AGTA
20TH ANNIVERSARY
CONFERENCE
30 October – 2 November 2022
Novotel Twin Waters
Sunshine Coast, Queensland

Identifying candidate RNA biomarkers for coronary artery disease by deep RNA-Sequencing in human plasma

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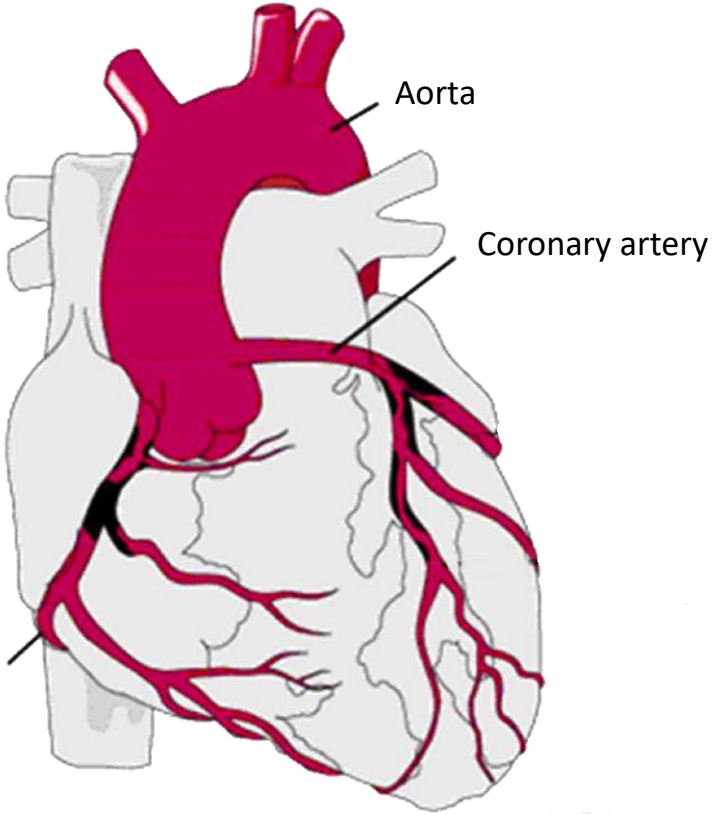
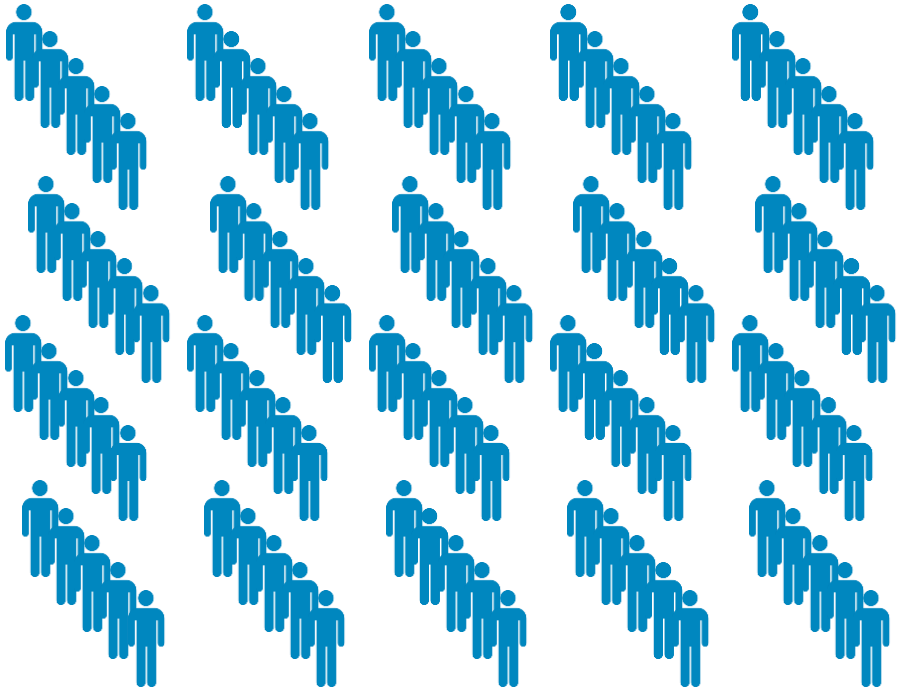
It started with pain in his jaw while he cycled to work

"Family History Cannot Be Ignored"
Heart Foundation *Heart Life* newsletter, Winter 2015

Coronary artery disease occurs when the coronary arteries become blocked



Cardiovascular risk prediction isn’t perfect



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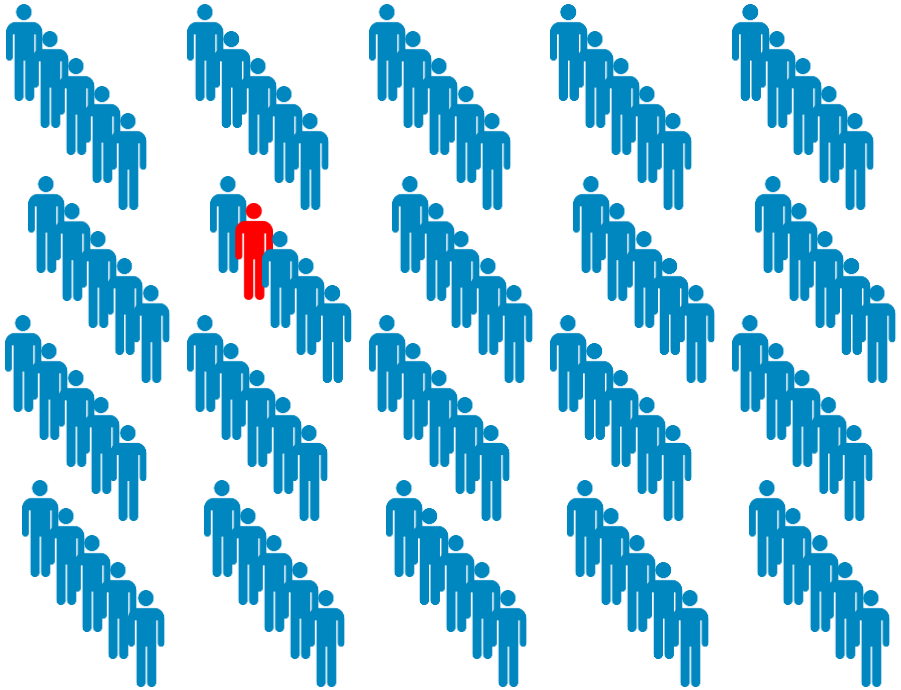
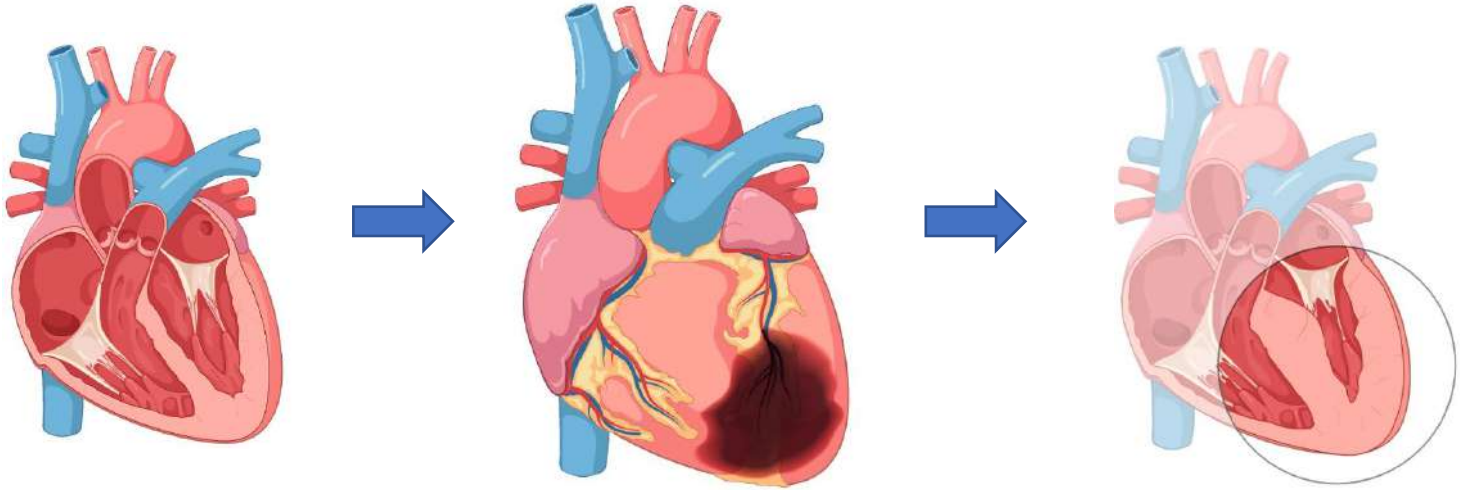


Aim: to develop a short-read RNA-Seq protocol to detect mRNA, lncRNA and circRNA in plasma

Sample selection: three groups spanning health and disease

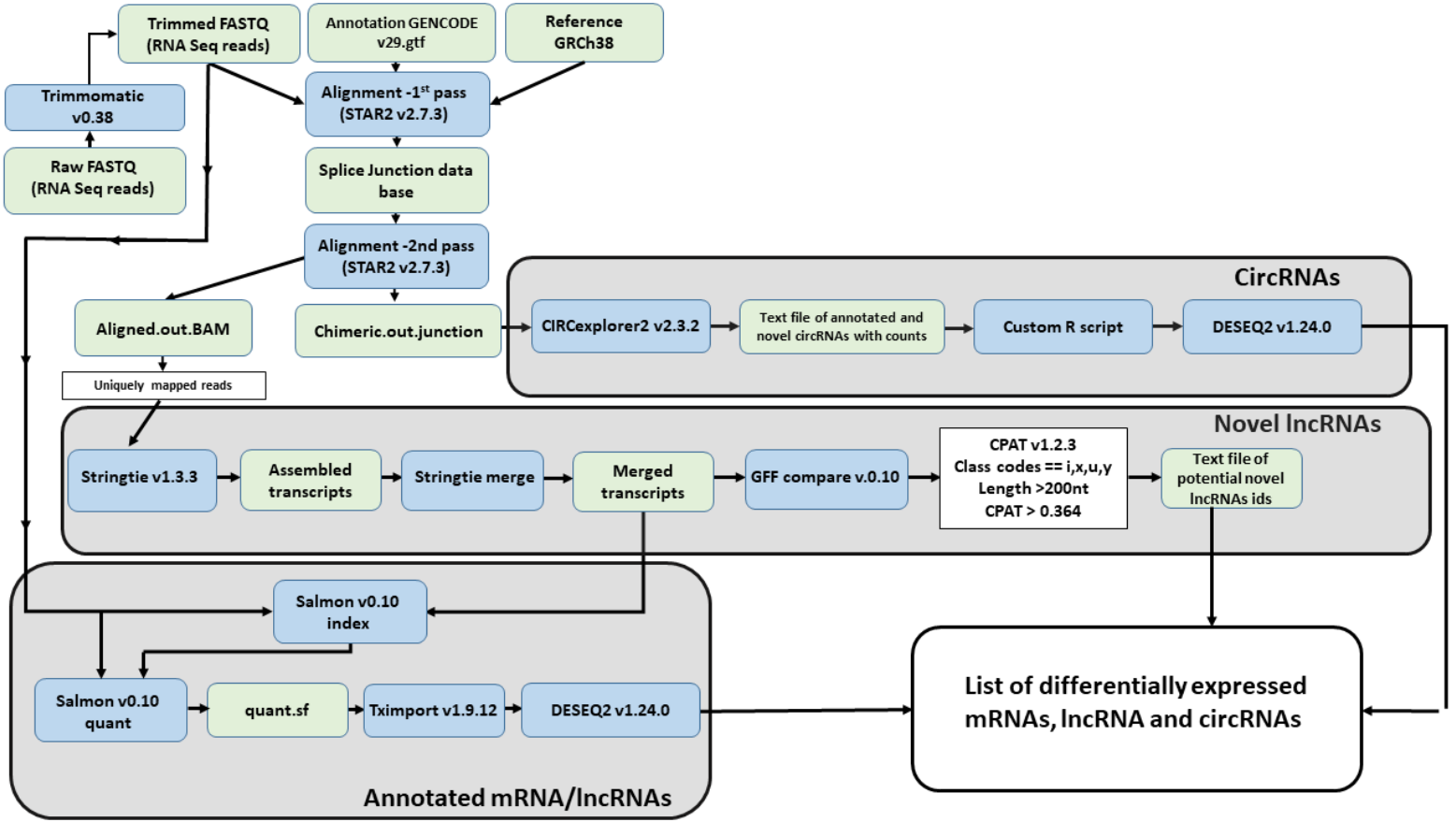


Controls
n=30

Stable coronary artery disease
without heart failure
n=30

Stable coronary artery disease
with heart failure
n=29

Bioinformatic pipeline available at https://github.com/zoeward-nz/PhD



Fewer than 20% of reads uniquely mapped to the human genome

Median and IQR	Controls (n=30)	CAD, HF- (n=30)	CAD, HF+ (n=29)
Total reads (M) *	111 (100-113)	102 (92-117)	110 (101-118)
Reads uniquely mapped (M) *	24 (9-33)	22 (8-29)	15 (9-30)
Reads uniquely mapped (%)*	19 (9-30)	19 (8-28)	14 (9-30)

- Potential for confounding with sample storage time

Comparing plasma RNA fragments: patients vs controls

	mRNAs	lncRNAs	putative novel lncRNAs	circRNAs
Fragments identified:	3,986	164	405	227
Differed in patients vs controls*:	160	10	2	0

*No difference between patients with heart failure vs those without heart failure

Gene name	Transcript type	Log 2 fold change (standard error)	P-value*
STAG2	Protein coding	1.61 (0.12)	1.62E-36
NEUROD2	Protein coding	1.89 (0.15)	1.48E-31
MT-ND3	Protein coding	2.09 (0.18)	5.75E-29
MT-ND5	Protein coding	2.13 (0.19)	5.80E-28
MT-CO2	Protein coding	2.08 (0.18)	1.00E-27
MT-ND6	Protein coding	2.22 (0.20)	1.64E-27
MT-CYB	Protein coding	2.10 (0.19)	3.29E-27
MT-ND1	Protein coding	2.16 (0.19)	1.65E-26
MT-ATP6	Protein coding	2.00 (0.18)	3.33E-26
MT-ND4L	Protein coding	1.98 (0.18)	5.77E-26
AL035078.1	lncRNA	1.77 (0.16)	6.48E-26
CXCL14	Protein coding	1.98 (0.18)	1.40E-25
MT-CO1	Protein coding	2.04 (0.19)	1.84E-25
CCDC26	lncRNA	1.72 (0.16)	4.51E-25
MT-ND2	Protein coding	1.98 (0.18)	6.49E-25
MT-ND4	Protein coding	2.01 (0.19)	9.42E-25
MT-CO3	Protein coding	2.01 (0.19)	1.82E-24
BTN3A2	Protein coding	1.39 (0.13)	6.75E-24
MT-ATP8	Protein coding	2.05 (0.20)	3.25E-22
MTRNR2L12	Protein coding	2.13 (0.22)	8.15E-20

* adjusted for multiple comparisons

The most differentially abundant transcripts were enriched in mRNAs encoded by the mitochondrial genome

Conclusions

- Identified several promising candidate mRNA and lncRNA biomarkers for stable coronary artery disease
- Technical challenges of RNA-Seq in plasma can be overcome by ultra-deep sequencing
- Advances in library preparation kits, enrichment techniques and sequencing technologies may improve detection of cell-free RNA in plasma

Thank you to our team, study participants and funders

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