Using Targeted Sequencing for the Discovery of Intron Sequences with Baits Derived from Transcriptome Data

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MYBAITS

Introduction

- Genome sequences of many organisms of interest are unavailable. However, it is now economically feasible to deep sequence the transcriptome.
- Here, we demonstrate that transcriptome data can be used to generate MYbaits sequence capture kits targeting expressed sequences to enrich samples for exon sequences and flanking intron sequences.
- We illustrate the discovery of exon boundaries, complete genes including full introns as well as the capture of paralog genes.

Experimental design

Perennial ryegrass *Lolium perenne*

- A set of 28,867 bait sequences (100mer baits, tiled every 60bp) were designed from a mix of transcript and genomic sequences (2.25Mb total) from the perennial ryegrass. RNA baits were manufactured into a MYbaits kit.
- 454 Sequencing libraries were prepared from ryegrass genomic DNA with an average fragment length of 600 – 700bp.
- MYbaits kit was used to capture targets following the recommended protocol with the exception that the captured molecules were directly sequenced without any intermediary PCR amplification.
- Sequencing was performed either on a 454 Junior or a 454 FLX system.

Western terrestrial garter snake *Thamnophis elegans*

- A set of 14,468 bait sequences (80mer baits, tiled every 40bp) were designed from transcript sequences (692 Kb) from the western terrestrial garter snake.
- Two 454 Sequencing libraries were prepared from *T. elegans* genomic DNA with an average fragment length of 600 – 700bp.
- MYbaits kit was used to capture targets following the recommended protocol.
- Sequencing was performed on a 454 Junior yielding 180,470 reads (77Mb).
- 57% of reads were directly mapped to the reference transcript sequences.
- The reads were first assembled de-novo into contigs and subsequently mapped to the reference transcript sequences, we obtained a total of 2,556 reference contigs (defined as containing some transcript reference sequences).
- 85% of the reads can be mapped to the reference contigs
- Altogether, the reference contigs contain 615,338 bases mapped to reference transcripts and 2,161,616 unmapped bases. Thus, for 1 base of exon sequence, we gained an average 3.5 bases of new sequence.

2. Uncover exact exon structure

- Exon 9 (421bp) was incorrectly predicted by aligning this snake transcript to other species transcripts. Mapping of reads from captured sample to the reference sequence reveals that exon 9 should be split into 4 distinct exons.

Structure	Size (bp)
Predicted Exon 9	421
Exon 9a	153
Intron	93
Exon 9b	81
Intron	385
Exon 9c	86
Intron	259
Exon 9d	101
# novel bases per known base = 2.57	

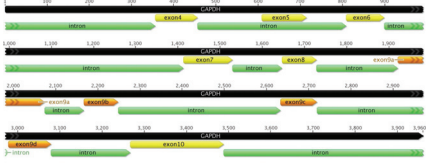


Figure shows delineation of exon 9 into 4 exons (orange) and discovery of novel intron sequences (green).

1. Identify exons and novel introns

- The entire coding region of the **ryegrass** SACDH1 gene (9,288bp) was captured using 44 baits designed from a transcript sequence without prior knowledge of the exon boundaries.
- Statistics:

	Exon	Intron
Number	25	24
Min. size (bp)	21	69
Max. size (bp)	312	1039
# novel bases per known base		1.84

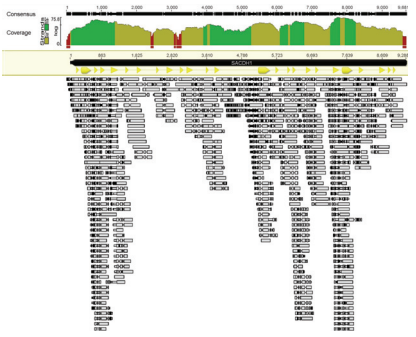
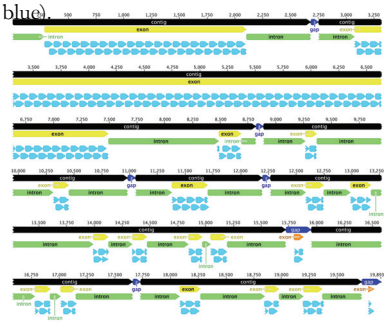


Figure shows the coverage and mapping of 454 reads along the assembled gene sequence. The reference sequence (exons) are shown in yellow.

- 212 baits (light blue) were designed from an uncharacterized **snake** transcript sequence (8,713bp) without prior knowledge of the exon boundaries.
- The reference sequence maps to 6 contigs (black) separated by gaps of unknown length inside introns (royal blue).
  - Identified at least 19 exons.
  - Fully sequenced 9 introns and partially sequenced 8 introns.
  - Largest sequenced intron is 996bp in length. Partially sequenced introns were likely larger.

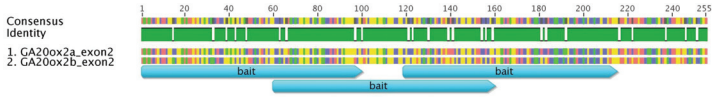


	Exon	Intron
Min. size (bp)	51	75
Max. size (bp)	4415	996
Average size range (bp)	100 - 200	200 - 500
# novel bases per known base		1.19

- Failure to capture two short exons (51 and 87bp) because of their relatively short length and limited sequencing depth.

3. Capture of gene paralogs

- 30 paralogs were identified for a set of 300 ryegrass transcripts targeted in this study.



- In this example, an exon was targeted with three baits and two paralogs were captured.

Conclusion

- It is possible to design baits from transcriptome sequence data to capture genomic sequence, characterize exons, and gain insight into some intron sequences.
- The identification of maximal exon-flanking (introns, upstream and downstream) sequences depends on intron length, exon length, and sequencing library fragment size.

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