

Background:

High Content Target Discovery and Drug Discovery Strategy:
Genomics and proteomics have identified large numbers of genes / gene products whose abnormal expression is associated with cancer (e.g. disease progression, maintenance of disease, drug response, relapse of disease). A strategy for drug discovery includes production of gene expression reporter cells for cancer-associated genes and screening with libraries of siRNAs and chemical compounds to identify regulatory pathways, drug targets and drug candidates (e.g. High Content Drug Discovery).

Kit Ligand:
Kit Ligand is expressed in colon, breast, ovarian, testicular, prostate, small-cell lung and gastric cancers. Binding of Kit Ligand to its receptor, c-Kit, activates oncology-relevant signaling through the Ras/ERK pathway. Kit Ligand stimulates anchorage independent growth of colon cancer cells, has been implicated in regulation of growth of cervical cancer and leukemia cells, and has been implicated in Schwann cell neoplasia, neuroblastomas, and gynecological tumors. The receptor for Kit Ligand, c-Kit, is also abnormally expressed in tumors. Imatinib (Gleevec) is an inhibitor of receptor tyrosine kinases that include platelet-derived growth factor alpha, c-ABL and c-Kit. Gleevec is active against gastrointestinal stromal tumor (GIST) where improved survival is associated with its effect on c-Kit activity.

Epiregulin:
Epiregulin is a member of the EGF family and binds to EGFR (ErbB1) and ErbB4. It is rarely expressed in adult tissues/cells but is often over-expressed in human cancer cell lines and can contribute to tumorigenesis in nude mice. Activated Ki-Ras appears to stimulate Epiregulin expression. EGFR is currently a target for drug discovery (e.g. EGFR-specific mAbs such as cetuximab (Erbix) and panitumumab (Vectibix)). The tyrosine kinase activity of the EGFR has also been targeted (e.g. gefitinib (Iressa), erlotinib (Tarceva), and lapatinib).

HMGA1:
HMGA1 is over-expressed in naturally occurring human cancers, including colon, prostate, thyroid, breast, pancreas, uterus, skin, intestine, brain, lung, and blood. It is a bonafide oncogene, since over-expression causes neoplastic transformation of nonmalignant cells and promotes progression from a low grade malignancy to a highly metastatic phenotype. Over-expression of HMGA1 can induce expression of Kit Ligand (by DNA array analysis).

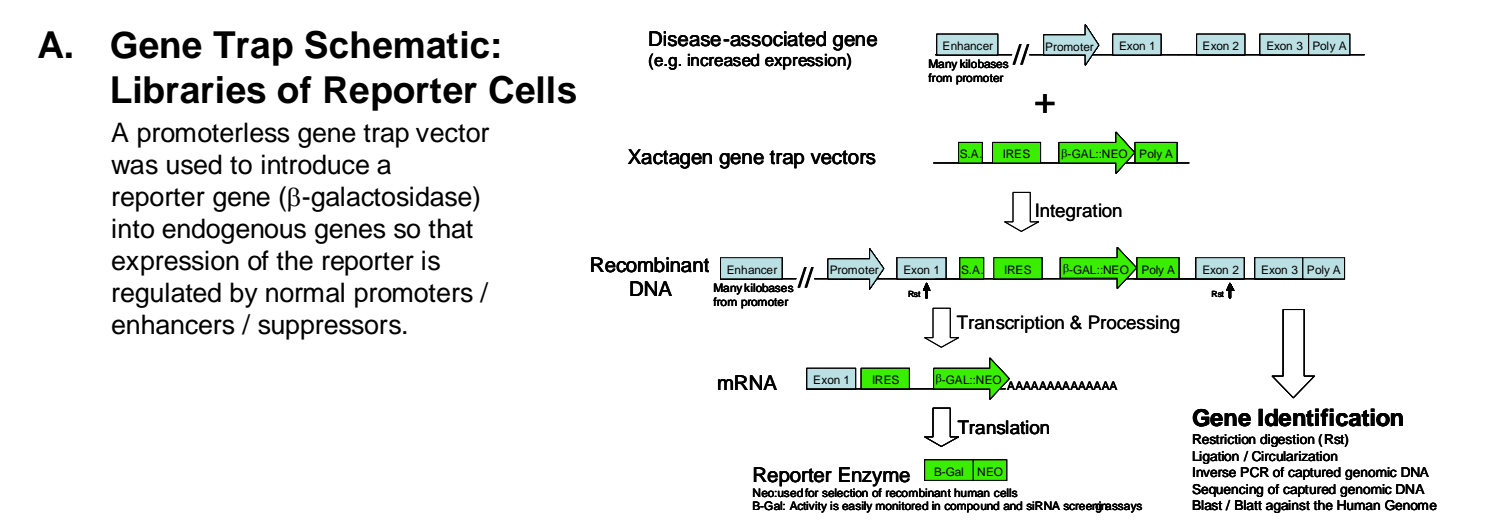
Results:

Gene Trapping and Gene Targeting Kit Ligand and Epiregulin:
Traditional gene trap and gene targeting vectors have limited success rates for production of gene expression reporter cells by transfection or infection (including AAV vectors). Using modified vectors designed to enhance this success rate, we demonstrate the trapping of 4 to 5-fold more transcriptional elements (in known, predicted and unpredicted genes) compared to traditional vectors. The modified gene trap and gene targeting vectors were used to generate gene expression reporter cells (β -galactosidase reporter) for Kit Ligand and Epiregulin in HCT116 human colon cancer and MCF-7 human breast cancer cells.

High Content Screening:
Screening reporter cells with a known bioactive library revealed agents that stimulated Kit Ligand expression in both HCT116 and MCF-7 cells. Further screening with a structurally diverse chemical library, including known antineoplastic agents, revealed additional families of compounds that augmented expression of Kit Ligand in HCT116 and MCF-7 cells by as much as ~30 fold and Epiregulin in HCT116 cells by as much as ~30 fold. Further investigation indicated that gambogic acid and tomatine induced both Kit ligand and Epiregulin reporter activity, as well as cytotoxicity at similar concentrations, suggesting that these agents may function within the regulatory pathways for Kit Ligand and Epiregulin. One possible target / pathway for inducing Kit Ligand reporter expression is HMGA1, since siRNAs against HMGA1 stimulated Kit Ligand expression in both HCT116 and MCF-7 cells. Gambogic acid has previously been shown to inhibit the catalytic activity of human topoisomerase II α , and induces apoptosis. Derivatives of gambogic acid are currently under investigation for cancer indications (Maxim Pharmaceuticals, San Diego, CA).

Gene Trapping and Gene Targeting Kit Ligand and Epiregulin

Production of Kit Ligand and Epiregulin reporter cells in HCT116 colon cancer cells by gene trapping.



B. Gene Trap Efficiency

Gene trap vectors used in this study capture 4 to 5 times more genes than traditional (standard) gene trap vectors.

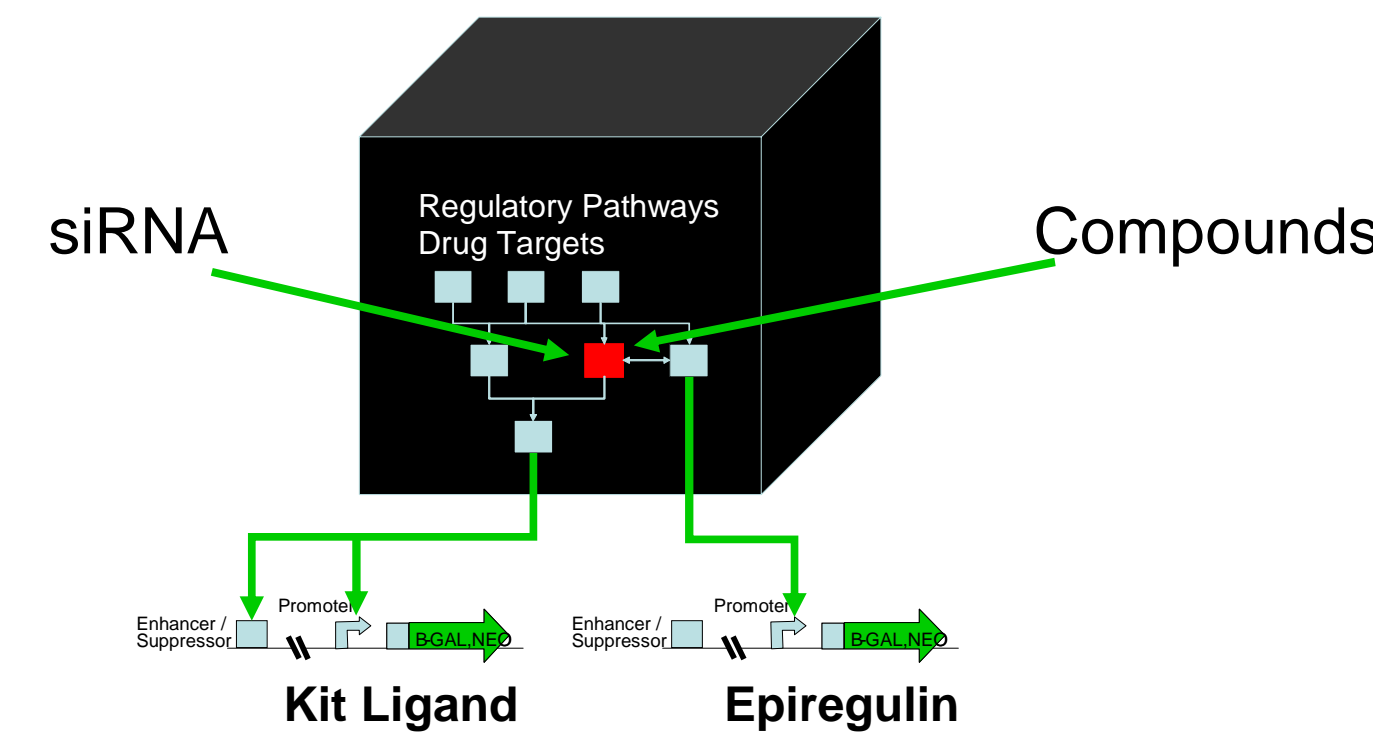
C. Reporter Cell Examples: KTLG and EREG
The resulting library of reporter cells contained known genes, predicted genes, and unpredicted transcription units. Included were reporters for Kit Ligand and Epiregulin.

High Content Screening of Kit Ligand and Epiregulin Reporter Cells

Targeting Genes that Cause Cancer

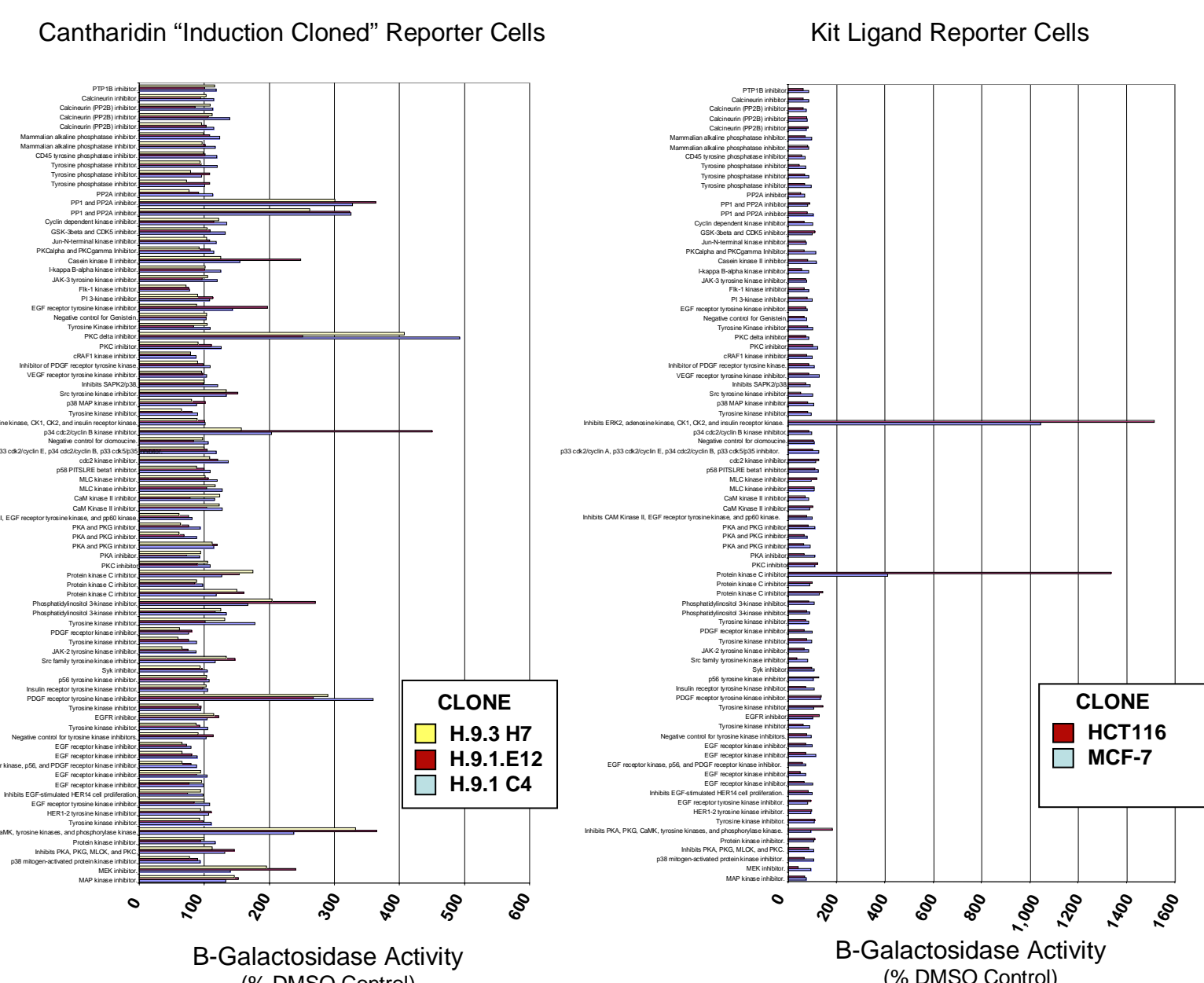
A. High Content Drug Discovery

By screening gene expression reporter cells, every gene product or interaction within a cell is a potential target for siRNA and compound intervention (e.g. High Content Screening). Screening with siRNA libraries identifies regulators of gene expression and potential drug targets, while screening with compounds identifies drug candidates



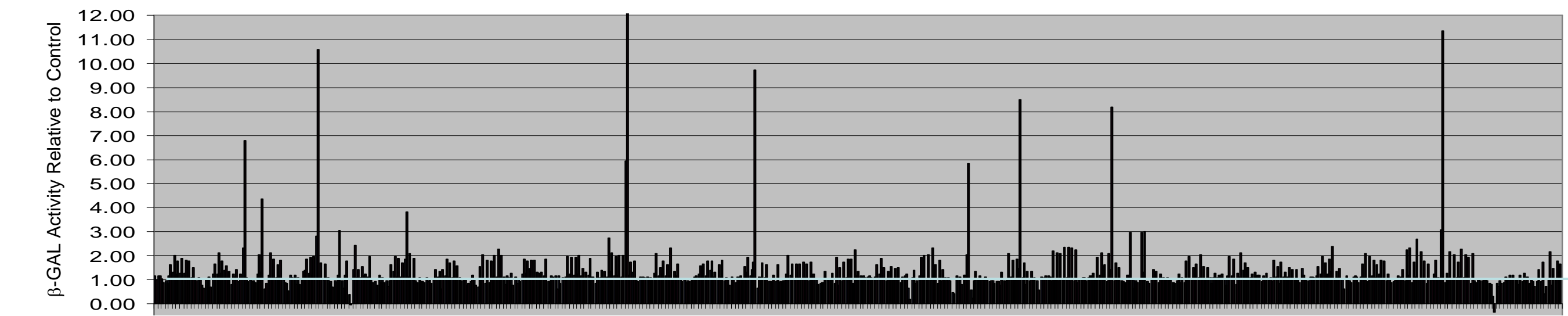
B. Stimulation of Kit Ligand Expression in HCT116 and MCF-7 Cells by Known Bioactives

Ro 31-8220 (an inhibitor of Protein Kinase C) and 5-Iodotubercidin (an inhibitor of ERK2, Adenosine Kinase, CK1, CK2, and Insulin Receptor Kinase) stimulated expression of Kit Ligand in both HCT116 and MCF-7 cells. Stimulation as high as 15 fold was observed. For comparison, the response of three reporter cell lines pre-selected ("Induction Cloned") for responsiveness to Cantharidin (an inhibitor of protein phosphatase 1 and 2A) is also shown. The data indicate that Kit Ligand is regulated by different pathways than those regulating cantharidin-selected clones.



C. Primary Kit Ligand Reporter Screen (HCT116 Colon Cancer & MCF-7 Breast Cancer Cells)

Kit Ligand reporter cells (HCT116 and MCF-7) were screened with a panel of structurally diverse compounds. Primary screening was at 20 μ M, 48 hour incubation. β -galactosidase activity is presented relative to activity in DMSO treated control cells.



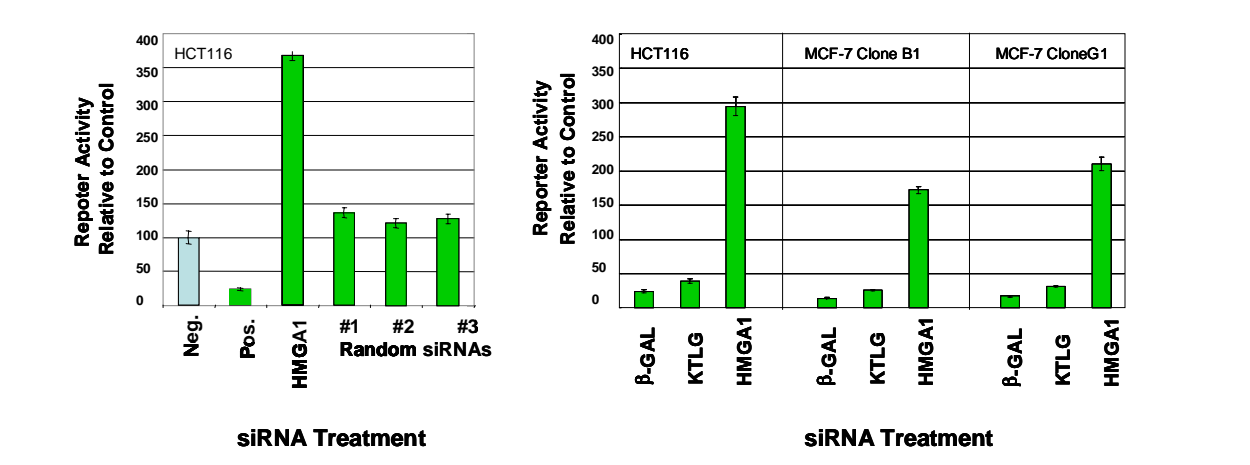
D. Synopsis of the Primary Screen: Stimulators of Kit Ligand Expression (Fold Induction)

Compound	Activities	HCT116	MCF-7
GAMBOGIC ACID AMIDE	antitumor, anti-inflammatory	28.0	8.2
POSTERON	antitumor, anti-inflammatory	23.7	6.0
TRIMESTROL	antitumor, preservative	22.8	3.9
POSTERONOL DIACETATE	antitumor, anti-inflammatory, cytotoxic	22.7	5.7
GAMBOGIC ACID	antitumor, anti-inflammatory	20.6	6.6
POSTERONOL	antitumor, anti-inflammatory	20.5	6.7
ETODOLAC	antitumor, anti-inflammatory	20.3	6.6
TETRAOL-DIISOPHTHALONITRILE	antitumor	17.7	5.3
LOFATADINE	anti-anthelmintic	17.4	6.0
ANTHOTHICOL	antitumor	17.2	4.7
OSIMERTINIB GAMBOGATE	anti-anthelmintic	16.9	6.2
PRESTIMEROL	antitumor	16.3	5.9
ALBENDAZOLE	antitumor	16.0	3.4
PHENYL MERCURIC ACETATE	antitumor	14.8	4.4
ACILACROMETON A	antitumor	14.4	4.8
CELASTROL	antitumor, anti-inflammatory, NO synthesis inhibitor, dopamine stimulant	13.2	2.6
ANDROSTERONE ACETATE	antitumor	11.3	3.1
DIBENZOYLGLUTARIC DIACETATE	antitumor	11.2	4.9
PFITHIONE ZINC	antitumor, antifungal, antiseborrheic	11.0	4.4
1-BENZYL-2-CARBONYL-LAMINO-2-NITRILE-CHLOROETHYL KETONE	antitumor, adenosine inhibitor	10.8	3.6
ENOSYAPPAHONE B 7'-O-CIMETHYL ETHER	antitumor	10.6	2.8
TOMATINE	antitumor, antibacterial, anti-inflammatory agent	9.9	3.5
DIBENZOYLGLUTARIC	antitumor	9.9	4.4

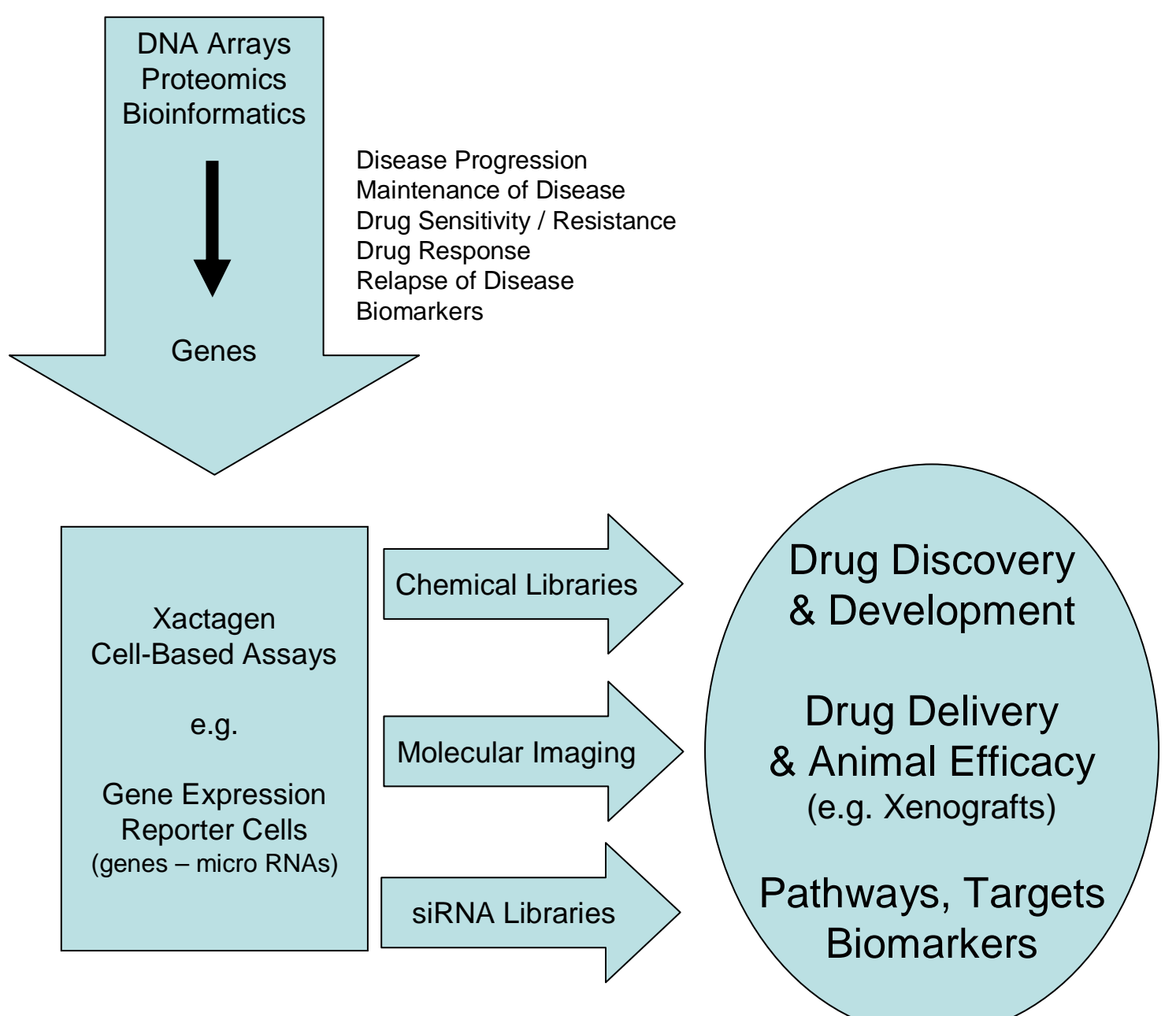
Compounds

F. siRNA Against High Mobility Group A1 (HMGA1) Induces Kit Ligand Expression in HCT116 and MCF-7 Cells

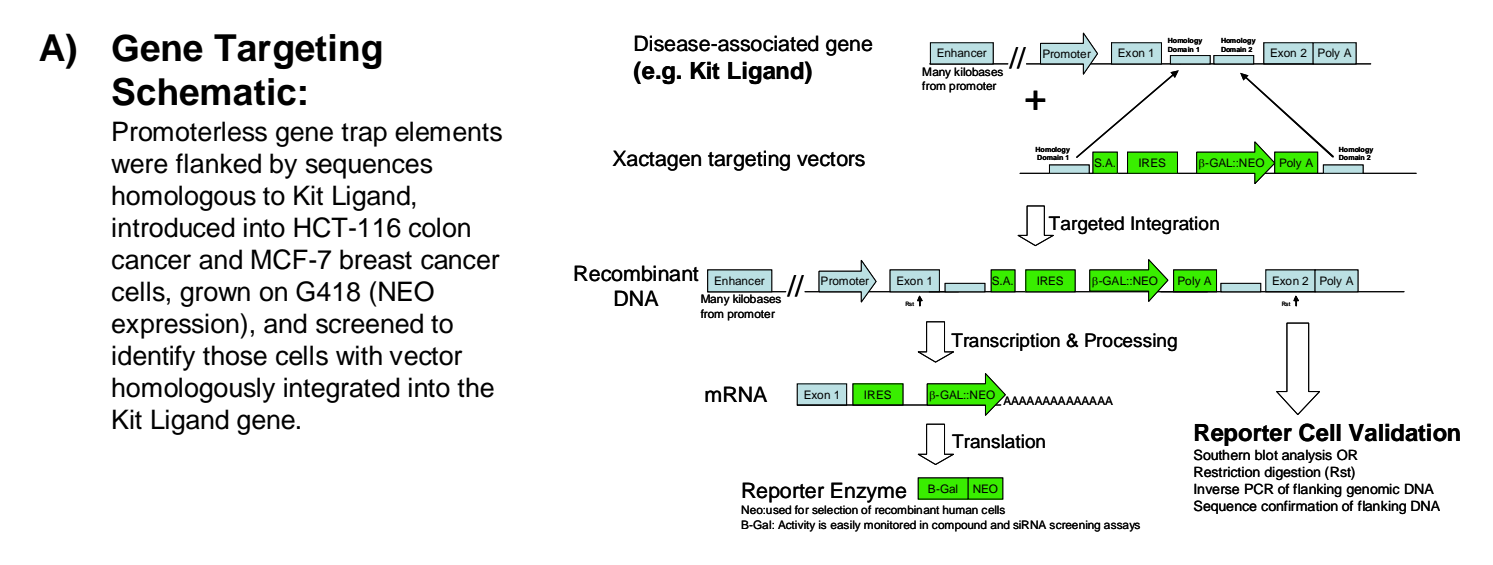
HMGA1 induced expression of Kit Ligand reporter activity in both HCT116 cells and MCF-7 cells indicating a potential regulatory role for HMGA1. In contrast, siRNA directed at β -galactosidase (Positive Control) suppressed reporter activity while "random" siRNA sequences had no effect.



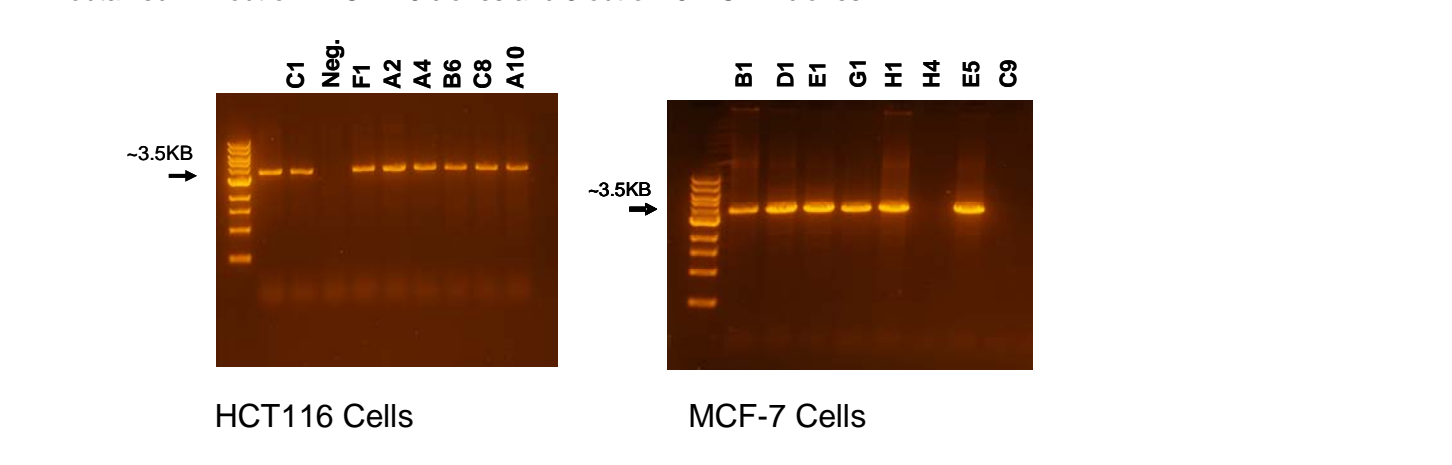
Xactagen Technologies From Genes to Drug Discovery / Development



Production of Kit Ligand reporter cells in HCT116 and MCF-7 cells by gene targeting.



B. PCR Validation of Gene Targeting



Conclusions:

- Improved vector design has increased the number of genes amenable to gene trapping and gene targeting 4 to 5 fold, thereby facilitating production of gene expression reporter cells.
- Kit ligand expression in HCT116 and MCF-7 cells was stimulated by an inhibitor of Protein Kinase C and by an inhibitor of ERK2, Adenosine Kinase, CK1, CK2, and Insulin Receptor Kinase.
- As indicated by reporter responses to chemical libraries, Kit Ligand and Epiregulin appear to be co-regulated. For example, gambogic acid and tomatine induce expression of both genes by as much as ~30 fold.
- In a dose-response study, induction of Kit Ligand and Epiregulin expression was correlated with cytotoxicity of gambogic acid and tomatine.
- siRNAs against HMGA1 also stimulated expression of Kit Ligand, suggesting a regulatory role of HMGA1.

