

# InPAS guide

Jianhong Ou, Sung Mi Park, Michael R. Green and Lihua Julie Zhu

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## 1 Introduction

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Alternative polyadenylation (APA) is one of the most important post-transcriptional regulation mechanisms which occurs in most human genes. APA in a gene can result in altered expression of the gene, which can lead pathological effect to the cells, such as uncontrolled cell cycle and growth. However, there are only limited ways to identify and quantify APA in genes, and most of them suffers from complicated process for library construction and requires large amount of RNAs.

RNA-seq has become one of the most commonly used methods for quantifying genome-wide gene expression. There are massive RNA-seq datasets available publicly such as GEO and TCGA. For this reason, we develop the InPAS algorithm for identifying APA from conventional RNA-seq data.

The workflow for InPAS is:

1. Calculate coverage from BEDGraph files
2. Predict cleavage sites
3. Estimate 3UTR usage

## 2 How to

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To use InPAS, BSgenome and TxDb object have to be loaded before run.

```
> library(InPAS)
> library(BSgenome.Mmusculus.UCSC.mm10)
> library(TxDb.Mmusculus.UCSC.mm10.knownGene)
> path <- file.path(find.package("InPAS"), "extdata")
```

Users can prepare annotation by `utr3Annotation` with a `TxDb` and `org` annotation. The 3UTR annotation prepared by `utr3Annotation` includes the gaps to next annotation region.

```
> library(org.Hs.eg.db)
> samplefile <- system.file("extdata", "hg19_knownGene_sample.sqlite",
+                             package="GenomicFeatures")
> txdb <- loadDb(samplefile)
> utr3.sample.anno <- utr3Annotation(txdb=txdb,
+                                     orgDbSYMBOL="org.Hs.egSYMBOL")
> utr3.sample.anno
```

GRanges object with 177 ranges and 6 metadata columns:

	seqnames	ranges	strand	feature	
	<Rle>	<IRanges>	<Rle>	<character>	
uc001bum.2_5 IQCC utr3	chr1	[ 32673684, 32674288]	+	unknown	
uc001fbq.3_3 S100A9 utr3	chr1	[153333315, 153333503]	+	unknown	
uc001gde.2_2 LRRC52 utr3	chr1	[165533062, 165533185]	+	unknown	
uc001hfg.3_15 PFKFB2 utr3	chr1	[207245717, 207251162]	+	unknown	
uc010psc.2_17 PFKFB2 utr3	chr1	[207252365, 207254368]	+	unknown	
...	...	...	...	...	
uc004dst.3_22 PHF8 CDS	chrX	[ 53965591, 53965679]	-	unknown	
uc004dsx.3_15 PHF8 CDS	chrX	[ 53969797, 53969835]	-	unknown	
uc004ehz.1_5 ARMCX3 CDS	chrX	[100879970, 100881109]	+	unknown	
uc004elw.3_6 FAM199X CDS	chrX	[103434289, 103434459]	+	unknown	
uc004fmj.1_10 GAB3 CDS	chrX	[153906455, 153906571]	-	unknown	
	id	exon	transcript	gene	symbol
	<character>	<character>	<character>	<character>	<character>
uc001bum.2_5 IQCC utr3	utr3	uc001bum.2_5	uc001bum.2	55721	IQCC
uc001fbq.3_3 S100A9 utr3	utr3	uc001fbq.3_3	uc001fbq.3	6280	S100A9
uc001gde.2_2 LRRC52 utr3	utr3	uc001gde.2_2	uc001gde.2	440699	LRRC52
uc001hfg.3_15 PFKFB2 utr3	utr3	uc001hfg.3_15	uc001hfg.3	5208	PFKFB2
uc010psc.2_17 PFKFB2 utr3	utr3	uc010psc.2_17	uc010psc.2	5208	PFKFB2
...	...	...	...	...	...
uc004dst.3_22 PHF8 CDS	CDS	uc004dst.3_22	uc004dst.3	23133	PHF8
uc004dsx.3_15 PHF8 CDS	CDS	uc004dsx.3_15	uc004dsx.3	23133	PHF8
uc004ehz.1_5 ARMCX3 CDS	CDS	uc004ehz.1_5	uc004ehz.1	51566	ARMCX3
uc004elw.3_6 FAM199X CDS	CDS	uc004elw.3_6	uc004elw.3	139231	FAM199X
uc004fmj.1_10 GAB3 CDS	CDS	uc004fmj.1_10	uc004fmj.1	139716	GAB3

```
-----
seqinfo: 27 sequences from hg19 genome; no seqlengths
```

Users can load mm10 and hg19 annotation from pre-prepared data. Here we loaded the prepared mm10 3UTR annotation file.

```
> ##step1 annotation
> # load from dataset
> data(utr3.mm10)
```

The coverage is calculated from BEDGraph file. The RNA-seq BAM files could be converted to BED-Graph files by bedtools genomecov tool (parameter: -bg -split). PWM and a classifier of polyA signal can be used for adjusting CP sites prediction.

[illegible]

GRanges object with 1 range and 28 metadata columns:

	seqnames	ranges	strand	transcript	gene	symbol
	<Rle>	<IRanges>	<Rle>	<character>	<character>	<character>
uc009eet.1	chr6	[128846245, 128850081]	-	uc009eet.1	232406	BC035044
	fit_value	Predicted_Proximal_APA	Predicted_Distal_APA		type	utr3start
	<numeric>	<numeric>	<numeric>	<character>	<numeric>	
uc009eet.1	128.8251	128849128		128846245	novalDistal	128849981
	utr3end	total.gp1	long.gp1	short.gp1	total.gp2	long.gp2
	<numeric>	<numeric>	<numeric>	<numeric>	<numeric>	<numeric>
uc009eet.1	128848044	39.59496	7.874827	31.72014	19.62749	21.95978
	total.mean.gp1	long.mean.gp1	short.mean.gp1	total.mean.gp2	long.mean.gp2	
	<numeric>	<numeric>	<numeric>	<numeric>	<numeric>	
uc009eet.1	39.59496	7.874827	31.72014	19.62749	21.95978	
	short.mean.gp2	test_status	PDUI.gp1	PDUI.gp2	dPDUI	pval
	<numeric>	<logical>	<numeric>	<numeric>	<numeric>	<numeric>
uc009eet.1	0	TRUE	0.1988846	1	0.8011154	2.28143e-10
	adjPval	filterPass				
	<numeric>	<logical>				
uc009eet.1	4.56286e-10	TRUE				

-----

seqinfo: 1 sequence from an unspecified genome; no seqlengths

The process described above can be done in one step.

```
> if(interactive()){
+   res <- inPAS(bedgraphs=bedgraphs, tags=c("Baf3", "UM15"),
+               genome=BSgenome.Mmusculus.UCSC.mm10,
+               utr3=utr3.mm10, gp1="Baf3", gp2="UM15",
+               txdb=TxDb.Mmusculus.UCSC.mm10.knownGene,
+               search_point_START=200,
+               short_coverage_threshold=15,
+               long_coverage_threshold=3,
+               cutStart=0, cutEnd=.2,
+               hugeData=FALSE,
+               shift_range=50,
+               PolyA_PWM=pwm, classifier=classifier)
+ }
```

InPAS can handle single group data.

```
> res <- inPAS(bedgraphs=bedgraphs[1], tags=c("Baf3"),
+             genome=BSgenome.Mmusculus.UCSC.mm10,
+             utr3=utr3.mm10, gp1="Baf3", gp2=NULL,
+             txdb=TxDb.Mmusculus.UCSC.mm10.knownGene,
+             search_point_START=200,
+             short_coverage_threshold=15,
+             long_coverage_threshold=3,
```

```
+          cutStart=0, cutEnd=.2,
+          hugeData=FALSE,
+          PolyA_PWM=pwm, classifier=classifier)
> res[1]
```

GRanges object with 1 range and 18 metadata columns:

	seqnames	ranges	strand	transcript	gene	symbol	
	<Rle>	<IRanges>	<Rle>	<character>	<character>	<character>	
uc009daz.2	chr6	[98018176, 98021358]	+	uc009daz.2	17342	Mitf	
	fit_value	Predicted_Proximal_APA	Predicted_Distal_APA		type	utr3start	
	<numeric>	<numeric>		<numeric>	<character>	<numeric>	
uc009daz.2	18177.92	98018524		98021358	distal	98018176	
	utr3end	total.gp1	long.gp1	short.gp1	test_status	PDUI.gp1	dPDUI
	<numeric>	<numeric>	<numeric>	<numeric>	<logical>	<numeric>	<numeric>
uc009daz.2	98021358	275.7874	293.1735	0	TRUE	1	1
	pval	adjPval	filterPass				
	<numeric>	<numeric>	<logical>				
uc009daz.2	4.678114e-05	9.356227e-05	TRUE				

-----

seqinfo: 1 sequence from an unspecified genome; no seqlengths

### 3 Session Info

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```
> toLatex(sessionInfo())
```

- R version 3.2.1 (2015-06-18), x86\_64-apple-darwin10.8.0
- Locale: en\_US.UTF-8/en\_US.UTF-8/en\_US.UTF-8/C/en\_US.UTF-8/en\_US.UTF-8
- Base packages: base, datasets, graphics, grDevices, methods, parallel, stats, stats4, utils
- Other packages: ade4 1.7-2, AnnotationDbi 1.30.1, Biobase 2.28.0, BiocGenerics 0.14.0, BiocParallel 1.2.14, Biostrings 2.36.1, BSgenome 1.36.3, BSgenome.Drerio.UCSC.danRer7 1.4.0, BSgenome.Mmusculus.UCSC.mm10 1.4.0, cleanUpdTSeq 1.6.1, DBI 0.3.1, e1071 1.6-6, GenomInfoDb 1.4.1, GenomicFeatures 1.20.1, GenomicRanges 1.20.5, InPAS 1.0.6, IRanges 2.2.5, org.Hs.eg.db 3.1.2, RSQLite 1.0.0, rtracklayer 1.28.6, S4Vectors 0.6.2, seqinr 3.1-3, TxDb.Mmusculus.UCSC.mm10.knownGene 3.1.2, XVector 0.8.0
- Loaded via a namespace (and not attached): acepack 1.3-3.3, BiocStyle 1.6.0, biomaRt 2.24.0, biovizBase 1.16.0, bitops 1.0-6, class 7.3-13, cluster 2.0.3, colorspace 1.2-6, dichromat 2.0-0, digest 0.6.8, foreign 0.8-65, Formula 1.2-1, futile.logger 1.4.1, futile.options 1.0.0, GenomicAlignments 1.4.1, ggplot2 1.0.1, grid 3.2.1, gridExtra 2.0.0, gtable 0.1.2, Gviz 1.12.1, Hmisc 3.16-0, lambda.r 1.1.7, lattice 0.20-33, latticeExtra 0.6-26, limma 3.24.14, magrittr 1.5, MASS 7.3-43, matrixStats 0.14.2, munsell 0.4.2, nnet 7.3-10, plyr 1.8.3, proto 0.3-10, RColorBrewer 1.1-2, Rcpp 0.12.0, RCurl 1.95-4.7, reshape2 1.4.1, rpart 4.1-10, Rsamtools 1.20.4, scales 0.2.5, splines 3.2.1, stringi 0.5-5, stringr 1.0.0, survival 2.38-3, tools 3.2.1, VariantAnnotation 1.14.6, XML 3.98-1.3, zlibbioc 1.14.0