Using Alternate References for Transcriptome Analysis of an Indigenous Australian Study Cohort

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Genomic Variability

The Indigenous Australian population contains a large amount of unique genetic diversity. Given that risk factors are increasingly being shown to be polygenic and dependent on the genetic background. ignoring diversity in a large cohort may limit our ability to address this contribution.

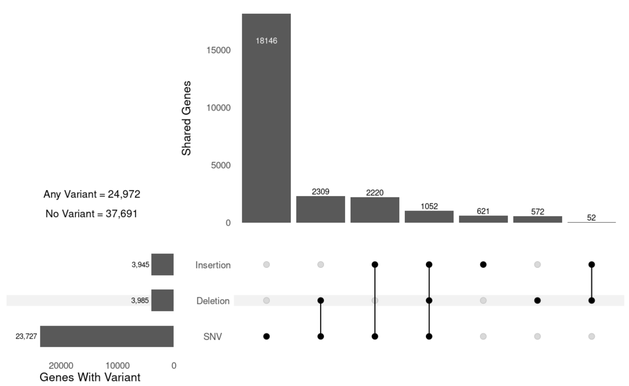
Genomic variants unique to Indigenous Australians[1]

The historical relationship between researchers and the Aboriginal community *has been problematic* and as such, this population is poorly represented in databases such as the 1000 Genomes Project (1000GP), and the Human Genome Diversity Project (HGDP).

Variants from the 1000 Genomes Project

Taking consensus 1000GP variants (>50%) as a *proof-of-principle*, a gene-level analysis (*STARconsensus*[2]) was prepared comparing the standard hg38 reference against a modified reference incorporating SNPs and InDels from the 1000GP.

Region	SNV	Insertion	Deletion	**Total**
No Classified Region	1,281,298	110,072	113,176	**1,504,546**
Promoter	151,537	13,775	14,616	**179,928**
Upstream Promoter	100,273	9,848	9,845	**119,966**
Exon	68,628	4,682	4,814	**78,124**
UTR	24,213	2,197	2,261	**28,671**
Splice Junction	1,628	236	265	**2,129**
Stop Codon	36	1	5	**42**
Start Codon	16	3	2	**21**



Genes with variants overlapping an exon

The PROPHECY Study

The PROPHECY study (**P**reventing **R**enal, **OP**hthalmic and **H**eart **E**vents in **C**ommunit**Y**) consists of ∼1400 indigenous participants drawn from regional, remote and urban locations within South Australia. Amongst community, the study is colloquially known as the *Aboriginal Diabetes Study*.

The PROPHECY Study is a multi-omics study including genomic variants, DNA methylation, bulk RNA-Seq, proteomics, lipidomics, metabolomics and multiple other layers, all derived from blood samples taken from the same participants.

Haploid Reference Strategies

Haploid reference strategies are well understood making these the first starting point for adressing issues of unique diversity. We are also exploring *pan-genome, graph-based approaches*[3].

	Reference Type	
	Genome	Transcriptome
Gene-Level Analysis	✔	✔
Transcript-Level Analysis[4]	✖	✔
Alignment Co-Ordinates	✔	✖
Alignment Visualisation	✔	✖
Speed of Workflow	✖	✔
Storage Requirements	✖	✔

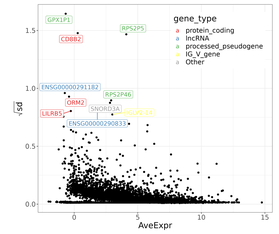
Strategies for modifying the reference include: 1) 1000GP consensus variants; 2) 1000GP AFR variants; 3) PROPHECY consensus variants and 4) personalised references. *Data sovereignty* with participants retaining control of their data impacts which approaches are viable.

Pilot samples will be assembled using Trinity and assemblies compared to variant-modifed references to assess the most appropriate strategy.

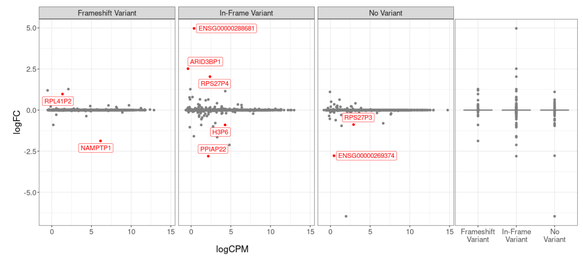
STAR Consensus Results

6 Pilot Samples were aligned to hg38 along with the 1000GP-modified hg38. Standard DGE Analysis performed along with key explorations.

- **1 in 500 reads** aligned to different locations
- Direction of change **inconsistent** between samples
- Some key genes were highly sensitive



Variability against average signal



Differential Gene Expression results showing genes which passed an FDR of 0.05 for consideration as differentially expressed

Developing a Variant-Modified Reference Transcriptome

The R package `transmogR` has now been developed for using variants to modify a standard reference trancriptome, including decoy transcripts.[5] This approach is coordinate-agnostic $\Longrightarrow$ mapping back to regulatory features and GWAS results remains uncomplicated

References

[1] Simon Easteal et al. "Equitable Expanded Carrier Screening Needs Indigenous Clinical and Population Genomic Data". en. In: *Am. J. Hum. Genet.* 107.2 (Aug. 2020), pp. 175–182.

[2] Benjamin Kaminow et al. "Pan-human consensus genome significantly improves the accuracy of RNA-seq analyses". en. In: *Genome Res.* 32.4 (Apr. 2022), pp. 738–749.

[3] Jonas A Sibbesen et al. "Haplotype-aware pantranscriptome analyses using spliced pangenome graphs". en. In: *Nat. Methods* (Jan. 2023).

[4] Pedro L Baldoni et al. "Dividing out quantification uncertainty allows efficient assessment of differential transcript expression with edgeR". en. Oct. 2023.

[5] Avi Srivastava et al. "Alignment and mapping methodology influence transcript abundance estimation". en. In: *Genome Biol.* 21.1 (Sept. 2020), p. 239.