Using RAMPAGE to identify and annotate regulatory elements in insect genomes

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Abstract. Application of Transcription Start Site (TSS) profiling technologies, coupled with large-scale next-generation sequencing (NGS) has yielded valuable insights into the location, structure and activity of promoters across diverse metazoan model systems. In insects, TSS profiling has been used to characterize the promoter architecture of *D. melanogaster*, and, shortly thereafter, to reveal widespread transposon-driven alternative promoter usage.
In this chapter we highlight the utility of one TSS profiling method, RAMPAGE (RNA annotation and mapping of promoters for analysis of gene expression), for the precise, quantitative identification of promoters in insect genomes. We demonstrate this using our bioinformatics pipeline GoRAMPAGE, providing details instructions with the aim of taking the user from raw reads to processed results.

Keywords: *cis*-regulatory regions, promoter architecture, transcription initiation, transcription start sites (TSSs)

1 Introduction

1.1 TSS Profiling Identifies Promoters at Genome-Scale

The promoter, defined in eukaryotes as the genomic region bound by RNA Polymerase II immediately prior to transcription initiation [1], is the site where regulatory signals unite to direct gene expression. The identification of promoter regions is a valuable step for understanding the *cis*-regulatory signals that are present in an organism, and is important for genome annotation. However, despite the rapid accumulation of genome sequences across metazoan and arthropod diversity, accurate annotation of promoter regions remains sparse. This is because—empirical mapping of TSSs—precisely identifying sequence motifs that demarcate the promoter is unreliable. In contrast with current *in*

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silico approaches, direct mapping of TSSs identifies the location of the core promoter. Cap Analysis of Gene Expression (CAGE) [2], one of the first methods devised to identify 5‘-ends of mRNAs at large-scale, involves selective capture of 5‘-capped transcripts, first-strand reverse-transcription and ligation of a short oligonucleotide (CAGE tag). CAGE was initially utilized by the FANTOM (Functional Annotation of the Mammalian Genome) consortium to identify promoter architecture in human and mouse [3], providing the first glimpse of the global landscape of transcription initiation. At the onset of the NGS era, CAGE was coupled with massively-parallel sequencing to generate 5‘-ends of mRNAs at substantially higher scale. This advance provided more extensive coverage of the expressed transcriptome, and provided increased sensitivity for quantitative measurements *i.e.* measurement of promoter activity.

1.2 Promoter Architecture of *Drosophila melanogaster*

Hoskins and colleagues [4] performed CAGE in *D. melanogaster*, identifying promoters at large-scale and characterizing the promoter architecture of an insect genome for the first time. Hoskins [4] indicated that TSS distributions at *Drosophila* promoters exhibit a range of shapes that can be generally grouped into two major classifications: *peaked* and *broad*. Peaked promoters have a single, major TSS position occupying a narrow genomic region, whereas broad promoters lack a single, major TSS and contain TSSs across a wider region [5][6]. The authors also showed a strong association between promoter class and motif composition (consistent with previous findings [5, 7]). Peaked promoters were associated with positionally-enriched *cis*-regulatory motifs including TATA, Inr and DPE, while broad promoters contained an enrichment of less-well characterized motifs, including *Ohler6* and *Ohler7* [8]. The existence of two promoter classes appears to be conserved among metazoans, and has been reported in insects, cladocerans [9], fish [10] and mammals [11, 6].

1.3 Promoter Structure of Insects

1.4 Paired-end TSS Profiling with RAMPAGE

2 Materials

3 Methods

4 Notes

Acknowledgments

Disclosure Declaration

The authors declare that they have no competing interests.

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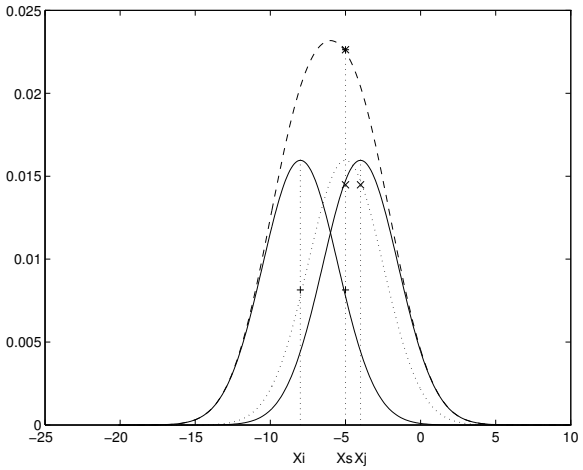

Fig. 1. One kernel at x_s (*dotted kernel*) or two kernels at x_i and x_j (*left and right*) lead to the same summed estimate at x_s. This shows a figure consisting of different types of lines. Elements of the figure described in the caption should be set in italics, in parentheses, as shown in this sample caption.

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Displayed equations or formulas are centered and set on a separate line (with an extra line or halfline space above and below). Displayed expressions should be numbered for reference. The numbers should be consecutive within each section or within the contribution, with numbers enclosed in parentheses and set on the right margin – which is the default if you use the *equation* environment, e.g.,

$$\psi(u) = \int_o^T \left[\frac{1}{2} \left(\Lambda_o^{-1} u, u \right) + N^*(-u) \right] dt \, . \tag{1}$$

Equations should be punctuated in the same way as ordinary text but with a small space before the end punctuation mark.

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5.3 Program Code

Program listings or program commands in the text are normally set in typewriter font, e.g., CMTT10 or Courier.

Example of a Computer Program

```
program Inflation (Output)
  {Assuming annual inflation rates of 7%, 8%, and 10%,...
   years};
   const
     MaxYears = 10;
   var
     Year: 0..MaxYears;
     Factor1, Factor2, Factor3: Real;
   begin
     Year := 0;
     Factor1 := 1.0; Factor2 := 1.0; Factor3 := 1.0;
     WriteLn('Year  7% 8% 10%'); WriteLn;
```

[1] The footnote numeral is set flush left and the text follows with the usual word spacing.

```
    repeat
      Year := Year + 1;
      Factor1 := Factor1 * 1.07;
      Factor2 := Factor2 * 1.08;
      Factor3 := Factor3 * 1.10;
      WriteLn(Year:5,Factor1:7:3,Factor2:7:3,Factor3:7:3)
    until Year = MaxYears
end.
```

(Example from Jensen K., Wirth N. (1991) Pascal user manual and report. Springer, New York)

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6 References

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