Lecture 17 Functional Genetics III

Functional genomics: Identify the function of each and every gene in the genome. Since the characterization of the function of a protein domain in one organism generally provides hint to its function in another organism, the first goal of functional genomics is to identify as many genes as possible in major model organisms

Basic Approaches

Gene expression profile (discussed already lectures 15-16)

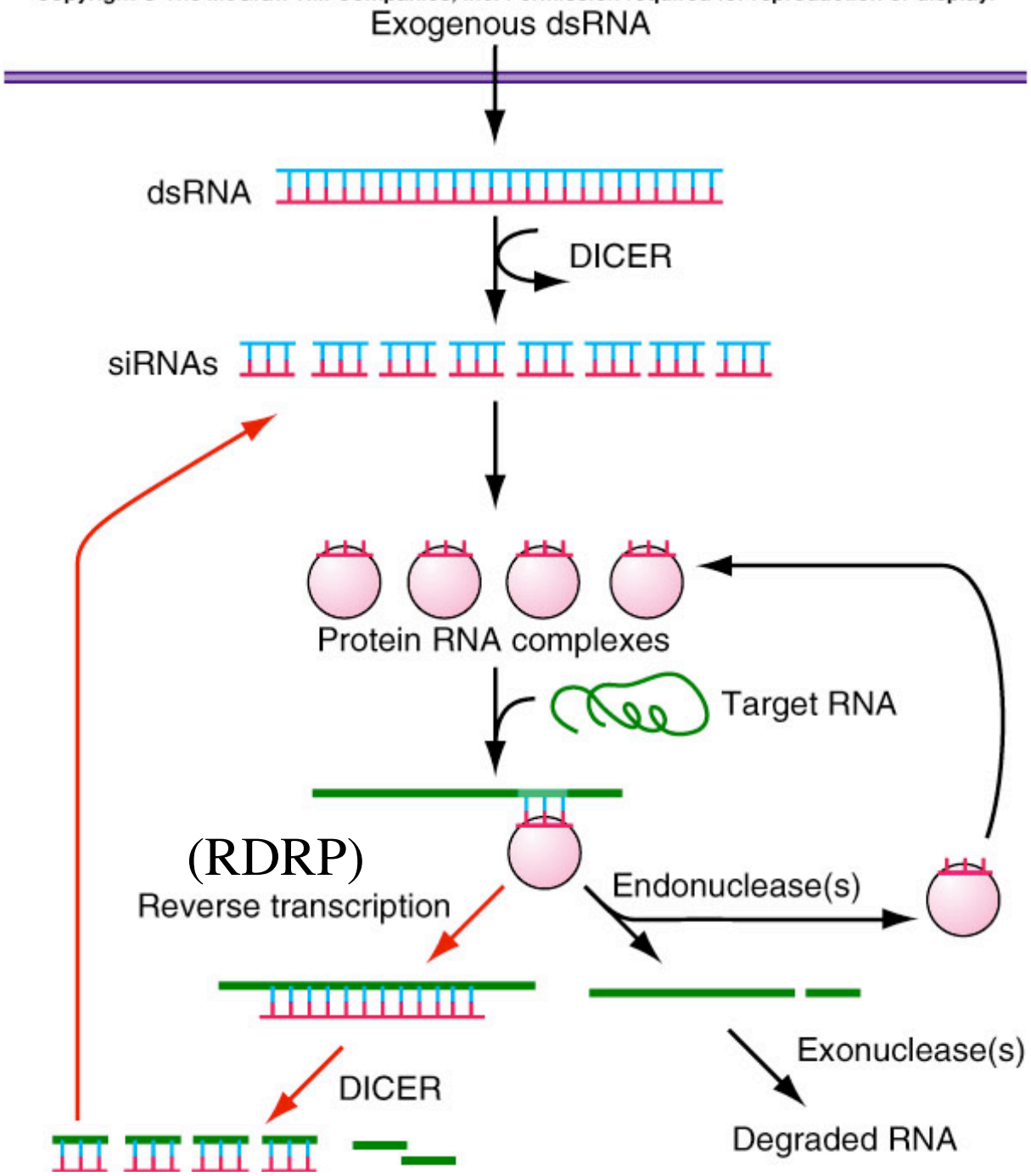
Protein-protein and protein-DNA interactions (lectures 15-16)

Reverse genetics: disrupt a particular gene or set of genes with known seq.

Reverse genetic methods

- RNA interference
- Delete genes by homologous recombination (yeast, moss, mouse and flies)
- Insertional or chemical mutagenesis
- Activation tagging and enhancer trap

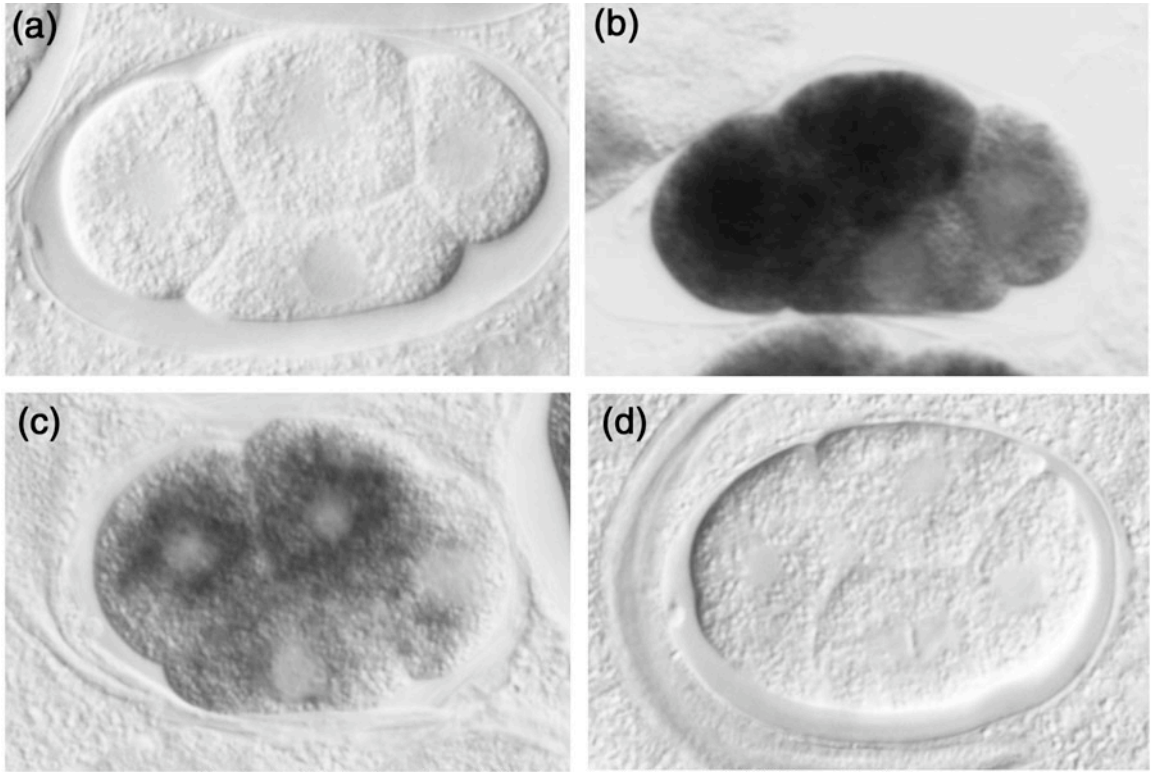
(most can be performed in high throughput manner)



Double-stranded RNA-induced RNA interference causes destruction of a specific mRNA in *C. elegans*

uninjected, no probe

uninjected, mex-3 probe



antisense mex-3 RNA, mex-3 probe

double-stranded mex-3 RNA injected, mex-3 probe

Guo, S. and Kemphues, K. J. *Cell* 81, 611-620 (1995)
Fire, A. et al. *Nature* 391, 809 (1998)

RNA interference

RNAi movie www.nature.com/focus/rnai/animations/index.html

- **Initially characterized in:**
  - *C. elegans*
    - Double-stranded RNA injection-named RNAi
  - **Plants**
    - Resistance to spread of virus
    - Suppression of transgene expression

- **Function of RNAi likely used to detect:**
  - genome-invading transposable genetic elements and double-stranded (ds) RNA viruses
  - Other abnormal gene expression

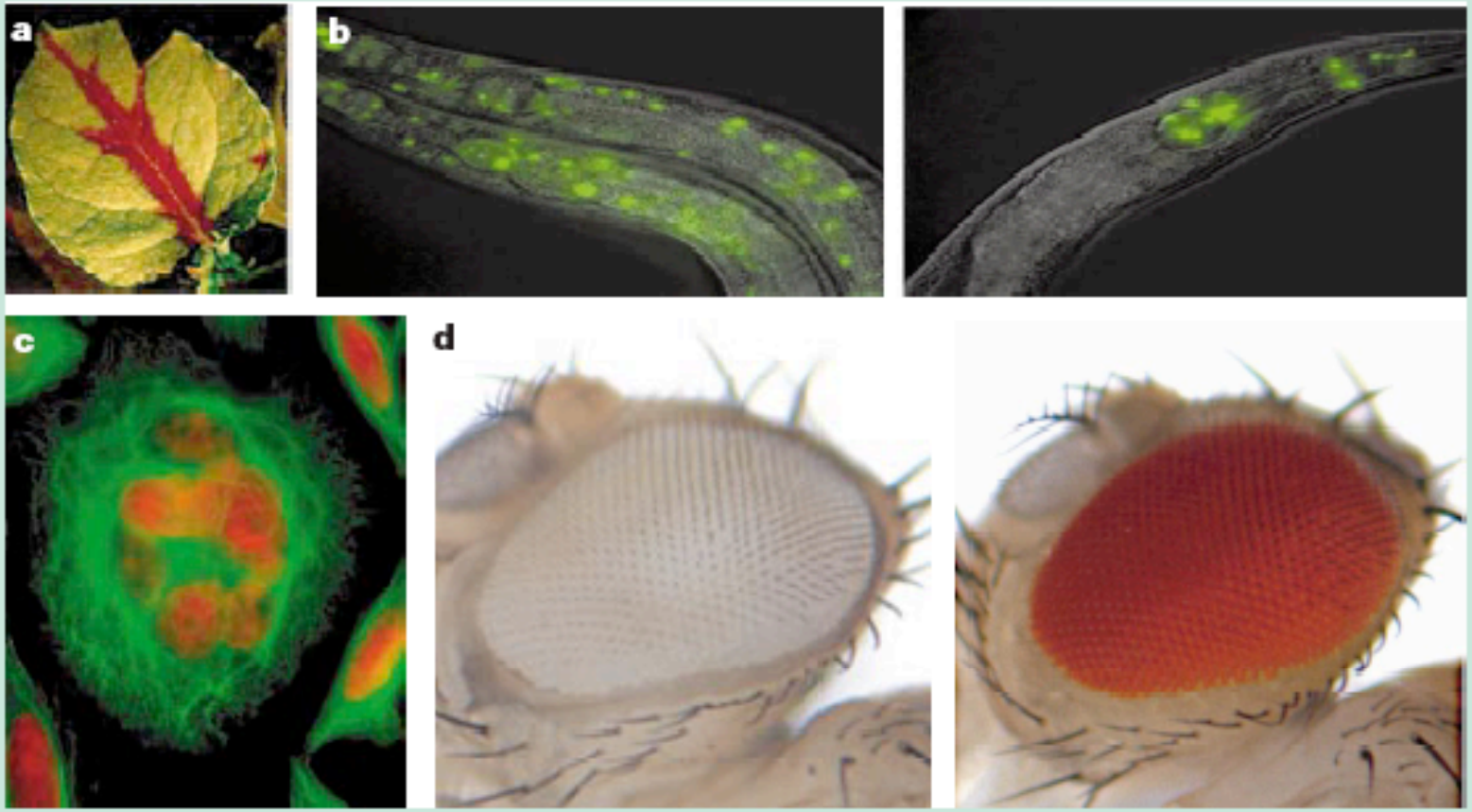
Diverse organisms display RNAi

- Model animals (*Drosophila*, *C. elegans*, mouse, etc.)
- Non-model animals (cnidaria, beetles, crickets, crustaceans)
- Protozoa (e.g. *Tetrahymena*)
- Dictyostelium
- Plants (e.g. *Arabidopsis*, maize)
- Fungi (e.g. *Neurospora*)

RNAi works in other organisms

silencing of GFP in leaf veins

silencing of GFP in *C. elegans* nuclei



depletion of ORC6 results in multinucleated HeLa cells

depletion of White results in unpigmented Drosophila eyes

Hannon, G. J. *Nature* 418, 244-251 (2002)

Potential Practical Applications of RNA Interference

- Control virus infection
- Analysis of cell biology by silencing specific gene
- Target validation for drug development
- Potentially new therapeutic approaches to treating diseases - a new approach to antisense and new possibilities for gene therapy

Systematic RNAi screens in *C. elegans* and mammalian cells

- **In the nematode, *C. elegans*, RNAi is easy to do**
  - Inject dsRNA
  - Feed bacteria expressing dsRNA
  - Or soak in solution of dsRNA
- **Makes systematic RNAi screens possible**

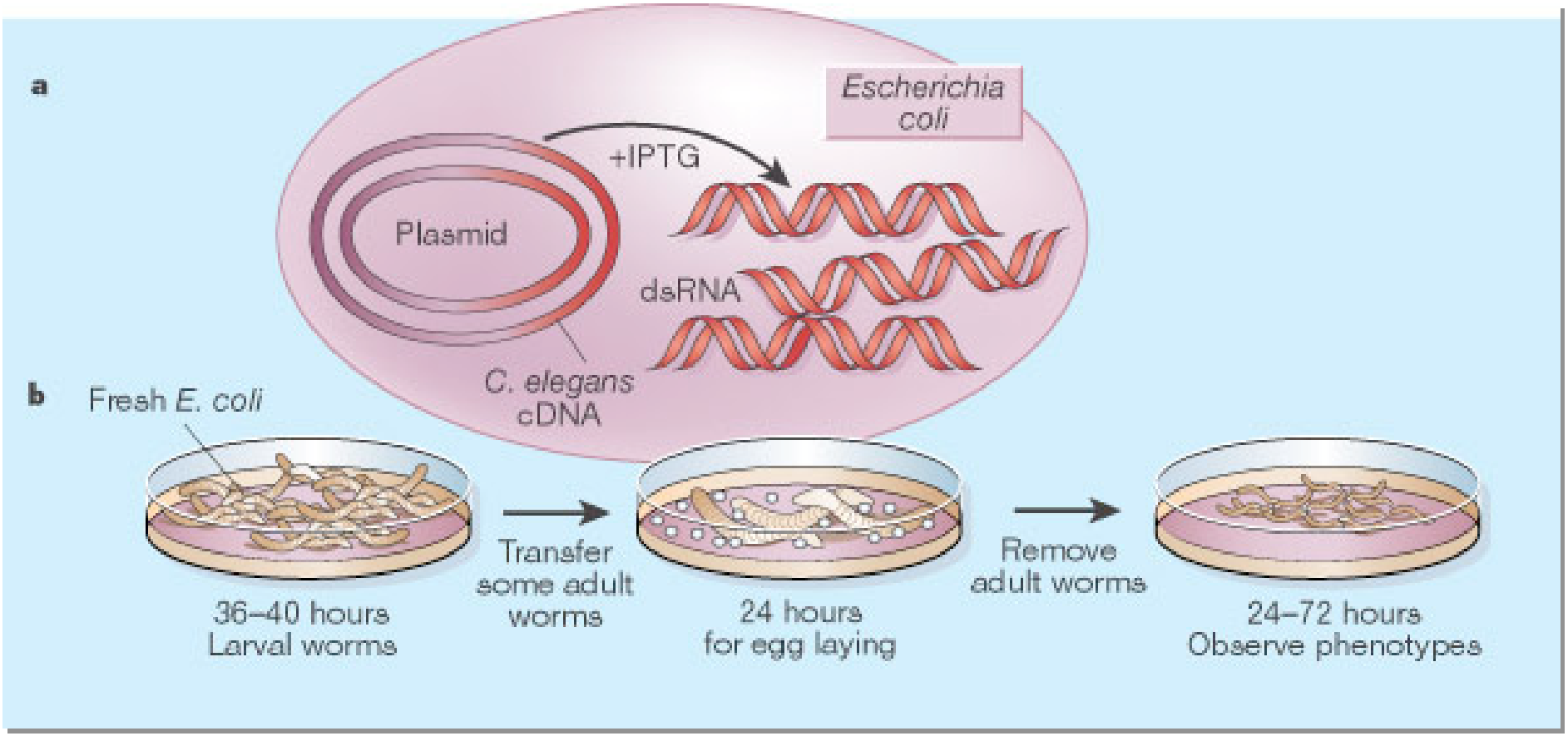
  -Fraser, 2000-Chromosome I-feeding

  -Gonczy, 2000-Chromsome III-injection

  -Kamath, 2003-Genome-wide feeding

  -Sonnichensen, 2005-Genome-wide injection

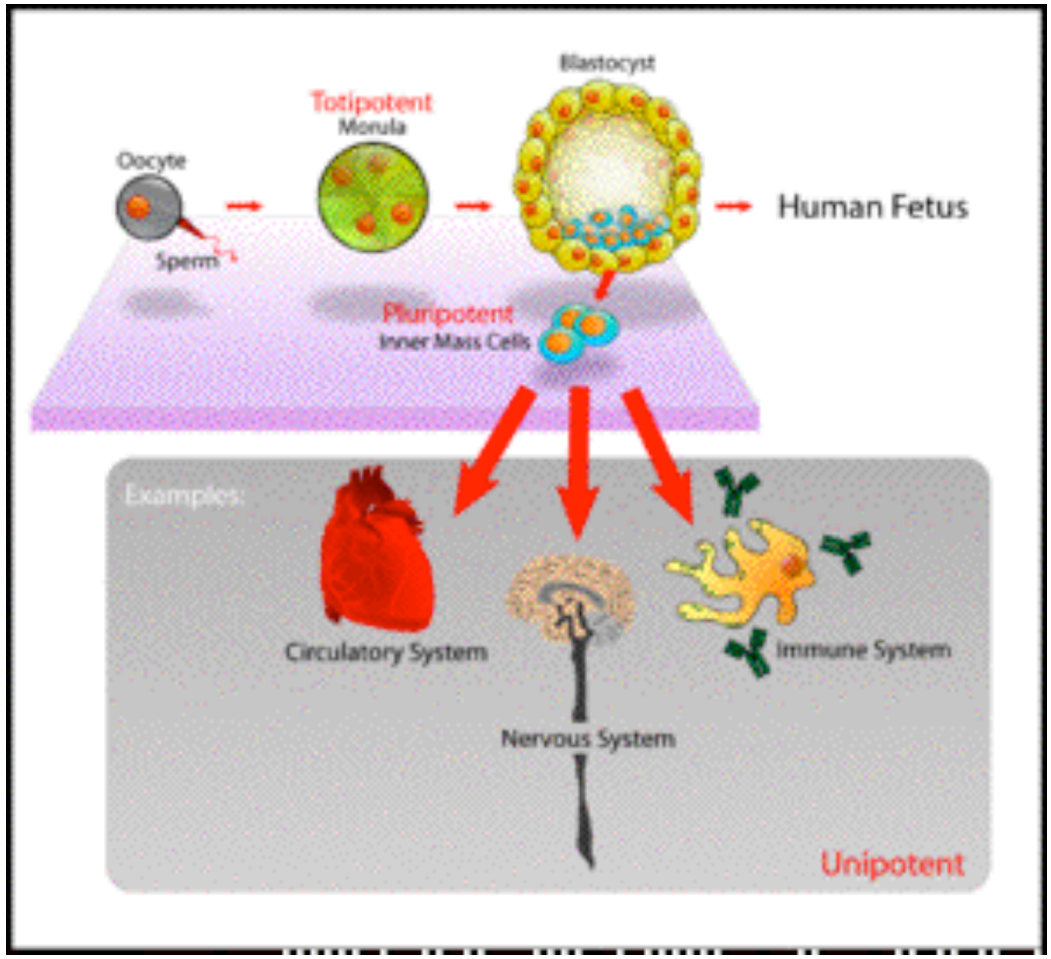
Genome screen by feeding worms with dsRNA expressing *E. coli*



Tuschl 2003 *Nature*

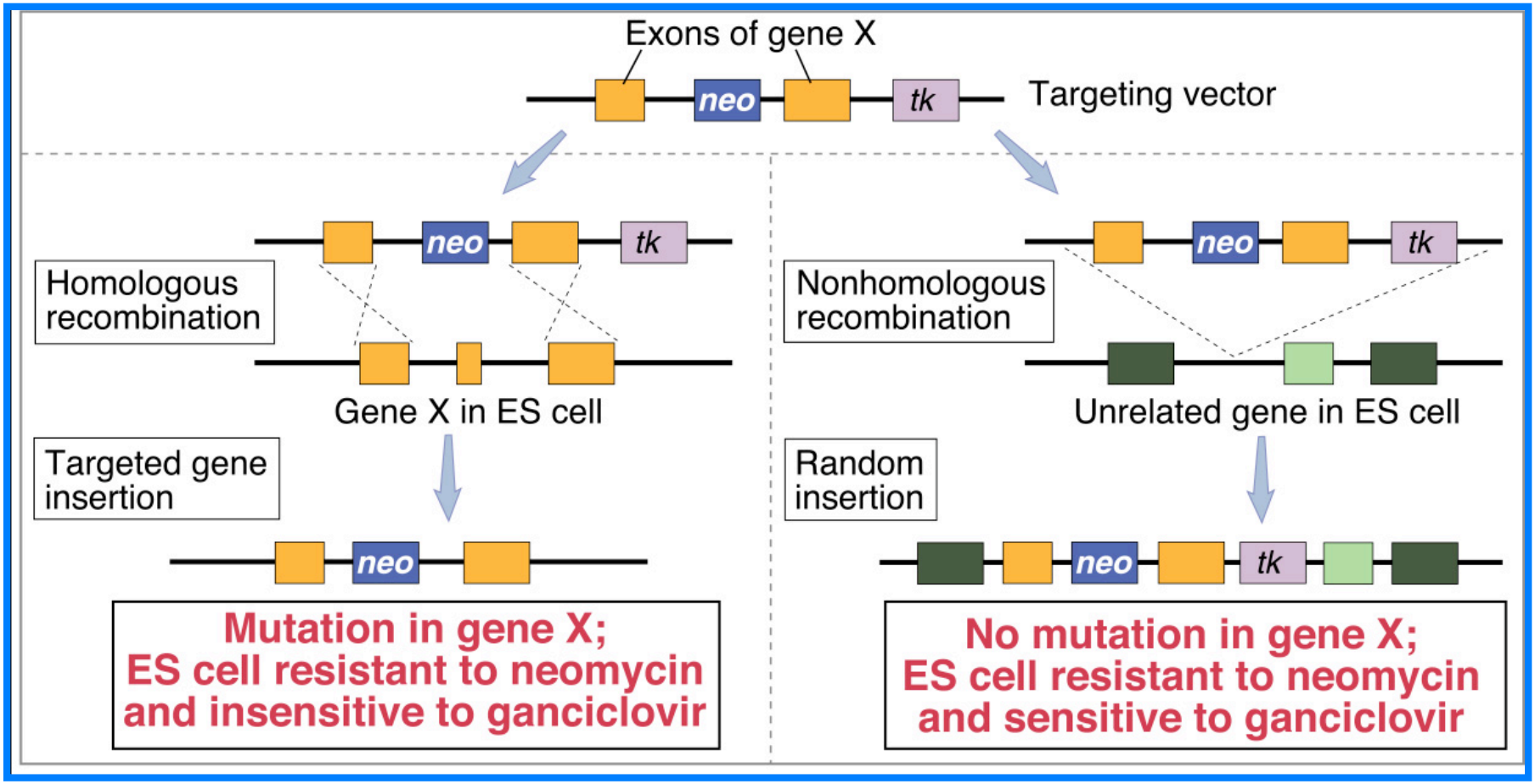
Homologous recombination and gene knock-out in yeast and mouse

Transgenic mouse depends on ES (embryonic stem) cells

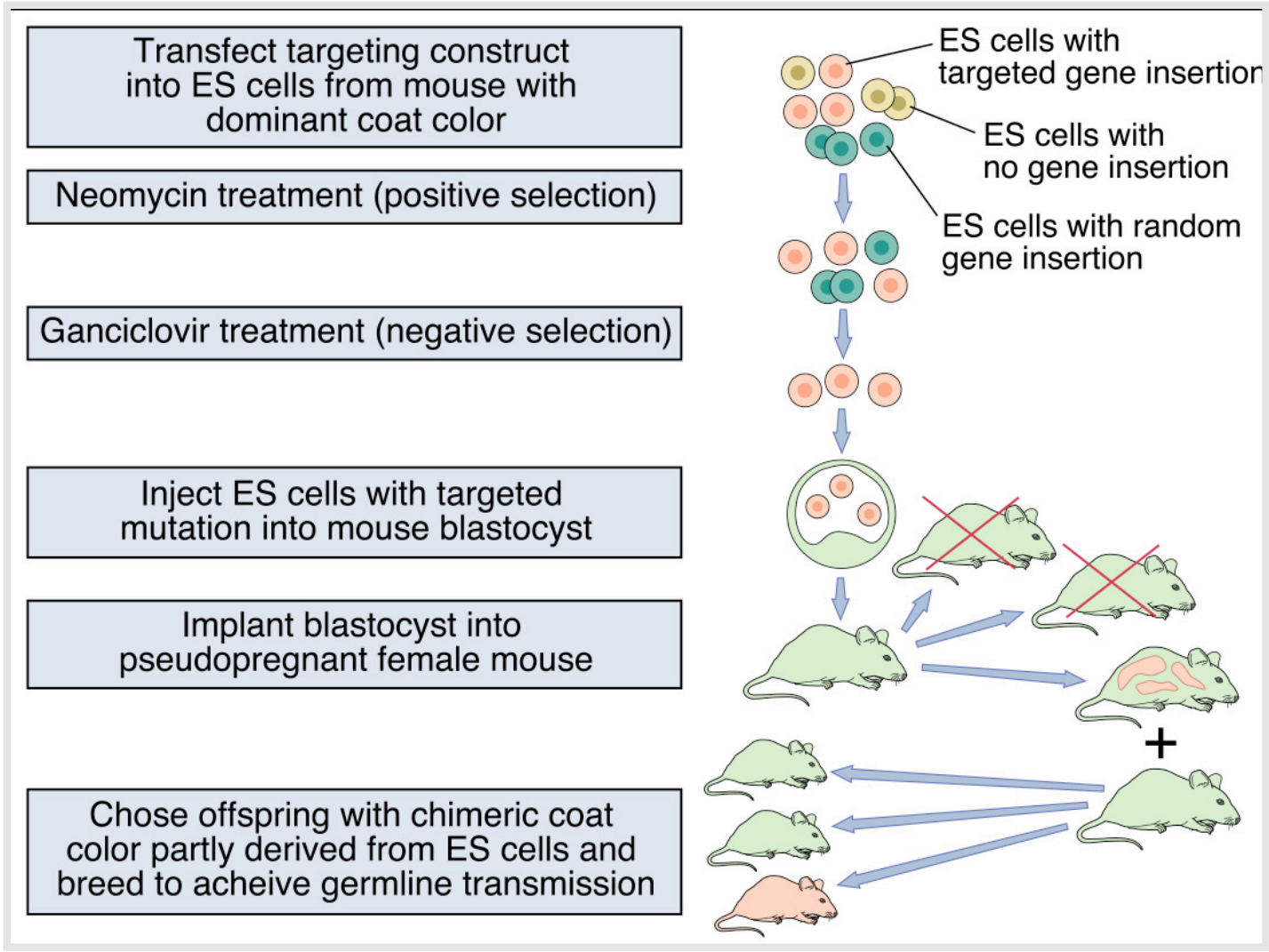


Pluripotent, embryonic stem cells originate as inner mass cells within a blastocyst. The stem cells can become any tissue in the body, excluding a placenta.

Knockout Mice Targeting Constructs for Homologous Recombination



Transgenic Mice
~Generation of Knockouts~

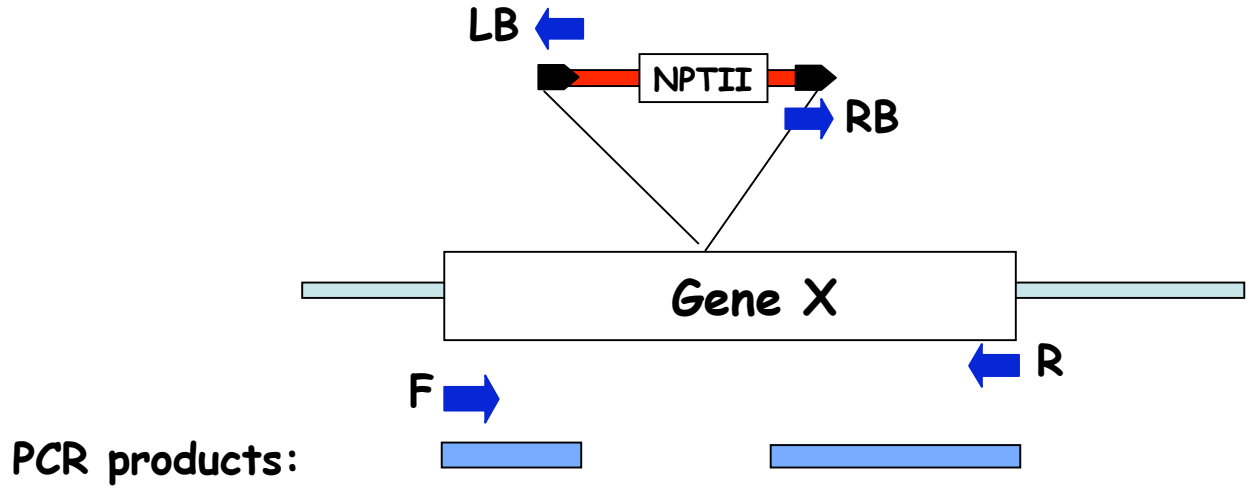


Identify gene function by insertional or chemical mutagenesis

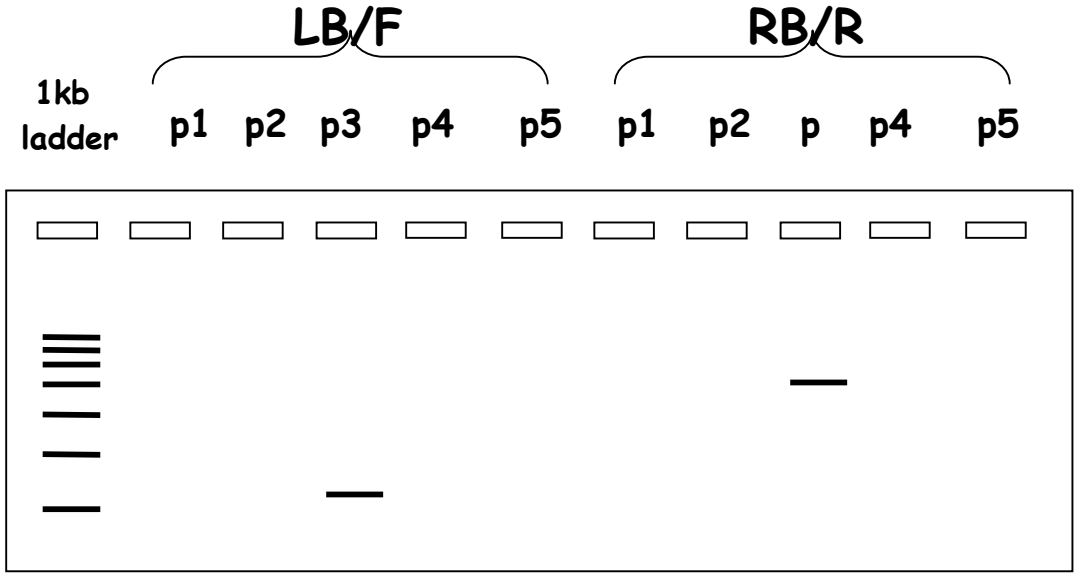
1) T-DNA or transposon insertions and PCR-based screens

2) Arabidopsis Tilling project

1. Screen for T-DNA (or Ds) insertion in specific genes

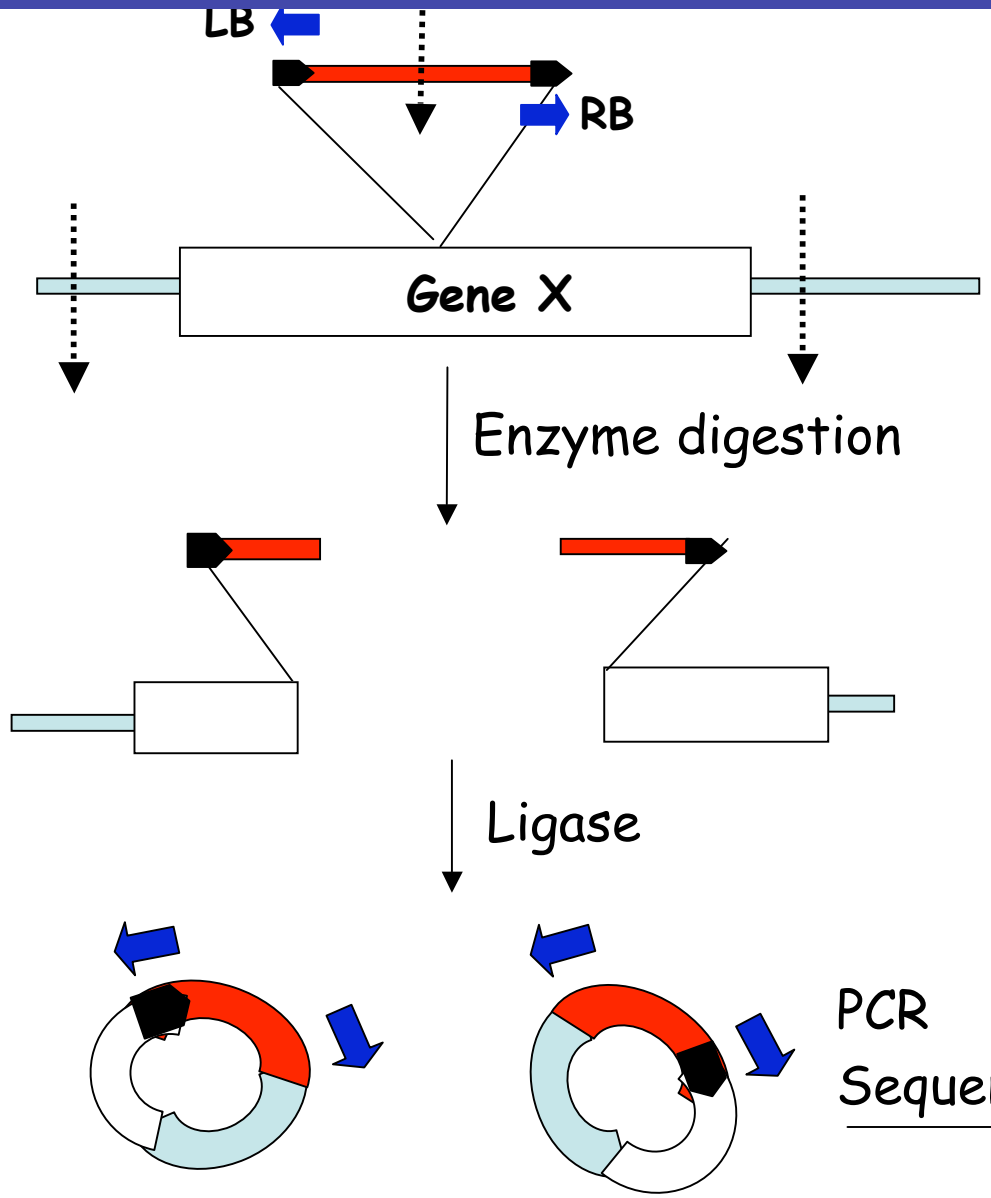


Screening pools (p1-p5)



Data-base searches for T-DNA insertions in the genes of interests

Salk Institute Genomic Group (http://signal.salk.edu/cgi-bin/tdnaexpress)



LEUNIG="At4g32550
SEU=At1g43850