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Overview of New and Developing Omic Technologies

Assessing Molecular Toxicity and Disease Susceptibility

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US Environmental Protection Agency

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Risk assessment challenges that 'omic technologies may help address

1. Relevance to human condition and disease etiology 
2. Susceptibility to disease 
3. Defining early key events and biomarkers of MOA 
4. Adverse vs. adaptive responses 

Genomics/genetics, epigenetics, proteomics, metabolomics

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Overview of genomic technologies

- Advances in sequencing and transcriptomics
  - –2nd generation sequencing

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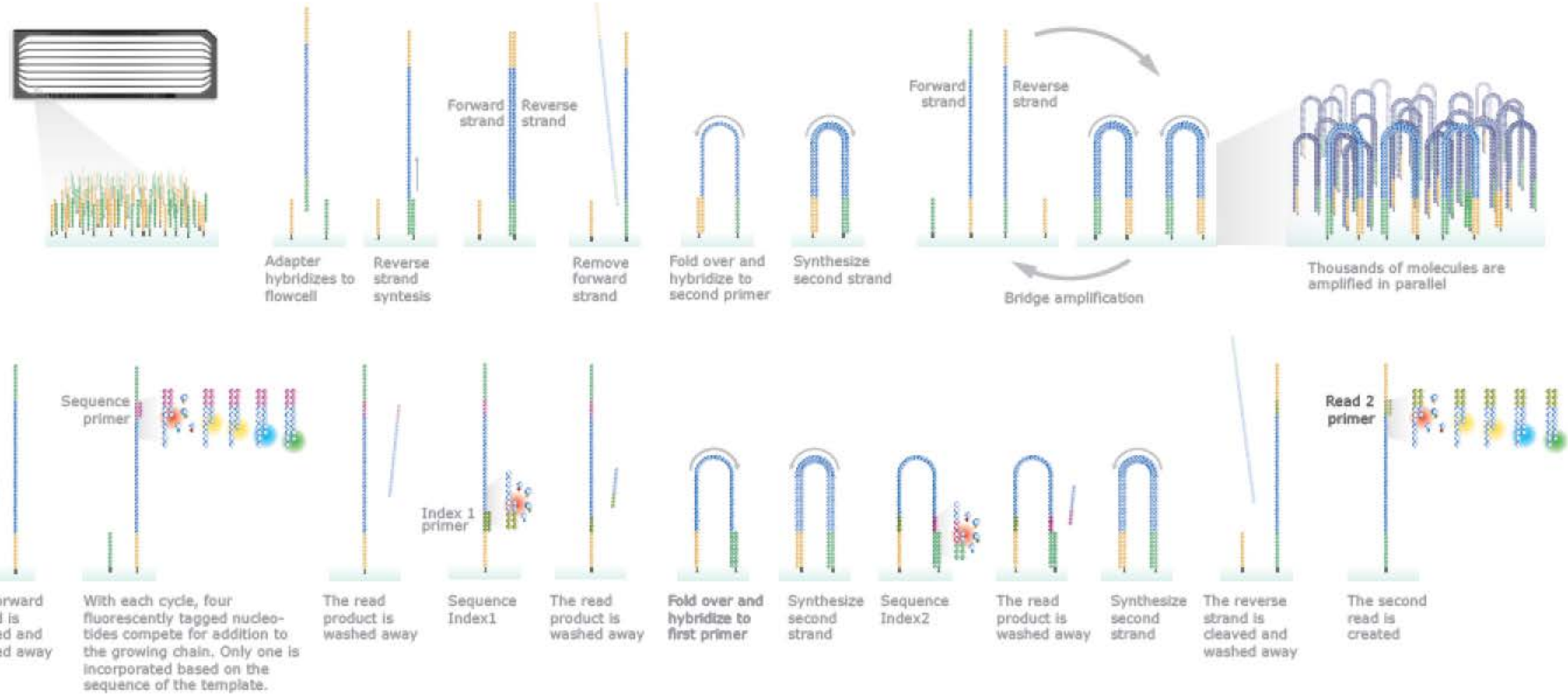
2nd generation high-throughput sequencing



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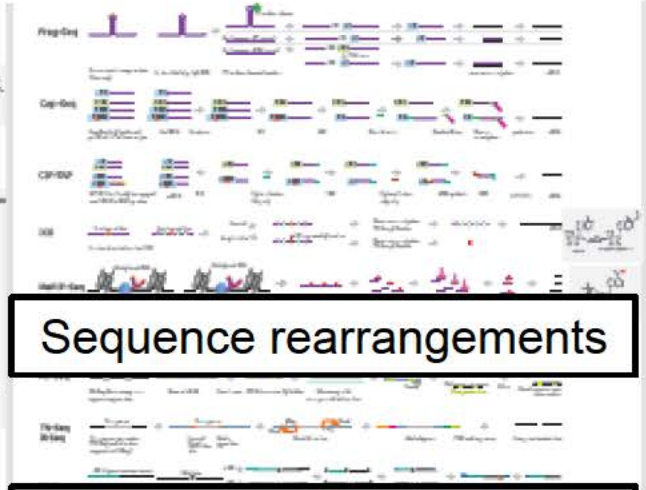
2nd generation sequencing: renaissance in genome-wide measurements

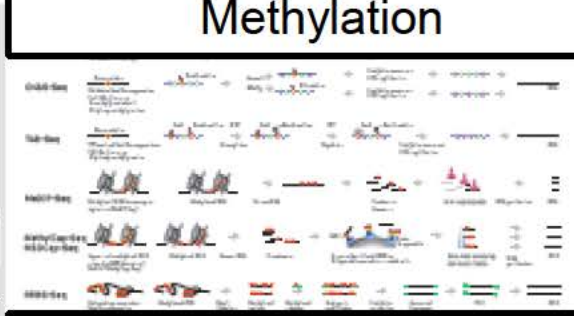
RNA transcription

Sequence rearrangements

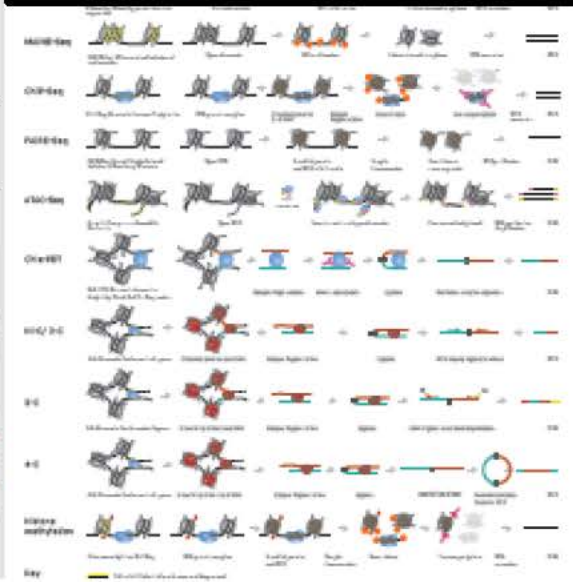


Low level detection

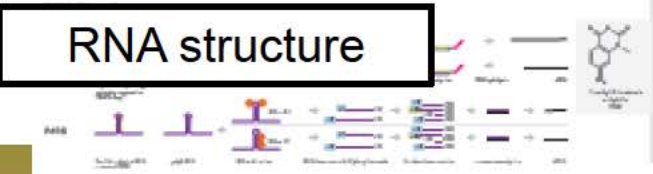
Methylation



DNA-protein interaction



RNA structure



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2nd generation sequencing: RNA-seq advantages over array

- Assess entire transcriptome (alternative splicing, allele-specific expression, rare, novel and non-coding RNA)
- High-throughput and lowering cost
- Increased detection levels compared to microarray
- Single cell threshold (Breakthrough of Year 2013 [Nature], Fluidigm)

Microarray vs. RNA-seq of Dose-Response Experiments

- Recent study: 5 doses of bromobenzene in F344/DuCrl rats
- RNA-seq and microarray liver gene expression; different normalization techniques applied
  - Ranked order of expressed genes were comparable between technologies
  - When fold-change (CTL vs. treatment) cut-off used, RNA-seq produced more hits
  - When p-values, used microarray produced more hits at lower doses; same at higher doses
  - Both fold change and p-value, the opposite was observed
  - Overlap of differentially expressed genes was 27.4%

Black et al., 2013 ToxSci

Microarray vs. RNA-seq of Dose-Response Experiments

- qRT-PCR validation using statistically significant genes was similar for both technologies
- 5 million read depth or higher for RNA-seq to achieve sensitivity to microarray.
- New gene discovery diminished above 10 million reads
- Gene- and pathway-based BMD showed moderate correlation between two technologies and was dependent on normalization method used (best was 0.406)
- **Differences in two technologies may be due to dynamic range and normalization correction of genes at high and low ends of expression**

Black et al., 2013 ToxSci

Single molecule sequencing: 3rd generation

- Increase throughput and lower costs; longer reads
- Focus on the use of DNA polymerase (for example, SMRT sequencing by PacBio)
- Can detect DNA modifications in real-time and unbiased
- Methods in development include nanopore DNA sequencing, microscopy-based techniques, and mass spec

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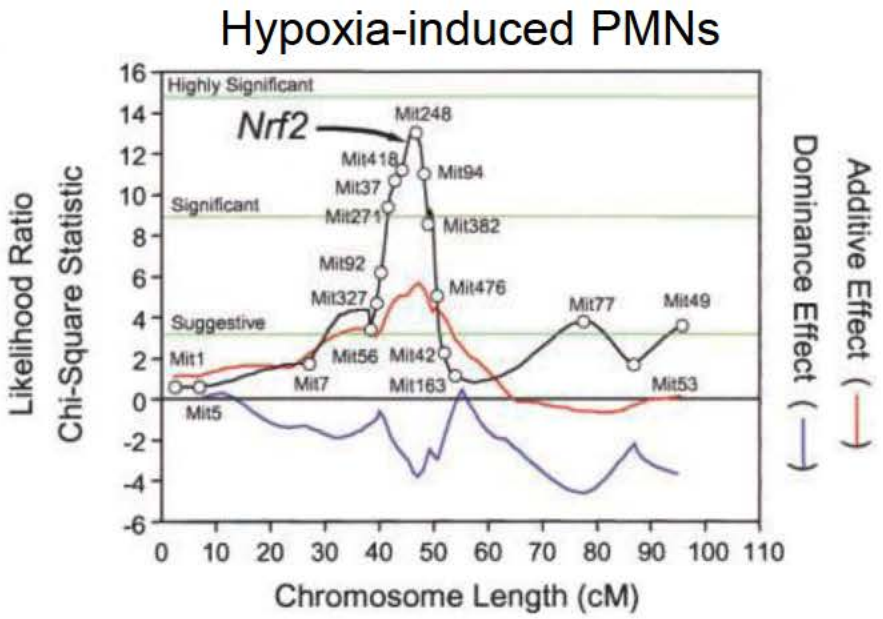
Genetic screens: delineating susceptibility

- Advances in inbred mouse screens
  - Collaborative Cross
- Combining genomic data
  - Chromatin immunoprecipitation and functional SNPs

Traditional inbred mouse screens: Quantitative Trait Loci (QTL) screens

- Genetic screen performed using susceptible (B6) and resistant (C3) strains exposed to hyperoxia
- Inflammatory endpoints used as quantitative traits
- Genetic linkage performed in $B6C3F_2$ mice
- Suggestive QTLs were identified on chr. 2 and 3
- Nrf2 identified as a susceptibility gene candidate



Cho et al. AJRCMB 2002

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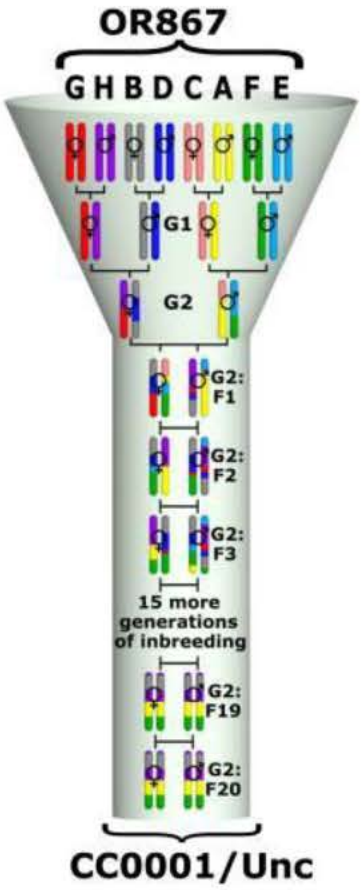
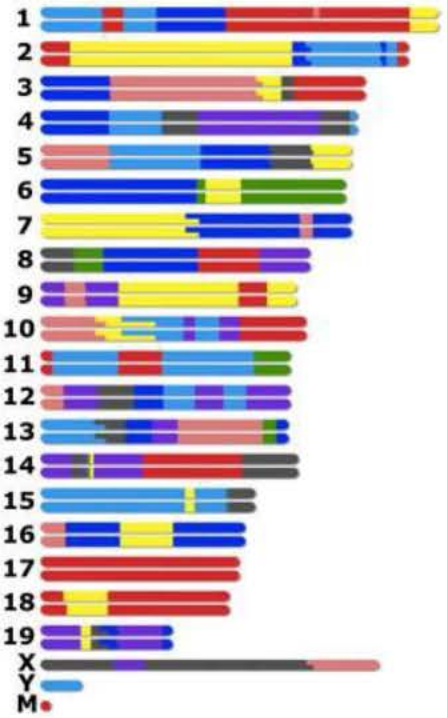
'Next generation' mouse genetic screens: Collaborative Cross

- Recombinant inbred mouse panel, 8 founders represent ~90% of variation present in all laboratory mice

- Advantages will be high mapping resolution (single gene), absence of gametic disequilibrium, whole genome sequence imputed from founder lines, representative of human variation





Threadgill and Churchill, G3 2012: PMID 22384393

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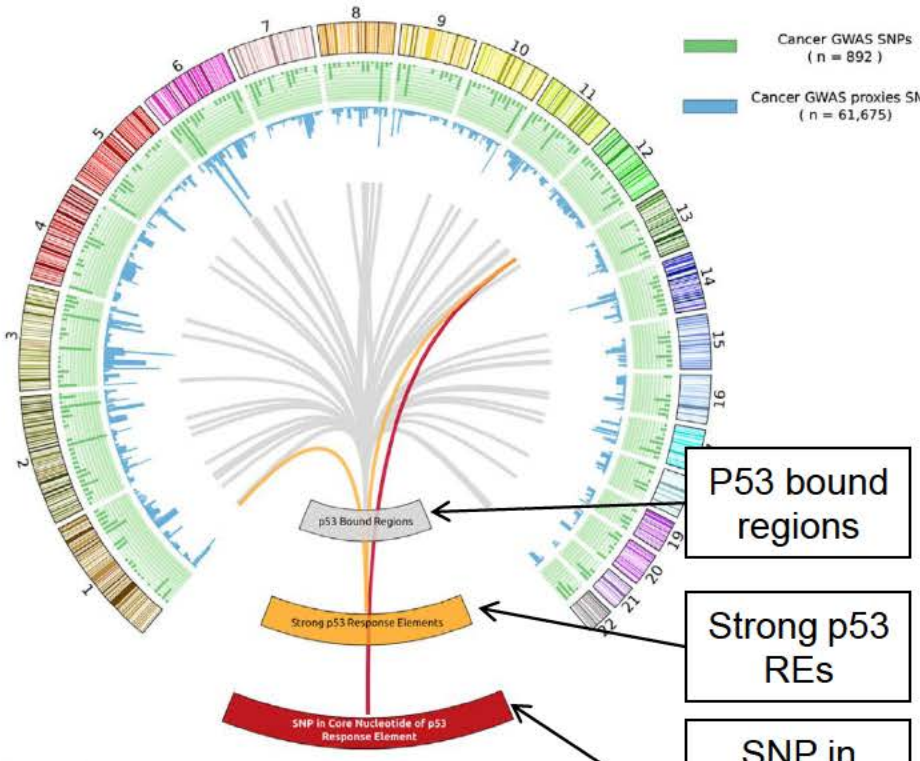
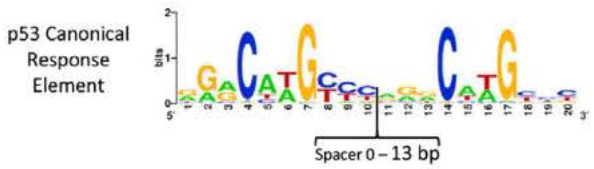
Causal SNP discovery with translational potential

- Combining genetic information with genomic discovery
- ~62K SNP in linkage with cancer associated SNPs; ~17K p53 binding regions identified by ChIP-seq
- 86 SNPs fall within p53 bound regions; 1 SNP is in key binding nucleotide for p53
- P53 RE regulates *KITLG* was associated with testicular cancer (but associates also with UV protection)





Zeron-Maedina, Wang et al. 2013 Cell

Modern definition of Epigenetics

"The structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states." Adrian Bird (2007)

- Not a genetic change
- Potential to be long-lasting
- Can be heritable
- Can be measured in accessible matrices

Epigenetics: key adverse and adaptive events; biomarkers of change

- New technologies to assess epigenetics alteration:
  - Chromatin changes and access
    - DNase-seq
  - DNA methylation
    - Arrays, sequencing
  - Non-coding RNA (miRNA, lncRNA, etc.)
    - Arrays, sequencing
    - Biomarkers, accessible matrices

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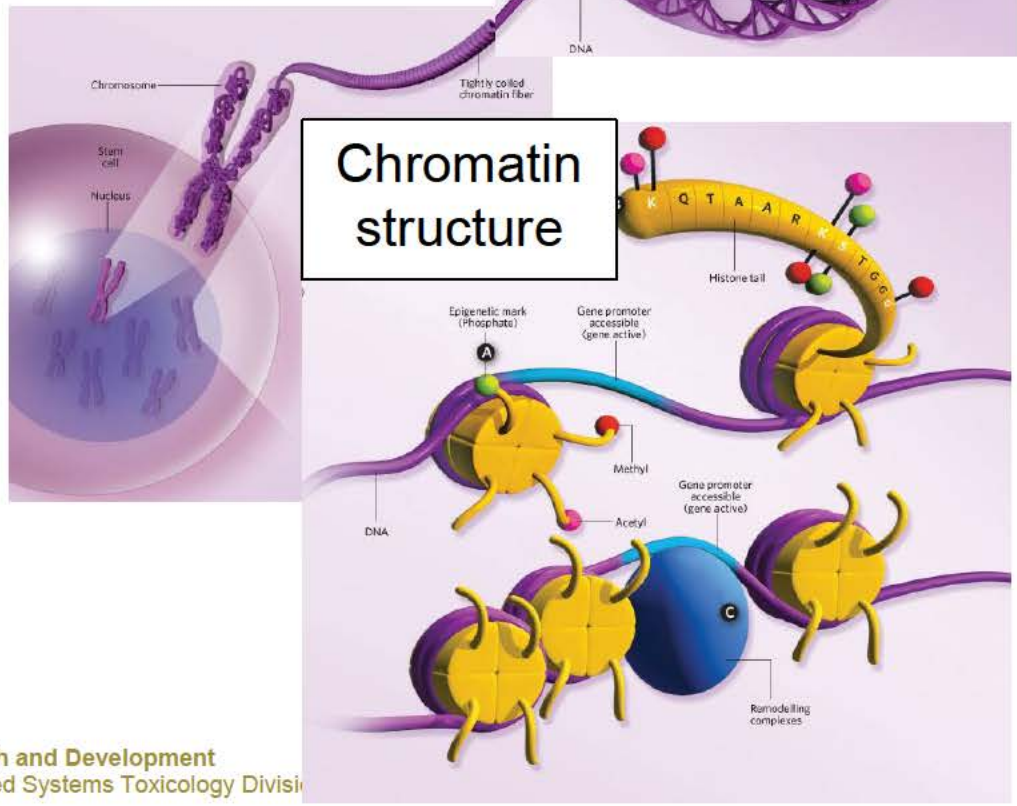
DNA methylation

Chromatin structure



Kubicek *The Scientist March 1,* 2011

DNA methylation and histone changes in cancer

- Hypermethylation of tumor suppressors
- Global hypomethylation and activation of oncogenes
- Loss of monoacetylation and trimethylation of histone H4

- Genomic assessment
  - Arrays
  - 2nd generation sequencing (bisulfite conversion, antibody capture, etc.)
  - ChIP-seq (histone modifications)

DNase-seq

- Digest
- Biotinylate and purify
- Ligate linkers
- PCR amp
- Sequence
- Map to genome

Song and Crawford, CSH Protocols 2010

A snapsot of accessible, open chromatin

When combined with gene expression, ChIP-seq data, motif analyses and other *in silico* profiling methods, we can determine:

- Binding of known and purported transcription factors, as well as other DNA binding proteins.
- Active and *poised* gene transcription
- Direction of transcription
- Use of alternative start sites
- Cell and exposure specific regions of the active chromatin
- Identification of specific transcription factors involved or activated after exposure
- Globally delineate cellular processes set into motion after exposure.

Crawford et al. *Genome Res* 2006,
Boyle et al. *Genome Res* 2011

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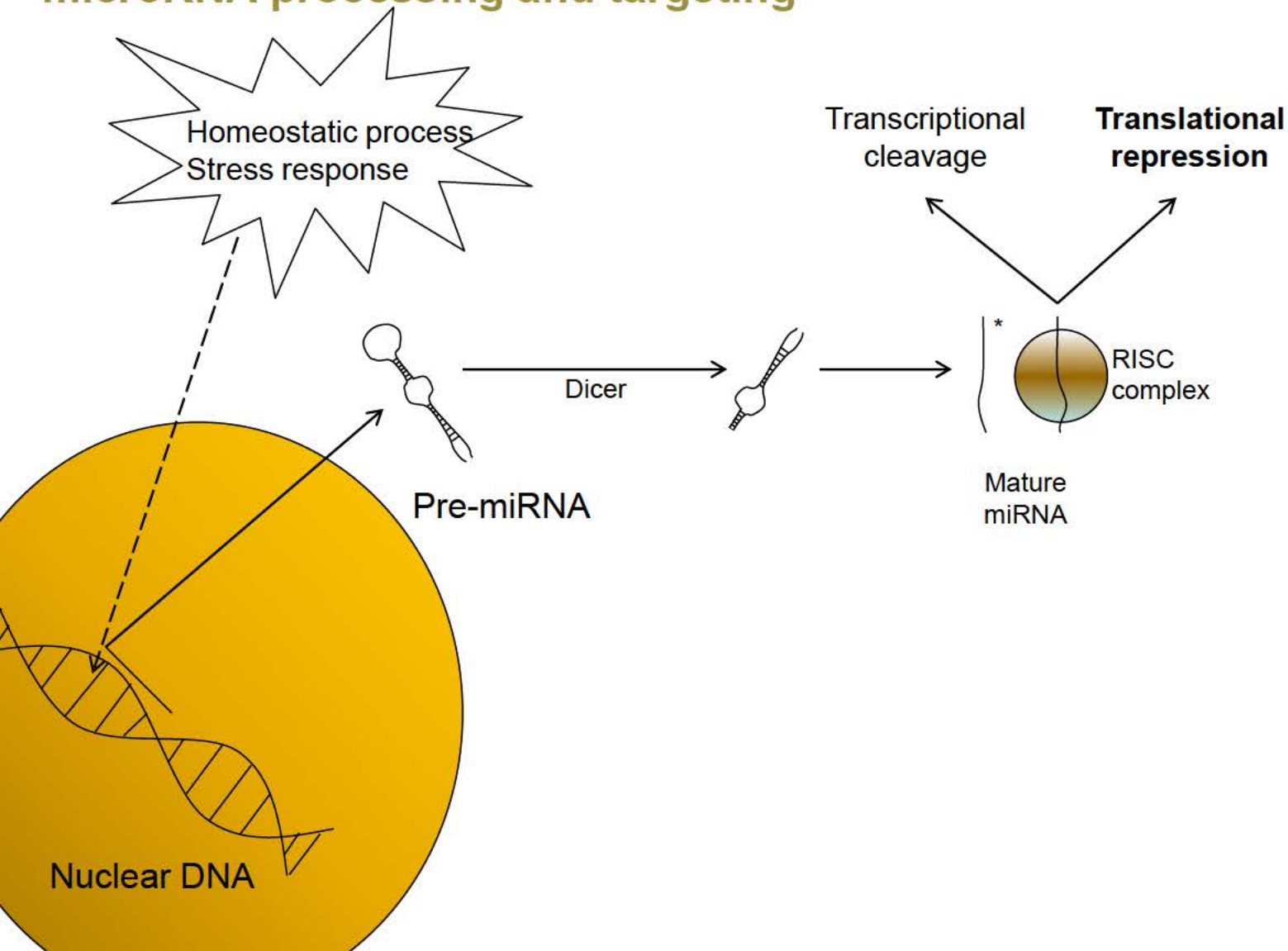
microRNA processing and targeting



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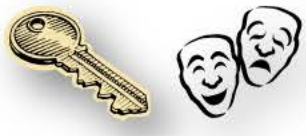
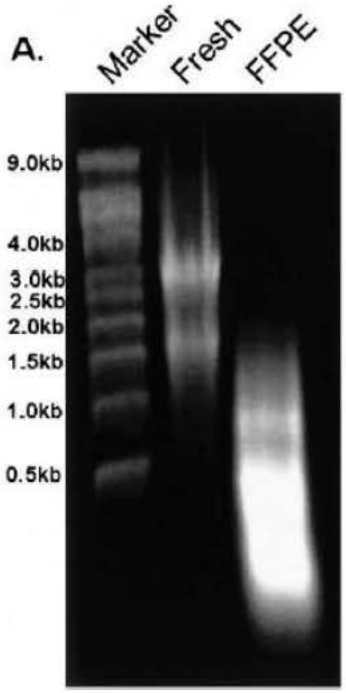
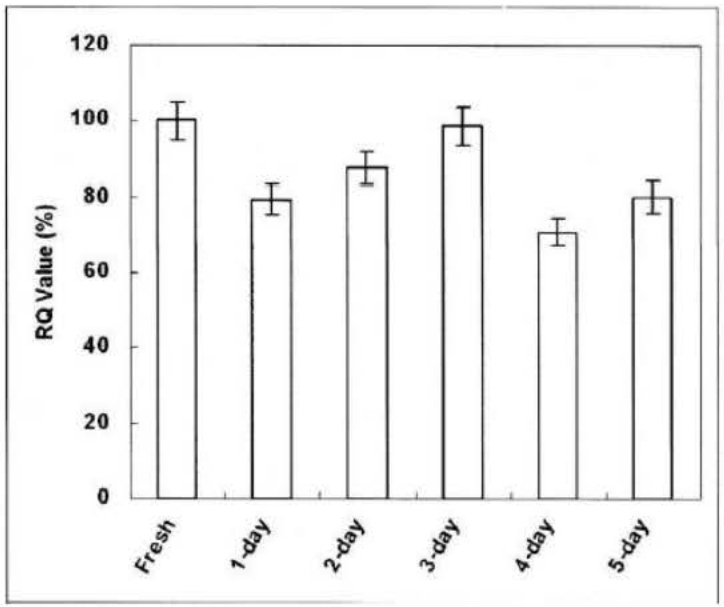
microRNA highly stable in archived and degraded samples

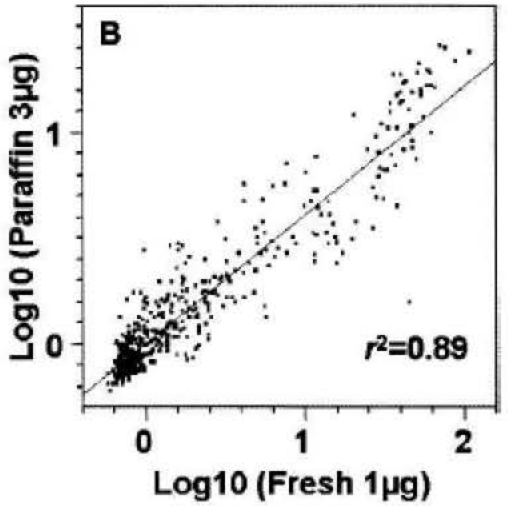


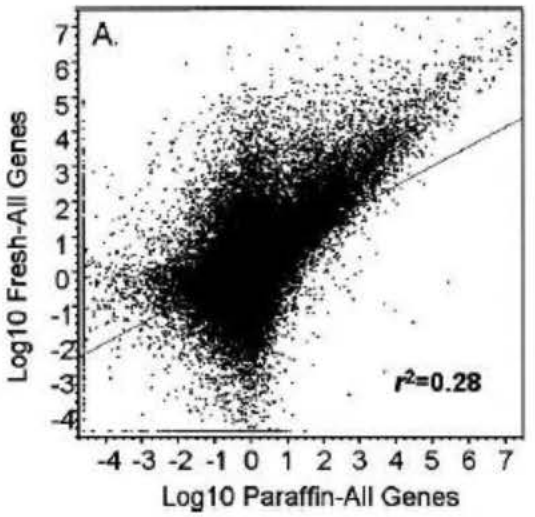


RNA on agarose gel



hsa-miR-16 expression in mouse liver





Xi Y, G Nokajima, et al, RNA 2007;13:1666-1674

Good microRNA correlations between fresh frozen (FF) and paraffin-embedded (FFPE) samples

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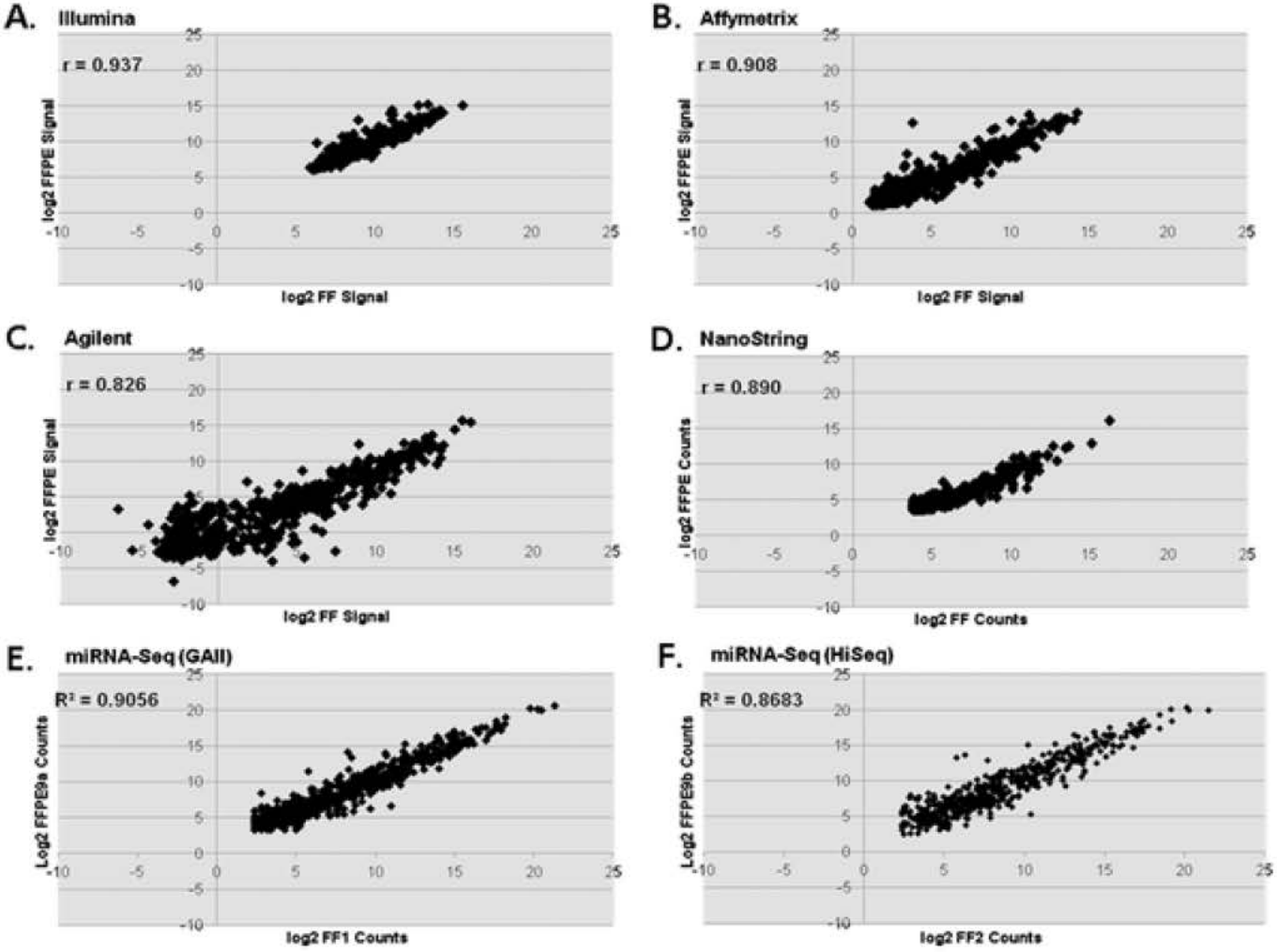

Kolbert CP, Feddersen RM, Rakhshan F, Grill DE, et al. (2013) Multi-Platform Analysis of MicroRNA Expression Measurements in RNA from Fresh Frozen and FFPE Tissues. PLoS ONE 8(1): e52517. doi:10.1371/journal.pone.0052517

Conservation patterns of known and predicted human miRNAs

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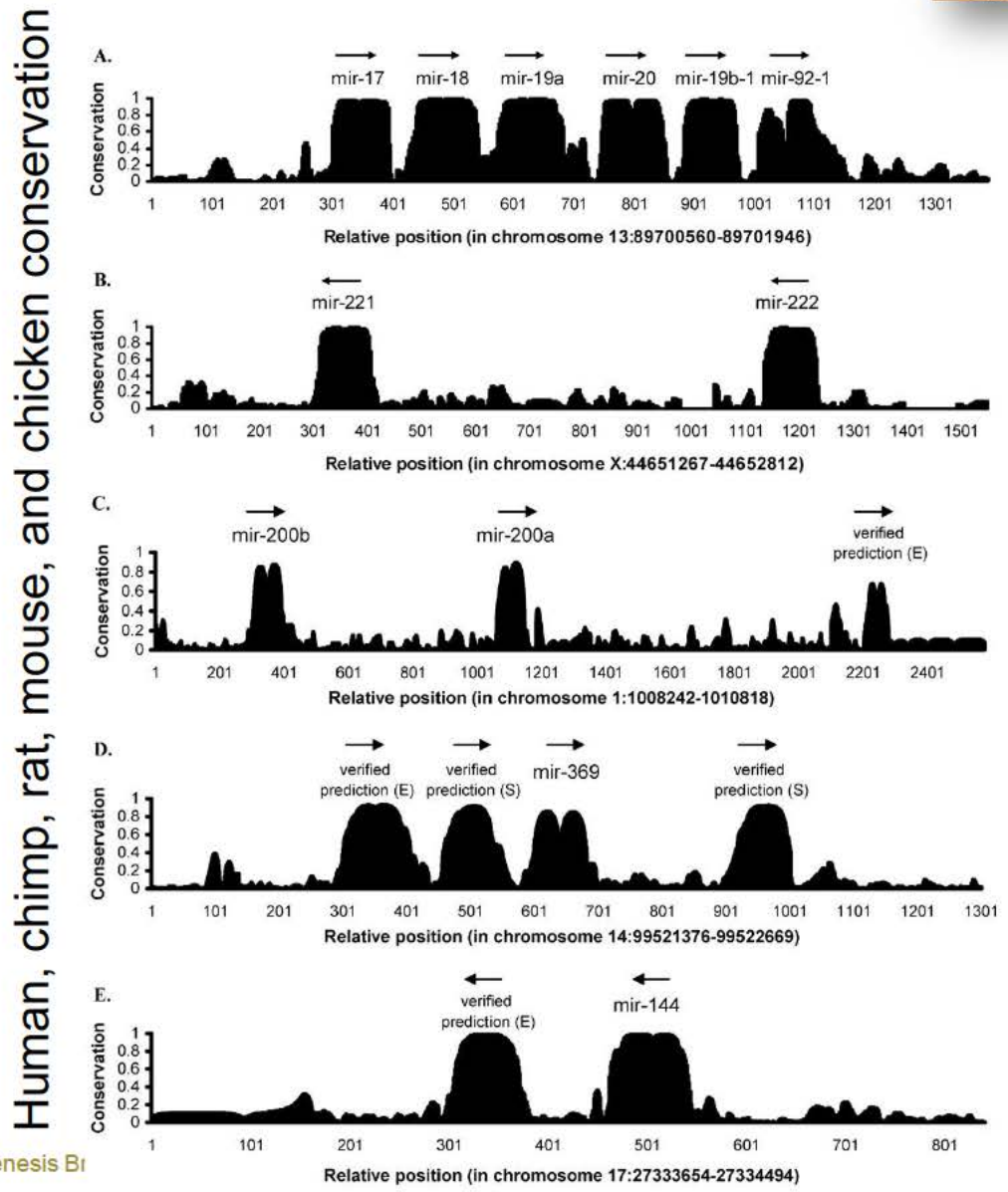
- ~42% of human microRNAs reside in genomic clusters (e.g., chr 14q32 region)
- These microRNA species exhibit conservation

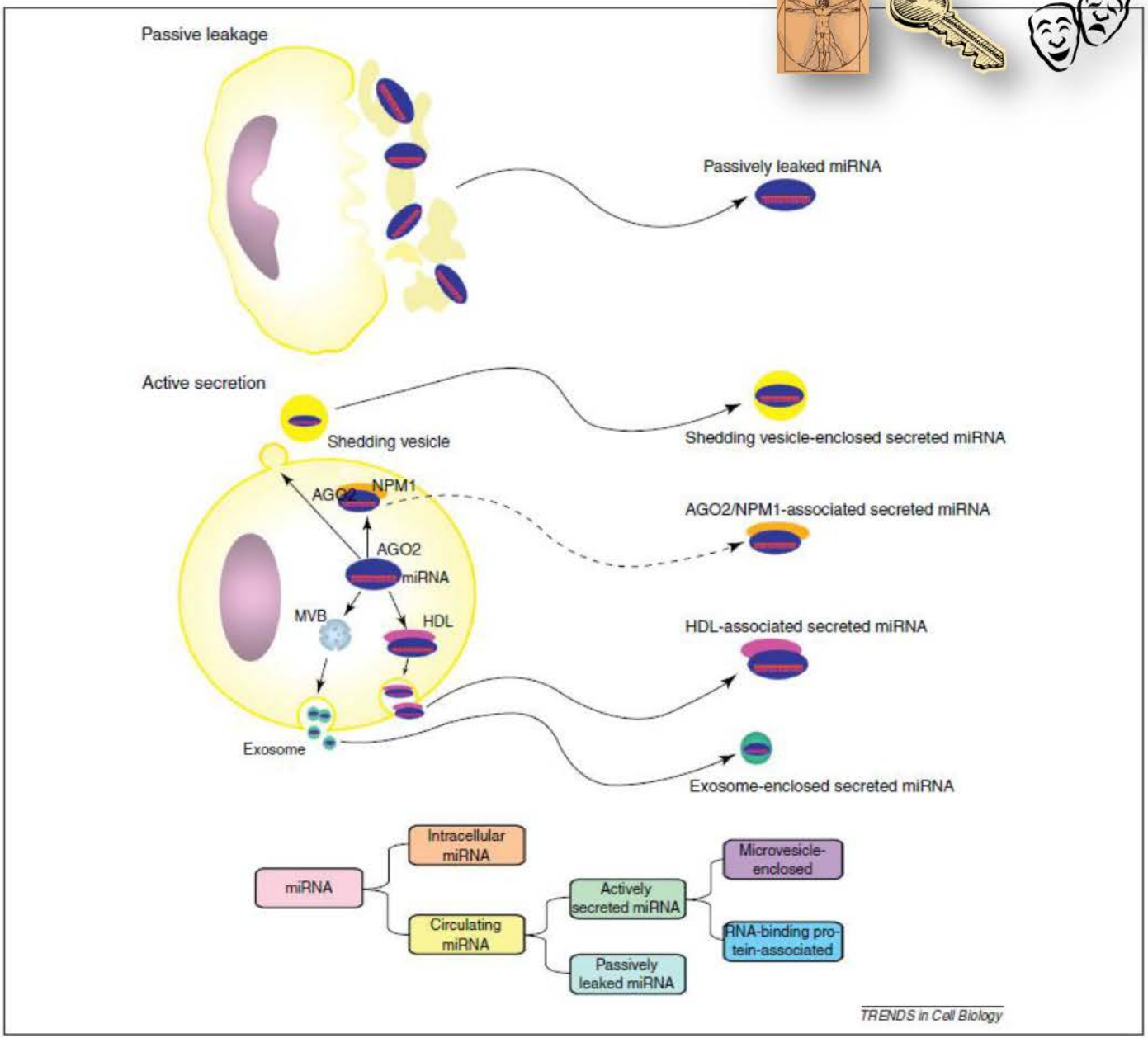


Altuvia Y et al. Nucl. Acids Res. 2005;33:2697-2706

miRNA alterations in lung cancer

- miRNA expression has been extensively studied in lung cancers
  - Especially in non-small-cell lung cancer
  - "OncomiRNAs"
    - miR-17-92 group, favor tumor development (Hayashita et al. Cancer Res. 2005)
    - miR-31; represses tumor suppressor genes (Liu et al. J Clin Invest. 2010)
    - miR-21; high in lung cancer regardless of smoking status (Seike et al. Nat Acad Sci USA 2009)
  - Diagnosis, stage of cancer, or response to therapy
  - Can be detected non-invasively

- Passive vs active release
- Methods of active release may be for communication (horizontal transfer)



Liang et al., *Trends in Cell Bio* 2012

2009 International Life Sciences Institute, Health and Environmental Sciences Institute (ILSI-HESI) Epigenetics Workshop

- Concluded that a gap in knowledge linking specific changes in epigenetic parameters to adverse public health outcomes exists
- Recommendations included
  - improved efforts to define appropriate public health concerns related to epigenetic effects
  - better defined epigenetic models and endpoints/targets
  - develop better tools for interpreting epigenetics data
  - improve expertise in applying epigenetics data within current risk assessment paradigms
  - better characterize the normal epigenome

Goodman et al. *Tox Sci* 2010

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Summary

- Current and developing technologies have made huge strides in ‘omic assessments
  - Genomics
  - Genetics
  - Epigenetics
- Real potential of utilizing information for chemical risk assessment
  - Current challenges may be met with ‘omic information
- Many obstacles until this can be done however…
  - Standardization
  - Reproducibility
  - Acceptance

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Thank you for your attention!