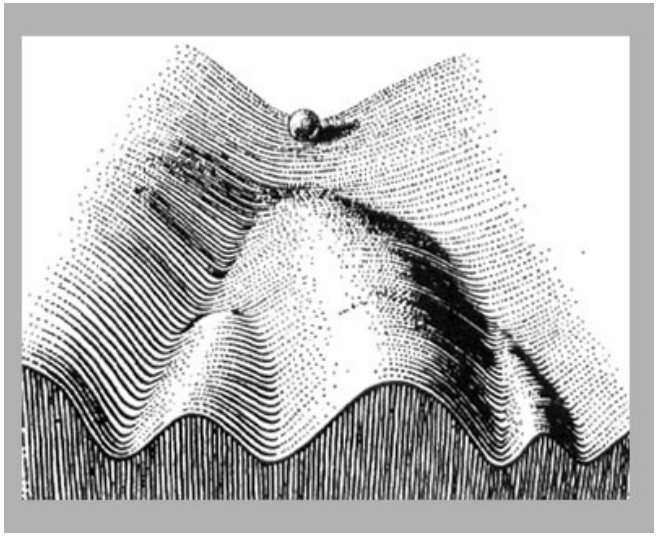
Towards targeted epigenetic therapies in breast cancer

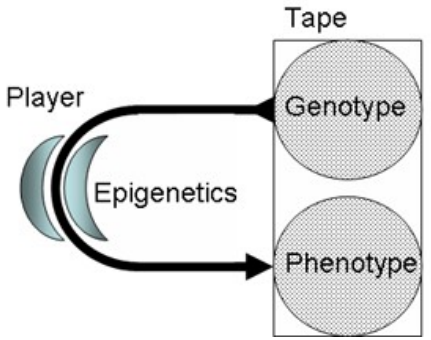
Epigeniuses:

Annie Moradian, Martin Hirst, Yvonne Li, Sorana Morrissy, Maziar Rahmani, Krithika Selvarajan, Kate Slowski, Lucas Swanson, Kevin Yang

History of Epigenetics

Conrad Hal Waddington





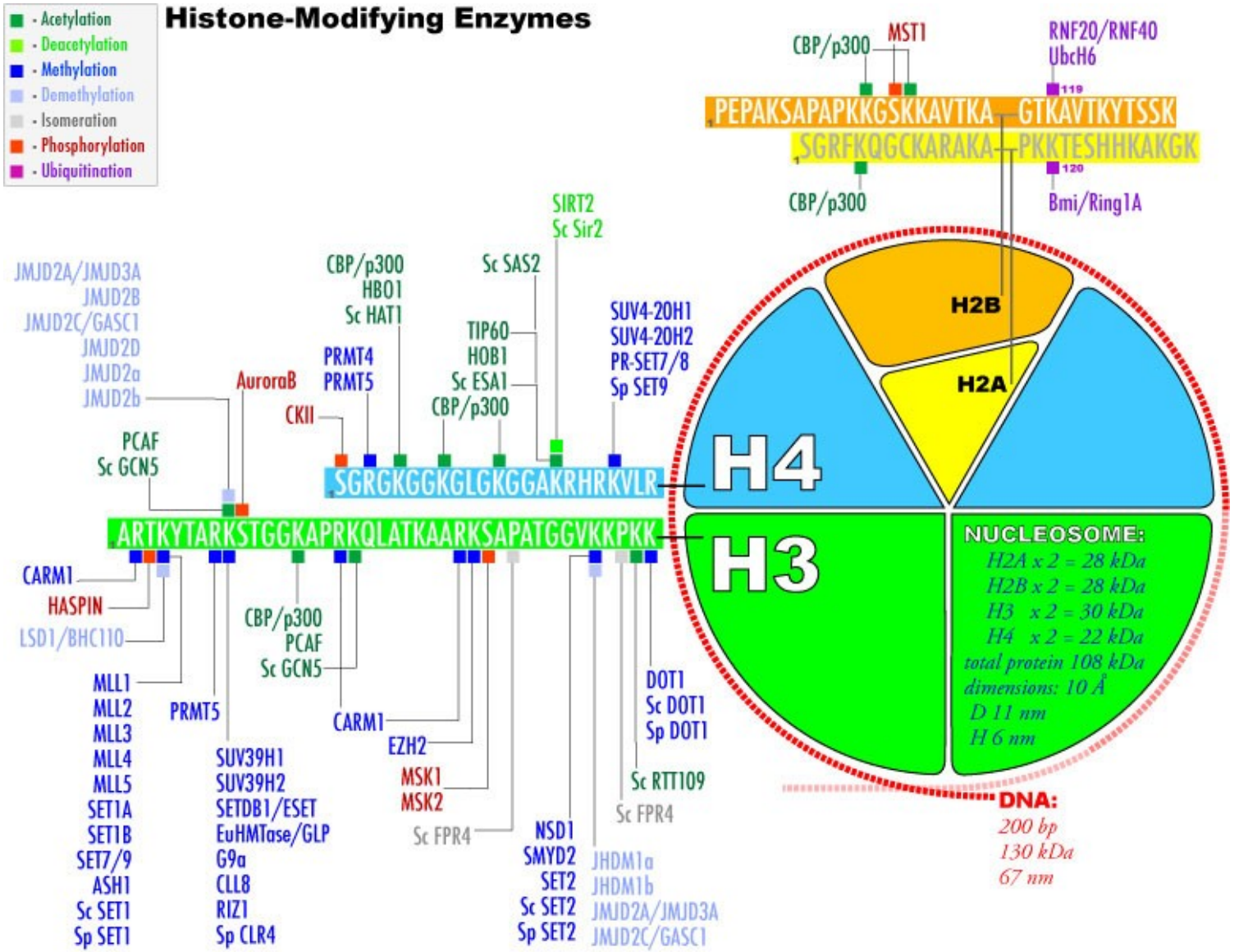
Bryan Turner

- Originally:
  - Cell differentiation during development
  - Cell fates become restricted as development proceeds
  - Balls, valleys, peaks
- Now:
  - Heritable traits not involving changes to DNA sequence
  - Tape (genome), player (epigenetics)

Background - Epigenetics

- Covalent modifications to DNA and histones impact gene expression
- Changes in DNA methylation and altered histone modification patterns have been associated with cancer
  - Can be through mutation of epigenetic modification enzymes
  - Can be through alterations in the targeting of epigenetic modifications

Background – Histone Modification Complexes (HMC)



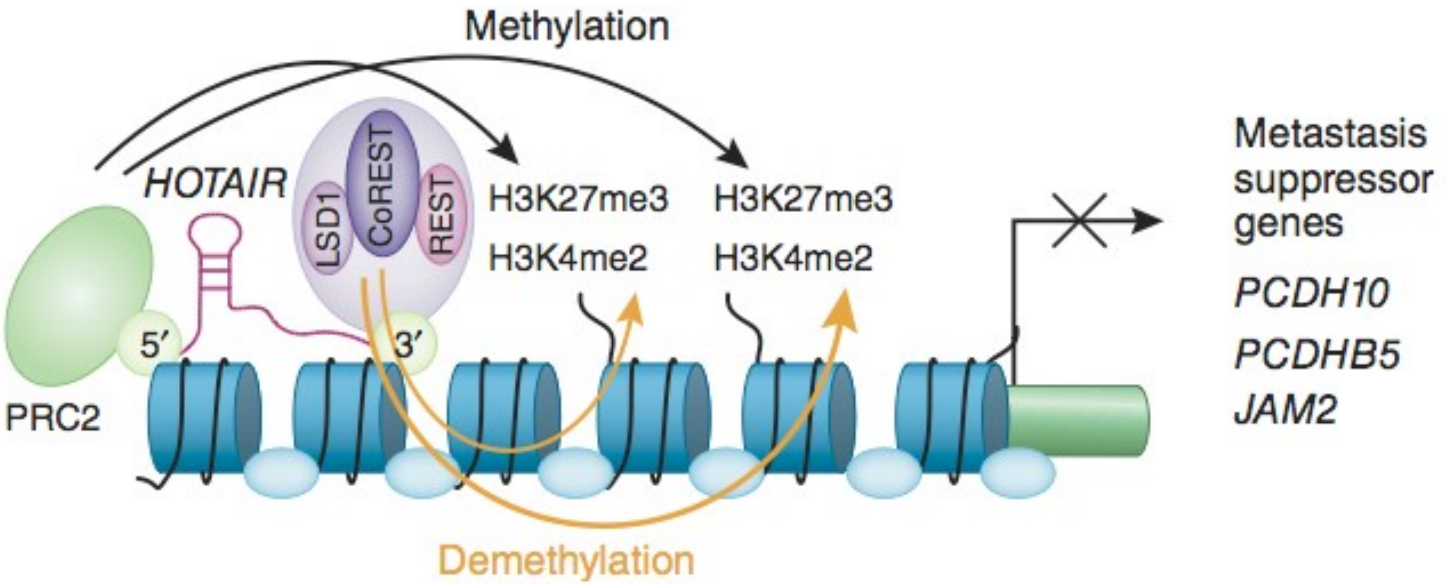
Background – lincRNA

- Long intergenic non-coding RNAs
- >3000 actively transcribed and highly conserved in human genome
- Implicated in the targeting of histone-modifying enzymes
- Act as a scaffold between multiple histone modification complexes
- Scaffolded complexes have increased binding specificity

Background – HOTAIR

- Transcribed from the HOXC cluster
- Represses (metastasis repressor) genes in the HOXD cluster
- Scaffolds PRC2 complex with LSD1/REST/CoREST complex
- Overexpressed in breast cancer
  - Causes an aggressive and metastatic phenotype

Background – HOTAIR Mechanism



Background – Motivation

- Current epigenetic cancer treatments directly target epigenetic modification enzymes
  - Effect gene expression globally across the genome
- Use insights from HOTAIR lincRNA/HMC-scaffolding mechanism to design treatments targeted to specific genes

Objectives

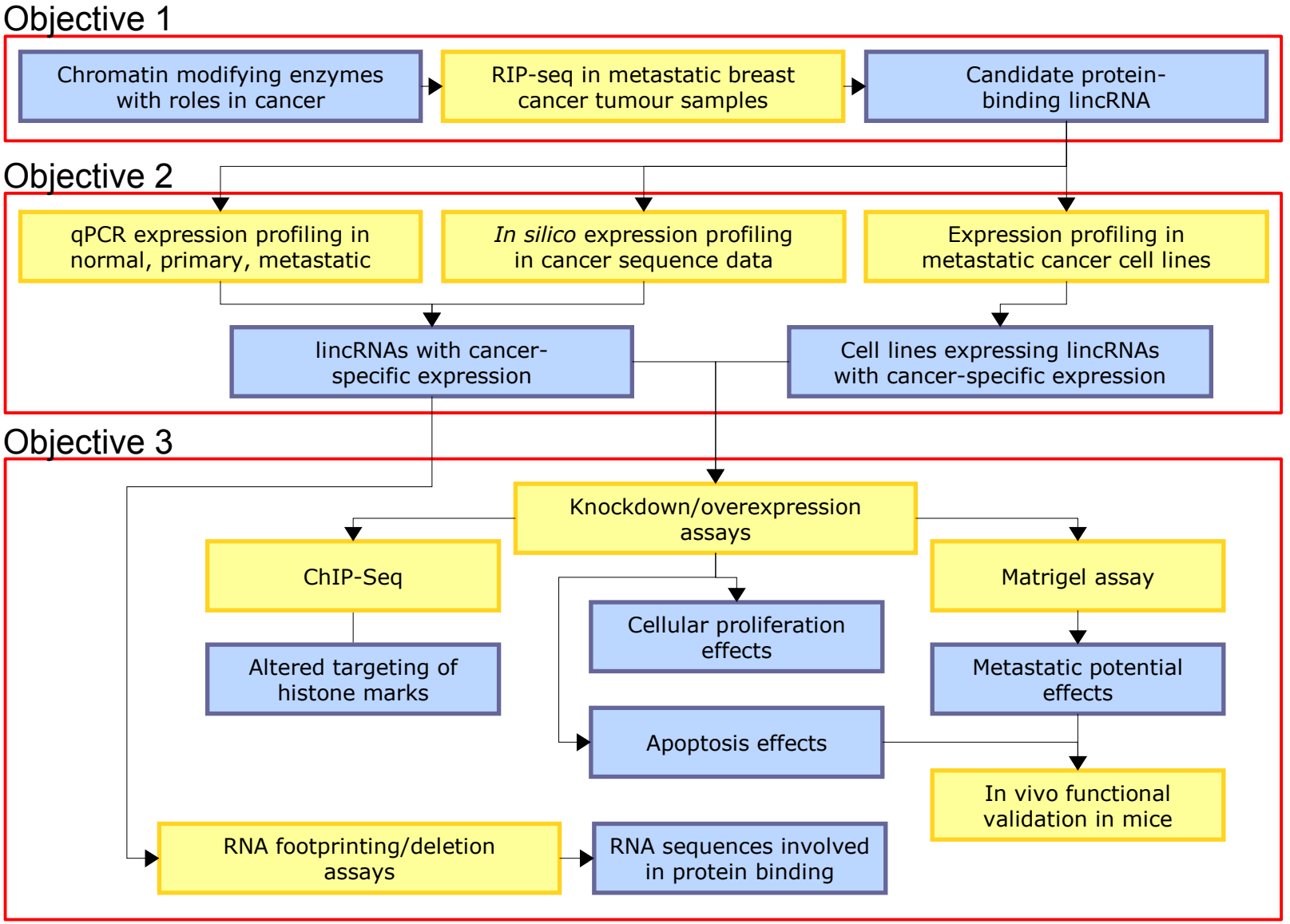
1) Identify lincRNAs associated with chromatin modifying enzymes in metastatic cells

2.1) Evaluate the cancer-specificity of these lincRNA by examining their expression in matched normal and primary cells

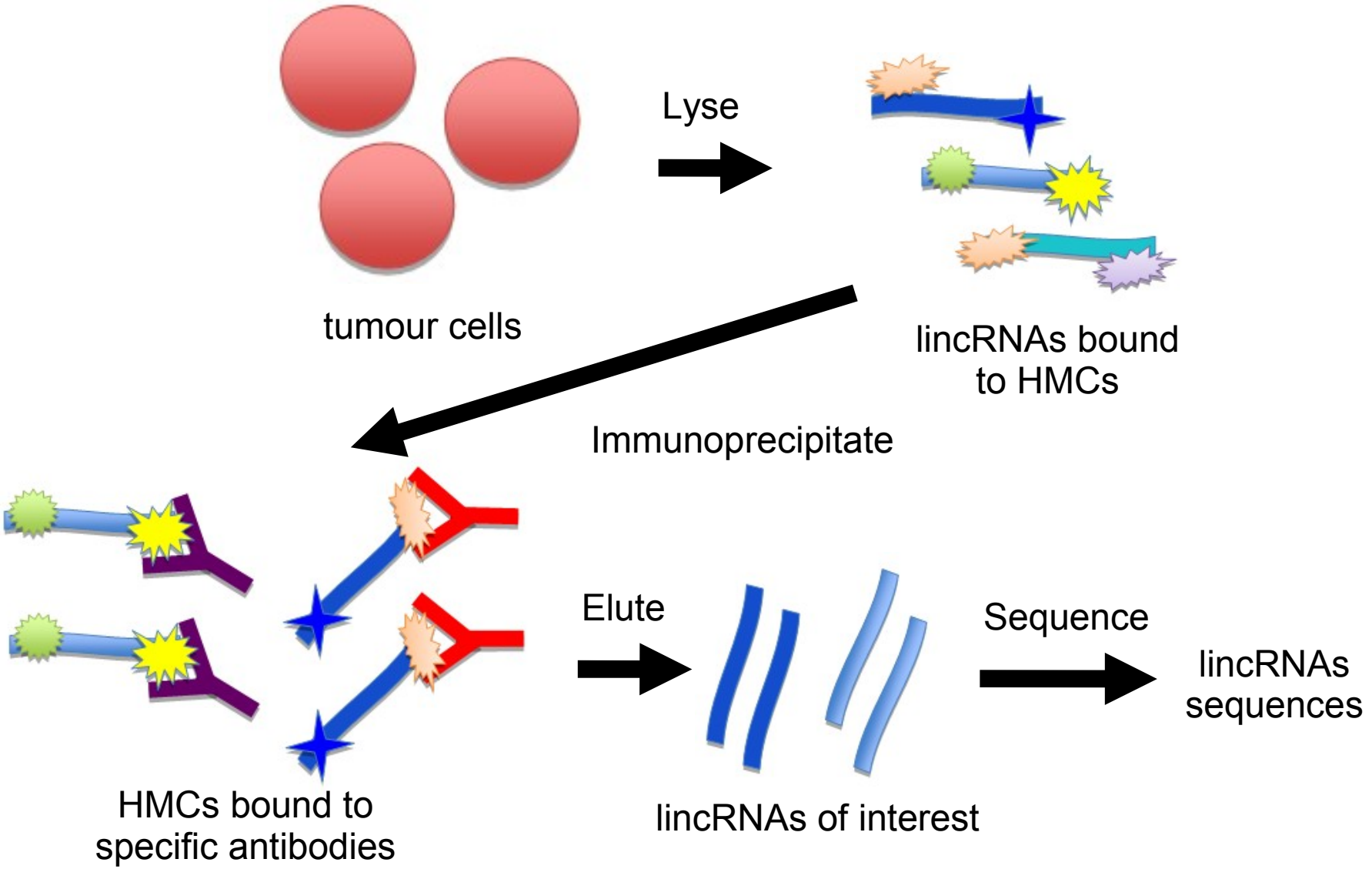
2.2) Identify cell lines showing abnormal expression of those lincRNAs showing cancer-specific expression

3) Perform functional validation through targeted knockdown and overexpression studies

Methods – Overview



Methods – Objective 1
RIP-Seq Discovery of lincRNAs

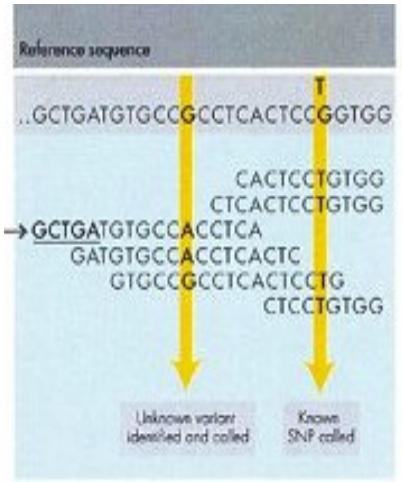


Methods – Objective 2
Expression Profiling – Cancer Specificity

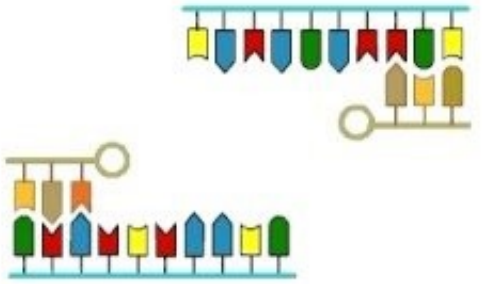
Develop lincRNA primers from RIP-Seq hits

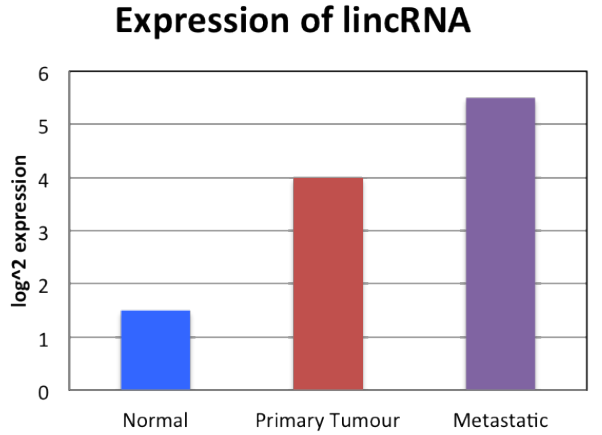
qPCR of lincRNAs

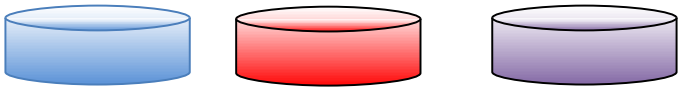
Compare normal vs primary vs metastatic expression



Perform qPCR with primers specific to lincRNAs of interest







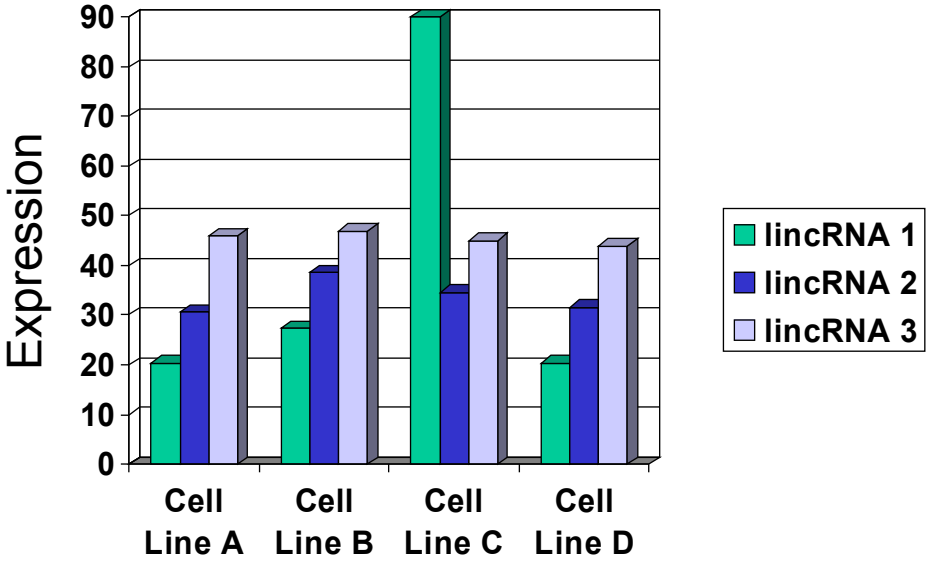
Normal Tissue

Primary Tumour

Metastatic Tumour

Methods – Objective 2
Expression Profiling – Cell Lines

- Measure expression of candidate lincRNAs in metastatic breast cancer cell lines
  - MB-231, SK-BR3, MCF7
- Look for cell lines that express lincRNAs with cancer-specific expression



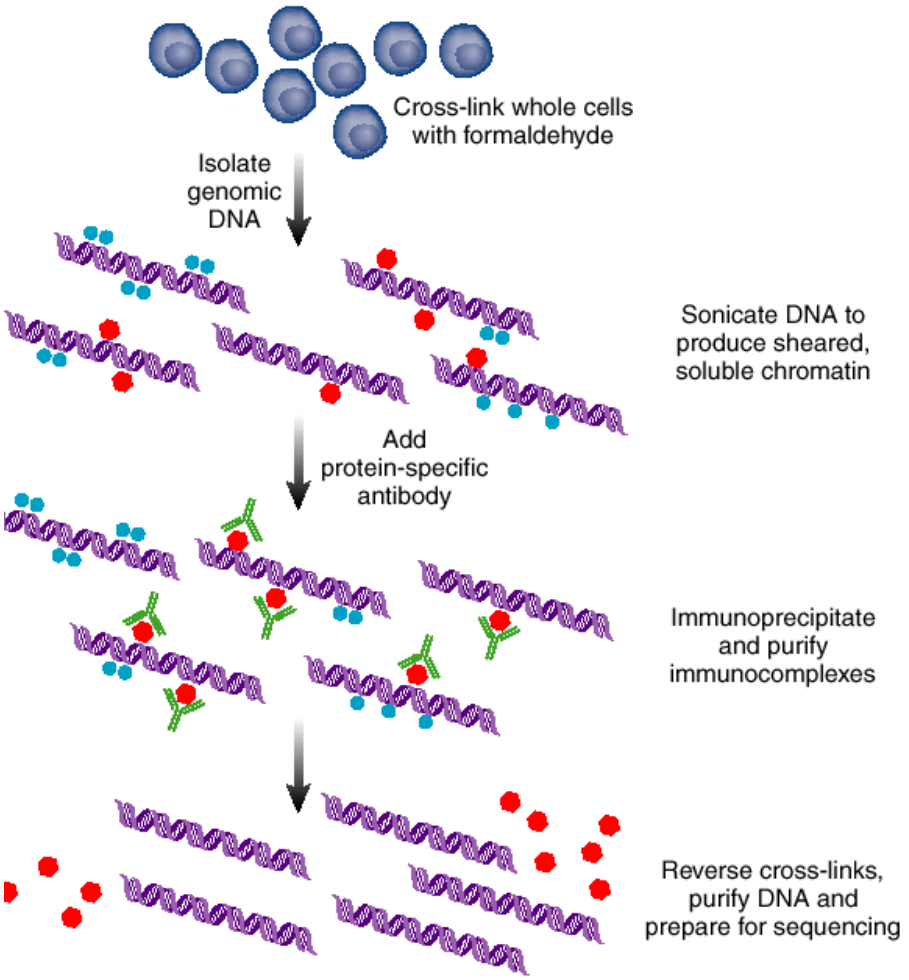
Methods – Objective 3

Biological and functional validation:

- ChIP-Seq for altered histone markers
- Matrigel Invasion Assay for *in vitro* effects on metastatic potential in breast cancer cell lines
- Proliferation assay/apoptotic assay/FACS
  - BrDU incorporation into DNA of cells in S phase
  - Activated caspase 3 as marker for apoptosis
  - FACS analysis for detailed cell cycle analysis
- *In vivo* evaluation of candidates on invasion of breast carcinoma cells

ChIP-Seq Validation of lincRNA Targets

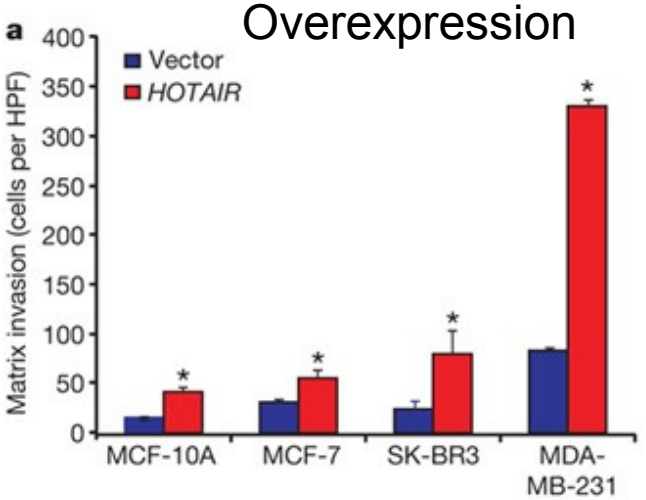
- Compare effects of knockdown or overexpression of lincRNA on chromatin occupancy
- Use antibodies specific to lincRNA-binding HMCs of interest
- Sequence DNA to find genes being targeted



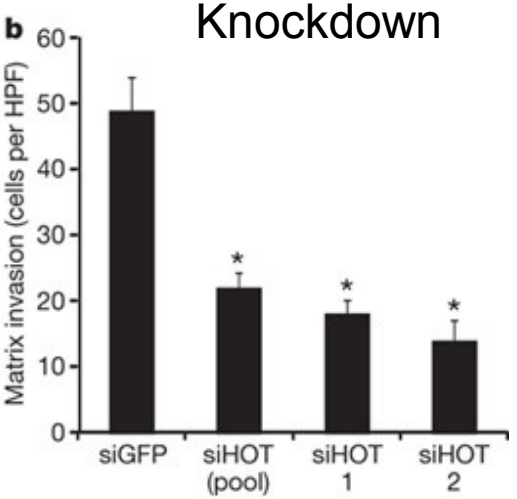
Elaine R Mardis. “ChIP-seq: welcome to the new frontier.” *Nature Methods* - **4**, 613-614 (2007).

In vitro Biological/Functional Validation

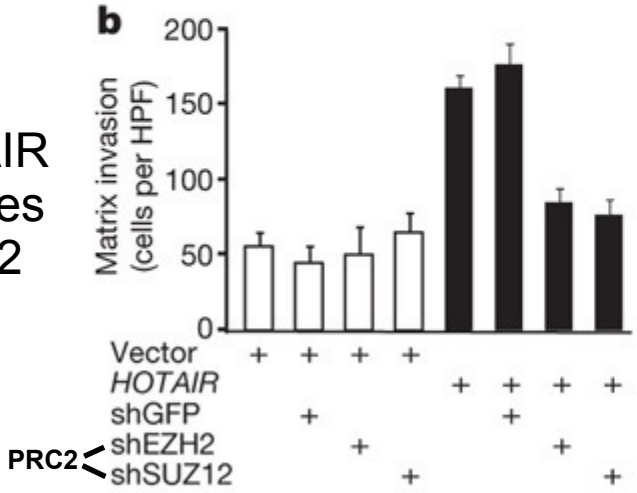
Overexpression

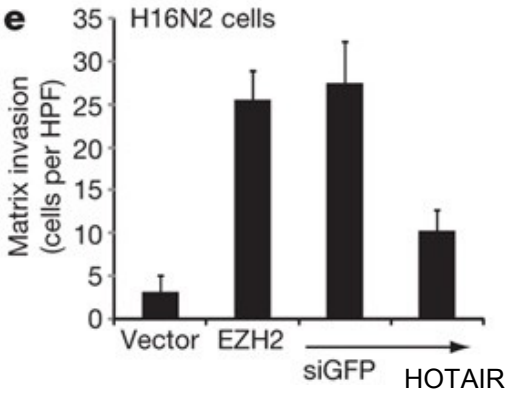


Knockdown



HOTAIR requires PRC2





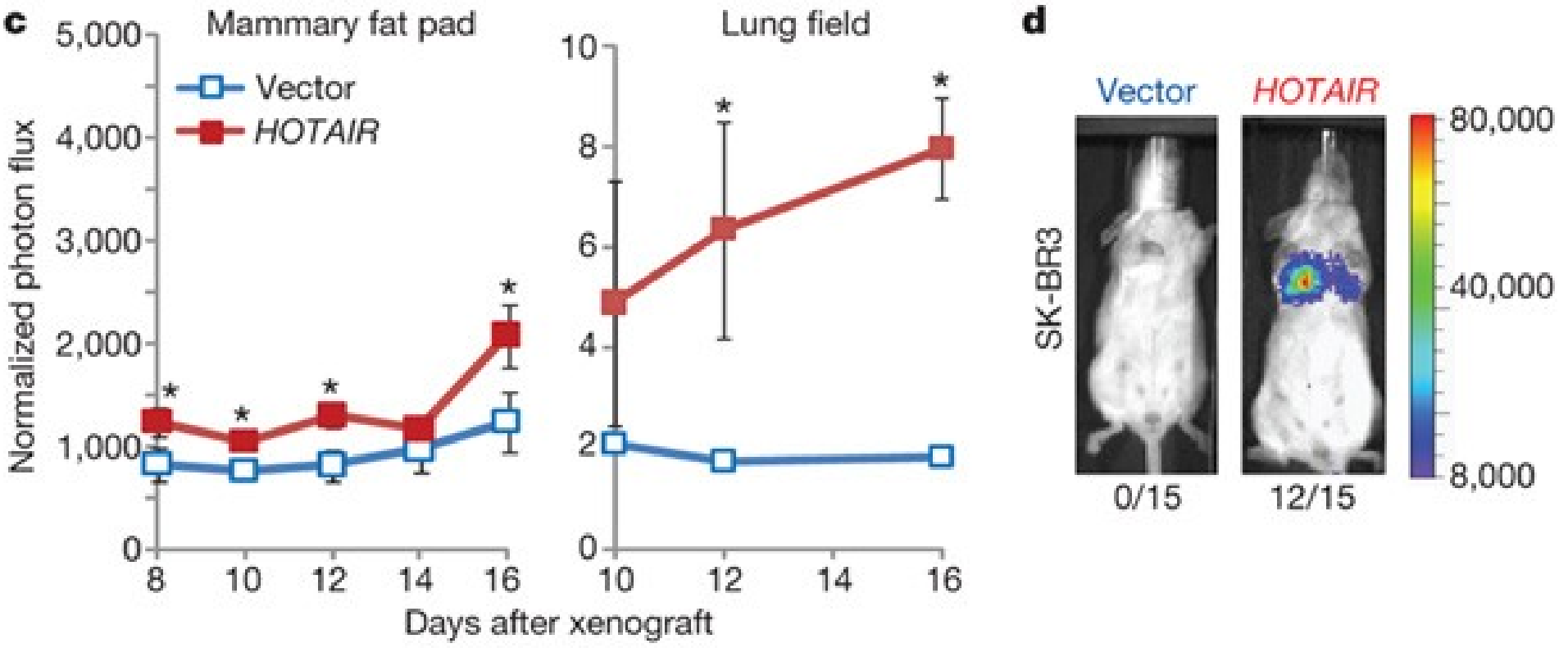
PRC2 requires HOTAIR

Rajnish A. Gupta, Nilay Shah, Kevin C. Wang, Jeewon Kim, Hugo M. Horlings, David J. Wong, Miao-Chih Tsai, Tiffany Hung, Pedram Argani, John L. Rinn, Yulei Wang, Pius Brzoska, Benjamin Kong, Rui Li, Robert B. West, Marc J. van de Vijver, Saraswati Sukumar & Howard Y. Chang
Nature 464, 1071-1076(15 April 2010)

In vivo Validation

Example: *HOTAIR* expression increases orthotopic growth in mammary fat pads and metastasis to lung

Example: *HOTAIR* promotes transient lung colonization



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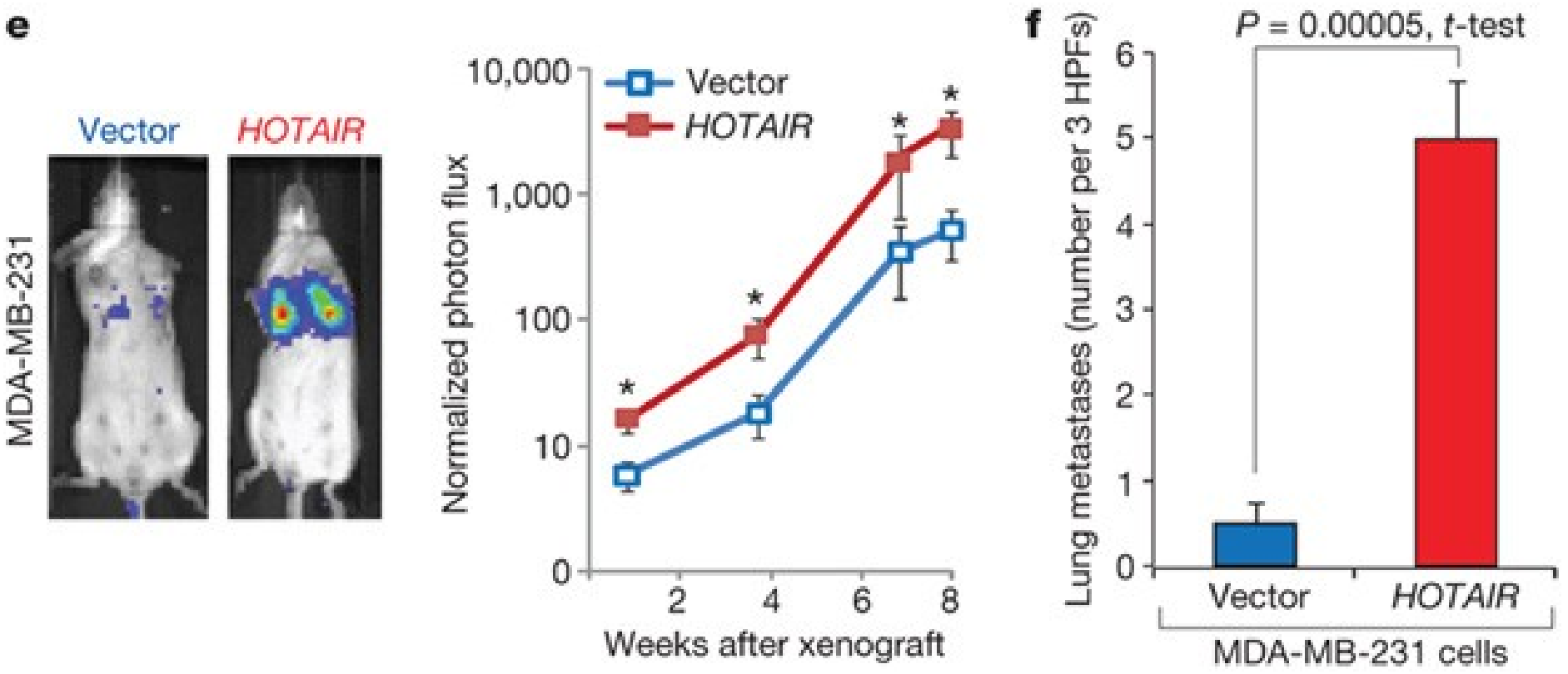
In vivo Validation

Example: *HOTAIR* causes lung colonization of MDA-MB-231

Example: *HOTAIR* leads to increased number of lung metastases by histological analysis.



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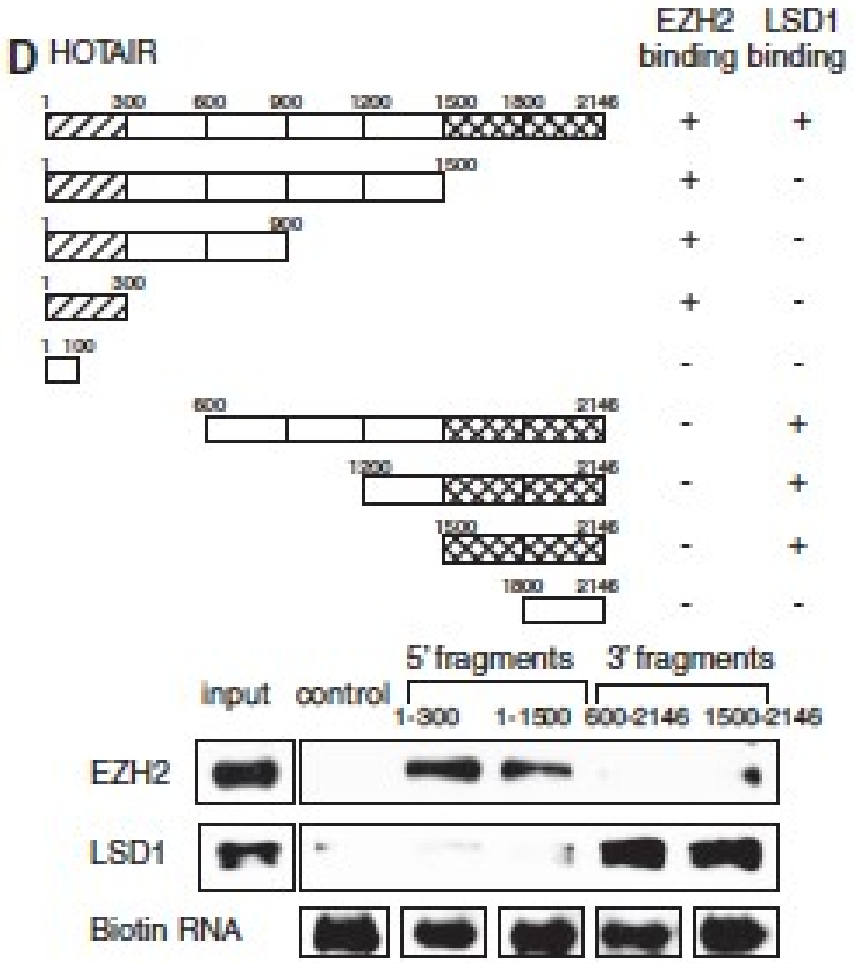
RNA-Footprinting to Discover lincRNA Binding Sites



- Divide RNA into fragments and evalute binding of chromatin modifying enzymes (e.g. HOTAIR shown left)
- Determine the region of lincRNA which binds to protein
- Binding region of RNA could be a therapeutic target

Tsai et al. “Long Noncoding RNA as Molecular Scaffold of Histone Modification Complexes.” *Science* **329**, 689 (2010) 689-694.

Significance

- Approach is designed to identify a set of novel lincRNA-protein interactions that affect breast cancer progression or metastasis
- Will allow design of targeted therapies aimed at correcting epigenetic abnormalities due to specific aberrantly expressed lincRNAs
- Methods can be adapted to similar studies in other cancer types

Thank you!