

Contents

Glossary	xxv
1 Introduction	1
1.1 Alternative Splicing	3
1.2 High-throughput technologies to characterise transcriptome complexity	7
1.2.0.1 PacBio Iso-Seq sequencing	9
1.3 Approaches for isoform quantification and differential isoform usage	14
1.4 Functional impact of isoform regulation	16
1.4.0.1 Functional impact on protein properties	16
1.4.0.2 Functional impact on UTR properties	18
1.4.0.3 Nonsense-mediated decay	19
2 Motivation, Aims, and Contributions	21
2.1 Motivation	23
2.2 Aims	25
2.3 Main contributions	27
2.3.1 Journal papers	27
2.3.2 Conferences	28
2.3.3 Awards	30
2.3.4 Software	31
2.3.5 Master's Thesis Supervisions	31
2.3.6 Teaching	31

3 Extensive characterization and quality control of long-read sequencing transcriptomes	33
3.1 Introduction	35
3.2 Data	38
3.2.1 Neural System in mouse	38
3.2.2 Public datasets	39
3.2.2.1 Maize PacBio	39
3.2.2.2 MCF-7 Human PacBio	39
3.2.2.3 B-cell mouse nanopore	40
3.3 Methods	41
3.3.1 Transcriptome definition using Iso-Seq PacBio long-reads	41
3.3.2 Iso-Seq PacBio evaluation of isoform quantification and detection	42
3.3.3 Classification of transcripts to describe long-read captured novelty	43
3.3.4 Extensive isoform characterisation as a means for quality control	45
3.3.5 Using quality control features to build a filter of isoform artefacts	47
3.3.6 Open reading frame prediction benchmarking and assessment of UTR/ORF variability in PacBio-defined transcriptomes	49
3.4 Results	51
3.4.1 PacBio Iso-Seq sequencing quality	51
3.4.2 Transcriptome complexity and transcript full-lengthness assessment across alternative pipelines	51
3.4.3 Characterisation of ToFU-defined novel calls reveals enrichment in artefacts	61
3.4.4 Experimental validation of ToFU results verifies the presence of novel-isoform artefacts	65

3.4.5	Machine learning enables accurate filtering of novel-isoform artefacts	67
3.4.6	PacBio sequencing unable to accurately quantify low-medium expressed isoforms but capturing most transcriptional signal	71
3.4.7	Novel transcripts have a major impact on the accuracy of transcriptome quantification by short reads	73
3.4.8	Open reading frame prediction in long-read defined transcriptomes	76
3.4.9	Open reading frame diversity generated by novel long-read defined isoforms	77
3.4.10	SQANTI tool	79
3.4.10.1	SQANTI quality control	80
3.4.10.2	SQANTI filter	81
3.4.10.3	Diagnostic plots	81
3.4.11	Generalization of the SQANTI approach	82
3.5	Discussion	85
4	In-silico annotation of isoforms with functional and regulatory features	89
4.1	Introduction	91
4.2	IsoAnnot pipeline	94
4.2.1	Input data	94
4.2.2	Functional annotation at transcript isoform resolution	94
4.2.2.1	Cis-acting UTR regulatory elements	96
4.2.2.2	Upstream open reading frame prediction	96
4.2.2.3	Repeats and low-complexity elements	97
4.2.2.4	miRNA binding sites	97
4.2.2.5	RNA binding protein binding sites	101
4.2.2.6	Polyadenylation signals	102
4.2.2.7	Nonsense-mediated decay	103
4.2.3	Functional annotation at protein isoform resolution	103
4.2.3.1	Pfam domains	103

4.2.3.2 Transmembrane domains	104
4.2.3.3 Signal peptide	104
4.2.3.4 Coiled regions	104
4.2.3.5 Disordered regions	105
4.2.3.6 Nuclear localization signals	105
4.2.3.7 Coordinate-based and in-frame transference of protein functional features	106
4.2.4 Annotation of non-positional functional information	111
4.2.5 IsoAnnot output	111
4.3 Results	112
4.3.1 Functional annotation of PacBio-defined neural transcriptomes	112
4.3.2 Functional annotation of reference transcriptomes and proteomes	121
4.4 Discussion	125
5 Comprehensive framework for the functional analysis of alternative isoform usage	129
5.1 Introduction	131
5.2 Methods	134
5.2.1 Input data	134
5.2.2 Module 1: Isoform functional diversity	134
5.2.2.1 Structural diversity	135
5.2.2.2 Feature diversity	137
5.2.2.3 Overall rate of diversity	138
5.2.3 Module 2: Transcriptome dynamics	139
5.2.3.1 Differential expression	139
5.2.3.2 Differential isoform usage	140
5.2.4 Module 3: Functional impact triggered by isoform regulation	145
5.2.4.1 Differential feature inclusion	145
5.2.4.2 Differential polyadenylation and UTR lengthening	148
5.2.5 TappAS software implementation	152

5.3 Data	154
5.4 Results	155
5.4.1 The impact of neural transcriptome complexity on functional diversity	155
5.4.2 Multi-layer analysis of alternative isoform usage	164
5.4.3 Impact of differential isoform usage on functional properties	169
5.4.4 Impact of APA events in UTR modulation and containing features	177
5.5 Discussion	181
6 Functional consequences of differential isoform usage in neural fate determination	185
6.1 Introduction	187
6.2 Methods	189
6.2.1 Experimental design	189
6.2.2 RNA-seq by single molecule and short-read sequencing .	189
6.2.3 De novo discovery of neural isoforms by Iso-Seq and transcriptome curation	190
6.2.4 Isoform quantification and normalisation	191
6.2.5 Principal component analysis for lineage characterisation	191
6.2.6 Relevance of novel isoforms in defining cell identity	192
6.2.7 Transcriptional and post-transcriptional dynamics in neural differentiation systems	193
6.2.8 Isoform functional diversity in neural differentiation systems	194
6.2.9 Differential feature inclusion between glial and neuronal differentiation	195
6.2.10 Differential polyadenylation in differentiating glial and neuronal cells	195
6.3 Results	197
6.3.1 Widespread novel post-transcriptional diversity in neural systems captured by long-read sequencing	197

6.3.2 System characterisation defines oligodendrocyte and motor neuron progenitors as the most mature differentiation stages	201
6.3.3 Novel isoforms are not prevalent but define cell-lineage and differentiation dynamics	203
6.3.4 Membrane trafficking among the strongest processes specifically regulated by alternative isoform usage in neural determination	207
6.3.5 Functional impact of differential isoform usage on neural fate determination	214
6.4 Discussion	231
7 Conclusions	239
Appendix 1: SQANTI attributes at transcript level	247
Appendix 2: SQANTI attributes at splice junction level	251
References	255