Lecture 15 Functional Genetics

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

A. Forward genetics: Random mutagenesis, screen for traits of interests

Chromosome walking or transposon-tagging

B. Gene expression profile (analyses of transcriptome)

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

D. Fine structure genetics

Forward and reverse genetics

- **Forward genetics** starts with identification of interesting mutants
  - Then aims to discover the function of genes defective in mutants

- **Reverse genetics** starts with a known gene and alters its function
  - Then aims to determine the role of the gene from the effects on the organism

Reverse genetic methods

- **RNA interference**
- **Identify gene affected by**

  Insertional or chemical mutagenesis

  Then screen for the mutation
- **Delete genes by homologous recombination**

  Can be done in yeast, mouse and flies

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

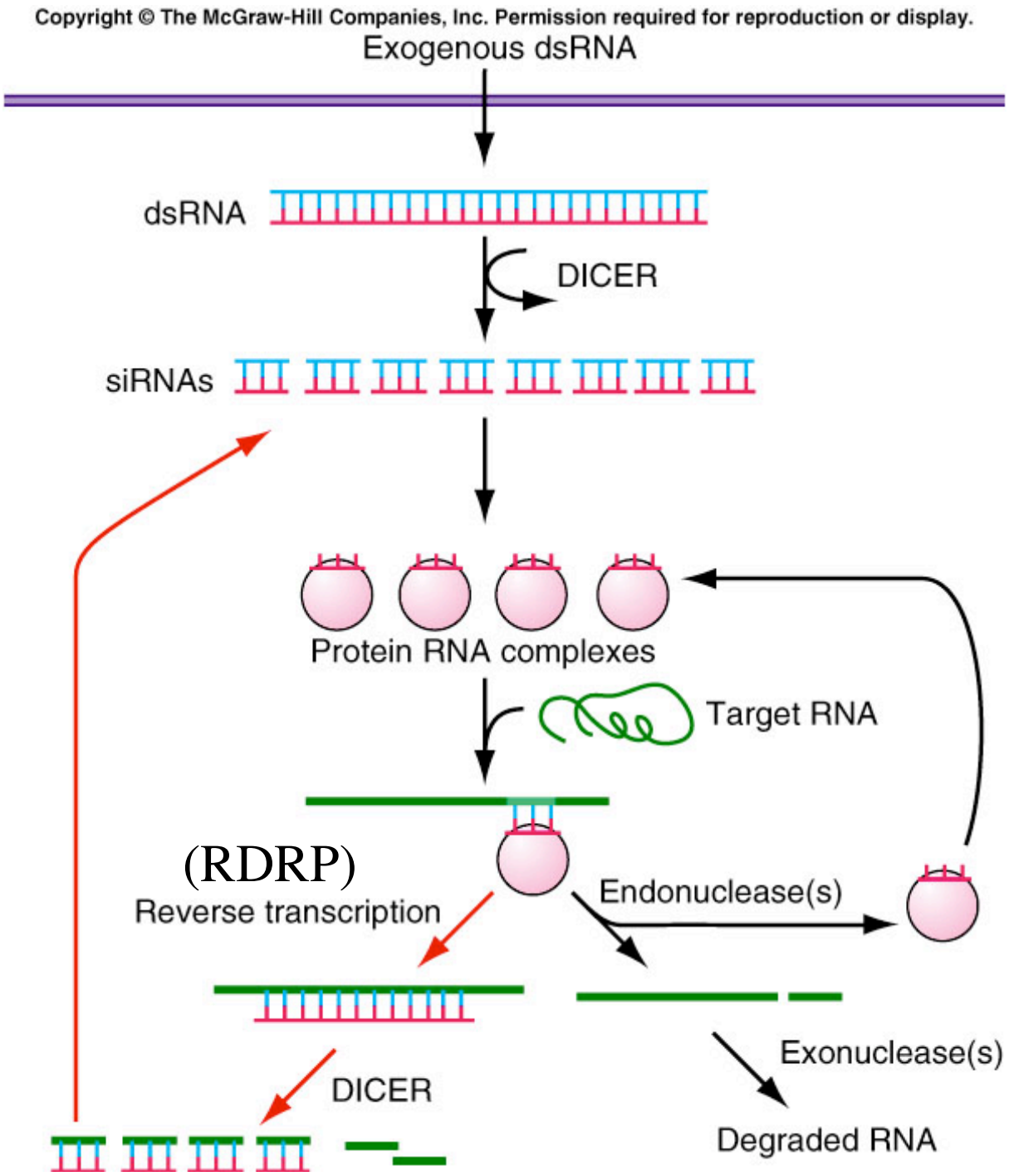
      post-transcriptional gene suppression (PTGS)

- **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*)**

Fig. C.9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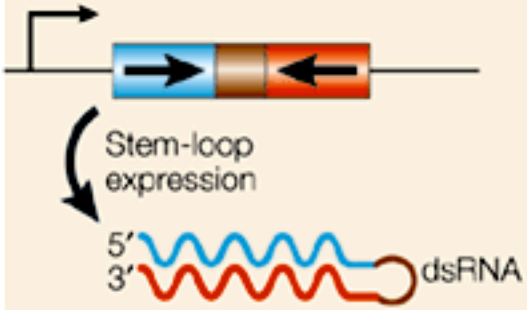
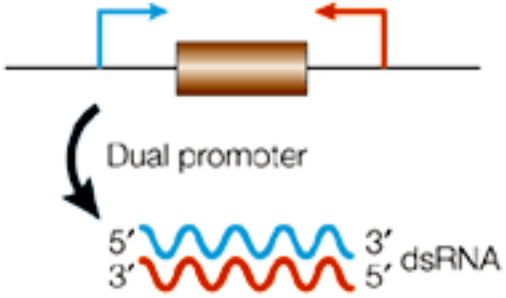
Exogenous dsRNA
dsRNA
DICER
siRNAs
Protein RNA complexes
Target RNA
(RDRP)
Reverse transcription
Endonuclease(s)
Exonuclease(s)
DICER
Degraded RNA

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

Method	Organism	Pros	Cons
 dsRNA	*C. elegans* *Drosophila* Trypanosomes	• Fast • Effective • Works in many systems	• Non-inducible • Most effective in embryonic systems
 Stem-loop expression 5′ 3′ dsRNA	*C. elegans*[77] *Drosophila*[55] Trypanosomes[78] Plants[79]	• Stable • Inducible • Tissue specific	• Time consuming to generate • Cloning can be problematic
 Dual promoter 5′ 3′ 3′ 5′ dsRNA	Trypanosomes[78,80]	• Stable • Inducible • Tissue specific	• Time consuming to generate • Promoters can silence each other
 VIRUS	Plants	• Most common technique in plants	• Limited to plants at present

Challenges for siRNAs as a therapeutic agent

Cellular uptake in tissue under physiological conditions

a) **Chemical modifications to improve**
   1) Stability in blood
   2) Tissue redistribution and cellular transport
   3) Efficient silencing of gene

b) **Specific target site delivery**
   Examples, eye and CNS

c) **Delivery as a complex**

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

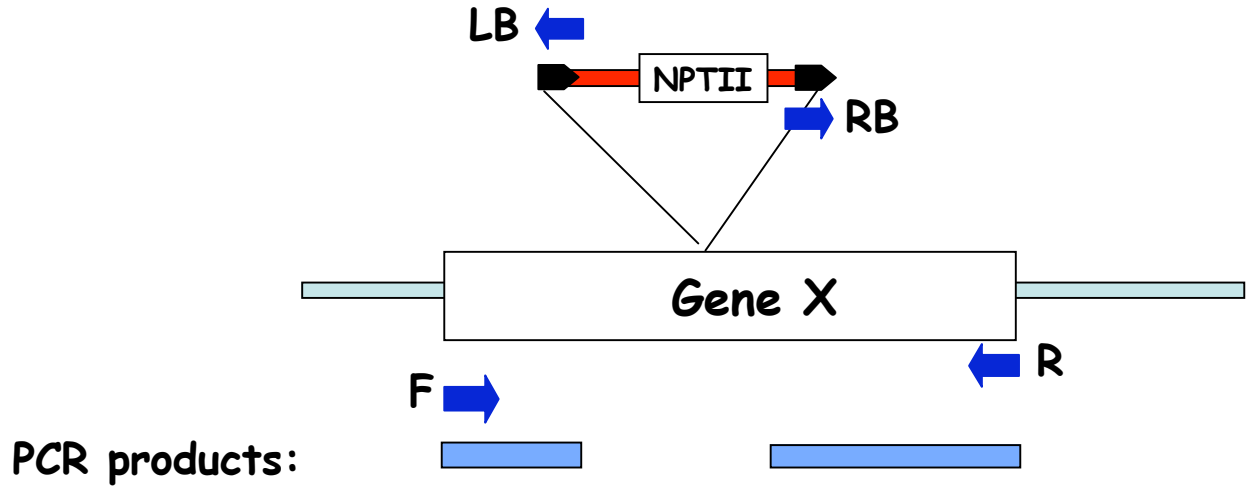
  Sonnichensen, 2005-Genome-wide injection

Identify gene function by insertional or chemical mutagenesis

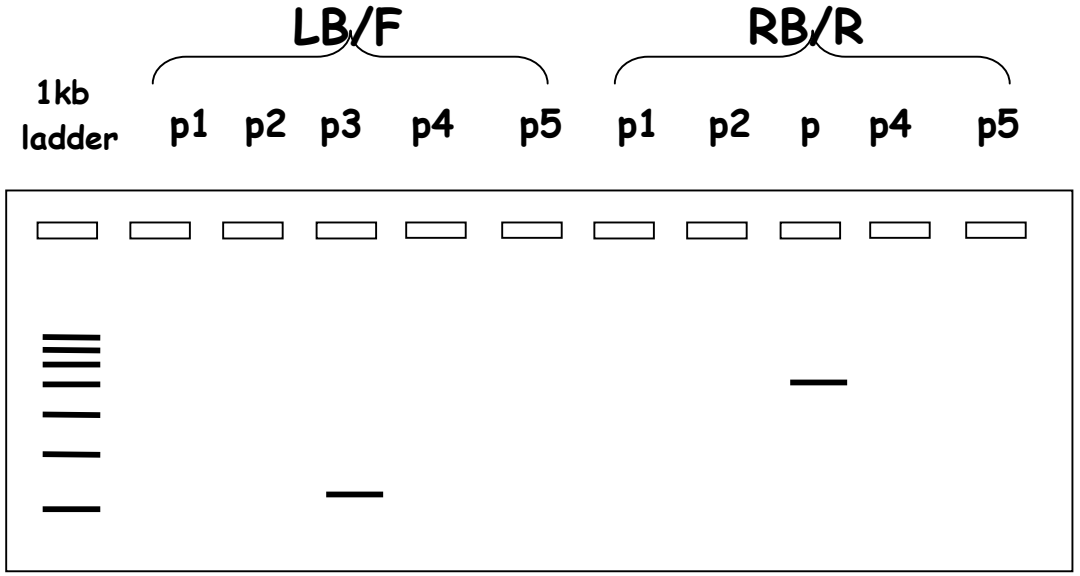
1) T-DNA or transposon insertions and PCR-based screens

2) Arabidopsis Tilling project

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

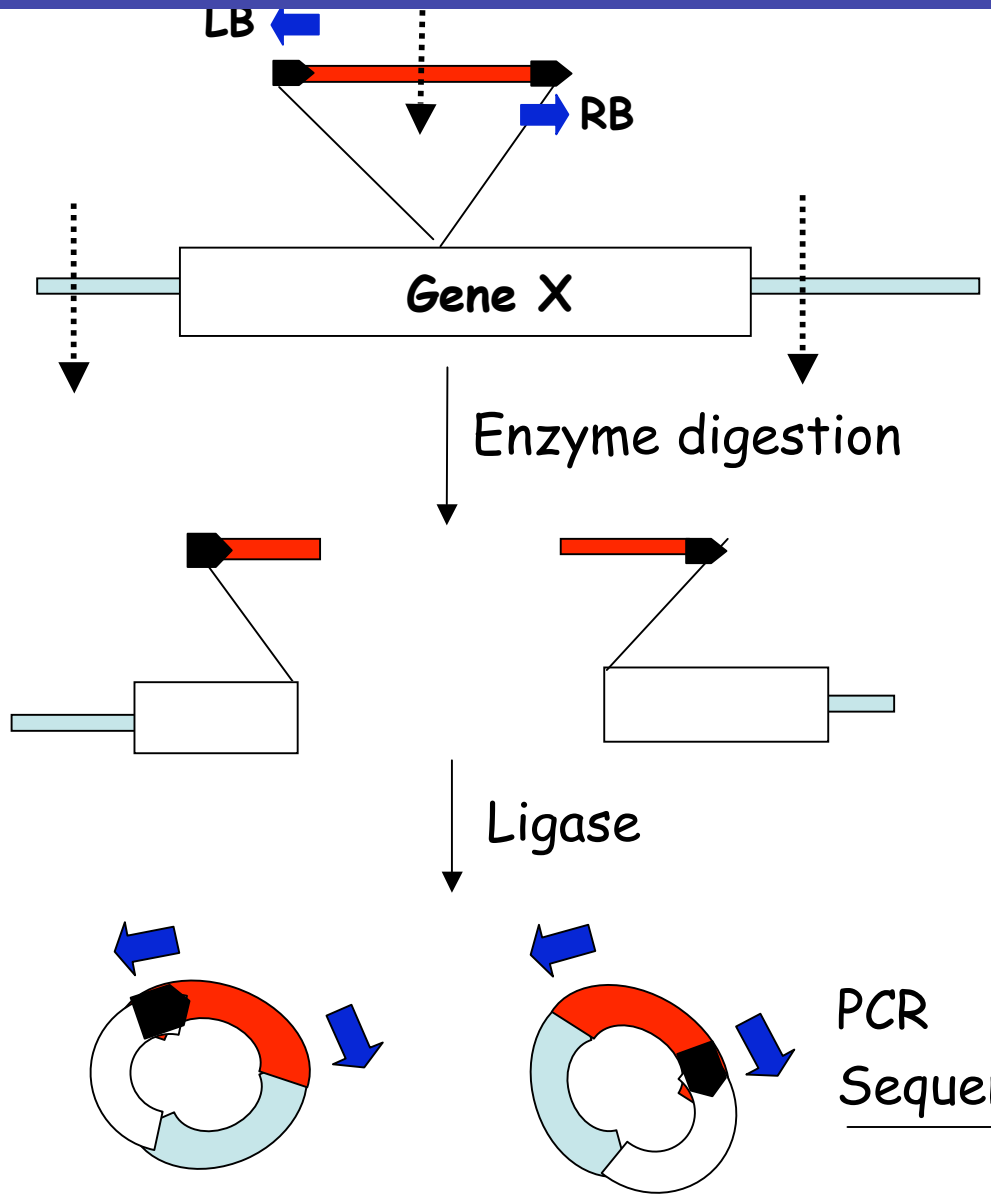


Screening pools (p1-p5)



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

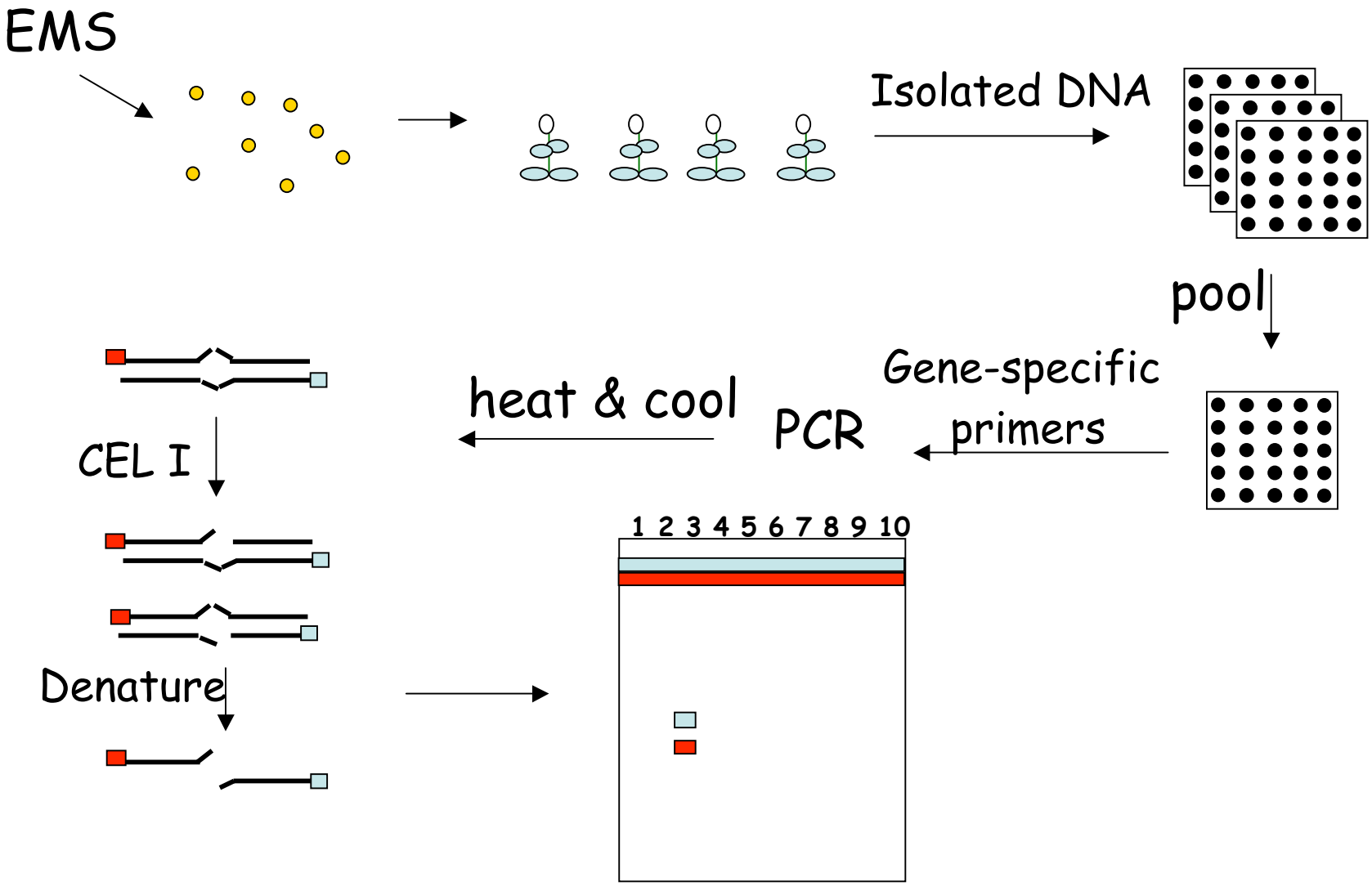
Salk Institute Genomic Group (http://signal.salk.edu/cgi-bin/tdnaexpress)



LEUNIG="At4g32550
SEU=At1g43850

2. TILLING (Targeting Induced Local Lesions IN Genomes)

Arabidopsis Tilling website:http://tilling.fhcrc.org:9366/



Homologouse recombination and gene knock-out in yeast and mouse

(Page 744-746, 852, A.7; A.8; E.8)

Fig. A.8

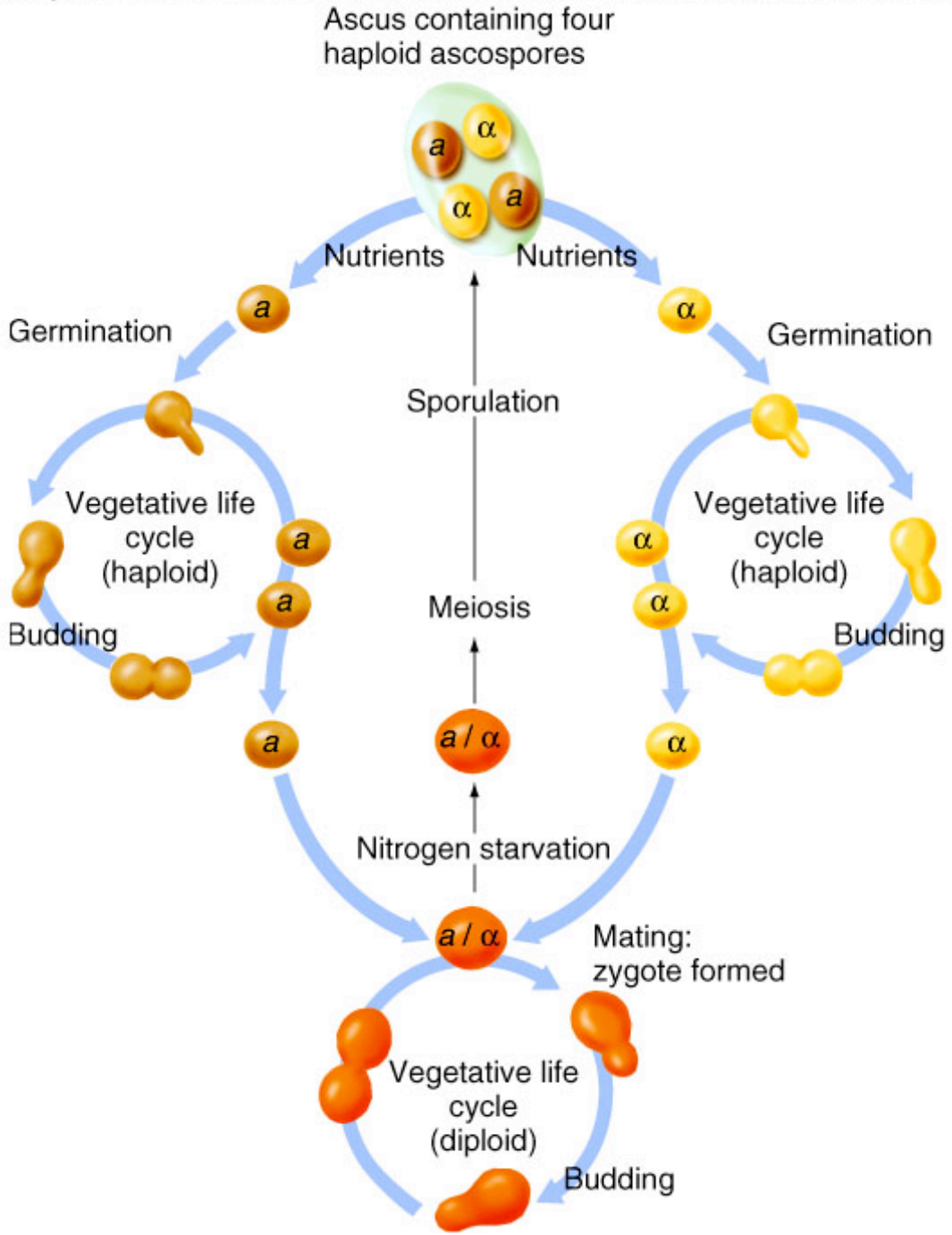


Fig. A.7

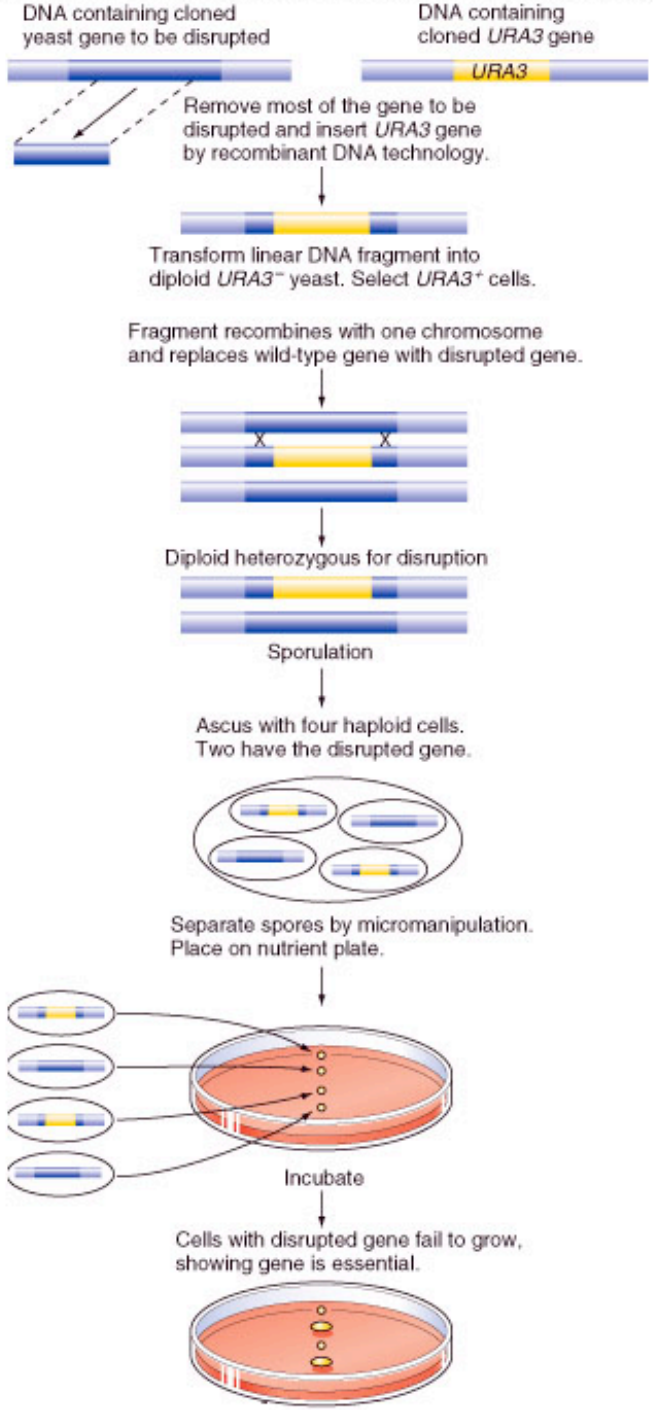


Fig. E.8