The following Instructions of Use come from an 'Application Note' for the journal Bioinformatics 2015.

OfftargetFinder: a web tool for species-specific RNAi design authored by R.T.Good, T. Varhese, J.F. Golz, D.A. Russell, A.

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1. Enter Query Sequence

The user pastes a DNA sequence in the FASTA format (with no white spaces in the definition line) that they believe may be a good RNAi target (e.g. a gene that is essential for pest insect viability). Users then specify how many mismatches (0,1,2,3) will be tolerated in each window of 21 nucleotides. Once the 'Find Off Targets' button is chosen the query appears in the History table and the status of the job is reported. For multiple queries, a separate window can be opened for each query sequence that is submitted. The user can check for changes in query status by reloading their browser. They can view results when query status is completed. The green colored text indicates query has returned hits and red text indicate the same table. The program takes about 15 minutes to run, and when completed results can be viewed by clicking on the motif in the Show Result column.

2. Results page

A view of three panels is seen in the results tab. The top panel shows the query sequence and the panel on the left (*Organism classification panel*) shows a cladogram of taxonomic ranks in which hits are observed. The cladogram splits 'Arthropods' (most of the species considered) from 'Others' (which includes unclassified) and shows Order, Family, Genus and Species names that are based on 1KITE species classifications. The colour of the box next to the taxa names reflects the number of 'hits' observed in that taxa (purple = 1 hit, dark blue= 2-5, light blue =6-10, green 11-20, yellow 21-50, orange 51-100, red >101). The plus and minus signs on the nodes of the cladogram can be clicked to collapse and expand those nodes. If the coloured boxes are clicked the analysis in the Query Result panel is limited to the species of that clade.

The Query Result panel houses a variety of different views that can be toggled between by clicking on view button.

3. View: Browser

Among these views is 'Browser' which lists each species that has at least one 'hit' with the input sequence and indicates the location of the hits in a cartoon, much like the first figure of an NCBI Blast search. A feature of this view is that the user can zoom in to nucleotide resolution to see the exact 21mer sequences responsible for the off-target hit/s.

4. View: Query Hit Regions

The view provided by the "Query Hit Regions" paints the query sequence in colours according to the number of 21mers in the database (or the portion of the database delimited by the Organism Classification panel) that match that particular 21bp window. With this tool the user will identify stretches of nucleotides that are highly specific to the targeted species. This output will be useful in ensuring that a particular dsRNA molecule has minimal off-target hits.

5. View: Raw data

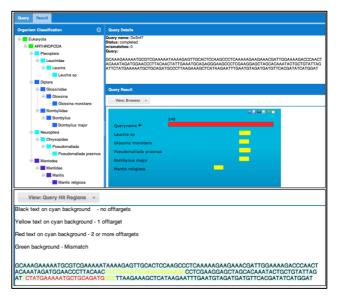
It is also possible to obtain a table of the number of 21mer 'hits' observed in each species ('Raw data'). In this, as in all other views, clicking on the "Eukaryote" box in the left panel will display all the results, whereas clicking on any subordinate box restricts the data in the view box to the subset of taxa selected.

6. View: Bar graph

The number of hits in each species can also be observed as bar charts with the x-axis having the species and the y-axis the hit count.

7. An example

Fig. 1. Offtarget hits using the snf7 gene. The 240nt sequence of the Snf7 gene from the beetle Diabrotica virgifera that was experimentally tested for cross-species hits by Bachman et al. (2013) was used as the query sequence in OffTargetFinder. Five hits were observed (see cladogram on the left panel), and they are distributed across the orders Plecoptera (a stonefly), Diptera (specifically a tsetse fly and a bee-fly), Neuroptera (a lacewing) and Mantodea (a praying mantis). This could motivate risk assessments, like the Bachman et al. (2013) experiments, on these particular 'off target' species. The large blue panel to the right of the cladogram shows which parts of the query contains at least one 21-mer hit across the five species. Note that in each of the cases here the hits are limited to one contiguous hit region. Note also that one region in the query sequence hits four distantly related non-target species. The



specific nucleotides that are hitting the off target species are observed in the bottom panel. Together these views demark sequences that should be avoided in RNAi designs to ensure taxonomically-limited RNAi pesticides.

>DvSnf7_ Sourced from Transgenic Research volume 22 issue 6 page 1219 Fig 2
GCAAAGAAAAATGCGTCGAAAAATAAAAGAGTTGCACTCCAAGCCCTCAAAAAGAAGAAACGATTGGAAAAGAC
CCAACTACAAATAGATGGAACCCTTACAACTATTGAAATGCAGAGGGAAGCCCTCGAAGGAGCTAGCACAAATAC
TGCTGTATTAGATTCTATGAAAAAGCTGCAGATGCCCTTAAGAAAGCTCATAAGAATTTGAATGTAGATGTT
CACGATATCATGGAT