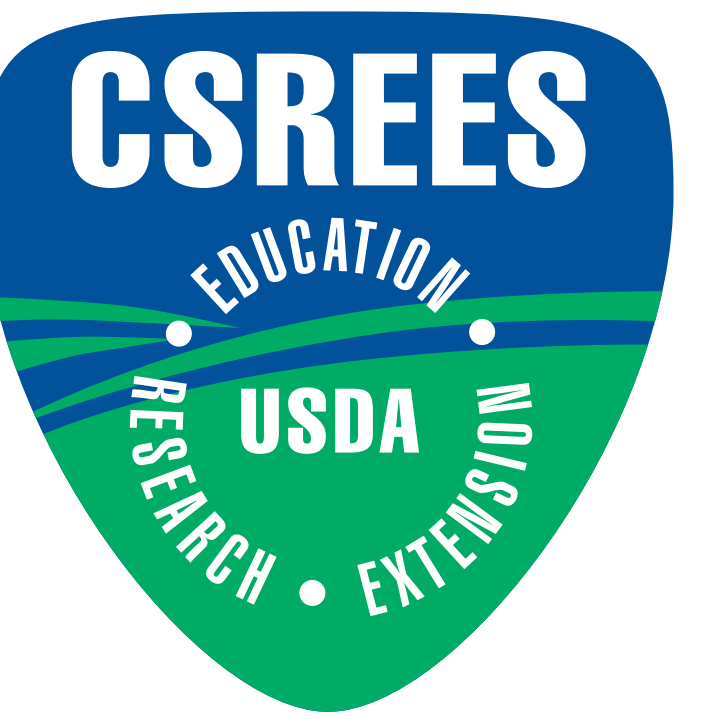


Antibiotic Resistance, Gene Transfer, and the Horizontal Gene Pool of Agriculture-Associated Soils and Waters



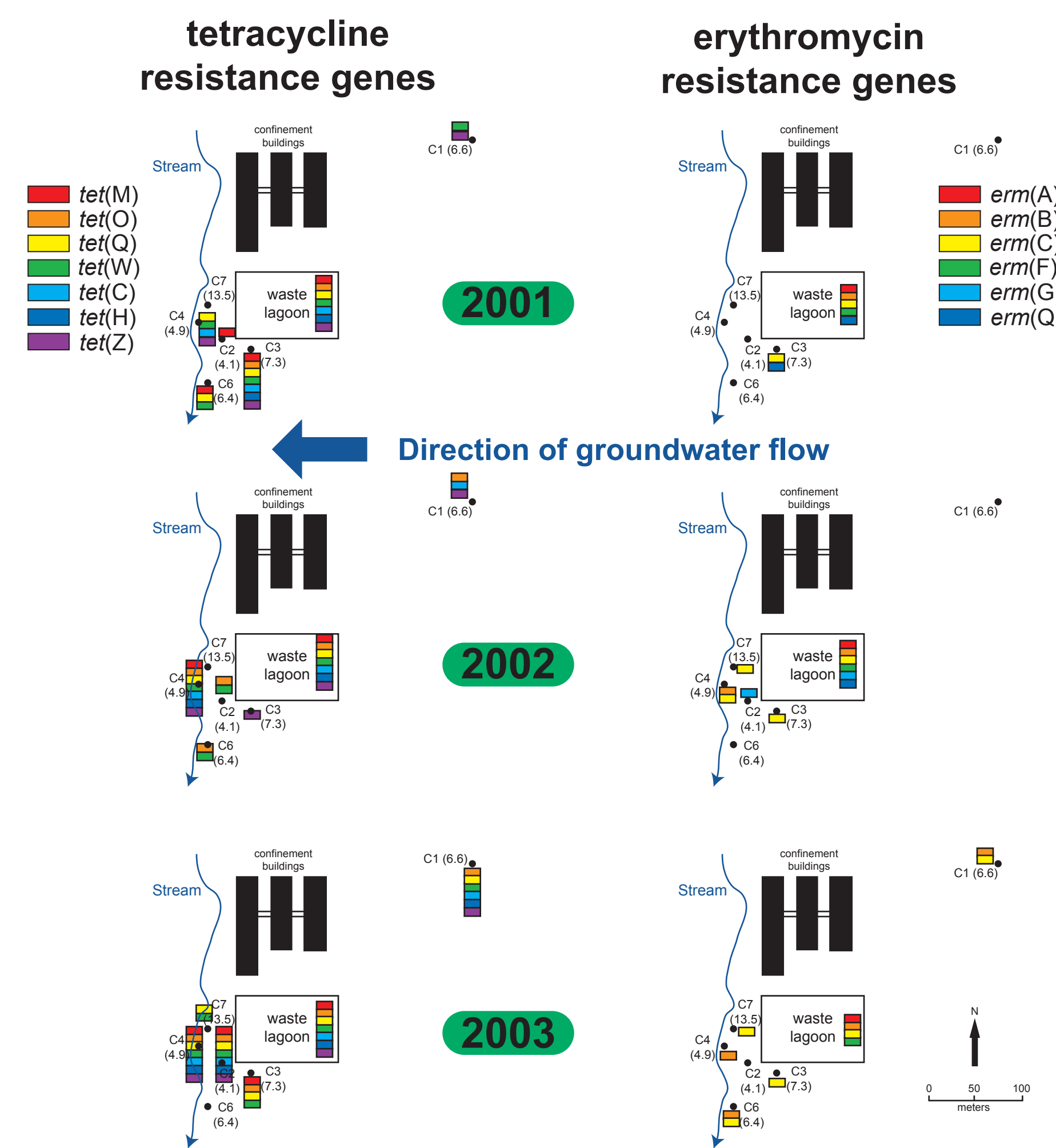
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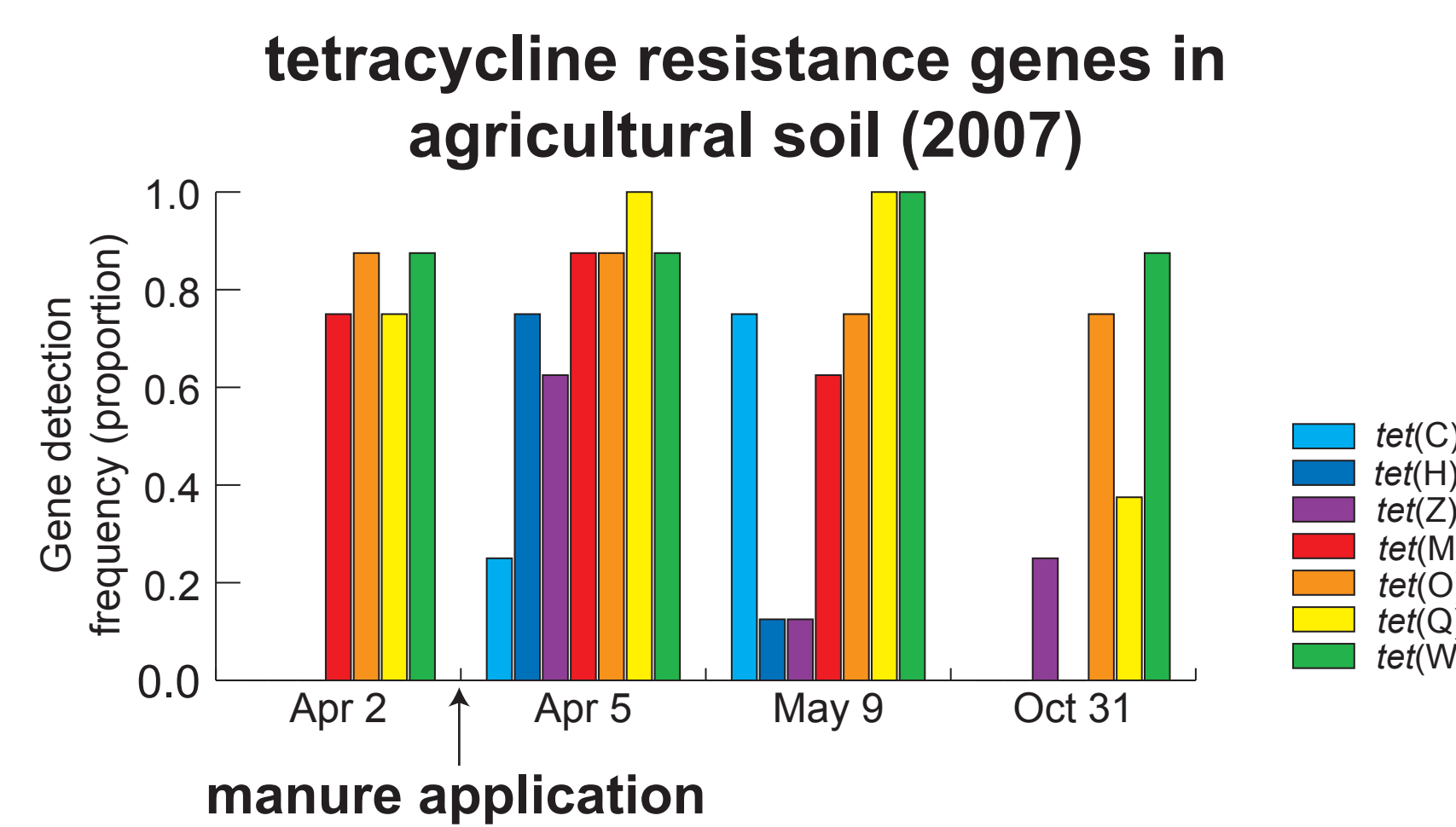
Background

- Horizontal gene transfer refers to the movement of genes from one bacterial cell to another. This results in novel genetic combinations, and it allows the recipient bacteria to acquire new traits, including **pathogenicity** and **resistance to antibiotic compounds**.
- The movement of genes is facilitated by **mobile genetic elements**. Examples include:
 - plasmids.
 - transposons.
 - integron gene cassettes.
 - viruses.
- The genes on all mobile genetic elements in an environment constitute a transferable **horizontal gene pool** that is potentially available to all bacteria in the environment.
- Outside of clinical settings, we do not understand the role of this horizontal gene pool in the emergence of microbial threats to human and animal health.
- How does horizontal gene transfer affect microbial evolution when human activities introduce **enteric microorganisms** and **antibiotic-resistant bacteria** to species-rich **soil** and **water** environments?

Antibiotic resistance genes in animal agriculture

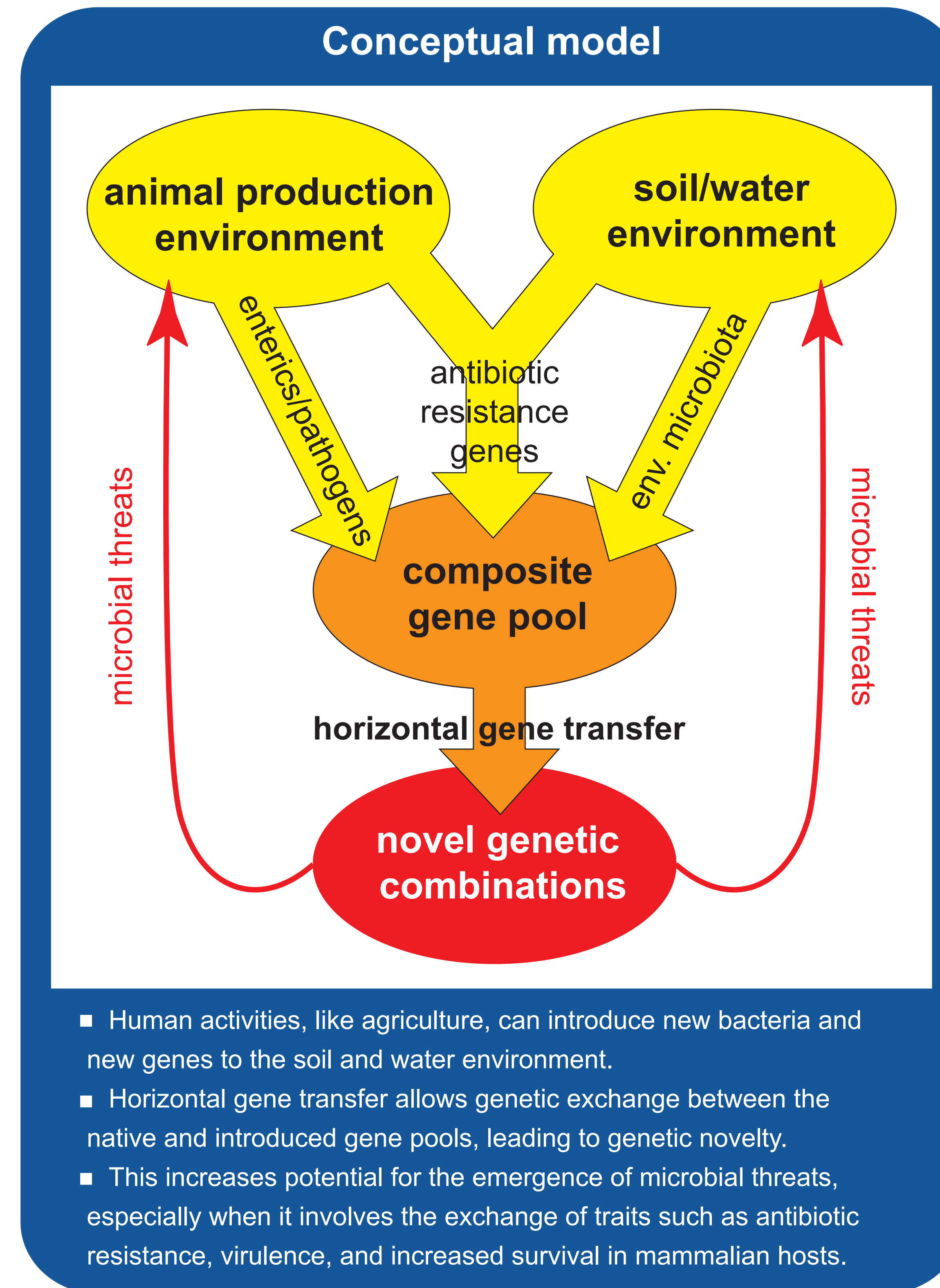


- Antibiotic use in animal agriculture can introduce antibiotic-resistant bacteria to nearby soils, streams, and groundwaters.
- Since 2001 we have monitored the presence of antibiotic resistance genes in groundwater wells near swine farms using antibiotics.
- Leakage from waste treatment lagoons was the primary determinant of gene presence in wells, but **spatial patterns** varied over time.
- Different genes were distributed individually.
- Waste lagoons containing antibiotic resistance genes are periodically dredged and used to fertilize crop fields.
- Gene detection frequencies in soils increase after manure application. The temporal variation of detection frequency was different for different genes.
- There is spatial and temporal variation of the **antibiotic resistance genes** available to the horizontal gene pool.



Purpose

Characterize the mobile gene pool of a hog farm environment, especially in relation to antibiotic resistance genes.



- Human activities, like agriculture, can introduce new bacteria and new genes to the soil and water environment.
- Horizontal gene transfer allows genetic exchange between the native and introduced gene pools, leading to genetic novelty.
- This increases potential for the emergence of microbial threats, especially when it involves the exchange of traits such as antibiotic resistance, virulence, and increased survival in mammalian hosts.

Approach

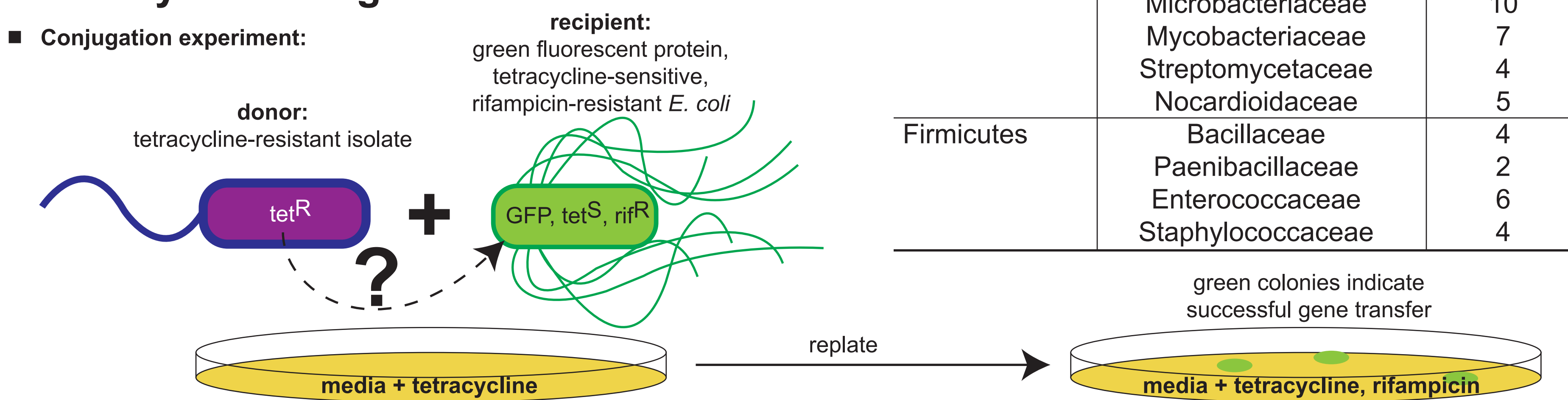
Techniques with bacterial isolates

What antibiotic-resistant organisms are in agricultural environments, and what genes do they have?

- From one swine farm, we have isolated over 200 bacteria that are resistant to tetracycline and/or erythromycin. These isolates cover a broad range of animal-associated and environmental taxa.
- PCR and gene sequencing will be used to characterize the antibiotic resistance genes in these isolates. We are particularly interested in identifying different bacterial species with identical genes.

Are any of these genes transferable?

Conjugation experiment:



Phylum	Subdivision of Family	Isolates
Proteobacteria	alpha-Proteobacteria	31
	beta-Proteobacteria	41
	gamma-Proteobacteria	80
	epsilon-Proteobacteria	1
Bacteroidetes	Flexibacteriaceae	4
	Sphingobacteriaceae	4
	Flavobacteriaceae	8
Actinobacteria	Corynebacteriaceae	5
	Microbacteriaceae	10
	Mycobacteriaceae	7
	Streptomycetaceae	4
	Nocardioideaceae	5
Firmicutes	Bacillaceae	4
	Paenibacillaceae	2
	Enterococcaceae	6
	Staphylococcaceae	4

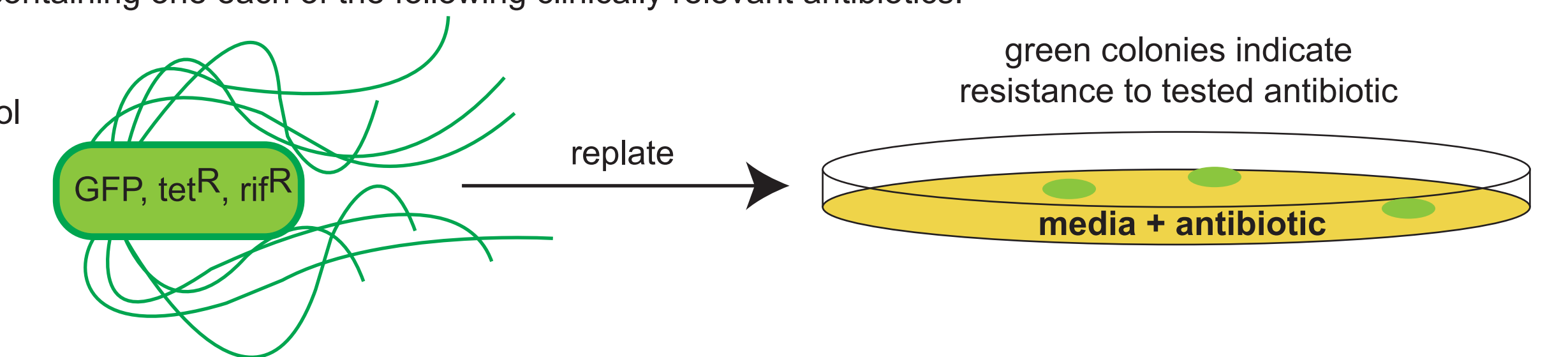
For transferable genes, what is the mobile genetic element responsible?

- Plasmid preps for successful transconjugants.
- Integron PCR (see below) for transconjugants that do not contain plasmids.
- For transconjugants without plasmids or integrons, use donor gene sequence to design primers for chromosome walking, and sequence 3 kb on either side of the donor gene.

Do any of these mobile genetic elements confer multidrug resistance?

Transconjugants will be grown on media plates containing one each of the following clinically relevant antibiotics:

- amoxicillin
- sulfadiazine
- streptomycin
- gentamycin
- ampicillin
- chloramphenicol
- kanamycin
- trimethoprim

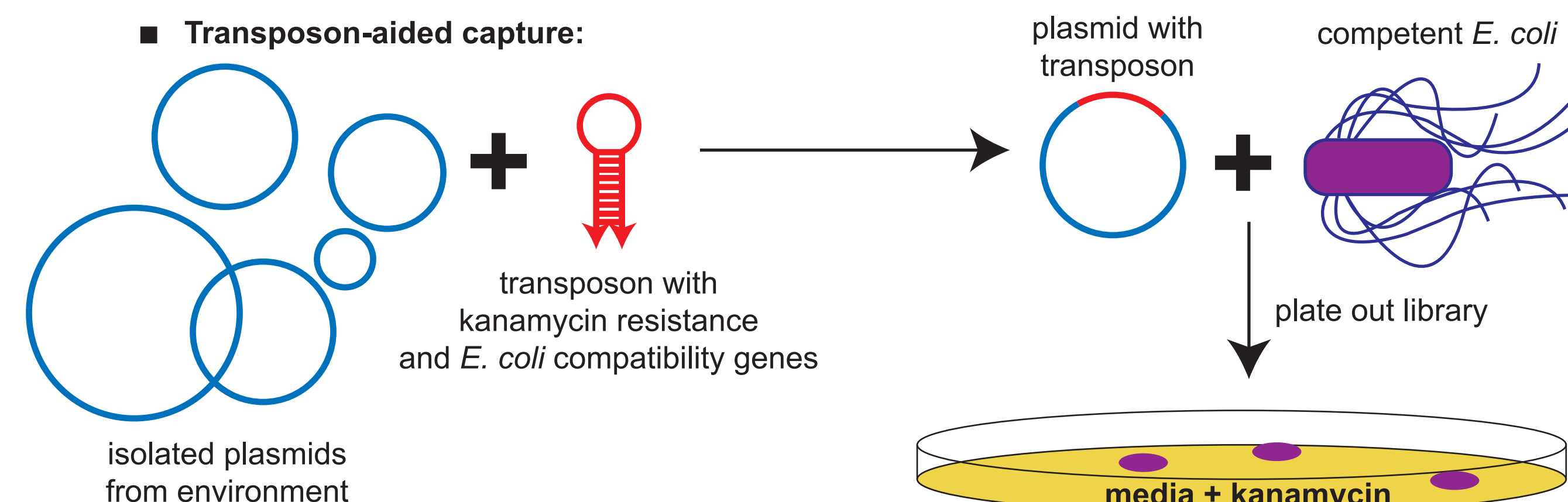


Cultivation-independent techniques

What is the plasmid pool of hog waste, soil, and water?

- Bacterial plasmids often contain accessory genes, e.g. antibiotic resistance and virulence factors.
- Not all plasmids are mobile, and not all are compatible with all bacterial species.

Transposon-aided capture:



What integron gene cassettes are present in hog waste, soil, and water?

- Integrons are conserved recombination sites on bacterial chromosomes.
- Strings of genes, called **integron gene cassettes**, can enter and leave a bacterial chromosome at these sites.
- Sequence conservation at the integron recombination site allows for PCR amplification of integron gene cassettes from bulk DNA isolated from hog waste, soil, and water.

