

5.3 Poly-dT Priming and DNase Digestion (*S.cerevisiae*)

The simulation of RNA-Seq in *Saccharomyces cerevisiae* joins a reverse transcription model by poly-dT primers with subsequent fragmentation by DNaseI. Sequence biases that have been reported for the DNaseI fragmentation process (Hansen et al. 2010) are captured in the simulation by a position weight matrix (DNaseI.pwm).

Input

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[Reference Annotation](#)

[Parameter File](#)

[Reference Genome](#)

Parameter

Expression		
NB_MOLECULES	5,000,000	Number of RNA molecules initially in the experiment
TSS_MEAN	25	Average deviation from the annotated transcription start site (TSS)
POLYA_SCALE	80	Scale of the Weibull distribution, shifts the average length of poly-A tail sizes
POLYA_SHAPE	2	Shape of the Weibull distribution describing poly-A tail sizes
Reverse Transcription		
RTRANSCRIPTION	YES	Switch on the reverse transcription
RT_PRIMER	PDT	Use poly-dT primers used for first strand synthesis
RT_LOSSLESS	YES	Flag to force every molecule to be reversely transcribed
RT_MIN	500	Minimum length observed after reverse transcription of full-length transcripts
RT_MAX	2,500	Maximum length observed after reverse transcription of full-length transcripts
Fragmentation		
FRAG_SUBSTRATE	DNA	Specifies DNA as the substrate of fragmentation
FRAG_METHOD	EZ	Enzymatic digestion as fragmentation method
FRAG_EZ_MOTIF	DNaseI.pwm	Fragmentation by enzymatic digestion
Amplification and Size Segregation		
PCR_DISTRIBUTION	default	Default PCR distribution with 15 rounds and 20 bins
GC_MEAN	0.5	Mean value of a gaussian distribution that reflects GC bias amplification probability
GC_SD	0.1	Standard deviation of a gaussian distribution that reflects GC bias amplification probability
FILTERING	YES	Enables size filtering of fragments
SIZE_SAMPLING	MH	The Metropolis-Hastings algorithm is used for filtering
Sequencing		
READ_NUMBER	1,000,000	Produce 1 million reads
READ_LENGTH	36	Each read sequence is 36nt long
PAIRED_END	NO	Single reads are simulated, one per fragment

Output

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[INFO] I am collecting information on the run.
      initializing profiler *****
[INFO] Checking GTF file
*[WARN] Unsorted in line 5 - cannot perform gene clustering: chrI + YAL069W @ 335 after YAL012W @ 130799
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***** OK (00:00:02)
[GTF FILE] The GTF reference file given is not sorted, but we found a sorted version.
[GTF FILE] The Simulator will use /Users/micha/Desktop/sacCer3_SGDGenes_fromUCSC120515_sorted.gtf
[GTF FILE] You might want to update your parameters file
[PROFILING] I am assigning the expression profile
***** OK (00:00:02)
  Reading reference annotation *[WARN] merging exon (31229,35248) with exon (29935,31227) in transcript
YBL100W-B because intervening intron has 4 or less nt.
[WARN] merging exon (222636,226598) with exon (221330,222634) in transcript YBL005W-B because intervening
intron has 4 or less nt.
*****[WARN] merging exon (-854953,-856257) with exon (-850989,-854951) in transcript YPR158C-D because
intervening intron has 4 or less nt.
  OK (00:00:01)
    found 6664 transcripts
[PROFILING] Parameters
  NB_MOLECULES      5000000
  EXPRESSION_K      -0.6
  EXPRESSION_X0     5.0E7
  EXPRESSION_X1     9500.0
  PRO_FILE_NAME     /Users/micha/Desktop/sacCer3_enzyme.pro
  profiling ***** OK (00:00:00)
  Updating .pro file ***** OK (00:00:00)
  molecules        4999971
[LIBRARY] creating the cDNA library
  Initializing Fragmentation File ***** OK (00:00:04)
  4999971 mol initialized
[LIBRARY] Reverse Transcription
[LIBRARY] Configuration
  Mode: PDT
  PWM: No
  RT MIN: 500
  RT MAX: 2500
  Processing Fragments ***** OK (00:00:15)
  4999971 mol: in 4999971, new 0, out 4999971
  avg Len 969.7831, maxLen 2500
  preparing transcript sequences *[WARN] merging exon (31229,35248) with exon (29935,31227) in transcript
YBL100W-B because intervening intron has 4 or less nt.
*****[WARN] merging exon (-854953,-856257) with exon (-850989,-854951) in transcript YPR158C-D because
intervening intron has 4 or less nt.
  OK (00:00:02)
[LIBRARY] Enzymatic Digestion
[LIBRARY] Configuration
Left Flank : 100
Right Flank : 300
Motif: DNaseI.pwm
  Processing Fragments ***** OK (00:02:38)
  60604099 mol: in 4999971, new 55604128, out 60604099
  avg Len 80.00923, maxLen 2500
  initializing Selected Size distribution
[LIBRARY] Segregating cDNA (MCMC Filter)
  Processing Fragments ***** OK (00:01:47)
  60604099 mol: in 60604099, new 0, out 25719279
  avg Len 47.310493, maxLen 276
  start amplification
[INFO] Loading default PCR distribution
[LIBRARY] Amplification
[LIBRARY] Configuration
  Rounds: 15
  Mean: 0.5
  Standard Deviation: 0.1
  Processing Fragments ***** OK (00:01:05)
  Amplification done.
  In: 25719279 Out: 693695450
  25719279 mol: in 25719279, new 0, out 693695450
  avg Len 47.319595, maxLen 266
  Copied results to /Users/micha/Desktop/sacCer3_enzyme.lib
  Updating .pro file ***** OK (00:00:00)
[SEQUENCING] getting the reads
  Initializing Fragment Index
  Indexing ***** OK (00:00:14)
  13804020 lines indexed (693695450 fragments, 6534 entries)

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sequencing *[WARN] merging exon (31229,35248) with exon (29935,31227) in transcript YBL100W-B because
intervening intron has 4 or less nt.
*****[WARN] merging exon (-854953,-856257) with exon (-850989,-854951) in transcript YPR158C-D because
intervening intron has 4 or less nt.
OK (00:14:03)
693695450 fragments found (13804020 without PCR duplicates)
998612 reads sequenced
226528 reads fall in poly-A tail
407504 truncated reads
Moving temporary BED file
Updating .pro file ***** OK (00:00:00)
[END] I finished, took me 1305 sec.
```