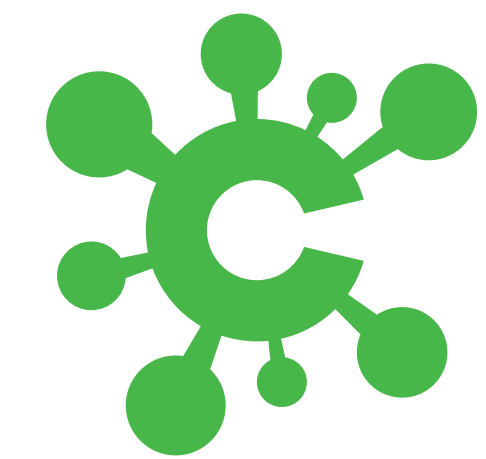




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Identification of potential cancer drug targets in prostate, blood, and breast cancer cells using HT RNAi screening with pooled shRNA libraries

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Introduction

In 2010, leukemia, lymphoma, and myeloma were estimated to cause the deaths of 54,020 people in the U.S., in part due to chemotherapy toxicity to non-hematopoietic tissues. One strategy to circumvent problems with conventional chemotherapy is to develop drugs against more specific cancer targets. The “targeted therapy” approach has resulted in a paradigmatic shift in modern cancer treatment.

We sought to identify genes required for survival of hematopoietic cell lines *en bloc* and for survival of specific hematopoietic cancer cell lines, yet not critical for survival of other cells. These gene products can be used as drug targets for development of novel targeted myeloablative and anticancer therapies.

We employed loss-of-function RNAi genetic screens using a pooled lentiviral shRNA library (DECIPHER™ shRNA Library) to identify viability genes of hematopoietic cancer cell lines. This was followed by arrayed validation of shRNA “hits” identified in the pooled screen to confirm blood- and cell line-specific cytotoxic shRNAs.

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Materials & Methods

A pooled shRNA library consisting of 27,500 shRNA targeting 5,043 human pathway-associated genes (DECIPHER 27K Human Module 1 Library) was generated by cloning a pooled oligonucleotide library (Agilent Technologies) into a lentiviral vector expressing RFP and Puro resistance gene. The plasmid shRNA library was packaged into lentiviral particles for use in the screen.

The following cells of hematopoietic origin were used: K-562 (CML), Jurkat (acute T-cell leukemia), and Raji (Burkitt's lymphoma, EBV). HeLa (cervix adenocarcinoma) and DU145 (prostate carcinoma) lines were used as controls. Cells were transduced by the viral library in triplicate at MOI ~0.3, with a minimum of 2.7×10^6 cells transduced per replicate. After selection using puromycin, transduced cells were collected at “early” (18 hrs or 2 days) and one or more “late” time points (e.g. 9 days post-transduction), with the early time point or DECIPHER plasmid library serving as baseline control. Genomic DNA was extracted from cell populations, and shRNA/bar-codes PCR amplified and subjected to HT sequencing (Illumina GAIIX) (Figs. 1, 2)

The shRNAs found to be significantly depleted were considered “hits” and subjected to further validation and analysis by qRT-PCR (Fig. 3) and other bioassay (apoptosis, cell cycle progression, cytotoxicity, etc.).

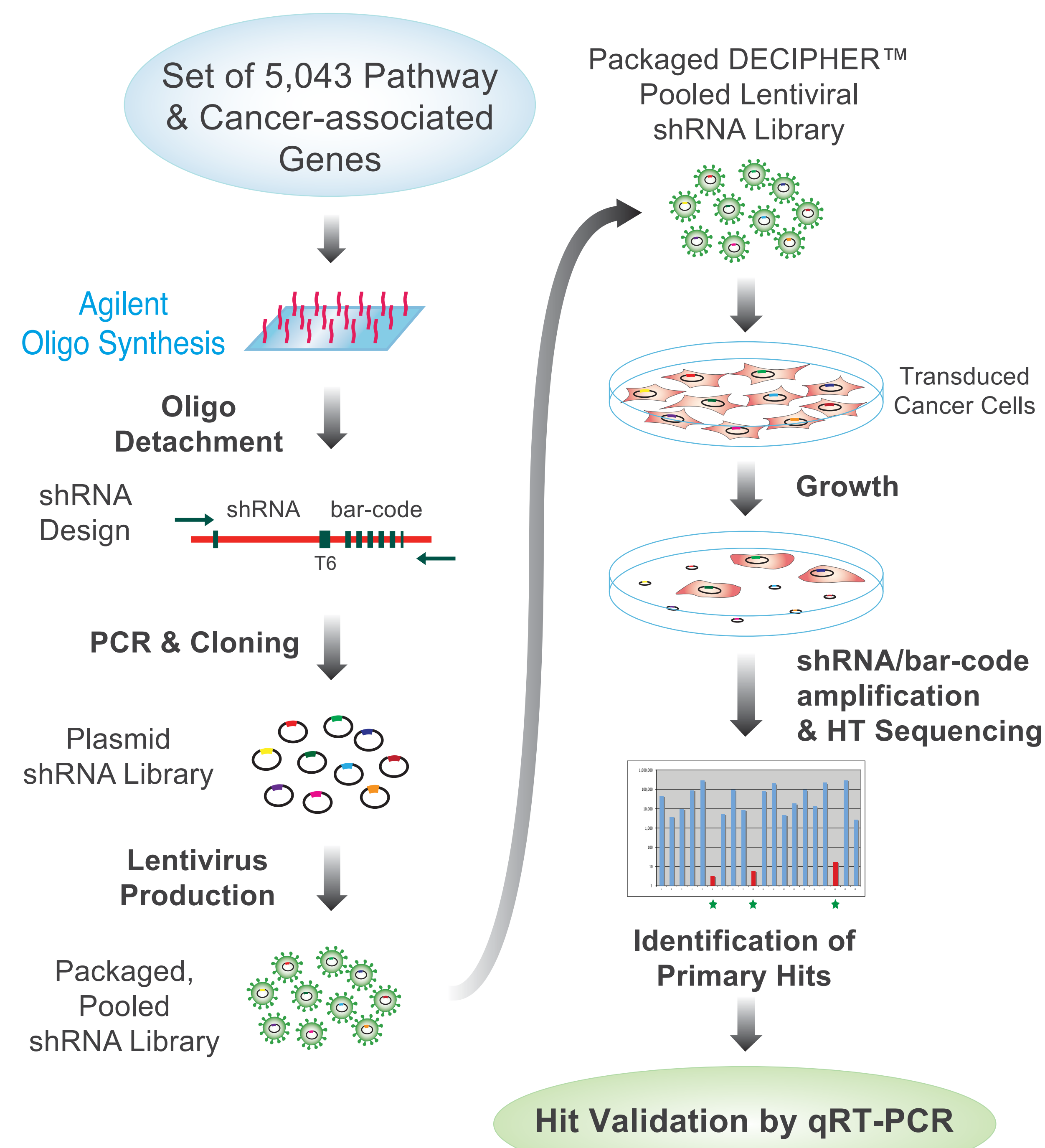
DECIPHER Pooled Lentiviral shRNA Libraries

The DECIPHER Human and Mouse shRNA Libraries consist of three 27K modules:

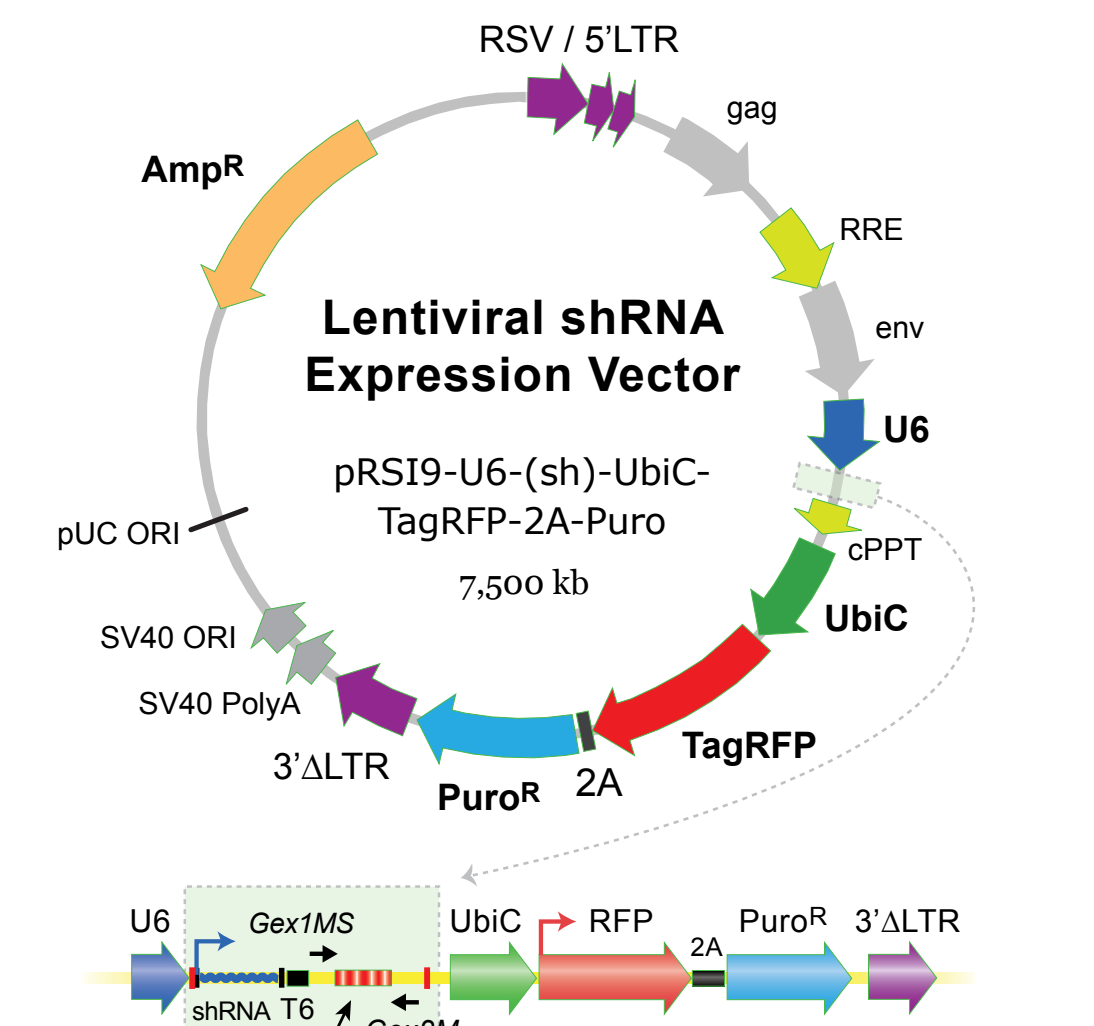
- Pathway Module 1** (used in this study) consists of ~5,000 target genes, including:
 - (1) members of the major canonical and non-canonical pathways, covered by KEGG, Reactome, and other expert-curated pathway databases;
 - (2) genes on the CSHL Cancer 1000 list and 2,500 top-ranking genes from the Cancer Genome Atlas;
 - (3) approximately 900 known FDA drug targets; and
 - (4) the most important genes involved in top MeSH ranking diseases.
- Disease Module 2** consists of ~5,000 disease-associated genes that are not included in Module 1.
- Cell Surface Module 3** consists of ~5,000 Cell Surface, Extracellular, and DNA Binding Targets (*currently available for human only*)

Libraries are distributed freely to academia via the DECIPHER Project. More information can be found on www.decipherproject.net, including gene lists and annotations.

BCR-ABL1 Synthetic Lethality Screen in Chronic Myelogenous Leukemia



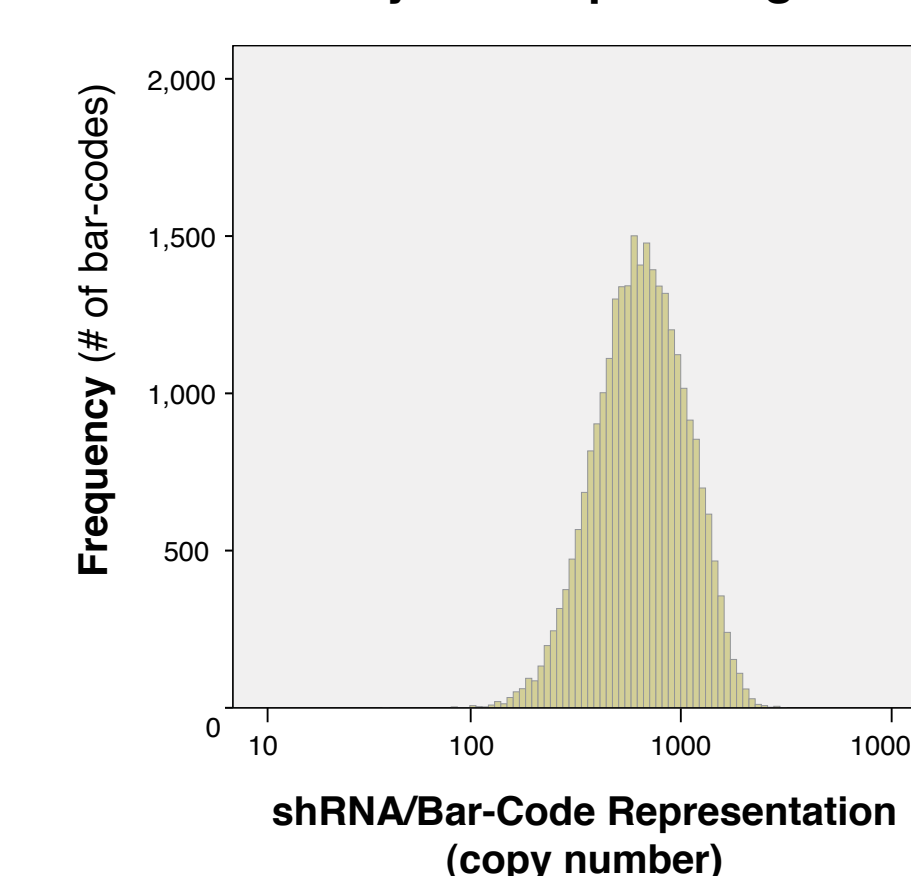
Library Vector Design



The pooled shRNA library was constructed by cloning a pool of shRNA inserts (Agilent) into a lentiviral vector expressing TagRFP and Puro and packaging the pool of constructs into lentiviral particles. Each shRNA insert, expressed from a U6 wild-type promoter, contains an HT sequencing primer binding site and bar-code sequence downstream of the hairpin and termination site (T6) for identification of shRNA constructs via the Illumina GAIIX or HiSeq 2000.

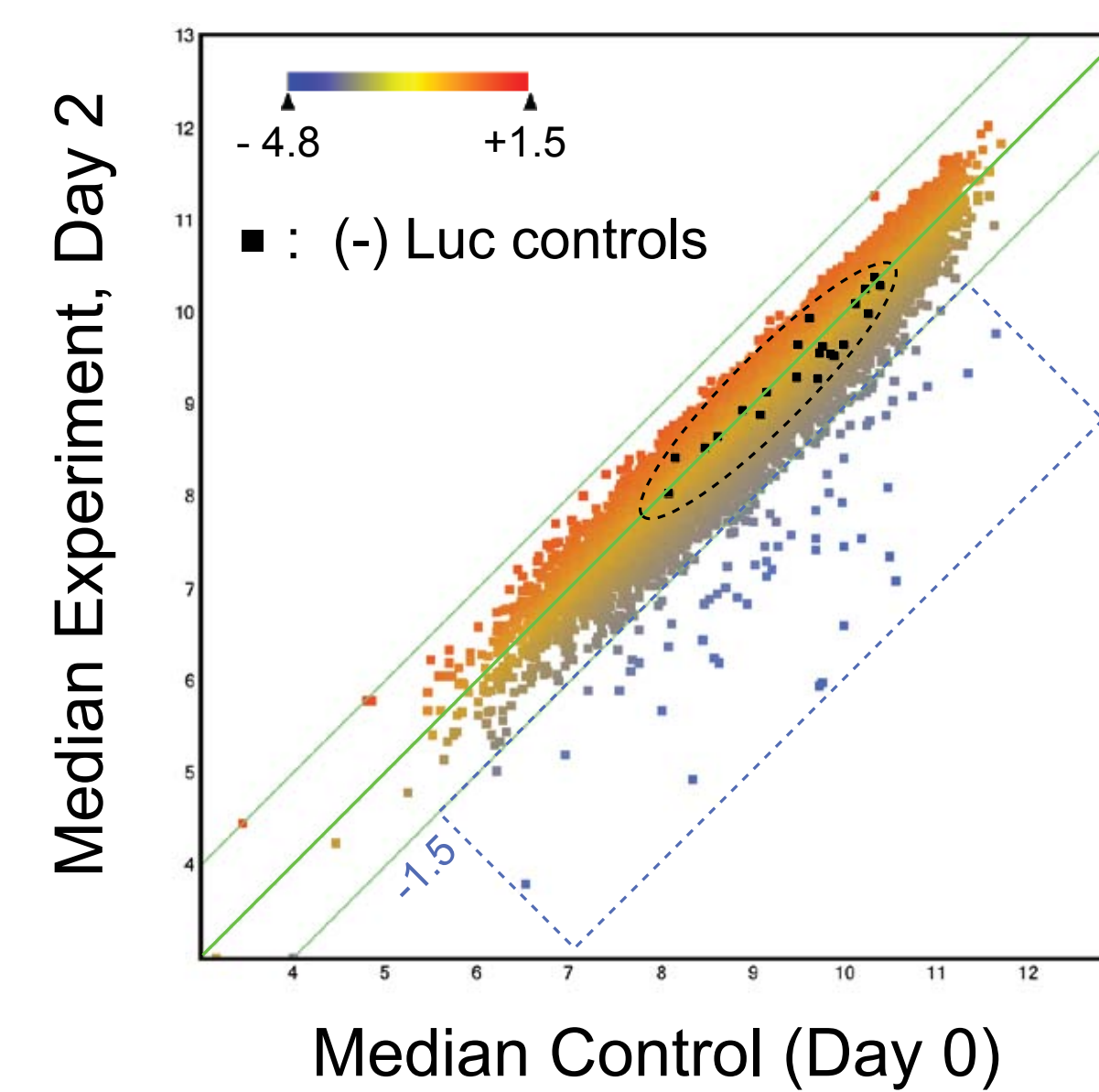
shRNA Representation

Pooled shRNA Library Q.C. by HT Sequencing



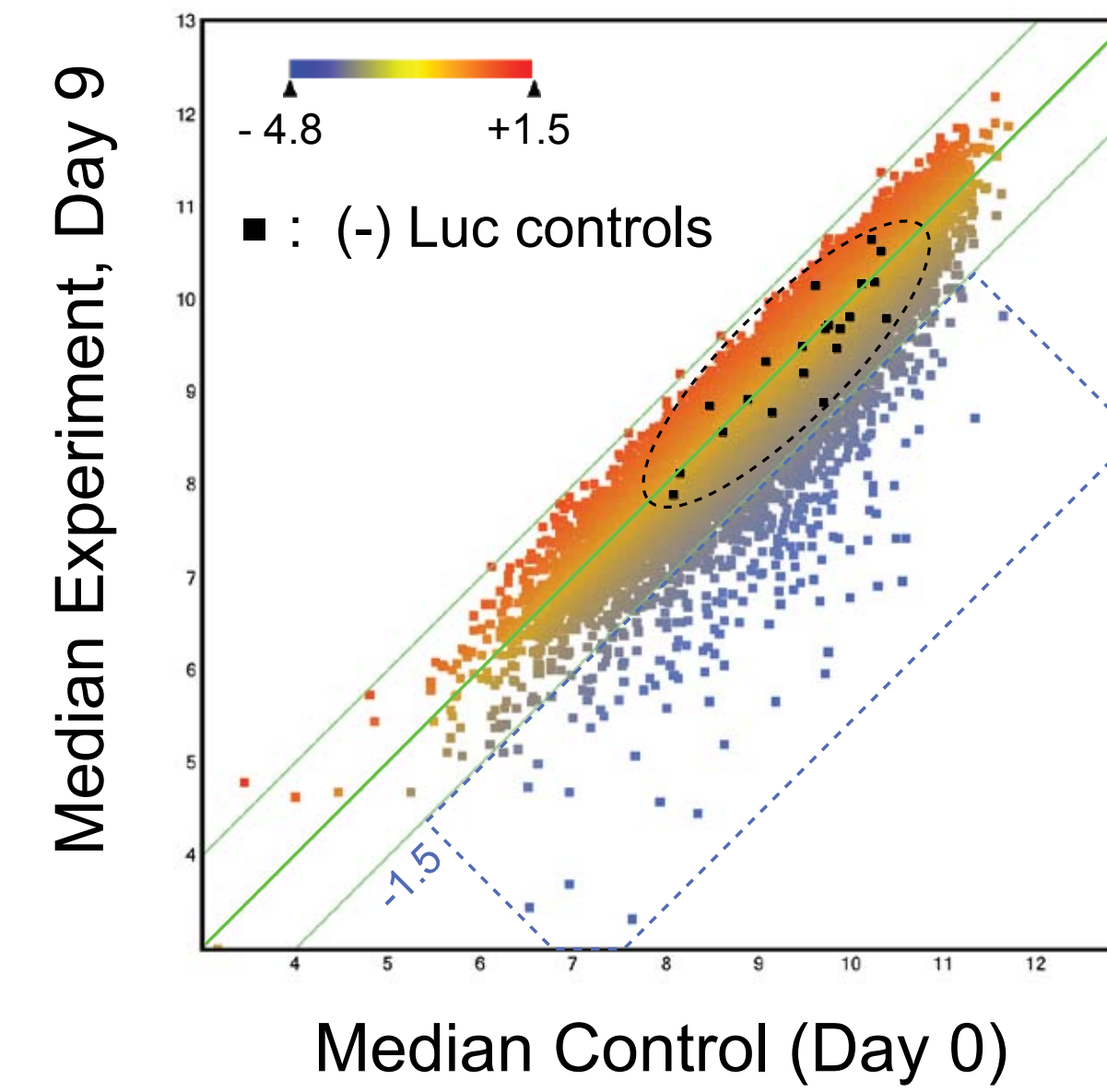
The histogram shows HT sequencing Q.C. data for the DECIPHER 27K Human Module 1 pooled shRNA library with good representation. Virtually all shRNA are present in more than 100 copies and most are around 1,000 copies per 20 million HT sequencing “reads”. Also, there is less than 10-fold difference between the most and least represented for 90% of the shRNA, so the library has a relatively balanced representation of all shRNA.

Viability Screen Time-Course



(A) Day 2

- 198 shRNAs targeting 177 genes
- 18 genes with multiple shRNAs
- 0 genes ≥ 6 shRNAs
- 0 genes ≥ 5 shRNAs
- 0 genes ≥ 4 shRNAs
- 3 genes ≥ 3 shRNAs
- 15 genes ≥ 2 shRNAs



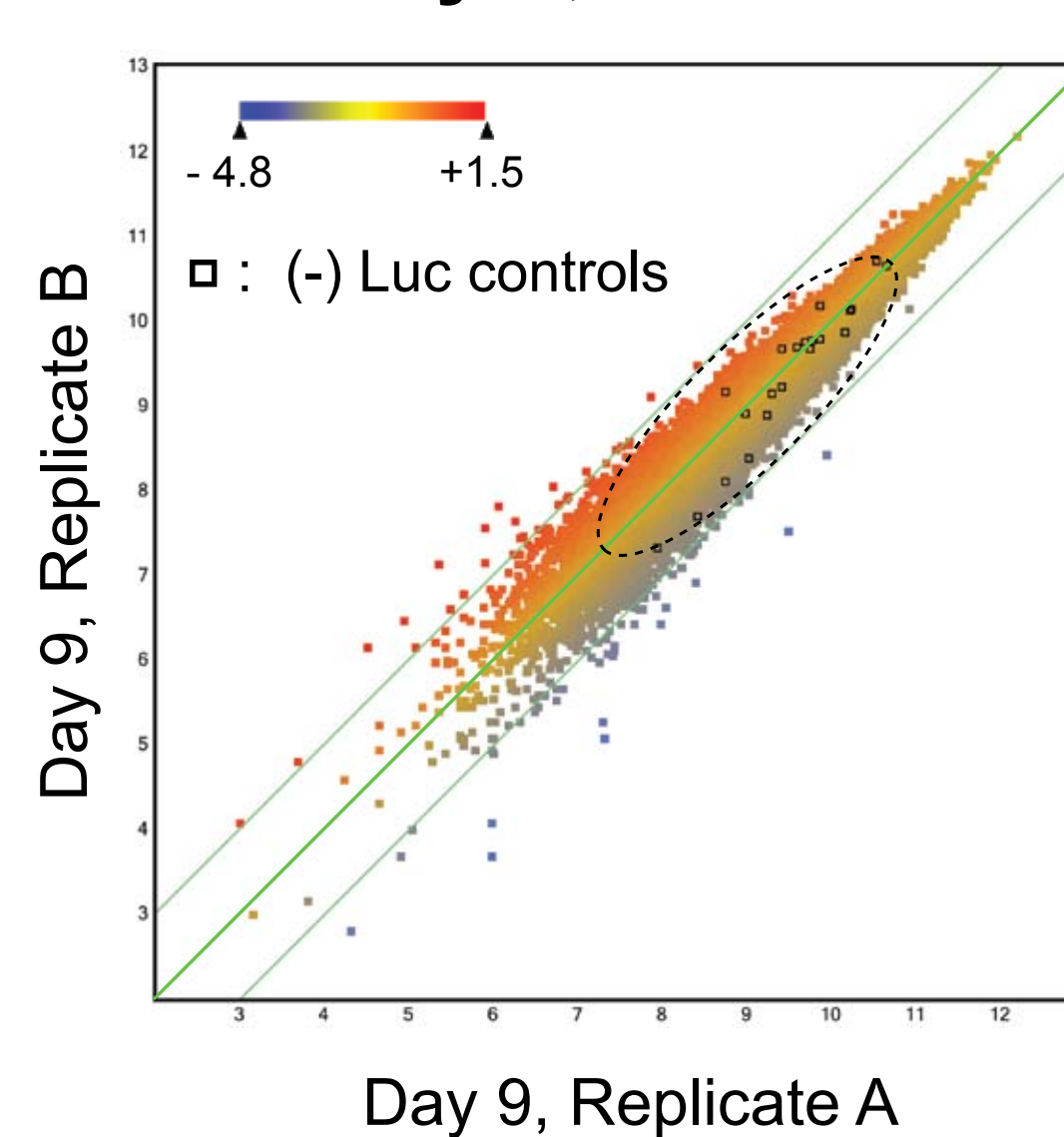
(B) Day 9

- 759 shRNAs targeting 529 genes
- 138 genes with multiple shRNAs, 22 associated with leukemia
- 3 genes ≥ 6 shRNAs
- 7 genes ≥ 5 shRNAs
- 13 genes ≥ 4 shRNAs
- 26 genes ≥ 3 shRNAs
- 89 genes ≥ 2 shRNAs

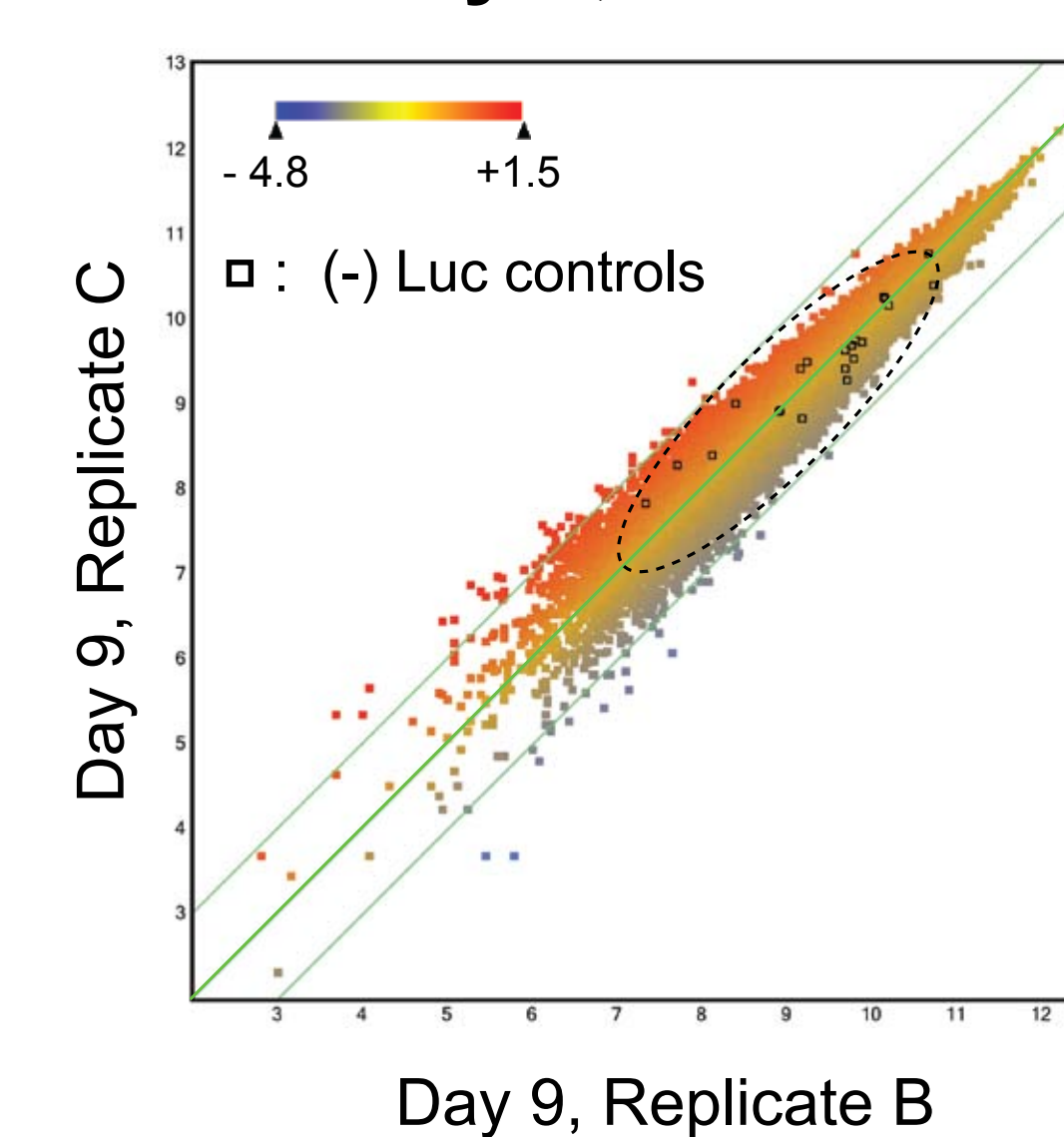
Figure 1. Viability screen in K562 cell line with a DECIPHER 27K Human Module 1 pooled shRNA library constructed in the pRS19-U6-(sh)-UbiC-TagRFP-2A-Puro vector. K562 cells were transduced at MOI ~0.3 in triplicate and selected with puromycin for 2 days. Three (3) cell samples were grown for 2 days (A) or 9 days (B) without splitting. Bar-codes were amplified from genomic DNA and HT sequenced, and toxic shRNAs were identified by depletion in cells grown for 2 days (A) or 9 days (B). Black spots in close proximity with diagonal (within yellow/orange area) correspond to 20 luciferase negative control shRNAs.

Reproducibility in Replicates

Day 9, A vs. B



Day 9, B vs. C



Day 9, A vs. C

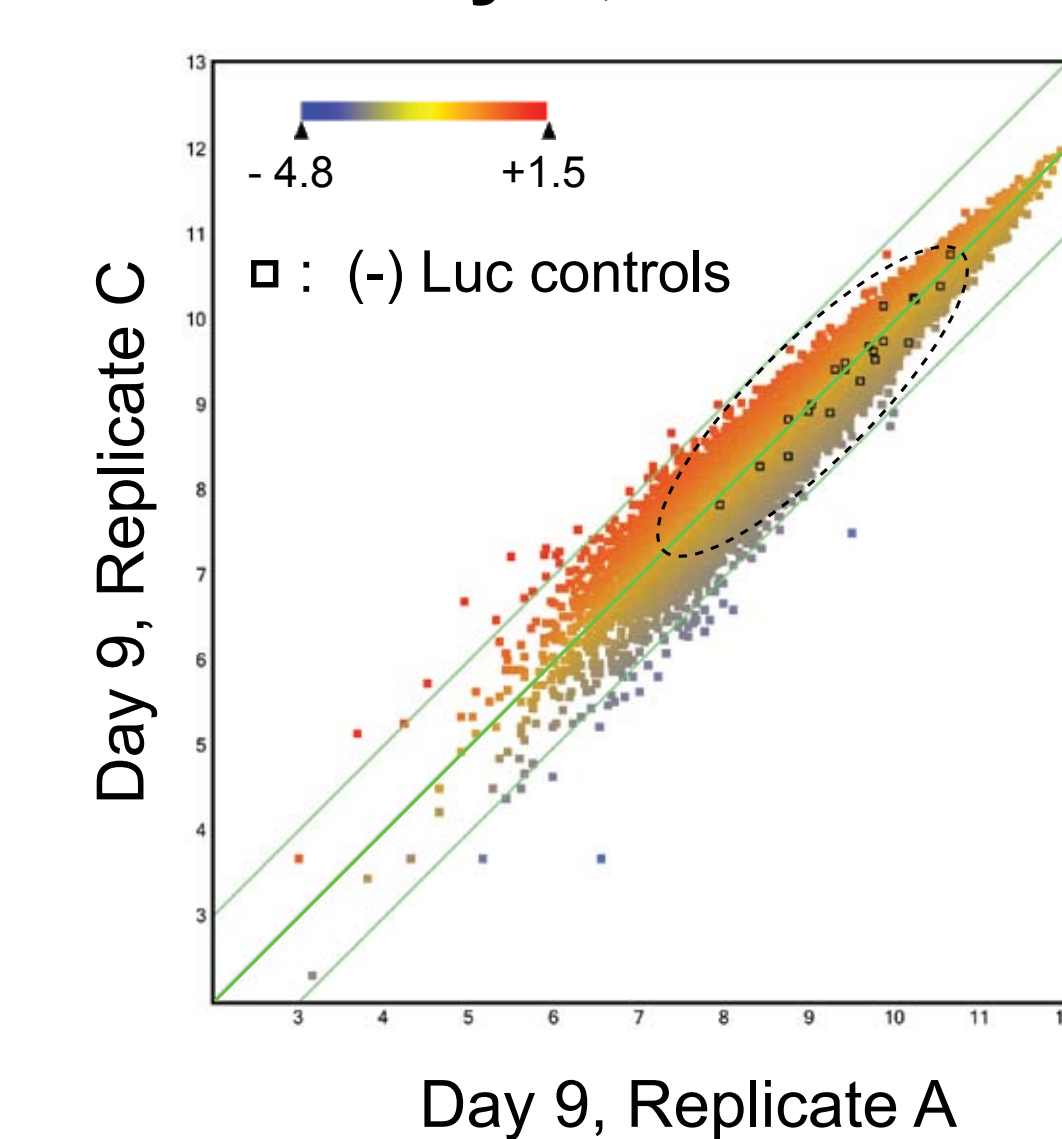


Figure 2. Reproducibility in triplicate cell samples for the day 9 time point. Black spots correspond to luciferase negative control shRNAs.

Confirmation by qRT-PCR

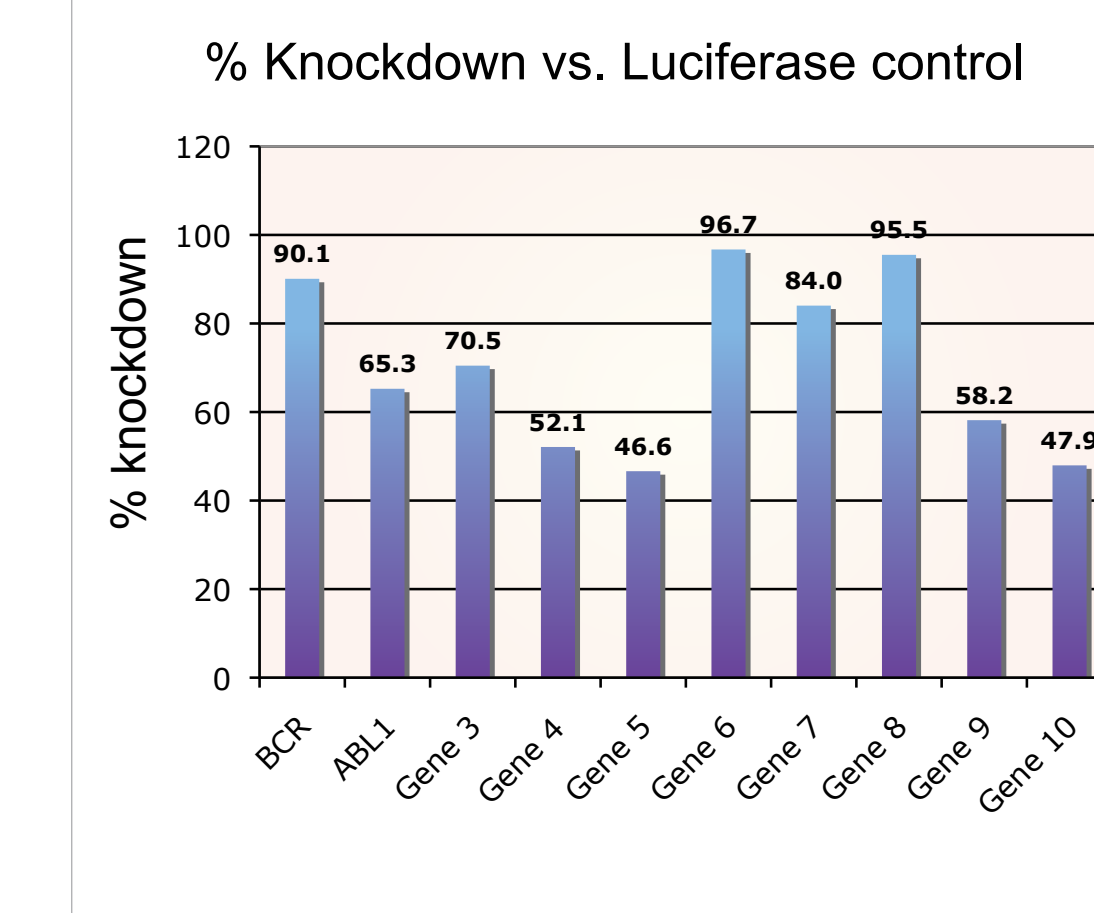
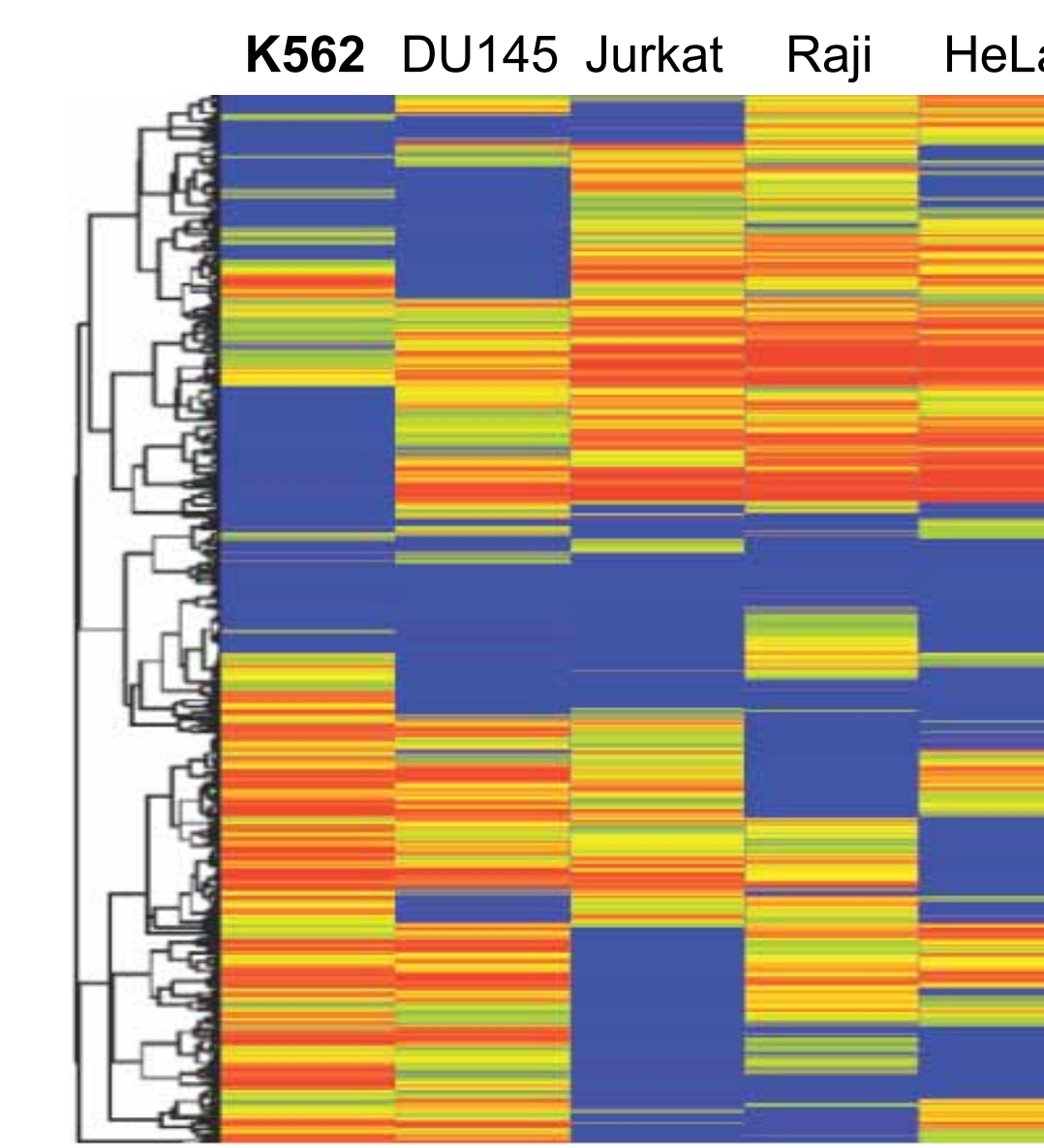


Figure 3. Representative qRT-PCR experiment showing validation of 10 shRNAs identified in the primary pooled screen as being significantly depleted. Range of mRNA knockdown is from 46.6 to 96.7%, with 5 shRNAs suppressing target gene expression by > 70%. Additional qRT-PCR analyses are in progress.

Cell-Specific Viability Signatures



Depleted SL Genes from K562

ABL1*
ATIC
BCR*
BRD4
CDC16
CDC23
CDC40
CDK1
MAPKAPK5
SOS1

Figure 4. Heat map showing shRNA signatures across several cell lines. Hierarchical clustering with Euclidean Squared-distance metric. Blue denotes highly statistically significant shRNAs that are depleted as determined by HT sequencing. K562-specific SL genes were identified, and at least two were also identified in a similar study by the Broad Institute*.

*Luo B, et al. Highly parallel identification of essential genes in cancer cells. Proc Natl Acad Sci U S A. 2008 Dec 23;105(51):20380-5.

Pathway Analysis and Comparison with other Published Screens

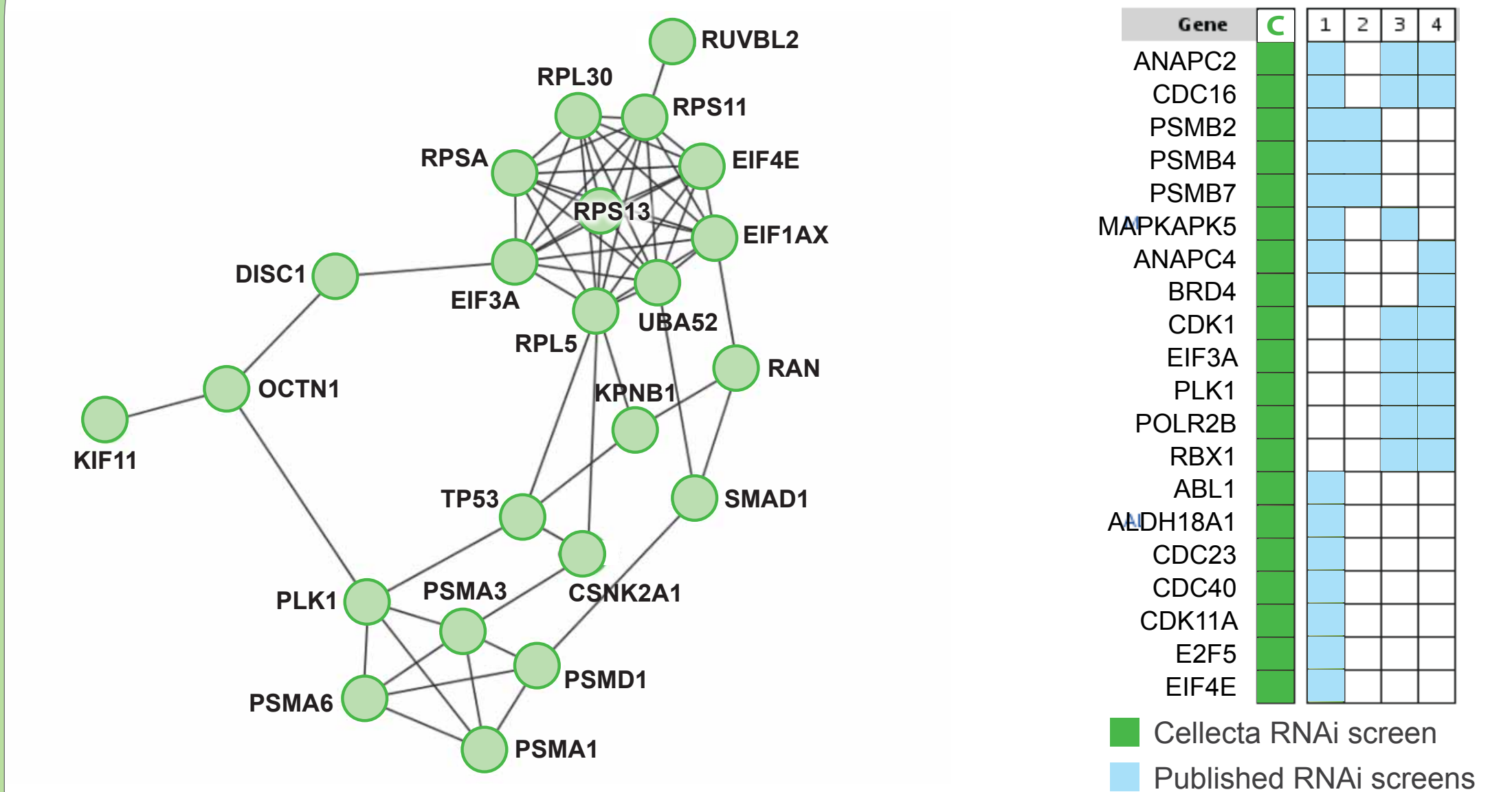


Figure 5A. PPI network model generated using essential gene signatures identified in Fig. 4.

Figure 5B. Comparative analysis of a number of essential genes identified in Fig. 4 and compared to published general lethality screens deposited in Oncomine.

Synthetic Lethality in HMEC

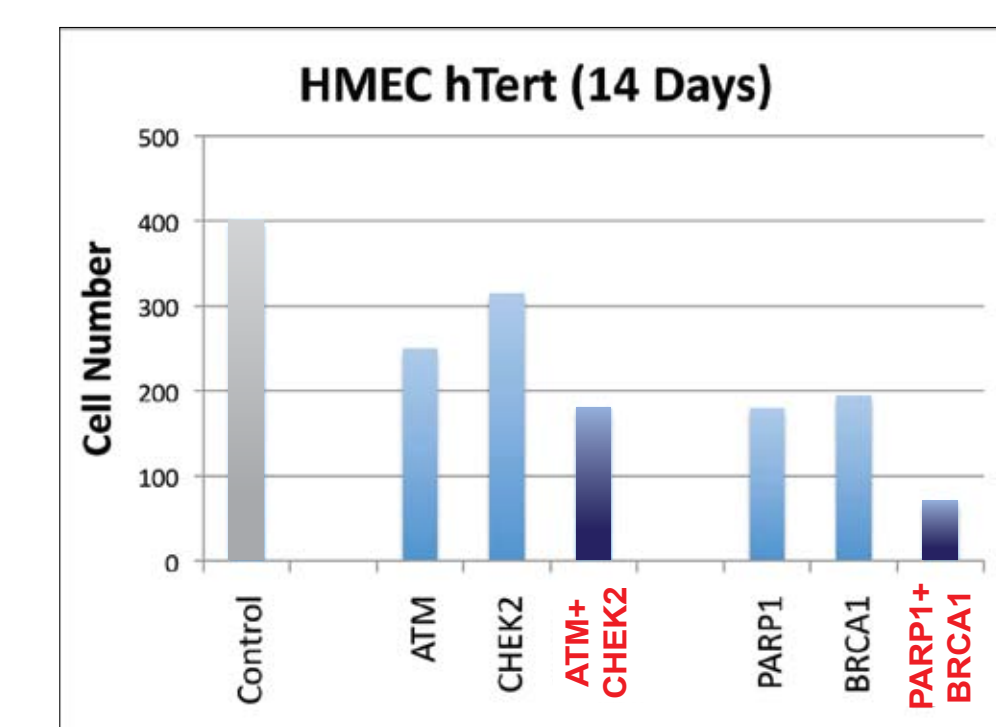


Figure 6. Enhanced toxicity resulting from dual expression of shRNA targeting two different DNA Damage Response (DDR) genes from a single lentiviral expression vector. This construction enables combinatorial screens of hundreds of shRNA for synthetically lethal effects.

Results & Conclusion

- Based on our RNAi genetic screens, we identified general lethality and hematopoietic-cancer-specific viability genes (Fig. 4).
- Genetic screens revealed cell-line specific viability gene signatures or “fingerprints” as well as general cancer lethality signatures reported in several published studies (Fig. 5B).
- Further validation is planned, using an expanded cell line panel of 12 hematopoietic and 12 non-hematopoietic cell lines.
- We have developed a dual shRNA expression vector in order to perform synthetic lethality screens (Fig 6). It is currently being used to screen HMECs.

The primary goal of our study was to identify lethal genes for hematopoietic lineages not critical for survival of other cell types of the human body, and neoplasias arising from these cells.

We believe these newly discovered hematopoietic-specific SL genes represent potentially novel drug targets. Moreover, they can be used to develop and establish both novel cancer targeted therapies and myeloablative conditioning regimens with decreased toxicity.

If you are interested in having a similar screening done, contact Cellecta Custom Services at orders@cellecta.com.