Ordering information

Product	Quantity	Cat. no.
BLOCK-iT™ HiPerform™ Lentiviral PolII miR RNAi Expression System with EmGFP	20 rxns	K4934-00
BLOCK-iT™ Pol II miR RNAi Expression Vector Kit	20 rxns	K4935-00
BLOCK-iT™ Pol II miR RNAi Expression Vector Kit with EmGFP	20 rxns	K4936-00
BLOCK-iT™ Lentiviral Pol II miR RNAi Expression System	20 rxns	K4937-00
BLOCK-iT™ Lentiviral Pol II miR RNAi Expression System with EmGFP	20 rxns	K4938-00
BLOCK-iT™ Inducible Pol II miR RNAi Expression Vector Kit w/EmGFP.	20 rxns	K4939-00
BLOCK-iT™ Pol II miR-lacZ Validated miRNA Control Vector	10 µg	V49350-00
BLOCK-iT™ Pol II miR-luc Validated miRNA Control Vector	10 µg	V49351-00
BLOCK-iT™ Pol II miR-LMNA Validated miRNA Control Vector	10 µg	V49352-00
BLOCK-iT™ Pol II miR-AAK1 Validated miRNA DuoPak	1 DuoPak	V49300-01
BLOCK-iT™ Pol II miR-ACVR1 Validated miRNA DuoPak	1 DuoPak	V49300-02
BLOCK-iT™ Pol II miR-ALPK1 Validated miRNA DuoPak	1 DuoPak	V49300-03
BLOCK-iT™ Pol II miR-BRD3 Validated miRNA DuoPak	1 DuoPak	V49300-04
BLOCK-iT™ Pol II miR-CAMK1 Validated miRNA DuoPak	1 DuoPak	V49300-05
BLOCK-iT™ Pol II miR-CAMK2A Validated miRNA DuoPak	1 DuoPak	V49300-06
BLOCK-iT™ Pol II miR-CDK2 Validated miRNA DuoPak	1 DuoPak	V49300-07
BLOCK-iT™ Pol II miR-CLK1 Validated miRNA DuoPak	1 DuoPak	V49300-08
BLOCK-iT™ Pol II miR-CSNK1G1 Validated miRNA DuoPak	1 DuoPak	V49300-09
BLOCK-iT™ Pol II miR-CSNK1G3 Validated miRNA DuoPak	1 DuoPak	V49300-10
BLOCK-iT™ Pol II miR-DAPK2 Validated miRNA DuoPak	1 DuoPak	V49300-11
BLOCK-iT™ Pol II miR-EEF2K Validated miRNA DuoPak	1 DuoPak	V49300-12
BLOCK-iT™ Pol II miR-EPHA2 Validated miRNA DuoPak	1 DuoPak	V49300-13
BLOCK-iT™ Pol II miR-FES Validated miRNA DuoPak	1 DuoPak	V49300-14
BLOCK-iT™ Pol II miR-IRAK3 Validated miRNA DuoPak	1 DuoPak	V49300-16
BLOCK-iT™ Pol II miR-IRAK4 Validated miRNA DuoPak	1 DuoPak	V49300-17
BLOCK-iT™ Pol II miR-JAK2 Validated miRNA DuoPak	1 DuoPak	V49300-18
BLOCK-iT™ Pol II miR-JAK3 Validated miRNA DuoPak	1 DuoPak	V49300-19
BLOCK-iT™ Pol II miR-MAP3K14 Validated miRNA DuoPak	1 DuoPak	V49300-20
BLOCK-iT™ Pol II miR-MAP3K4 Validated miRNA DuoPak	1 DuoPak	V49300-21
BLOCK-iT™ Pol II miR-MAP4K3 Validated miRNA DuoPak	1 DuoPak	V49300-22
BLOCK-iT™ Pol II miR-MAP4K5 Validated miRNA DuoPak	1 DuoPak	V49300-23
BLOCK-iT™ Pol II miR-MAPK13 Validated miRNA DuoPak	1 DuoPak	V49300-24
BLOCK-iT™ Pol II miR-MAPK6 Validated miRNA DuoPak	1 DuoPak	V49300-25
BLOCK-iT™ Pol II miR-MAPK8 Validated miRNA DuoPak	1 DuoPak	V49300-26
BLOCK-iT™ Pol II miR-MGC42105 Validated miRNA DuoPak	1 DuoPak	V49300-27
BLOCK-iT™ Pol II miR-MKNK1 Validated miRNA DuoPak	1 DuoPak	V49300-28
BLOCK-iT™ Pol II miR-NEK10 Validated miRNA DuoPak	1 DuoPak	V49300-29
BLOCK-iT™ Pol II miR-NEK6 Validated miRNA DuoPak	1 DuoPak	V49300-30
BLOCK-iT™ Pol II miR-PAK2 Validated miRNA DuoPak	1 DuoPak	V49300-31
BLOCK-iT™ Pol II miR-PCTK1 Validated miRNA DuoPak	1 DuoPak	V49300-32
BLOCK-iT™ Pol II miR-PCTK2 Validated miRNA DuoPak	1 DuoPak	V49300-33

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Get control of your knockdown experiments

BLOCK-iT™ Pol II miR RNAi expression vectors

invitrogen™

BLOCK-iT™ Pol II miR RNAi expression vectors

- → Screen fewer sequences than when using shRNA vectors, with a higher design predictability that provides over 70% knockdown success
- → Regulate RNAi experiments for more flexibility with inducible miR RNAi expression
- → Generate up to 5-fold higher virus titers and EmGFP expression with a new *in vivo* application–suitable HiPerform™ lentiviral expression system containing an mRNA stabilizing sequence (WPRE) and a nuclear import sequence (cPPT)
- → Save time and gain confidence with guaranteed knockdown using predesigned miR RNAi hairpins

BLOCK-iT™ Pol II miR RNAi expression vectors allow researchers to perform stable or transient RNAi expression experiments more efficiently, experiments that are challenging with synthetic duplex options. Additionally, difficult-to-transfect cells are no longer a barrier to RNAi as knockdown can be performed using BLOCK-iT™ RNAi vectors and viral delivery systems. The versatility of BLOCK-iT™ Pol II miR RNAi vectors allows the use of different promoters to control the initiation, duration, and specificity of RNAi in cells or tissues. Invitrogen's family of BLOCK-iT™ Pol II miR RNAi expression vectors and viral delivery systems give researchers a solution for any vector-based RNAi experiment.

The pcDNA™6.2-GW/miR and pcDNA™6.2-GW/EmGFP (Emerald Green Fluorescent Protein)-miR vectors included in the BLOCK-iT™ Pol II miR RNAi Expression Vector Kits are designed to express artificial miRNAs that are engineered to have 100% homology to your target sequence, resulting in target cleavage (Figures 1 and 2). These vectors contain flanking and loop sequences from an endogenous miRNA and direct excision of your RNAi sequence from Pol II transcripts. The vectors are designed to use cellular machinery to process knockdown sequences, resulting in more efficient processing of expressed RNAi hairpins. Using Invitrogen's award-winning BLOCK-iT™ RNAi Designer software, over 70% of constructs produce more than 70% knockdown.

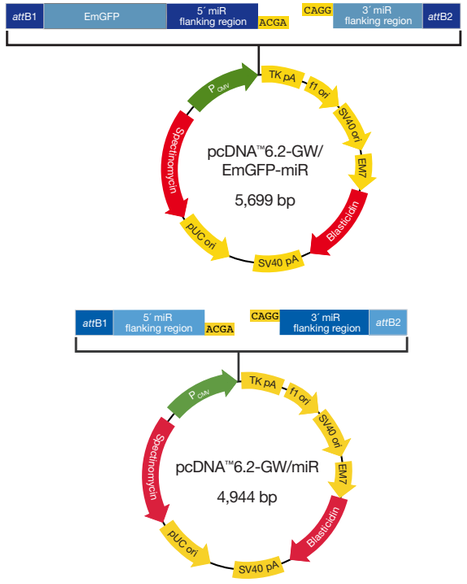


Figure 1—The BLOCK-iT™ Pol II miR RNAi expression vectors. The pcDNA™6.2-GW/miR vector is driven by the CMV promoter, has the blasticidin resistance marker, and is available with or without cocistronic Emerald GFP as a reporter.

High success rate with BLOCK-iT™ RNAi Designer

BLOCK-iT™ RNAi Designer software maximizes your chances of knockdown success by identifying an optimal target site within a gene for the miR RNAi sequence to induce gene knockdown. The Designer provides the sequences of two DNA oligos that you will need to hybridize and clone into the BLOCK-iT™ Pol II miR RNAi expression vector. miR RNAi knockdown success is easier to predict than shRNA knockdown because the endogenous miRNA pathway is utilized for processing of the RNAi guide strand from the miRNA precursor. The process for producing highly effective miR RNAi inserts is very simple:

1. Go to the BLOCK-iT™ RNAi Designer at www.invitrogen.com/rnaidesigner and choose the miR RNAi Design option.
2. Input the accession number or the sequence of your target of interest, and the Designer automatically generates DNA duplexes for cloning into the expression vector.
3. Hybridize the oligos to form a 60-bp duplex with 4-nucleotide 5′ overhangs.
4. Cohesively ligate the duplex into the prelinearized vector.
5. After expression and processing in cells, the vectors produce artificial miRNAs that have 100% homology to your target and cleave it using the RNAi pathway.

With the BLOCK-iT™ Pol II miR RNAi Designer, you'll get maximum knockdown for a diverse range of target sequences.

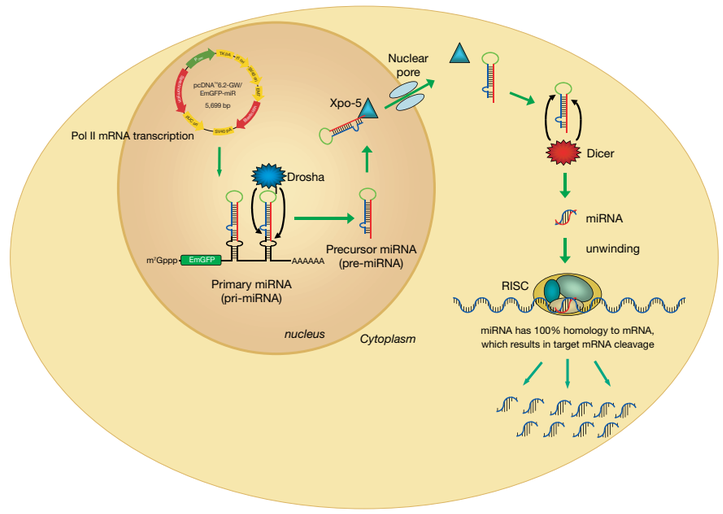


Figure 2—Expression of a miR RNAi sequence using the BLOCK-iT™ Pol II miR RNAi Expression Vector Kits. BLOCK-iT™ Pol II miR RNAi Expression Vector Kits utilize the endogenous miRNA pathway. Artificial miRNAs expressed from the pcDNA™6.2-GW/miR and pcDNA™6.2-GW/EmGFP-miR vectors are transcribed by RNA Polymerase II, which enables cocistronic expression of EmGFP and multiple miRNA hairpins on the same transcript. The primary miRNA (pri-miRNA) transcript contains the EmGFP sequence on the 5′ end, followed by one or more precursor miRNAs (pre-miRNAs). The RNase type III enzyme Drosha recognizes the flanking sequences of each pre-miRNA and excises them from the pri-miRNA transcript. Each precursor miRNA is then actively transported out of the nucleus by Xpo-5. Once in the cytoplasm, the pre-miRNA hairpins are further processed by Dicer (which converts them into miRNAs). Finally, the miRNAs load into RISC (RNA Induced Silencing Complex), unwind, and hybridize with their mRNA target. While most endogenous mammalian miRNAs do not perfectly complement the target mRNA sequence and thus result in translational inhibition (moderate knockdown effect), the artificially designed miRNAs used in this system are 100% homologous to the target mRNA sequence and result in target cleavage (strong knockdown effect).

Knockdown of multiple targets for experimental flexibility

BLOCK-iT™ Pol II miR RNAi expression vectors provide an easy and effective way to knock down more than one gene in a single experiment. The Pol II promoter enables cocistronic expression of two or more miR RNAi sequences, allowing you to suppress multiple targets with a single construct (Figure 3). Using a simple restriction enzyme procedure, you can link two or more miR RNAi sequences in any order. This is ideal for knockdown of more than one pathway component or splice variant in your cell type or for using miR RNAi to produce synthetic phenotypes.

Reliable tracking of your RNAi cassette

Easily determine which cells are expressing your miR RNAi of interest. Just use the pcDNA6.2™GW/EmGFP-miR vector in the BLOCK-iT™ Pol II miR RNAi Expression Vector Kit with EmGFP (Emerald Green Fluorescent Protein), the BLOCK-iT™ Inducible Pol II miR RNAi Expression Vector Kit with EmGFP, or the BLOCK-iT™ HiPerform™ Lentiviral Pol II miR RNAi Expression System with EmGFP. Because EmGFP is expressed cocistronically with your miR RNAi insert, you will see essentially 100% correlation of EmGFP expression with the expression of your miR RNAi(s) (Figure 4).

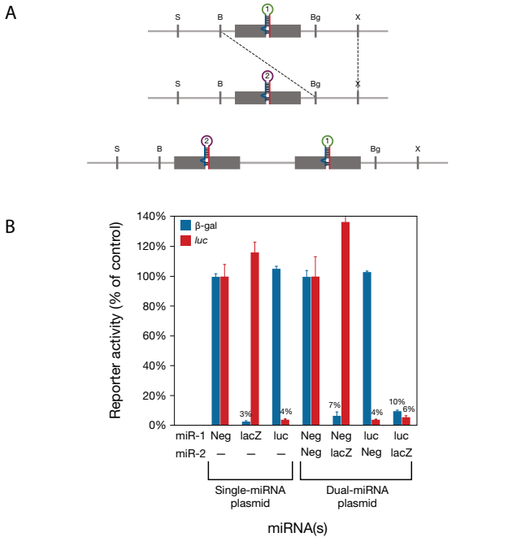
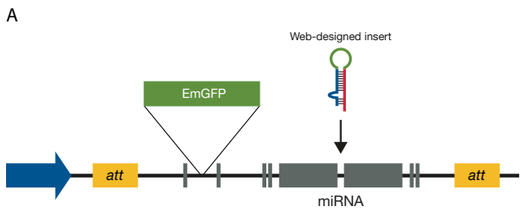


Figure 3—Knockdown of multiple targets with BLOCK-iT™ Pol II miR RNAi expression vectors. **A.** Example of restriction digestion/ligation scheme for combining miRNAs from different vectors. *Sal* I (S), *Bam*H I (B), *Bgl* II (Bg), and *Xho* I sites (X) around the miRNA flanking regions (bars) are indicated. By cloning the *Bam*H I–*Xho* I fragment containing miRNA 1 into the *Bgl* II–*Xho* I fragment of the vector containing miRNA 2, a dual-miRNA plasmid is created. The original restriction site pattern is recreated (restriction sites between the miRNA sequences are destroyed), and additional miRNAs can be added in the same manner. Alternatively, miRNAs can be added in front of miRNA 1 by combining *Sal* I–*Bgl* II and *Sal* I–*Bam*H I fragments. **B.** Results of an experiment cotransfecting luciferase and *lac*Z reporter plasmids with single- or dual-miRNA vectors with the indicated inserts. Luciferase and β-galactosidase activities are normalized to the single (neg)- or dual (neg/neg)-miRNA negative control inserts, which form a pre-miRNA but are not predicted to target any known vertebrate genes. Knockdown is slightly attenuated in the dual-miRNA vectors but remains very potent at ≥90%.



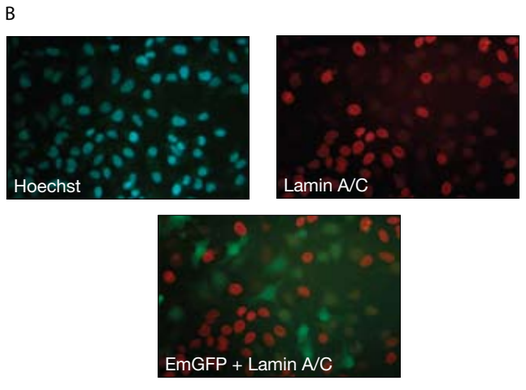


Figure 4—100% correlation of EmGFP and miRNA expression. **A.** Map of the BLOCK-iT™ Pol II miR RNAi expression cassette with EmGFP between *Dra* I restriction sites for easy removal. **B.** Cells were transfected with pcDNA™6.2-GW/EmGFP-miR (directed against lamin) and Lipofectamine™ 2000 Reagent at an expected 50% efficiency to demonstrate the 100% tracking of EmGFP and miRNA expression. After 48 hr, cells were stained with Hoechst nuclear stain (which stains all cells), stained with a red lamin stain, and monitored for GFP expression. Approximately half of the cells highly express the lamin protein. When cells are imaged simultaneously for EmGFP expresssion and lamin A/C stain, it is clear that cells expressing GFP do not appear to have lamin A/C present, and cells stained red for lamin A/C do not appear to have any GFP expression. This demonstrates that cells expressing EmGFP are also greatly reduced in lamin expression due to the presence of the miRNA that is cocistronically expressed.

Pol II promoter flexibility allows tissue-specific expression

The BLOCK-iT™ Pol II miR RNAi expression systems are compatible with virtually any Pol II promoter, allowing regulated and tissue-specific knockdown studies to be performed (Figure 5).

Inducible expression for control over your experiment

The BLOCK-iT™ Inducible Pol II miR RNAi Expression Vector Kit with EmGFP provides the ability to regulate RNAi experiments. Changes can be observed over time by controlling the initiation of the RNAi response with an inducible system. The kit contains a pT-REx™-DEST30 Gateway® vector which, after simple cloning and shuttling techniques, produces a miR RNAi expression vector suitable for inducible knockdown (Figure 5B). The pT-REx™-DEST30 Gateway® vector contains the CMV promoter with two copies of the tetracycline operator (tetO2) sequence, allowing high-level regulated expression. This permits the study of loss of function in a stably transfected cell line even if the gene of interest is essential. In addition, induction of miR RNAi expression can be halted so that phenotypic changes can be measured during recovery of gene function.

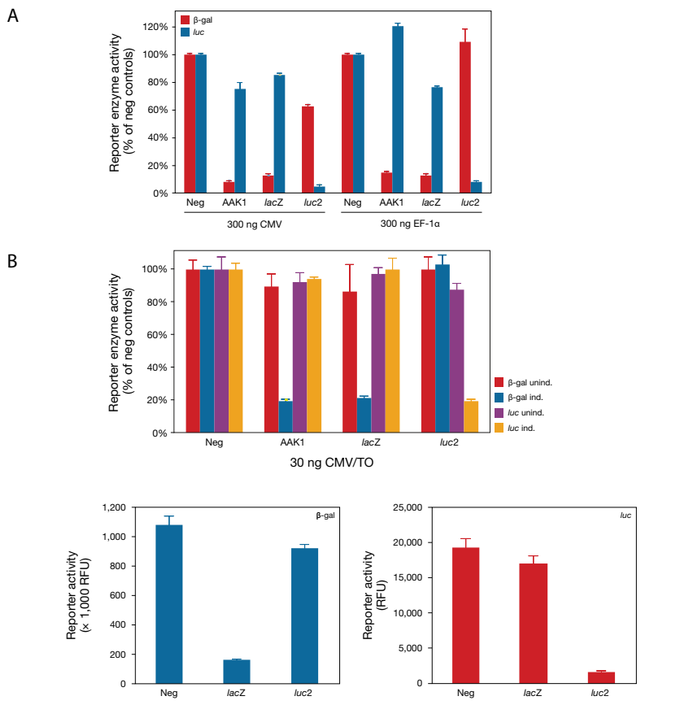


Figure 5—BLOCK-iT™ Pol II miR RNAi vectors are compatible with multiple promoters. **A.** Normalized reporter activities from lysates of GripTite™ 293 cells cotransfected with 100 ng each of luciferase and *lacZ* reporter plasmids and 300 ng of pcDNA™6.2-GW/EmGFP-miR (CMV) or pEF-GW/EmGFP-miR (EF-1α) expression vectors per well. **B.** Knockdown of pSCREEN-iT™/*lacZ*-AAK1 and luciferase reporters in T-REx™ 293 cells with and without induction with 1 µg/ml tetracycline at 30 ng pT-REx™-GW/EmGFP-miR (CMV/TO) plasmid per well. **C.** Knockdown of cotransfected *lacZ* and luciferase reporter genes in HepG2 cells using MultiSite Gateway® EmGFP-miR constructs with the αI anti-trypsin promoter as the 5´ element and the poly(A)+ signal from the HSV thymidine kinase gene as the 3´ element.

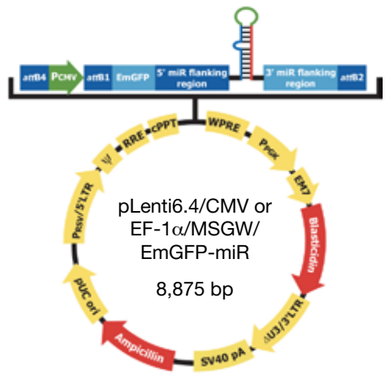
Combine with lentivirus for stable, long-term expression

The BLOCK-iT™ Lentiviral Pol II miR RNAi Expression Systems combine the BLOCK-iT™ Pol II miR RNAi Expression Vector Kit with ViraPower™ Lentiviral Expression Vectors, enabling stable delivery of engineered miRNAs into nondividing, primary, and hard-to-transfect cells. The new BLOCK-iT™ HiPerform™ Lentiviral Pol II miR RNAi Expression System with EmGFP contains an mRNA-stabilizing sequence, the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE), and a nuclear import sequence, the central polypurine tract (cPPT), which generate up to 5-fold higher virus titers and EmGFP expression levels in many cell lines when compared to vectors without these sequences. Blasticidin resistance is expressed from the mouse PGK promoter to avoid shutdown after multiple passages. Additionally, MultiSite Gateway® technology allows you to express your EmGFP/miR RNAi cassette from CMV, EF-1α, or your own tissue-specific or other *in vivo*–appropriate promoter.

Unlike other lentiviral vector systems that utilize the HIV-1 promoter to express the viral genome during packaging, the ViraPower™ lentiviral expression vectors are third-generation vectors that do not depend on Tat protein for activation. Tat protein can efficiently translocate across nuclear and plasma membranes. During HIV-1 infection, the Tat protein enters neighboring cells where it affects various cellular targets to change the expression patterns of host genes. Use of the heterologous RSV promoter to drive viral genome expression in the ViraPower™ Lentiviral Expression System, Tat will never be present in the viral supernatant, where it could be cotransduced into target cells leading to nonspecific effects.

These features make this a powerful and flexible RNAi vector (Figure 6).

A



B

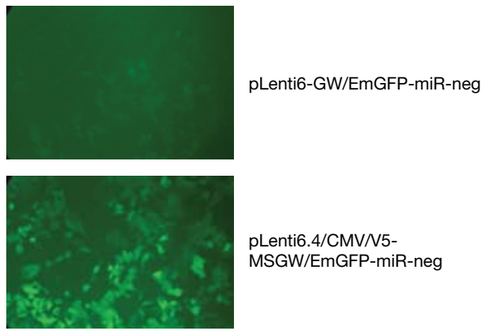


Figure 6—The BLOCK-iT™ HiPerform™ Lentiviral Pol II miR RNAi Expression System with EmGFP. **A.** pLenti6.4/CMV or EF-1α/MSGW/EmGFP-miR map. The EmGFP-miR vector is driven by the CMV promoter, has the blasticidin resistance marker, and is available with cocistronic EmGFP as a reporter. **B.** Images taken four days following transduction of GripTite™ 293 MSR cells at an MOI of 3 with lentiviral particles generated using the indicated vectors.

Bench-tested BLOCK-iT™ Pol II miR Validated miRNA Vector DuoPaks

In addition to designing your own miR RNAi knockdown sequence, you can choose validated vectors available for human kinases and control genes. Bench-tested by Invitrogen scientists, each BLOCK-iT™ Pol II miR Validated miRNA Vector DuoPak contains two independent EmGFP vectors with pre-miRNA inserts designed to nonoverlapping target sites on the same gene. These validated DuoPaks are designed to all known splice variants, have been proven to knock down their targets by at least 70%, and are guaranteed.* Validated DuoPaks offer unique convenience, since two sequences are necessary to confirm the knockdown phenotype and to meet publication requirements. The list of available vectors can be checked by visiting www.invitrogen.com/rnaiexpress.

Let Invitrogen Custom Services do the work for you

Services are available for every step of the RNAi experimental workflow. Please contact us today at custom.services@invitrogen.com.

For more information on Invitrogen's RNAi portfolio of products and services, visit www.invitrogen.com/rnai.

BLOCK-iT™ miR RNAi Select predesigned hairpins targeting most human, rat, and mouse genes

Now it is easier than ever to harness the power of the BLOCK-iT™ Pol II miR RNAi expression vectors with predesigned BLOCK-iT™ miR RNAi Select hairpins targeting most human, rat, and mouse genes (Table 1). Each BLOCK-iT™ miR RNAi Select 4 Set contains four hairpin designs, which are provided as DNA oligos ready to anneal and clone into BLOCK-iT™ Pol II miR RNAi expression vectors. Two of the four designs are guaranteed to produce 70% transcript knockdown, given at least 80% transfection efficiency. They are designed using the award-winning BLOCK-iT™ RNAi Designer utilizing BLAST and an additional Smith-Waterman mismatch alignment for further reduction of off-target effects. Visit www.invitrogen.com/mirselect for more information.

Table 1—Genome coverage of BLOCK-iT™ miR RNAi Select 4 Sets.

Species	% Genome coverage
Human	72
Mouse	67
Rat	78