

**FORWARD GENETICS**

Starting with a known function/phenotype → find the gene(s) responsible.

**REVERSE GENETICS**

Start with a gene(s) → find the gene(s) function or cellular role.

**GENOME PROJECT GOALS:**

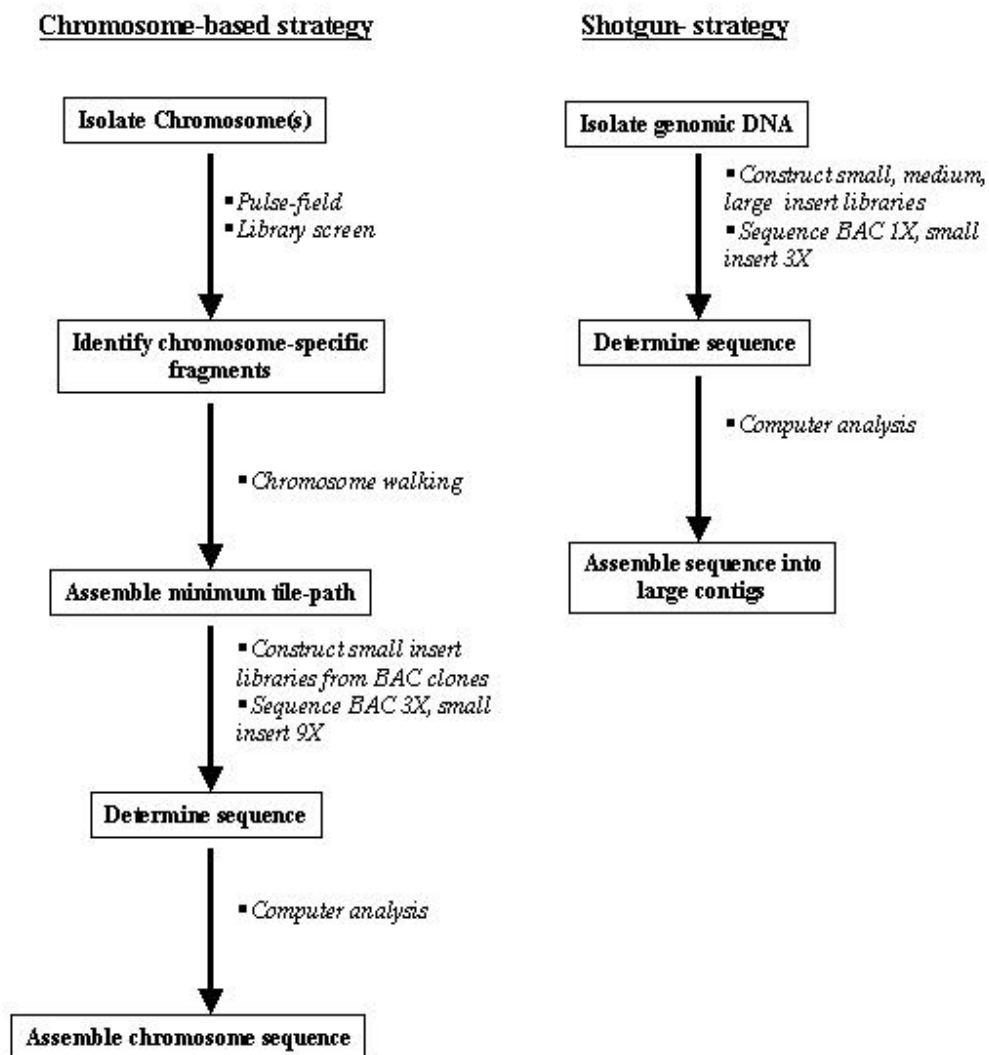
Gene Discovery

- Comparative genomics—Reverse Genetics
- Functional genomics (study of the expressed genome)

Genetic Mapping

- Physical maps--Chromosome ontology
- Molecular basis of genetic variability
- /inheritance of human and animal disease—Forward Genetics

**TOXOPLASMA GONDII GENOME PROJECT:**



## PULSE-FIELD ELECTROPHORESIS

Method for resolving large DNA fragments.

Range of separation=50 kb-to-10 MB

Switch-time governs resolution

field inversion= $180^\circ$  switch

standard pulse-field= $106^\circ$ -to- $120^\circ$

Preparative and analytical tool

## LIBRARY VECTORS

### **YACs=**Yeast Artificial Chromosome

insert sizes=0.2—to---2 Mb,

Positive features—large insert size

Negative features--large chimerism—10-60%

### **BACs=**Bacterial Artificial Chromosome

insert sizes=50-150 Kb

F-episome/plasmid based

Positive—E. coli system, relatively large inserts, major library model

Negative—empty vectors, new negative selection models (levan sucrose, sacB gene sucrose poison)

### **Cosmids=**cos site containing plasmid

cos sites are lambda phage packaging signals, 49 nt region with 12 nt sticky ends,

lambda phage head can accommodate 37-52 kb, therefore insert sizes for cosmid vectors are ~40 kb

Optimal cosmid vector contains:

cos sites

conventional selectable markers—ampicillin, tetracycline

multiple cloning site flanked by rare, 8 bp restriction sites

for restriction mapping or tagging

MCS flanked by promoters for bacteriophage RNA synthesis, SP6/T7

to identify overlapping clones in order to assemble a clone tile

Positive—clones are packaged into an infectious phage, low background and chimerism. Maintained as a plasmid

Negative—relatively small insert size

### **$\lambda$ -phage vectors (37-52 kb DNA):**

DASH II—average 15 kb insert used for medium insert genomic libraries

gt10, gt11—average 1-8 kb used for cDNA

ZAPII—average 1-8 kb used for cDNA

Positive—extracellular preservation of recombinants, cloning/packaging efficiency

>1X10<sup>6</sup>/ng DNA, propagation and screening methods are superior to plasmids

Negative—DNA yields are low due to low copy number, direct phage sequencing poor, must convert to plasmid prior to sequencing

### **Plasmid-based libraries:**

Bluescript II—1-8 kb insert size

Positive—can be directly sequenced, DNA yields are high due to copy number,

Negative—cloning efficiency may be poor, passage through bacteria results in lost sequences, more difficult format for library propagation and screening

SCREENING DNA LIBRARIES: see Genome lecture #2

## CHROMOSOME RESTRICTION MAPPING/WALKING

### **Fingerprinting**

Steps: cut inserts with 6 bp cutter, label ends, cut a second time with a 4 bp cutter, run out on a gel and compare patterns.

### **Comparative Restriction mapping**

Steps: linearize clones with rare cutter, partially digest with a selection of 6 bp cutters, resolve fragments on agarose gel and probe with vector sequences.--

