CellPress
PARTNER JOURNAL

Molecular Plant
Letter to the Editor

The SGN VIGS Tool: User-Friendly Software to Design Virus-Induced Gene Silencing (VIGS) Constructs for Functional Genomics

Dear Editor,

Virus-induced gene silencing (VIGS) is a fast and powerful method to study gene function in plants (Burch-Smith et al., 2004). It is based on plant defense mechanisms against viral gene replication and allows high-throughput silencing of genes of interest (Senthil-Kumar and Mysore, 2014). The molecular mechanisms involved in post-transcriptional gene silencing (PTGS) have been studied intensively, and its steps are well known. The silencing process begins with the recognition through Dicer-like ribonucleases (DCL) of double-stranded RNA (dsRNA) that is generated during viral replication. Upon recognition, the dsRNA is processed into 21–24 nucleotide fragments, termed small interfering RNA (siRNA). Based on sequence homology, both viral siRNA and plant mRNA associate with an RNA-induced silencing complex (RISC) and are targeted for degradation (Llave, 2010).

The PTGS mechanism can be exploited in the laboratory by introducing a plant gene construct, together with virus elements, to achieve silencing of endogenous transcripts to study gene function (Burch-Smith et al., 2004). However, in the same way that a target gene is silenced by a construct based on similarity, unintended off-target genes can also be silenced and consequently obscure the interpretation of the observed phenotype (Senthil-Kumar and Mysore, 2011). For this reason, it is important to optimize the design of VIGS constructs to minimize similarity to off-target genes. In addition, the length of the construct should be between 200 and 400 nucleotides, since shorter fragments reduce the silencing efficiency and longer fragments increase the chance of silencing off-targets (Senthil-Kumar and Mysore, 2014). Fragments of similar size derived from different regions of the same target gene may result in different silencing efficiencies (Liu and Page, 2008). Consequently, each particular VIGS construct requires experimental follow-up to verify the extent of silencing.

Despite the widespread use of VIGS, no specific tool for selecting target regions is currently available. Some tools, such as BLAST, are not appropriate for this purpose as they were not developed for this task, and the researcher would need to spend a considerable amount of time to manually check each alignment and some relevant ones could easily be missed (Senthil-Kumar and Mysore, 2011). Other tools such as pssRNAit (http://plantgrn.noble.org/pssRNAit/) are very useful for designing constructs for RNAi silencing, but do not identify a construct of 200–400 nucleotides long required for VIGS. Therefore, at the Sol Genomics Network (SGN; Bombarely et al., 2011) we have developed the SGN VIGS tool to assist researchers with the selection of appropriate regions for silencing experiments (http://vigs.solgenomics.net).

The SGN VIGS tool algorithm simulates *in silico* the VIGS processes occurring in the plant cell (Figure 1A). In the first stage, the target gene is processed in all possible 21-nucleotide fragments (or any size chosen by the user between 18 and 24 nucleotides), to get short sequences equivalent to siRNAs produced by DCLs. Then, similar to the recognition between siRNA and mRNA directed by RISC, the siRNA are mapped on all gene models of the transcriptome using Bowtie (Langmead et al., 2009). The Bowtie result file is parsed to obtain the positions of targets and off-targets, and the number of targets and score values are assigned (Supplemental Information). Based on this score, a 300-nucleotide window (or any size defined by the user, between 100 nucleotides and the length of the query gene) with the maximum percentage covered by the target gene and the minimum presence of off-target matches is selected. Then, all alignments found are displayed on a graphical representation, showing the targets and off-targets, the best construct predicted by the tool, and the score graph (Figure 1C). The tool allows the user to upload a file with expression values for each gene, which are then visible alongside the corresponding gene. Once the results are displayed, any parameter, such as target number, construct size, n-mer size, and mismatches, can be redefined by the user (Figure 1B). If this occurs, the results and the graphical interface are recalculated on the fly without the need of repeating all the steps previously calculated by the algorithm (Figure 1A). To facilitate the inspection of the targets and off-targets, the output graph can be collapsed or expanded to either display only the coverage graph (regions covered by the targets and off-targets) or to show each of the siRNA fragments that individually align to the query gene. If the user is not satisfied with the automatic prediction, a custom region can be set by clicking on the "Set Custom Region" button (Figure 1B), which allows dragging and resizing the region to the desired location in the sequence. The information from the score graph and the expression values helps with the design of a custom VIGS construct. Even though the user is required to establish the desired fragment length (by default set at 300 nucleotides), the "Set Custom Region" feature allows easy adjustment of the region size if necessary. In addition, the graph can be zoomed in and out for a better observation of the data and delimitation of the custom construct ends. As a final result, the constructs are available in FASTA format for both the one predicted by the tool and the one defined by the user. Sequence overview information and the description of the genes with matches to the query are also displayed on the results page.

Published by the Molecular Plant Shanghai Editorial Office in association with Cell Press, an imprint of Elsevier Inc., on behalf of CSPB and IPPE, SIBS, CAS.

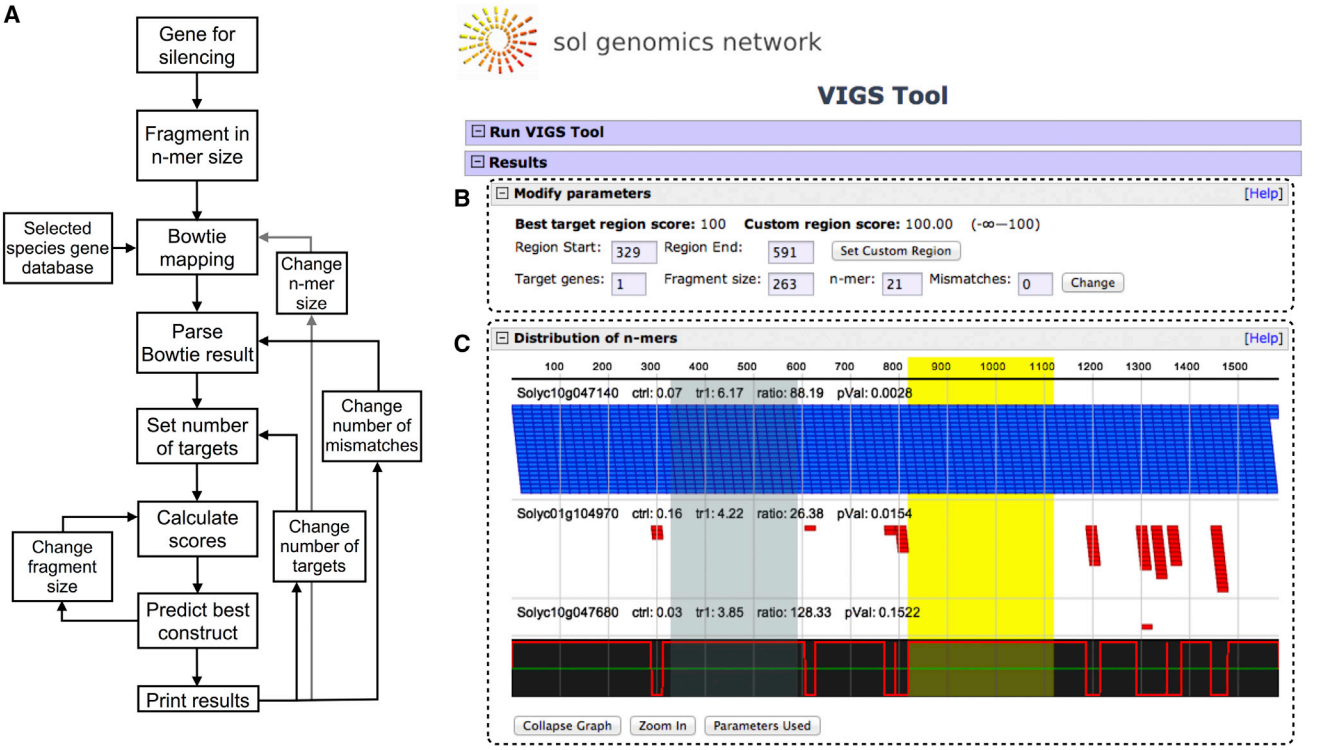

Figure 1. The SGN VIGS Tool.

(A) VIGS tool algorithm linear workflow. Arrows leaving from "Print results" box back to previous steps represent options to recalculate results with different parameters changed by the user.

(B) Control panel to recalculate the results with new parameters.

(C) Graphical representation of siRNA from targets (in blue) and off-targets (in red), suggesting the best construct predicted (in yellow), and allowing the user to define a custom construct (in transparent gray).

This web tool was developed using Perl, JavaScript, AJAX, and HTML5 canvas to allow a fast and interactive graphical representation of the results. BLAST (Altschul et al., 1990) and Bowtie are the only dependencies for the SGN VIGS tool. BLAST is used together with the BioPerl module Bio::BLAST::Database to get the functional descriptions, and sequences from query genes when using gene IDs as input. To install the tool independently of the SGN, a stand-alone version was developed that can be installed as a web tool running on a Catalyst server (https://github.com/solgenomics/VIGS).

Not all plant species are amenable to VIGS, and different species show different silencing efficiencies. *Nicotiana benthamiana* is the model species for VIGS, and is widely used to study plant–pathogen interactions and other biological processes (Senthil-Kumar et al., 2007). Because VIGS is especially efficient in *N. benthamiana*, this species can also be used to characterize genes identified in other plant species to study their function (Senthil-Kumar et al., 2007). The *N. benthamiana* genome was recently sequenced (Bombarely et al., 2012) and its gene models are now available on SGN, enabling a more precise construct design. The SGN VIGS tool includes databases from Solanaceae and other plant species: *N. benthamiana*, tomato, potato, pepper, eggplant, *A. thaliana*, cotton, grape, maize, rice, soy, and *Medicago truncatula*. Since the tool was developed and is hosted at the SGN, all updates on the gene models from *N. benthamiana* and the other Solanaceae species will be reflected in the databases available for selection. Importantly, other species could be added when their gene models are publicly available, to fulfill the needs of the plant research community. The e-mail address sgn-feedback@solgenomics.net is available for the users to suggest any other species of interest to be added to the tool.

The SGN VIGS tool is a fast, user-friendly interactive web tool, highly parameterizable and customizable. It will help researchers to design VIGS constructs faster, with more precision and enhanced gene-expression data. In addition, it will allow the identification of the most probable targets and off-targets. We recommend the use of the SGN VIGS tool as an aid in the selection of regions for silencing experiments. As with any gene silencing approach, it is necessary in each case to validate the silencing efficiency of target and putative off-targets (e.g. using quantitative RT–PCR). We have used the SGN VIGS tool to rapidly design several dozen silencing constructs. In the future, we plan to further enhance the tool to allow processing of large numbers of genes in bulk for a faster design of VIGS constructs for high-throughput functional genomics screens. In addition, we anticipate that this tool will be useful to conduct retrospective analysis of previous silencing experiments to identify the specificity of the gene fragment used and possible off-targets.

SUPPLEMENTAL INFORMATION

Supplemental Information is available at *Molecular Plant Online*.

FUNDING
This research was supported, in part, by National Science Foundation grant IOS-1025642 (GBM).

ACKNOWLEDGMENTS
No conflict of interest declared.

Received: October 9, 2014
Revised: November 21, 2014
Accepted: November 30, 2014
Published: December 30, 2014

Noe Fernandez-Pozo[1,], Hernan G. Rosli[1,2], Gregory B. Martin[1,3] and Lukas A. Mueller[1]*

[1]Boyce Thompson Institute for Plant Research, Ithaca, NY 14853, USA
[2]Instituto de Investigaciones Biotecnológicas – Instituto Tecnológico de Chascomús (IIB-INTECH), UNSAM-CONICET, Chascomús, B7130IWA Buenos Aires, Argentina
[3]Section of Plant Pathology and Plant-Microbe Biology, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, USA
***Correspondence: Noe Fernandez-Pozo (nf232@cornell.edu)**
http://dx.doi.org/10.1016/j.molp.2014.11.024

REFERENCES
Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990). Basic local alignment search tool. J. Mol. Biol. **215**:403–410.

Bombarely, A., Menda, N., Tecle, I.Y., Buels, R.M., Strickler, S., Fischer-York, T., Pujar, A., Leto, J., Gosselin, J., and Mueller, L.A. (2011). The Sol Genomics Network (solgenomics.net): growing tomatoes using Perl. Nucleic Acids Res. **39**:D1149–D1155.

Bombarely, A., Rosli, H.G., Vrebalov, J., Moffett, P., Mueller, L.A., and Martin, G.B. (2012). A draft genome sequence of *Nicotiana benthamiana* to enhance molecular plant-microbe biology research. Mol. Plant Microbe Interact. **25**:1523–1530.

Burch-Smith, T.M., Anderson, J.C., Martin, G.B., and Dinesh-Kumar, S.P. (2004). Applications and advantages of virus-induced gene silencing for gene function studies in plants. Plant J. **39**:734–746.

Langmead, B., Trapnell, C., Pop, M., and Salzberg, S.L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. **10**:R25.

Liu, E., and Page, J.E. (2008). Optimized cDNA libraries for virus-induced gene silencing (VIGS) using tobacco rattle virus. Plant Methods **4**:5.

Llave, C. (2010). Virus-derived small interfering RNAs at the core of plant-virus interactions. Trends Plant Sci. **15**:701–707.

Senthil-Kumar, M., and Mysore, K.S. (2011). Caveat of RNAi in plants: the off-target effect. Methods Mol. Biol. **744**:13–25.

Senthil-Kumar, M., and Mysore, K.S. (2014). Tobacco rattle virus-based virus-induced gene silencing in *Nicotiana benthamiana*. Nat. Protoc. **9**:1549–1562.

Senthil-Kumar, M., Hema, R., Anand, A., Kang, L., Udayakumar, M., and Mysore, K.S. (2007). A systematic study to determine the extent of gene silencing in *Nicotiana benthamiana* and other Solanaceae species when heterologous gene sequences are used for virus-induced gene silencing. New Phytol. **176**:782–791.