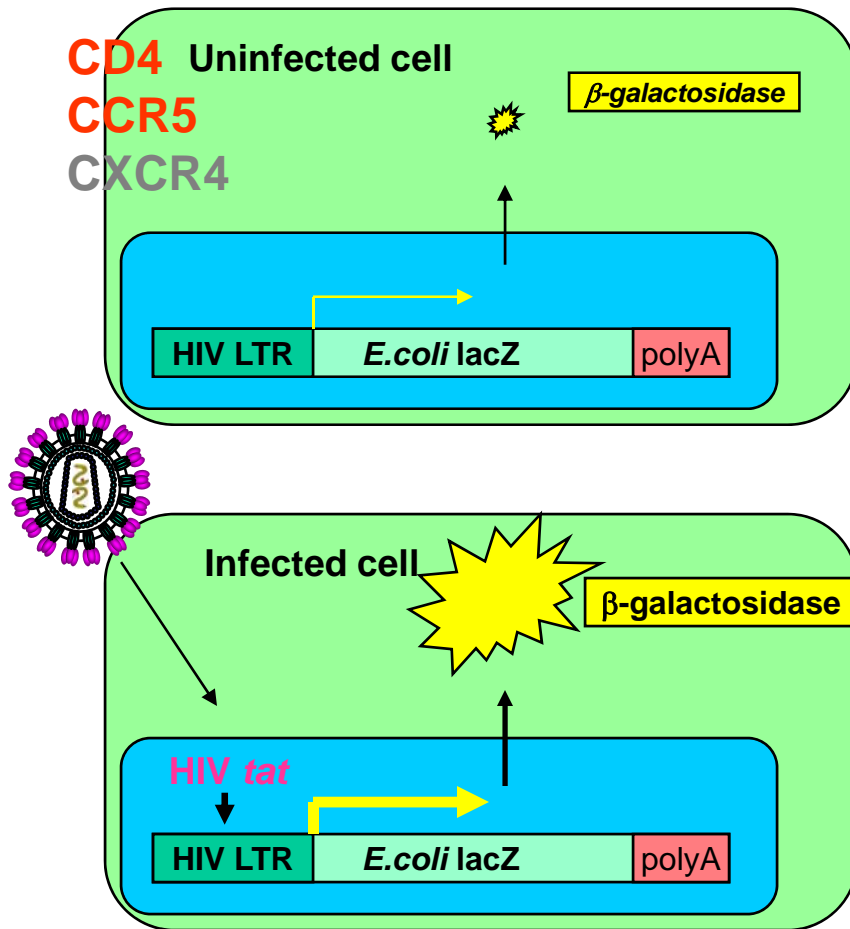




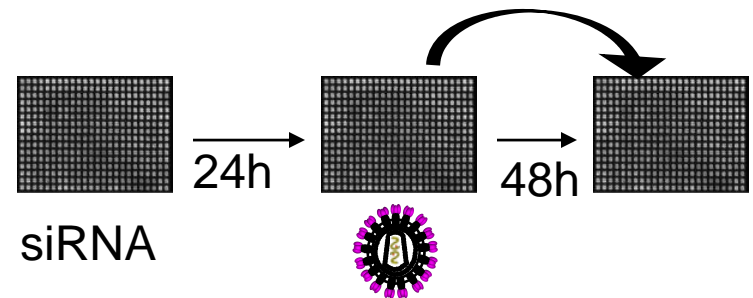
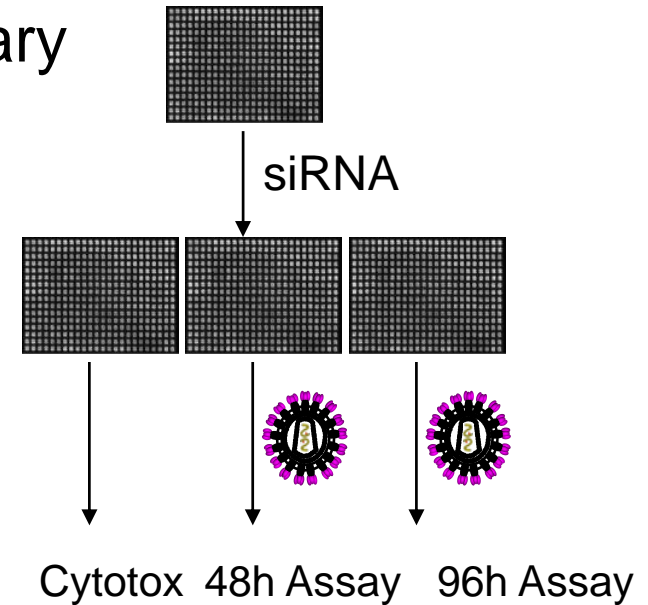
Genome-scale RNAi Screen for Host Factors Required for HIV Replication

Amy S. Espeseth
Merck Research Laboratories
December 12, 2008

siRNA screen for novel cellular factors for HIV replication in HeLa P4/R5 cells

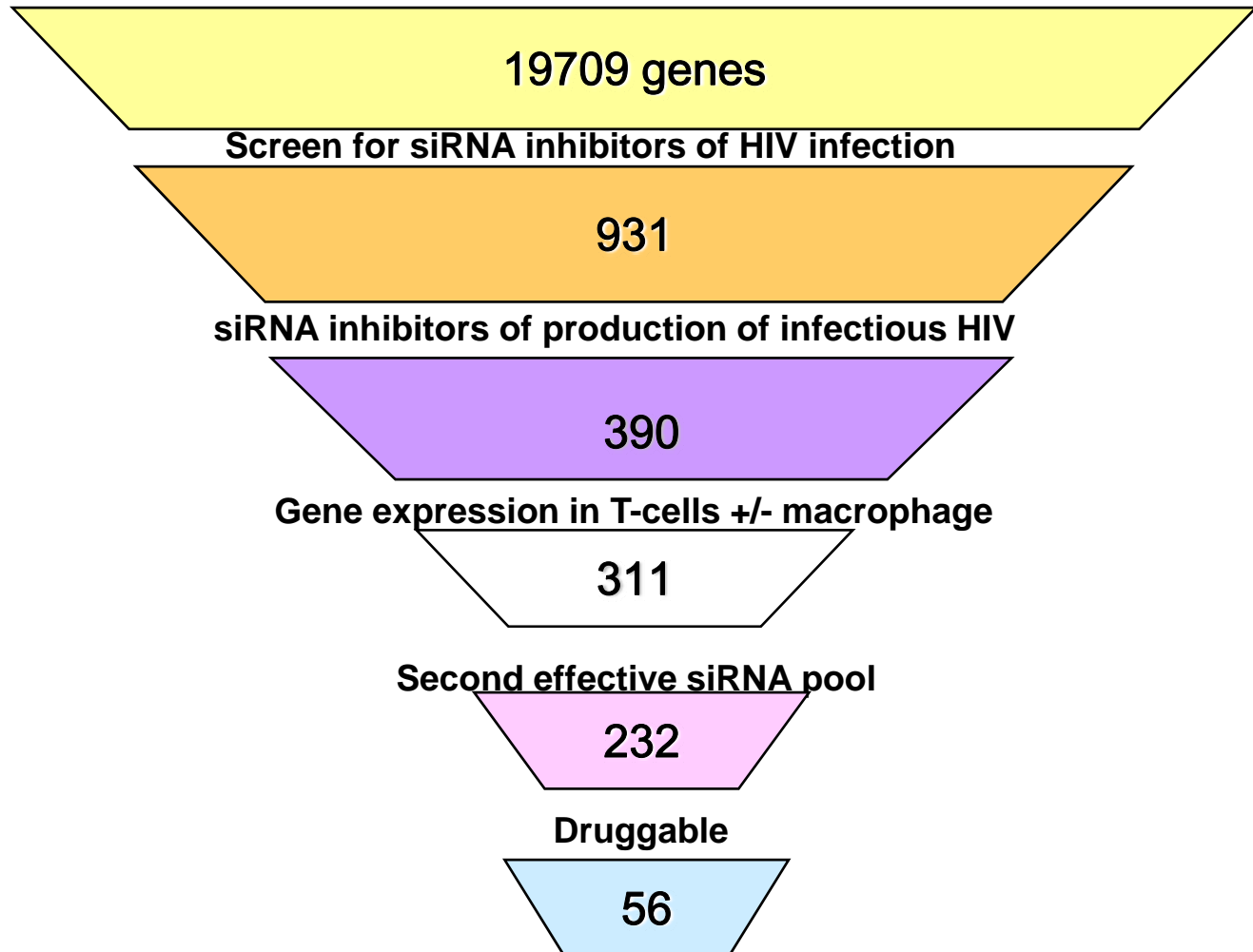


Primary

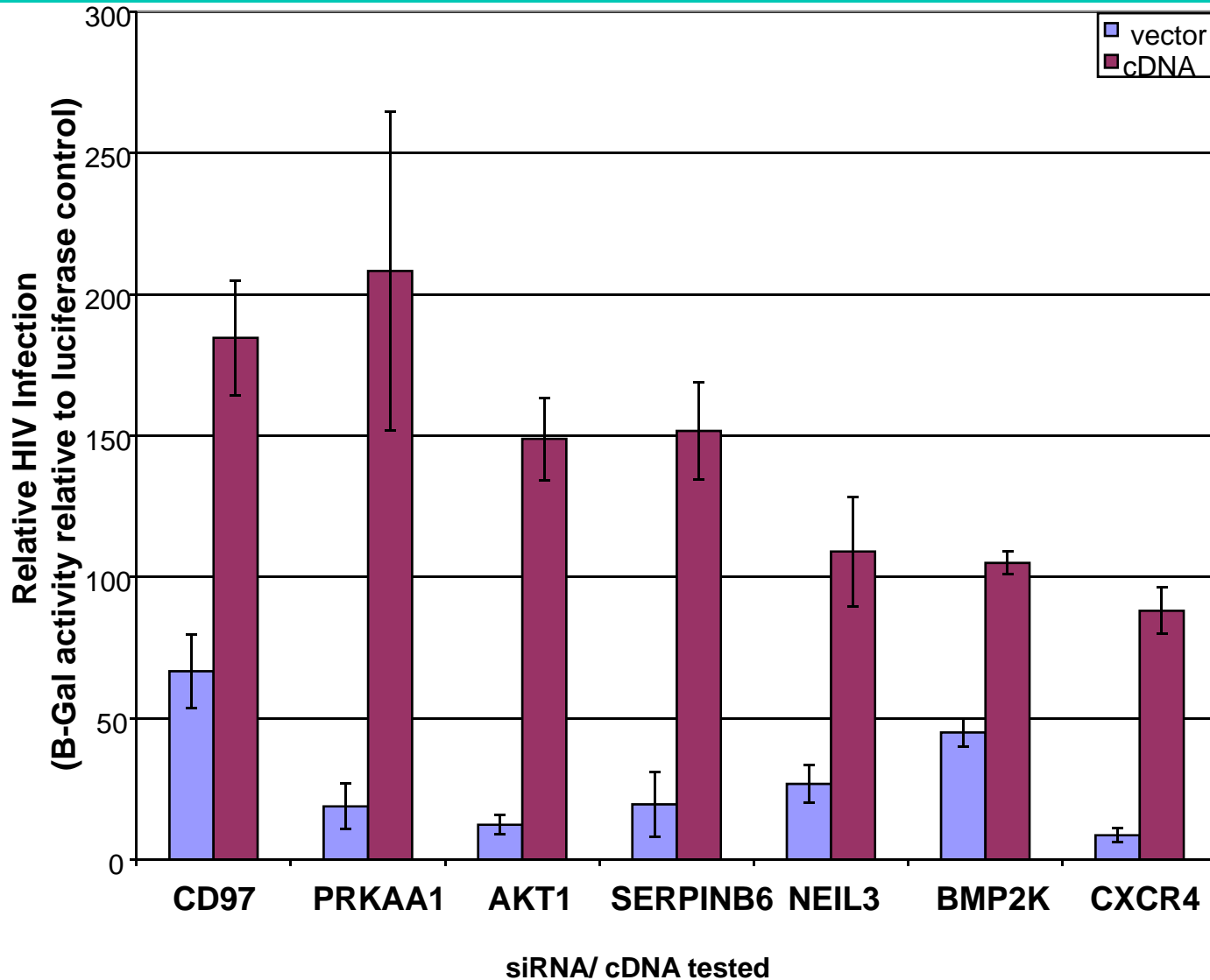


**Confirmation 1:
Production of infectious virus**

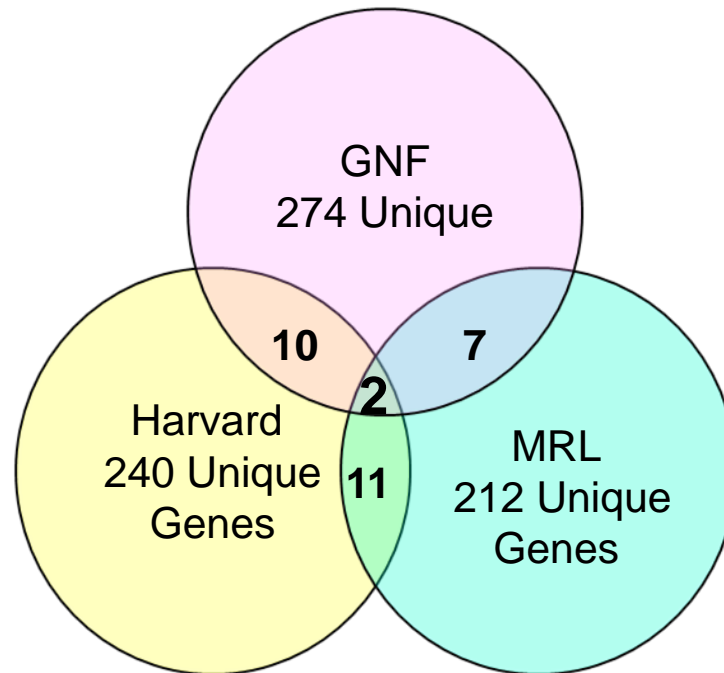
Screening Funnel



cDNA Rescue of HIV Infection



Little Overlap between Hits in Three Genome Scale Screens for HIV Co-Factors



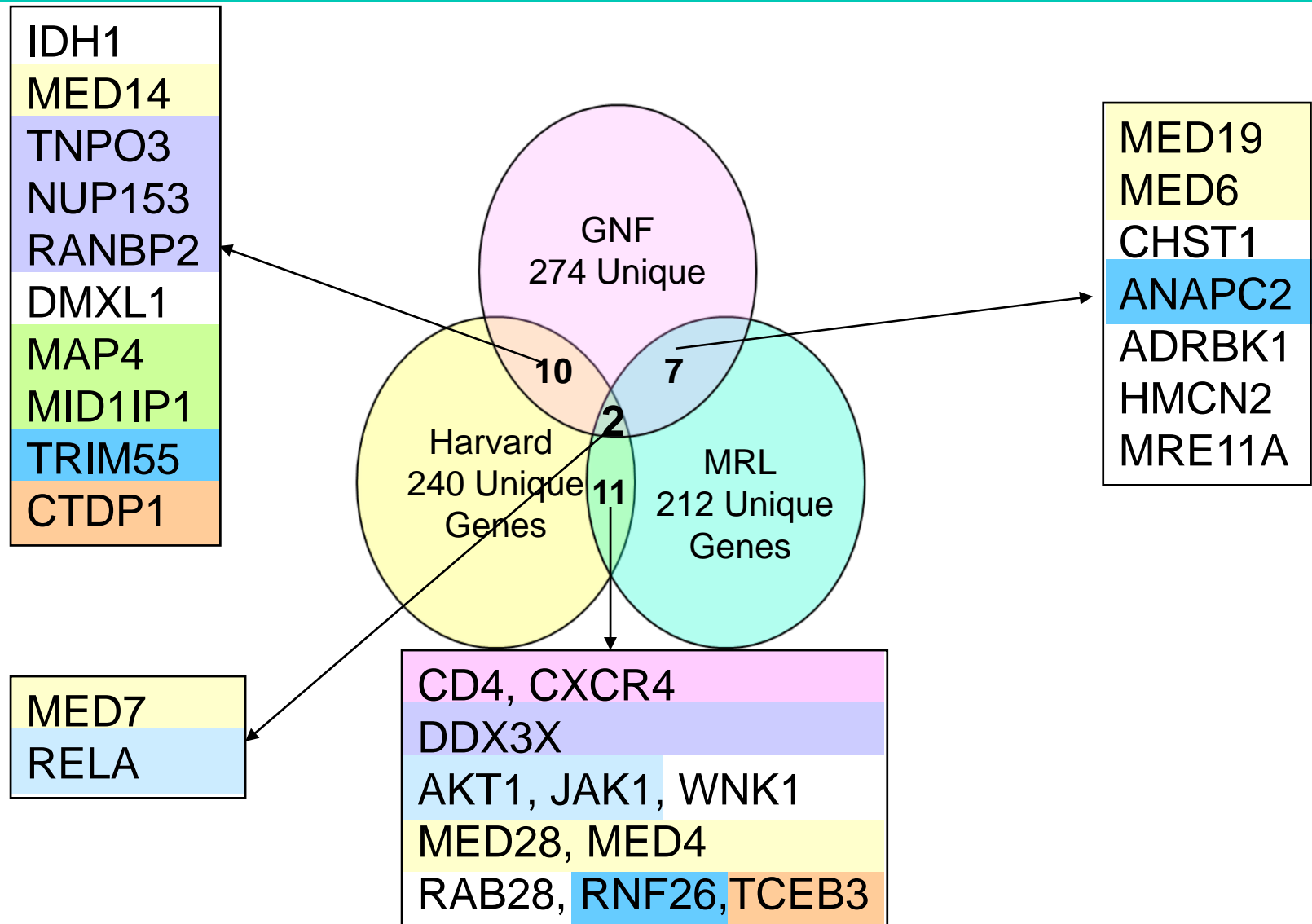
GNF= Chanda et al. Cell (2008) 135(1):49-60

Harvard = Brass et al. Science (2008) 319(5865):921-6

Variations between screening conditions

	Affects	MRL	Brass	Koenig
siRNA library	mRNA knockdown	Proligo	Dharmacon	Qiagen + GNF
Cell line	?Factors required?	HeLa (P4/R5)	HeLa (TZM-bl)	HEK-293T
Virus	Factors required	HXB2	HIV-IIIb	VSV-G/NL4-3
Time from transfection to virus addition	knockdown	24h	72h	48h
Time from virus addition to assay read	Factors required	48, 96h	48, 72h	24h

Despite Small Overlap, Common Pathways Emerge



Novel Pathways

	<u># of Genes</u>	<u>MRL</u>	<u>Brass</u>	<u>Koenig</u>
Global	SP1/Mediator Complex	9	3	5
	Oxidative Phosphorylation (GO)	7	2	3
	Transport RAN Regulation (GO)	1	8	7
Known	{ NF-kB Signalling	5	3	1
Source Of New Targets?	Antigen Presentation by MHC Class I (GO)	3	1	11
	DNA Repair (GO)	6	5	15
	Adipocytokine Signalling (KEGG)	8	4	1

Summary

- High throughput genome scale siRNA screening is likely to result in false positives and false negatives along with true positives
- True positives will include global (housekeeping) genes, components of known pathways, and components of previously unlinked pathways
- Our screen for HIV co-factors resulted in little hit overlap with screens carried out at Harvard (Brass et al) and GNF (Konig et al) but overlap between pathways/ functions was observed
- Using cDNA rescue, we confirmed the role of six novel host factors in HIV infection: CD97, PRKAA1, SERPINB6, AKT1, BMP2K, and NEIL3

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