An approach for quantifying allele-specific expression estimates on the X chromosome

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Background: X and Y share homologous regions

- Genetic males (46, XY) and females (46, XX) share highly similar genomes, only differing in the sex chromosomes (X and Y). To equalize dosage on the X chromosome, one of the two X chromosomes in genetic females is transcriptionally silenced; this process is called X chromosome inactivation (XCI)[1] (**Fig 1**).
- Between ~15-30% of genes on the inactive X chromosome escape silencing, resulting in biallelic expression of these genes on the X chromosome in genetic females[1].
- Allele-specific expression analysis requires heterozygous variants from DNAseq and counts from RNAseq. However, quantifying allele-specific expression on the X chromosome can be challenging due to the shared sequence homology between the X and Y chromosome that results in the mismapping of reads[2,3]. It is unknown how mismapping due to homologous regions between the X and Y chromosomes affects allele-specific expression analyses.
- **This work aims to quantify allele-specific expression estimates on the X chromosome by employing a sex chromosome complement alignment approach.**

Fig 1. X chromosome inactivation. Gray cells are expressing the paternal X copy (X^p). White cells are expressing the maternal X copy (X^m). Some tissues are patchy with sections of cells showing biased expression for either the maternal or paternal X copy, while other tissues are mosaic[4].

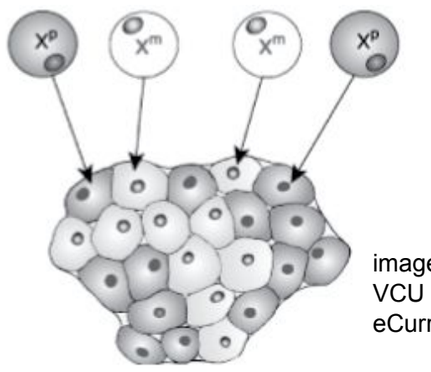


image credit: VCU eCurriculum

Methods: Sex chromosome complement alignment

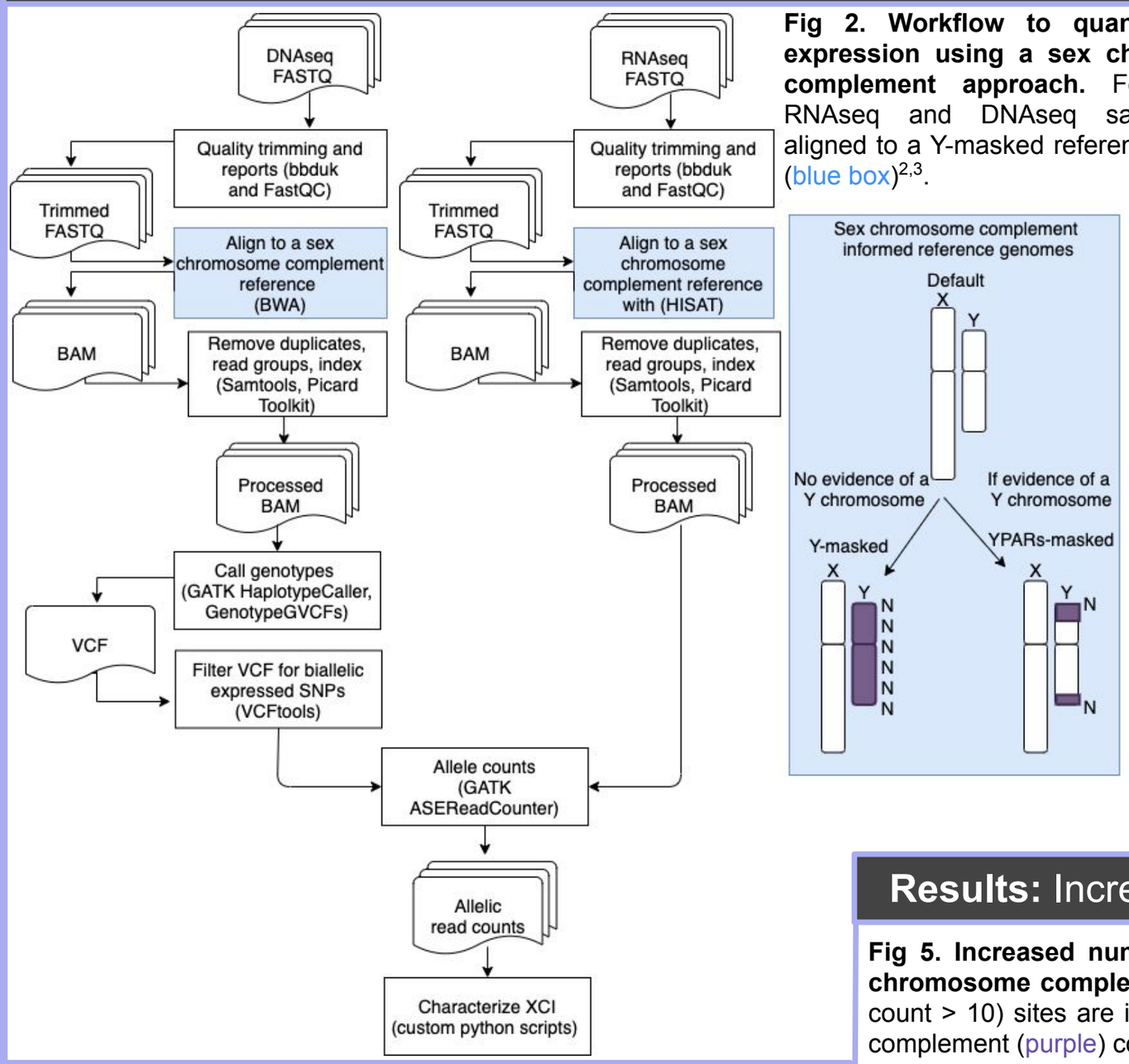
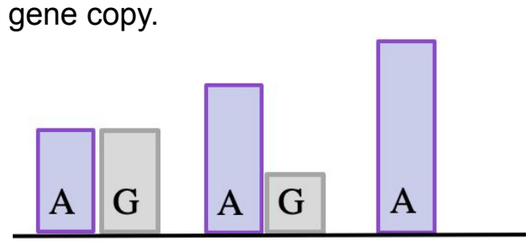


Fig 2. Workflow to quantify allelic expression using a sex chromosome complement approach. Female XX RNAseq and DNAseq samples are aligned to a Y-masked reference genome (blue box)[2,3].

Fig 3. Allele-specific expression estimates are used to determine if a gene is silenced or escapes XCI. If both alleles are expressed equally at heterozygous sites on the X chromosome, this gene escapes XCI. There may also be an allelic imbalance in which the gene escapes XCI but shows bias expression for one of the gene copies. A gene is silenced if it is only expressing one gene copy.

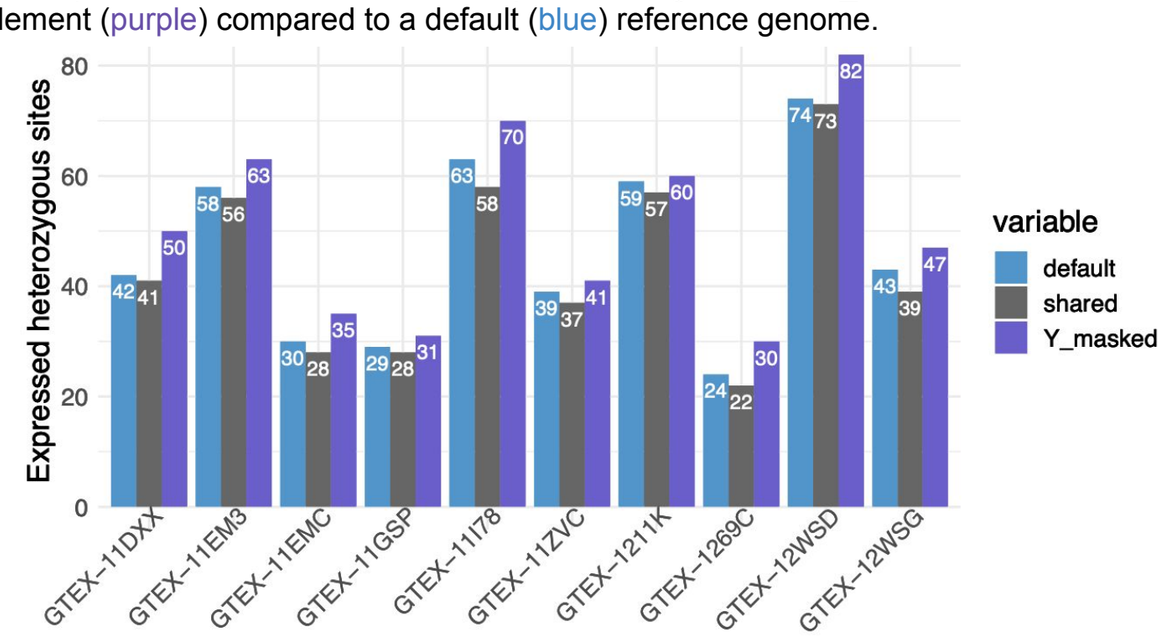


Equal expression (escaped) | Allelic imbalance (escaped) | Allele-specific expression (silenced)

- Here, we process ten female (46, XX) heart RNAseq and DNAseq samples from GTEx[5].
- RNAseq and DNAseq samples were aligned to both a default reference genome and a Y-masked reference genome (**Fig 2**).
- Compare the number of expressed (total allele count > 10) heterozygous sites between default and Y-masked approach (**Fig 5**).

Results: Increased number of expressed heterozygous sites

Fig 5. Increased number of heterozygous sites when samples are aligned to a sex chromosome complement reference genome. More heterozygous expressed (total allele count > 10) sites are identified when samples are aligned to a Y_masked sex chromosome complement (purple) compared to a default (blue) reference genome.



- Uniquely identified sites when using the sex chromosome complement approach are enriched in genes located within the pseudoautosomal regions (PAR1 and PAR2).

References

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