Genetic selection and screening

By:

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Selection:
- Exploitation of the genetics of a recombinant organism to enable desirable, recombinant genomes to be selected over non-recombinants during growth

Screening:
- Identification of a clone in a genomic or cDNA library (q.v.) by using a method that discriminates between different clones
Antibiotic resistance marker

- ampicillin resistance (Apr)
- tetracycline resistance (Tcr)
- kanamycine resistance (Kanr)
- Neomycine resistance (Neor)
- Hygromycine resistance (Hydr)
<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Mode of Action</th>
<th>Mechanism of Resistance</th>
<th>Working Concentration^a</th>
<th>Stock Solution^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>Inhibits cell wall synthesis by inhibiting formation of the peptidoglycan cross-link.</td>
<td>The resistance gene (bla) specifies an enzyme, β-lactamase, which cleaves the β-lactam ring of the antibiotic.</td>
<td>20 – 100 μg/ml (50 μg/ml)</td>
<td>100 mg/ml in H₂O Store at −20°C.</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Prevents peptide bond formation by binding to the 50S subunit of ribosomes.</td>
<td>The resistance gene (cat) specifies an acetyltransferase that acetylates and thereby inactivates the antibiotic.</td>
<td>25 – 170 μg/ml (100 μg/ml)</td>
<td>34 mg/ml in Ethanol Store at −20°C.</td>
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<tr>
<td>Kanamycin</td>
<td>Causes misreading of mRNA by binding to 70S ribosomes.</td>
<td>The resistance gene (kan) specifies an aminoglycoside phosphotransferase that inactivates the antibiotic.</td>
<td>10 – 50 μg/ml (30 μg/ml)</td>
<td>30 mg/ml in H₂O Store at −20°C.</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>Causes misreading of mRNA by binding to 30S subunit of ribosomes.</td>
<td>The resistance gene (str) specifies an enzyme that modifies the antibiotic and inhibits its binding to the ribosomes.</td>
<td>10 – 125 μg/ml (50 μg/ml)</td>
<td>50 mg/ml in H₂O Store at −20°C.</td>
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<tr>
<td>Tetracycline</td>
<td>Prevents protein synthesis by preventing binding of the aminoacyl tRNA to the ribosome A site.</td>
<td>The resistance gene (tet) specifies a protein that modifies the bacterial membrane and prevents transport of the antibiotic into the cell.</td>
<td>10 – 50 μg/ml (10 μg/ml in liquid culture – 12.5 μg/ml in plates)</td>
<td>12.5 mg/ml in Ethanol Store at −20°C.</td>
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<tr>
<td>Geneticin G 418</td>
<td>Interferes with the function of 80S ribosomes and blocks protein synthesis in eukaryotic cells.</td>
<td>The resistance gene (neo) encodes a bacterial aminoglycoside phosphotransferase that inactivates the antibiotic.</td>
<td>50 – 1000 μg/ml; optimal concentration is to be tested experimentally^b</td>
<td>5 – 50 mg/ml in culture medium or physiological buffers Store at −20°C</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>Inhibits protein synthesis by binding to the L6 protein of the 50S ribosomal subunit.</td>
<td>The resistance gene specifies an aminoglycoside phosphotransferase that inactivates the antibiotic.</td>
<td>100 μg/ml</td>
<td>10 – 50 mg/ml solution in H₂O. Store at 4°C</td>
</tr>
<tr>
<td>Hygromycin B</td>
<td>Inhibits protein synthesis of bacteria, fungi and eukaryotic cells by interfering with translocation and causing mistranslation.</td>
<td>The resistance gene (hyg or hph) codes for a kinase that inactivates Hygromycin B through phosphorylation.</td>
<td>50 – 1000 μg/ml; optimal concentration is to be tested experimentally^b</td>
<td>50 mg/ml in PBS Store at 4°C</td>
</tr>
</tbody>
</table>
use of chromogenic substrates

- Is a genetic screening method
- Use:
  - X-gal (5-bromo-4-chloro-3-indolyl-D-galactopyranoside)
  - Substrate of β-galactosidase
  - IPTG (iso-propyl-thiogalactoside)
- Screening method for cells or plaques
- System for the detection of tissue-specific gene expression in transgenics
Types of B/W screening system

• intact $\beta$-galactosidase gene ($lacZ$)
  – insertion vector Charon 16A
  – Blue is recombinant
  – Usually is selection method

• $\alpha$-complementation system ($lacZ'$)
  – only part of the $lacZ$ gene
  – $\alpha$–peptide
  – Needs $lacZ'$- host cell
(a) lacZ' \rightarrow \text{CS} \rightarrow \text{pUC/M13} \rightarrow \alpha^- \rightarrow \text{Functional } \beta\text{-galactosidase} \rightarrow \text{Blue colonies or plaques}

(b) lacZ' \rightarrow \text{CS} \rightarrow \text{pUC/M13} \rightarrow \text{Insert} \rightarrow \alpha^- \rightarrow \alpha\text{-Peptide not produced} \rightarrow \text{Non-Functional } \beta\text{-galactosidase} \rightarrow \text{Colourless colonies or plaques}
Insertional inactivation

- Insertion interrupts the coding sequence of a gene
- Can uses antibiotic resistance phenotype
- Can uses X-gal system
- Can uses Plaque morphology
  - Also a selection method
  - certain \( \lambda \)-vectors such as \( \lambda \text{gt}10 \)
  - \textit{cl gene}
Complementation of defined mutations

• Direct selection of cloned sequences is possible in some cases
• Needs mutant host for desired gene (auxotrophic mutants)
• mouse dihydrofolate reductase (DHFR)
• drug trimethoprim
Hybridization based screening methods

- Used to screen shotgun libraries
- cDNA
- Genomic DNA
- Oligonucleotides
PCR based screening protocols

- Vector specific PCR
- Insert specific PCR
- Both primer
- Can perform on pooled samples of clones
Immunological screening

• Use Ab to detect desired clone
• Insert must be in right orientation and frame
• Insert must be cDNA if cloned in bacteria
• often used for screening cDNA expression libraries
• Expression vectors such as λgt11
• Replica filter is used
Analysis of cloned genes

• Restriction mapping
• Blotting techniques
  – Southern blotting
• Sequencing
• Protein product detection methods based on translation of mRNA in vitro
  – hybrid-arrest translation (HART)
  – hybrid-release translation (HRT) or “hybrid-select translation”
HART & HRT

• hybridization
• In vitro translation
• Use a radioactive aminoacid (usually [35S]methionine)
• SDS-PAGE
Cloned DNA → HRT → Bind to filter → HART → Select → Translate in vitro → Released mRNA → Protein gel (autoradiogram)