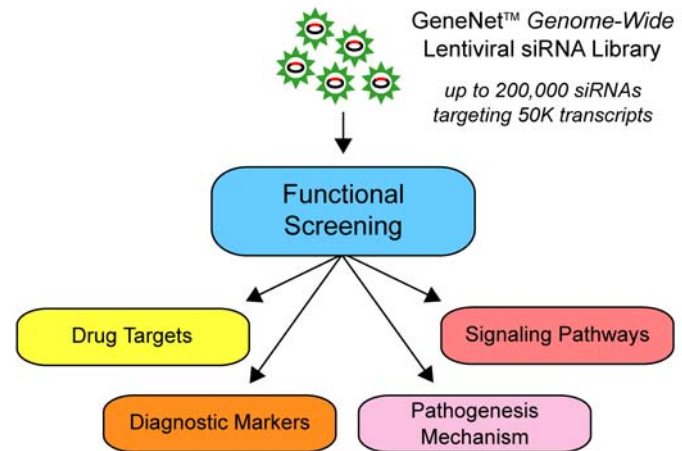


Profile Gene Function on a Genome-Wide Scale

Assay 200,000 siRNA sequences in a single screening

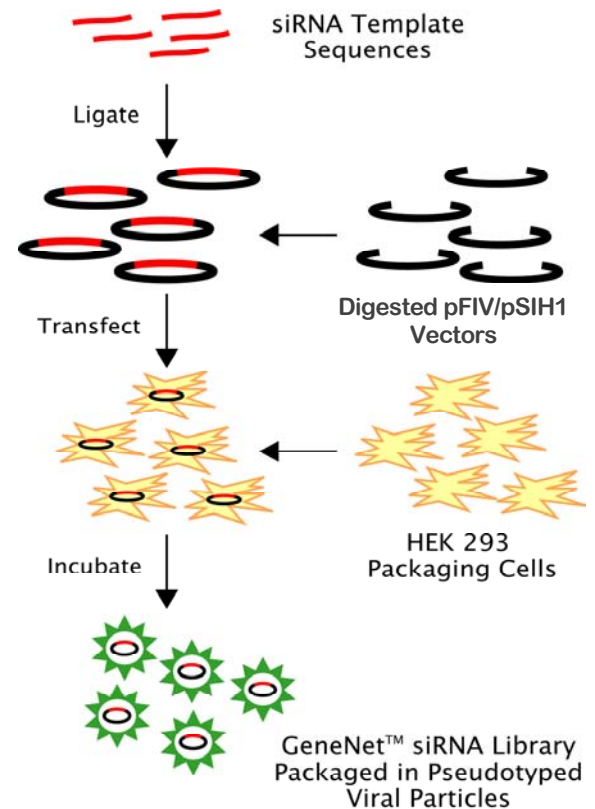
- Discover new therapeutic targets and diagnostic markers functionally involved in critical responses
- Decipher signaling pathways
- Take advantage of the only cost effective technology for genome-wide functional screening
- Efficiently screen in almost any cell type or *in vivo* biological system



With SBI's FIV- or HIV-based **GeneNet™ Lentiviral siRNA Libraries**, you can functionally profile tens of thousands of genes by introducing and screening hundreds of thousands of siRNA sequences in a single experiment. Similar to the revolutionary genome-wide siRNA screening approaches described in Nature (March 25, 2004, vol. 428, p. 427 and p. 431), this technology enables high-throughput simultaneous screening for genes that functionally inhibit any selectable response or phenotype.

GeneNet™ Libraries

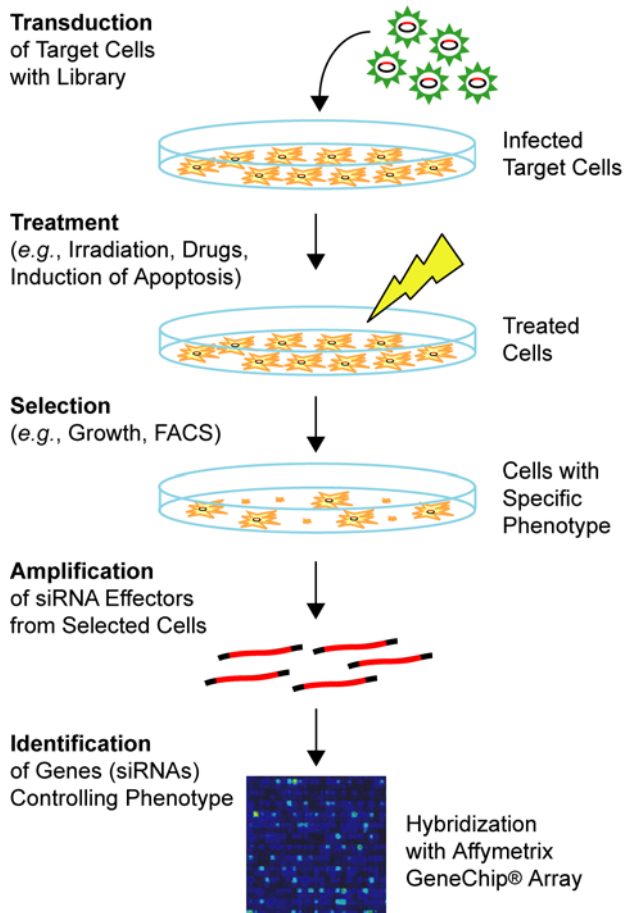
GeneNet™ libraries are constructed by ligating synthesized oligonucleotide siRNA templates with linearized pFIV or pSIH1 library vectors. The libraries are available as plasmid DNA and/or in a pre-packaged format; following construction, the library is packaged into highly transducible VSV-G pseudotyped lentiviral particles using HEK 293 packaging cells that are co-transfected with the pPACKF1 or pPACKH1 Lentivector Packaging Plasmids.



Design and Selection of siRNA Sequences

GeneNet™ siRNA Libraries consist of many thousands of siRNA vector constructs—4–5 different constructs per target gene. In addition to designing the gene-specific siRNA template sequences to include characteristics like favorable GC content, minimal secondary structure, and no termination signals, GeneNet™ Library siRNA sequences were designed to hybridize to probes present on Affymetrix GeneChip® Arrays. This design facilitates detection of the siRNA sequences selected in functional screening experiments.

Functional Profiling with GeneNet™ siRNA Libraries



Transduction

Transduce target cells with the packaged GeneNet™ Library. Transduction with high-efficiency, pantropic VSV-G pseudotyped lentiviral particles produces a target cell population where almost every cell expresses at least one siRNA.

Treatment (optional)

If necessary, treat transduced cells to induce the desired phenotype (e.g., radiation or chemical exposure, apoptosis induction, differentiation).

Selection

Select or isolate the population of cells with the desired phenotype after treatment (e.g., growth selection, cell sorting).

Amplification

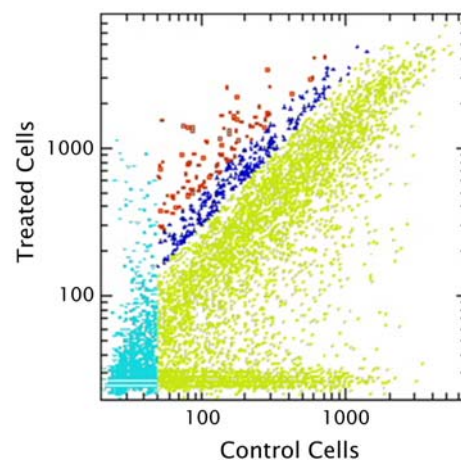
Recover siRNA template sequences from selected target cells using vector PCR primers to known vector sequences flanking the siRNA inserts.

Identification

Identify the specific species of siRNA recovered from the selected target cell population by sequencing or by hybridization to an Affymetrix GeneChip® Array that recognizes all the siRNA templates present in the library.

Screening for Genes Involved in Radiation Resistance

Cells were transduced with a library of 7,500 siRNA sequences targeting 1,500 genes. The expressed siRNA sequences were rescued from irradiated cells (treated) and control cells, and the siRNA template sequences present in each population were compared by microarray hybridization. Red indicates sequences enriched 5-fold and blue indicates those enriched 3-fold. These enrichments indicate siRNA targeting genes that make the cells radiation sensitive.



GeneNet™ siRNA Libraries

GeneNet™ Library	Vector	# Target Genes	# siRNAs	Cat. # (Packaged)	Cat. # (Plasmid)
Human 50K	pSIH1-H1-Puro	47,400	200,000	SI606B-1-SBI	SI606PB-1-SBI
Human 50K	pSIF-H1-Puro	47,400	200,000	SI206B-1-SBI	SI206PB-1-SBI
Mouse 40K	pSIH1-H1-Puro	39,000	150,000	SI622B-1-SBI	SI622PB-1-SBI
Mouse 40K	pSIF-H1-Puro	39,000	150,000	SI222B-1-SBI	SI222PB-1-SBI
Mouse 40K	pSIF-H1-copGFP	39,000	150,000	-	SI221PA-1-SBI