

ILLUMINA-based Sequencing of Yeast RNA

From a set of 13 billion based pairs corresponding to 82 million paired end reads we aligned 68 million reads to the *S. cerevisiae* genome using Novocraft novoalign V2.07.10 software. MapSplice* filtering was used to screen out likely sequence and alignment artifacts. MapSplice considers both the quality and diversity of read alignments and is not dependent on splice site features or intron length, allowing for the detection of non-canonical splicing events.

We identified 94% (268 total) of the previously detected transcripts spliced in mitotic cells. Preliminary analysis of the data provides evidence for low-level splicing in 23 genes not previously shown to have an intron and evidence for alternative splice site choice in roughly 25% of all known intron-bearing genes - 81 instances of novel alternative splice site use were found. This level of alternate splice site selection greatly exceeds previous estimates in yeast and includes many examples of novel 5' splice sites, alternative 3' splice sites, and combined alternative 5' and 3' splice sites. The levels of the alternatively processed RNA varies greatly, from <1% to >30% of the major splice product and, as might be anticipated, most alternative 5' or 3' splice site junctions are close matches to the authentic splice site in sequence composition and location.

* Wang et al., [Nucleic Acids Res.](#) 2010 Oct;38(18):e178. doi: 10.1093/nar/gkq622. Epub 2010 Aug 27.

Chrom: Yeast chromosome where the gene is located

Start/end: beginning and end of the intron

ORF: Open reading frame designation (unique identifier for yeast genes; YBL093 = Yeast; 2nd chromosome; Left of the centromere; 93 ORF from the centromere)

Gene: common name of gene

Exp: relative expression level (Low, Medium, High)

Alt. site: alternate 5' splice site, 3' splice site or both

Flank: 2 nucleotides of exon sequence before and after intron

In SGD? Was this annotated in the *Saccharomyces cerevisiae* genome database at the time of the experiment?

Ares 4.1: Was this annotated in the Dr. Manny Ares database at the time of the experiment?

WT depth: Number of times the sequence occurs in the wildtype yeast

H218A depth: Number of times the sequence occurs in the *prp43H218A* mutant yeast

ILLUMINA Sequence

chrom	start	end	ORF	Gene	Exp. Le	Alt. site	flank	In SGD?	Ares 4.1	WT depth	H2168A depth
chrXI	625543	625619	3' dnstm MLP	3' dnstm M			GTAG	n	n	396	356
chrXI	437480	437549	5' Upstrm DII	5' Upstrm i		3'	GTAG			262	298
chrVI	54377	54686	YFL039C	ACT1	H		CTAC	Y	Y	4264	3999
chrXV	565946	566195	ADE2	ADE2	L		CTAC	n	n	18	0
chrV	348197	348273	YER093C-A	AIM11	L		CTAC	Y	Y	141	129
chrVII	497369	497463	YGR001C	AML1	M		CTAC	Y	Y	412	319
chrVII	497941	498004	YGR001C	AML1	M		CTAC	Y	Y	207	117
chrXI	155276	155660	YKL157W	APE2	L		GTAG	y	y	18	17
chrX	396488	396566	YJL024C	APS3	L		CTAC	y	y	143	117
chrIV	216157	216505	YDL137W	ARF2	L	3'	GTAG	N	N	0	13
chrIV	216157	216490	YDL137W	ARF2	M		GTAG	Y	Y	541	472
chrIV	399359	399494	YDL029W	ARP2	L	3'	GTAG	N	N	11	0
chrIV	399359	399483	YDL029W	ARP2	M		GTAG	Y	Y	673	616
chrXIII	337816	337903	YMR033W	ARP9	M		GTAG	y	y	195	197
chrXIII	499877	500151	YMR116C	ASC1	H		CTAC	n	n	3916	2678
chrIX	348360	348492	YIL004C	BET1	M		CTAC	Y	Y	175	149
chrX	387343	387431	YJL031C	BET4	L		CTAC	y	y	50	70
chrVIII	315774	315862	YHR101C	BIG1	L		CTAC	Y	Y	106	72
chrIV	652778	653522	YDR099W	BMH2	M		GTAG	Y	Y	496	447
chrXII	286468	286558	YLR078C	BOS1	L		CTAC	y	y	92	90
chrXIII	206097	206204	YML036W	CGI121	M		GTAG	y	y	164	130
chrII	110422	110508	YBL059C-A	CMC2	M		CTAC	Y	Y	424	351
chrXIII	652774	652847	YMR194C-B	CMC4	L		CTAC	y	y	61	31
chrXI	83003	83087	YKL190W	CNB1	L		GTAG	n	n	20	16
chrXIII	559781	560157		YMR147W	L	new intron	GTAG	n	n	48	68
chrVII	62131	62184	YGL232W	TAN1	L	3'	GTAG	N	N	64	47
chrVII	62131	62197	YGL232W	TAN1	L	3'	GTAG	N	N	54	41
chrVII	62131	62215	YGL232W	TAN1	L	3'	GTAG	N	N	11	10
chrVII	62131	62190	YGL232W	TAN1	M		GTAG	Y	Y	280	251
chrIV	254955	255045	YDL115C	IWR1	L	3'	CTAC	N	N	46	29
chrIV	254974	255045	YDL115C	IWR1	L		CTAC	Y	Y	113	98
chrXIII	77308	77430	YML098W	TAF13	L	new intron	GTAG	n	n	7	20