0.05, unless large *n* | 50; 80% | 50; 80% | 1e-6; 0.01 | No, but correct p-values |
| Mutation Detection | No filter | Parents: 100%,  Offspring: 0% | Parents: 100%,  Offspring: 0% | 1e-6 | No |
| Metagenomics/eDNA | MAC > 4 | - | - | - | - |

**Supplementary Table 5**: Justifications for filtering threshold recommendations in Table S4.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Question/Approach** | **MAF/MAC** | **Individuals missing data: % genotypes Required** | **Loci missing data: % genotypes Required** | **HWP** | **LD** |
| Population Structure | Higher MAF values can reveal additional population structure | Poor quality individuals or loci may mask structure | Poor quality individuals or loci may mask structure | Need to keep a low pass filter for cryptic populations | Probably has no effect unless inversions or other factors drive clustering. |
| Demography | Any measures which depend on SFS estimates are extremely biased by the removal of low frequency variants. | Many demographic estimation approaches function well with small sample sizes but can be misled by poor quality individuals | Projection can reduce the impact of missing data. | Paralog removal | Non-independent loci can create misleading site frequency spectra. Not as essential for Tajima’s D as it is for ABC, etc. |
| Relatedness/  Pedigree Construction | Singletons don’t help | Including all individuals is important | Only a few loci needed to infer a relationships but they need to be high quality. | Only a few loci needed to infer a relationships but they need to be high quality. | Should not bias mean outcomes, but could change confidence intervals. |
| Selection | Don’t want to remove signal, but some methods require or recommend higher MAF (0.05) | Don’t want to remove signal | Don’t want to remove signal | Don’t want to remove signal; less strict filtering to keep more loci | Removing loci in LD can remove signals of hitchhiking/selective sweeps |
| Genetic Diversity | Note that some metrics (like # seg sites) are impacted differently from others (like HE) | Some metrics (# seg sites) can drop fast as you exclude too many individuals/loci (see Box 1 fig.) | Some metrics (# seg sites) can drop fast as you exclude too many individuals/loci (see Box 1 fig.) | This range will usually capture most of the changes due to filtering (see Box 1 fig). | *Usually* should cause major impacts, but can if regions in high LD vary (chromosomal inversion). Can skew confidence intervals. |
| Phylogenetics | Autapomorphies/Singletons are not informative |  |  | Don’t want anything with odd behavior throwing off signal | Need independent evolutionary histories (e.g., un-linked) |
| GWAS | Low frequency variants are typically uninformative unless sample sizes are very large. | If your method requires no missing data, use a high filter to avoid extra imputation | If your method requires no missing data, use a high filter to avoid extra imputation | Usual range, check effects. Selection on causal may cause deviations rarely. | Removal can cloud signals from linkage around causal genes. However, complex correction for *p*-values is needed for multiple testing–see (CITE) |
| Mutation Detection | Many (new) mutations have very low frequencies | Cannot have missing data in parents, but skip over missing data in offspring (can’t detect mutations but won’t cause problems) | Cannot have missing data in parents, but skip over missing data in offspring (can’t detect mutations but won’t cause problems) | Get rid of paralogs, otherwise don’t filter | Irrelevant; don’t want to remove potential mutations. |
| Metagenomics/eDNA | Need multiple reads from a region for confidence (5+ or so) | NA | NA | NA | NA |

**Supplementary Figures:**

**A collection of graphs showing different types of data

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**Figure S1**: Change in *F*ST, HO, *F*IS, Tajima’s D, He, 𝜋, Watterson’s 𝜃, Tajima’s 𝜃, the proportion of the total (highest) number of segregating sites, the proportion of the total (highest) number of private alleles, and Ne, observed in each population and dataset depending on MAF filtering stringency. Parameter values have been normalised to show change by subtracting off the value observed with no filter.

A collection of different colored lines

Description automatically generated with medium confidence

**Figure S2**: Raw change in *F*ST, HO, *F*IS, Tajima’s D, He, 𝜋, Watterson’s 𝜃, Tajima’s 𝜃, the proportion of the total (highest) number of segregating sites, the proportion of the total (highest) number of private alleles, and Ne, observed in each population and dataset depending on MAF filtering stringency.

A collection of different colored lines

Description automatically generated with medium confidence

**Figure S3**: Change in *F*ST, HO, *F*IS, Tajima’s D, He, 𝜋, Watterson’s 𝜃, Tajima’s 𝜃, the proportion of the total (highest) number of segregating sites, the proportion of the total (highest) number of private alleles, and Ne, observed in each population and dataset depending on HWP filtering stringency. Parameter values have been normalised to show change by subtracting off the value observed with no filter.

A group of colorful lines

Description automatically generated with medium confidence **Figure S4**: Raw change in *F*ST, HO, *F*IS, Tajima’s D, He, 𝜋, Watterson’s 𝜃, Tajima’s 𝜃, the proportion of the total (highest) number of segregating sites, the proportion of the total (highest) number of private alleles, and Ne, observed in each population and dataset depending on HWP filtering stringency.

A collection of diagrams showing different types of data

Description automatically generated with medium confidence **Figure S5**: Change in *F*ST, HO, *F*IS, Tajima’s D, He, 𝜋, Watterson’s 𝜃, Tajima’s 𝜃, the proportion of the total (highest) number of segregating sites, the proportion of the total (highest) number of private alleles, and Ne, observed in each population and dataset depending on missing data filtering stringency. Parameter values have been normalised to show change by subtracting off the value observed with no filter. Filtering stringencies for both loci and individuals are identical.

A group of different colored lines

Description automatically generated with medium confidence **Figure S6**: Raw change in *F*ST, HO, *F*IS, Tajima’s D, He, 𝜋, Watterson’s 𝜃, Tajima’s 𝜃, the proportion of the total (highest) number of segregating sites, the proportion of the total (highest) number of private alleles, and Ne, observed in each population and dataset depending on missing data filtering stringency. Filtering stringencies for both loci and individuals are identical.

A collection of different colored lines

Description automatically generated with medium confidence **Figure S7**: Change in *F*ST, HO, *F*IS, Tajima’s D, He, 𝜋, Watterson’s 𝜃, Tajima’s 𝜃, the proportion of the total (highest) number of segregating sites, the proportion of the total (highest) number of private alleles, and Ne, observed in each population and dataset depending on missing data filtering stringency. Loci were removed if genotyped in too few individuals. Parameter values have been normalised to show change by subtracting off the value observed with no filter.

A group of different colored lines

Description automatically generated with medium confidence **Figure S8**: Raw change in *F*ST, HO, *F*IS, Tajima’s D, He, 𝜋, Watterson’s 𝜃, Tajima’s 𝜃, the proportion of the total (highest) number of segregating sites, the proportion of the total (highest) number of private alleles, and Ne, observed in each population and dataset depending on missing data filtering stringency. Loci were removed if genotyped in too few individuals.

A collage of graphs showing different types of data

Description automatically generated with medium confidence **Figure S9**: Change in *F*ST, HO, *F*IS, Tajima’s D, He, 𝜋, Watterson’s 𝜃, Tajima’s 𝜃, the proportion of the total (highest) number of segregating sites, the proportion of the total (highest) number of private alleles, and Ne, observed in each population and dataset depending on missing data filtering stringency. Individuals were removed if genotyped in too few loci. Parameter values have been normalised to show change by subtracting off the value observed with no filter.

A group of diagrams showing different types of lines

Description automatically generated with medium confidence **Figure S10**: Raw change in *F*ST, HO, *F*IS, Tajima’s D, He, 𝜋, Watterson’s 𝜃, Tajima’s 𝜃, the proportion of the total (highest) number of segregating sites, the proportion of the total (highest) number of private alleles, and Ne, observed in each population and dataset depending on missing data filtering stringency. Individuals were removed if genotyped in too few loci.

A grid of blue and yellow lines

Description automatically generated **Figure S11**: Principal Component Analysis plots for the first two PCs for each dataset depending on MAF filter stringency. Points are coloured by population.

A grid of blue and yellow dots

Description automatically generated **Figure S12**: Principal Component Analysis plots for the first two PCs for each dataset depending on HWE filter stringency. Points are coloured by population.

A grid of blue and yellow dots

Description automatically generated **Figure S13**: Principal Component Analysis plots for the first two PCs for each dataset depending on missing data filter stringency. Locus and individual missing data thresholds were identical at each threshold. Points are coloured by population.

A grid of blue and yellow dots

Description automatically generated **Figure S14**: Principal Component Analysis plots for the first two PCs for each dataset depending on missing data filter stringency. Loci were removed if genotyped in too few individuals. Points are coloured by population.

A grid of blue and yellow dots

Description automatically generated

**Figure S15**: Principal Component Analysis plots for the first two PCs for each dataset depending on missing data filter stringency. Individuals were removed if genotyped in too few loci. Points are coloured by population.