

Using RAMPAGE to identify and annotate regulatory elements in insect genomes

R. Taylor Raborn^{*1,2} and Volker P. Brendel^{1,2}

¹Department of Biology, Indiana University

²School of Informatics and Computing, Indiana University

Department of Biology and School of Informatics and Computing,
Indiana University

212 S. Hawthorne Drive 205 Simon Hall, Bloomington, IN 47401, USA
<http://www.brendelgroup.org>

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

The promoter, defined in eukaryotes as the genomic region bound by RNA Polymerase II immediately prior to transcription initiation, 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, including those of insects, annotation of promoter regions remains sparse. This is because—absent empirical mapping of TSSs—precisely identifying sequence motifs that demarcate the promoter is unreliable. In contrast with *in silico* approaches, direct mapping of

* Correspondence: rtraborn@indiana.edu

12 TSSs identifies the location of the core promoter. Cap Analysis of Gene Ex-
 13 pression (CAGE) [?], the first method to identify 5'-ends of mRNAs at large-
 14 scale, involves selective capture of 5'-capped transcripts, first-strand reverse-
 15 transcription and ligation of a short oligonucleotide (CAGE tag). CAGE was
 16 initially utilized by the FANTOM (Functional Annotation of the Mammalian
 17 Genome) consortium to identify promoter architecture in human and mouse [?],
 18 providing the first glimpse of the global landscape of transcription initiation. At
 19 the onset of the NGS era, CAGE was coupled with massively-parallel sequenc-
 20 ing to generate 5'-ends of mRNAs at substantially higher scale. This advance
 21 provided more extensive coverage of the expressed transcriptome, and provided
 22 increased sensitivity for quantitative measurements *i.e.* measurement of pro-
 23 moter activity. Hoskins and colleagues [?] performed CAGE in *D. melanogaster*,
 24 revealing the promoter architecture in any insect.

25 2 Materials

26 3 Methods

27 4 Notes

28 Acknowledgments

29 Disclosure Declaration

30 The authors declare that they have no competing interests.

5 Figures

For L^AT_EX users, we recommend using the *graphics* or *graphicx* package and the `\includegraphics` command.

Please check that the lines in line drawings are not interrupted and are of a constant width. Grids and details within the figures must be clearly legible and may not be written one on top of the other. Line drawings should have a resolution of at least 800 dpi (preferably 1200 dpi). The lettering in figures should have a height of 2 mm (10-point type). Figures should be numbered and should have a caption which should always be positioned *under* the figures, in contrast to the caption belonging to a table, which should always appear *above* the table; this is simply achieved as matter of sequence in your source.

Please center the figures or your tabular material by using the `\centering` declaration. Short captions are centered by default between the margins and typeset in 9-point type (Fig. 1 shows an example). The distance between text and figure is preset to be about 8 mm, the distance between figure and caption about 6 mm.

To ensure that the reproduction of your illustrations is of a reasonable quality, we advise against the use of shading. The contrast should be as pronounced as possible.

If screenshots are necessary, please make sure that you are happy with the print quality before you send the files.

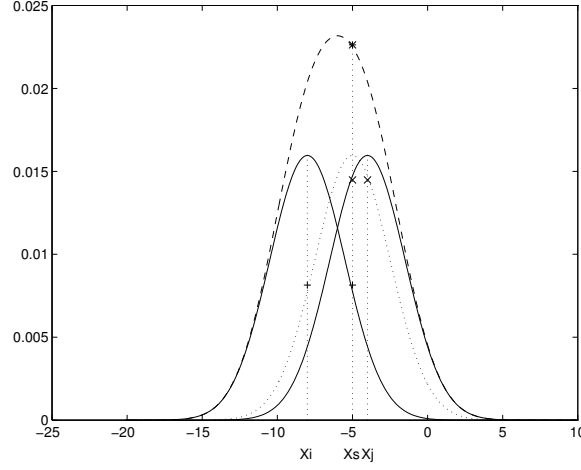


Fig. 1. One kernel at x_s (*dotted kernel*) or two kernels at x_i and x_j (*left and right*) lead to the same summed estimate at x_s . This shows a figure consisting of different types of lines. Elements of the figure described in the caption should be set in italics, in parentheses, as shown in this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

Please define figures (and tables) as floating objects. Please avoid using optional location parameters like “[h]” for “here”.

5.1 Formulas

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.,

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

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

5.2 Footnotes

The superscript numeral used to refer to a footnote appears in the text either directly after the word to be discussed or – in relation to a phrase or a sentence –

following the punctuation sign (comma, semicolon, or period). Footnotes should appear at the bottom of the normal text area, with a line of about 2 cm set immediately above them.¹

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;
  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)

5.4 Citations

For citations in the text please use square brackets and consecutive numbers: [?], [?], [?] – provided automatically by L^AT_EX's \cite ... \bibitem mechanism.

5.5 Page Numbering and Running Heads

There is no need to include page numbers. If your paper title is too long to serve as a running head, it will be shortened. Your suggestion as to how to shorten it would be most welcome.

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

6 References

References

1. R. Kodzius, M. Kojima, H. Nishiyori, M. Nakamura, S. Fukuda, M. Tagami, D. Sasaki, K. Imamura, C. Kai, M. Harbers, Y. Hayashizaki, and P. Carninci, "CAGE: cap analysis of gene expression." *Nature Methods*, vol. 3, no. 3, pp. 211–222, Mar. 2006.
2. P. Carninci, T. Kasukawa, S. Katayama, J. Gough, M. C. Frith, N. Maeda, R. Oyama, T. Ravasi, B. Lenhard, C. Wells, R. Kodzius, K. Shimokawa, V. B. Bajic, S. E. Brenner, S. Batalov, A. R. R. Forrest, M. Zavolan, M. J. Davis, L. G. Wilming, V. Aidinis, J. E. Allen, A. Ambesi-Impiombato, R. Apweiler, R. N. Aturaliya, T. L. Bailey, M. Bansal, L. Baxter, K. W. Beisel, T. Bersano, H. Bono, A. M. Chalk, K. P. Chiu, V. Choudhary, A. Christoffels, D. R. Clutterbuck, M. L. Crowe, E. Dalla, B. P. Dalrymple, B. de Bono, G. Della Gatta, D. di Bernardo, T. Down, P. Engstrom, M. Fagiolini, G. Faulkner, C. F. Fletcher, T. Fukushima, M. Furuno, S. Futaki, M. Gariboldi, P. Georgii-Hemming, T. R. Gingeras, T. Gojobori, R. E. Green, S. Gustincich, M. Harbers, Y. Hayashi, T. K. Hensch, N. Hirokawa, D. Hill, L. Huminiecki, M. Iacono, K. Ikeo, A. Iwama, T. Ishikawa, M. Jakt, A. Kanapin, M. Katoh, Y. Kawasawa, J. Kelso, H. Kitamura, H. Kitano, G. Kollias, S. P. T. Krishnan, A. Kruger, S. K. Kummerfeld, I. V. Kurochkin, L. F. Lareau, D. Lazarevic, L. Lipovich, J. Liu, S. Liuni, S. McWilliam, M. Madan Babu, M. Madera, L. Marchionni, H. Matsuda, S. Matsuzawa, H. Miki, F. Mignone, S. Miyake, K. Morris, S. Mottagui-Tabar, N. Mulder, N. Nakano, H. Nakauchi, P. Ng, R. Nilsson, S. Nishiguchi, S. Nishikawa, F. Nori, O. Ohara, Y. Okazaki, V. Orlando, K. C. Pang, W. J. Pavan, G. Pavesi, G. Pesole, N. Petrowsky, S. Piazza, J. Reed, J. F. Reid, B. Z. Ring, M. Ringwald, B. Rost, Y. Ruan, S. L. Salzberg, A. Sandelin, C. Schneider, C. Schönbach, K. Sekiguchi, C. A. M. Semple, S. Seno, L. Sessa, Y. Sheng, Y. Shibata, H. Shimada, K. Shimada, D. Silva, B. Sinclair, S. Sperling, E. Stupka, K. Sugiura, R. Sultana, Y. Takenaka, K. Taki, K. Tammoja, S. L. Tan, S. Tang, M. S. Taylor, J. Tegner, S. A. Teichmann, H. R. Ueda, E. van Nimwegen, R. Verardo, C. L. Wei, K. Yagi, H. Yamanishi, E. Zabarovsky, S. Zhu, A. Zimmer, W. Hide, C. Bult, S. M. Grimmond, R. D. Teasdale, E. T. Liu, V. Brusic, J. Quackenbush, C. Wahlestedt, J. S. Mattick, D. A. Hume, C. Kai, D. Sasaki, Y. Tomaru, S. Fukuda, M. Kanamori-Katayama, M. Suzuki, J. Aoki, T. Arakawa, J. Iida, K. Imamura, M. Itoh, T. Kato, H. Kawaji, N. Kawagashira, T. Kawashima, M. Kojima, S. Kondo, H. Konno, K. Nakano, N. Ninomiya, T. Nishio, M. Okada, C. Plessy, K. Shibata, T. Shiraki, S. Suzuki, M. Tagami, K. Waki, A. Watahiki, Y. Okamura-Oho, H. Suzuki, J. Kawai, Y. Hayashizaki, F. Consortium, R. G. E. R. Group, and G. S. G. G. N. P. C. Group, "The transcriptional landscape of the mammalian genome," *Science (New York, NY)*, vol. 309, no. 5740, pp. 1559–1563, Sep. 2005.
3. R. A. Hoskins, R. A. Hoskins, J. M. Landolin, J. M. Landolin, J. B. Brown, J. B. Brown, J. E. Sandler, J. E. Sandler, H. Takahashi, H. Takahashi, T. Lassmann, T. Lassmann, C. Yu, C. Yu, B. W. Booth, B. W. Booth, D. Zhang, D. Zhang, K. H. Wan, K. H. Wan, L. Yang, L. Yang, N. Boley, N. Boley, J. Andrews, J. Andrews, T. C. Kaufman, T. C. Kaufman, B. R. Graveley, B. R. Graveley, P. J. Bickel, P. J. Bickel, P. Carninci, J. W. Carlson, J. W. Carlson, S. E. Celniker, and S. E. Celniker, "Genome-wide analysis of promoter architecture in *Drosophila melanogaster*." *Genome Research*, vol. 21, no. 2, pp. 182–192, Feb. 2011.

In order to permit cross referencing within LNCS-Online, and eventually between different publishers and their online databases, LNCS will, from now on, be standardizing the format of the references. This new feature will increase the visibility of publications and facilitate academic research considerably. Please base your references on the examples below. References that don't adhere to this style will be reformatted by Springer. You should therefore check your references thoroughly when you receive the final pdf of your paper. The reference section must be complete. You may not omit references. Instructions as to where to find a fuller version of the references are not permissible.

We only accept references written using the latin alphabet. If the title of the book you are referring to is in Russian or Chinese, then please write (in Russian) or (in Chinese) at the end of the transcript or translation of the title.

The following section shows a sample reference list with entries for journal articles [?], an LNCS chapter [?], a book [?], proceedings without editors [?] and [?], as well as a URL [?]. Please note that proceedings published in LNCS are not cited with their full titles, but with their acronyms!

7 Checklist of Items to be Sent to Volume Editors

Here is a checklist of everything the volume editor requires from you:

- ☐ The final L^AT_EX source files
- ☐ A final PDF file
- ☐ A copyright form, signed by one author on behalf of all of the authors of the paper.
- ☐ A readme giving the name and email address of the corresponding author.