Transposon mutagenesis:

past, present and future approaches

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Transposon mutagenesis

PAST

- History and importance of transposons
- Transposon as molecular tool to study bacterial genetic
- A classical experiment of transposon mutagenesis

PRESENT

- Trasposon Insertion Sequencing approaches
- TraDIS

FUTURE

- TraDIShigella infecting human organoids Project:
  - A new tool to study host-pathogen interaction: enteroids model
  - Combination of TraDIS approach and human intestinal organoids infection
  - Project workflow: preliminary results and future steps

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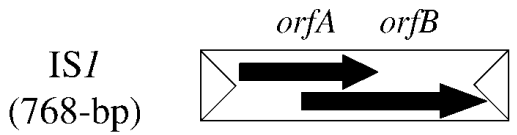
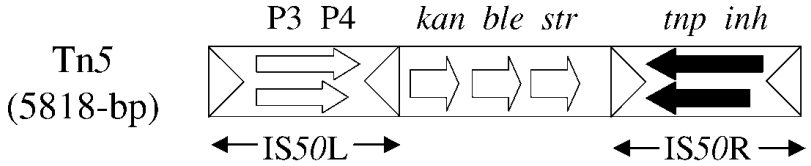
Transposons: what are they?

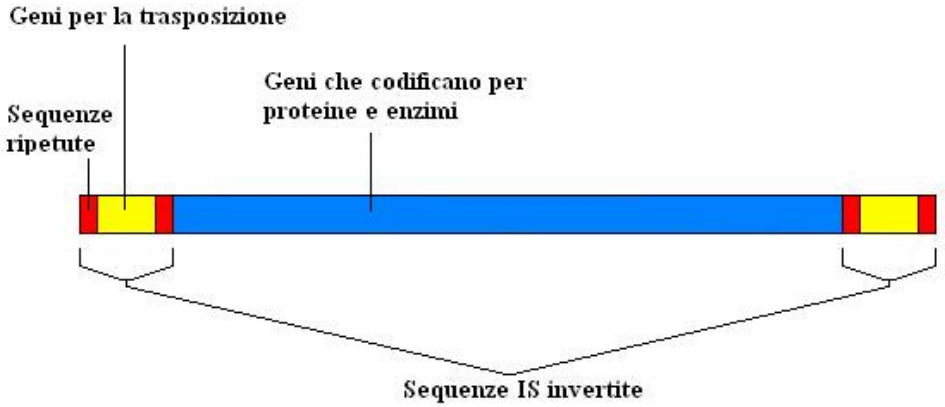
Transposable elements range from:

- simple insertion sequence (IS) elements



- composite transposons composed of a pair of IS elements that bracket additional genetic information for antibiotic resistance or other properties



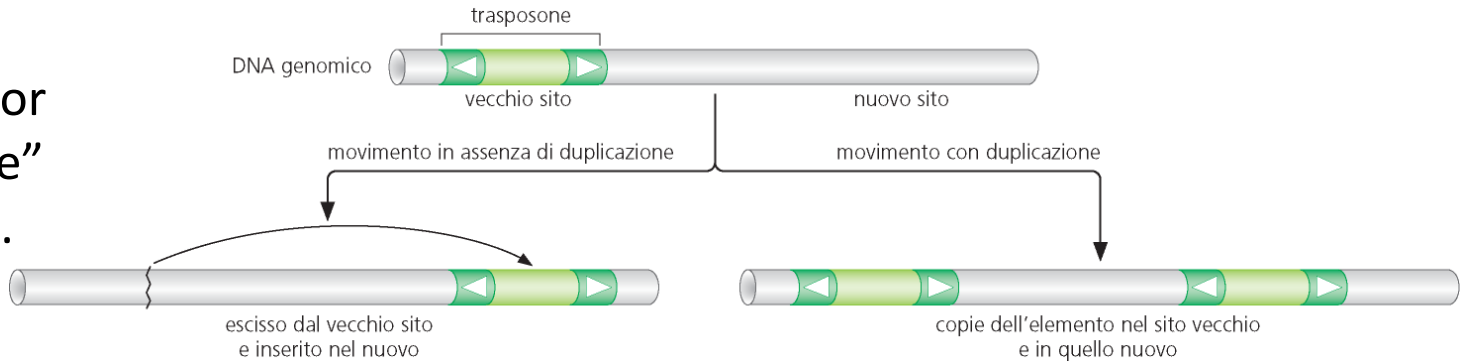


The transposase recognizes the inverted repeats at the end of the transposon and also recognize the target sequence, in which it makes a double-strand break and insert the transposon.

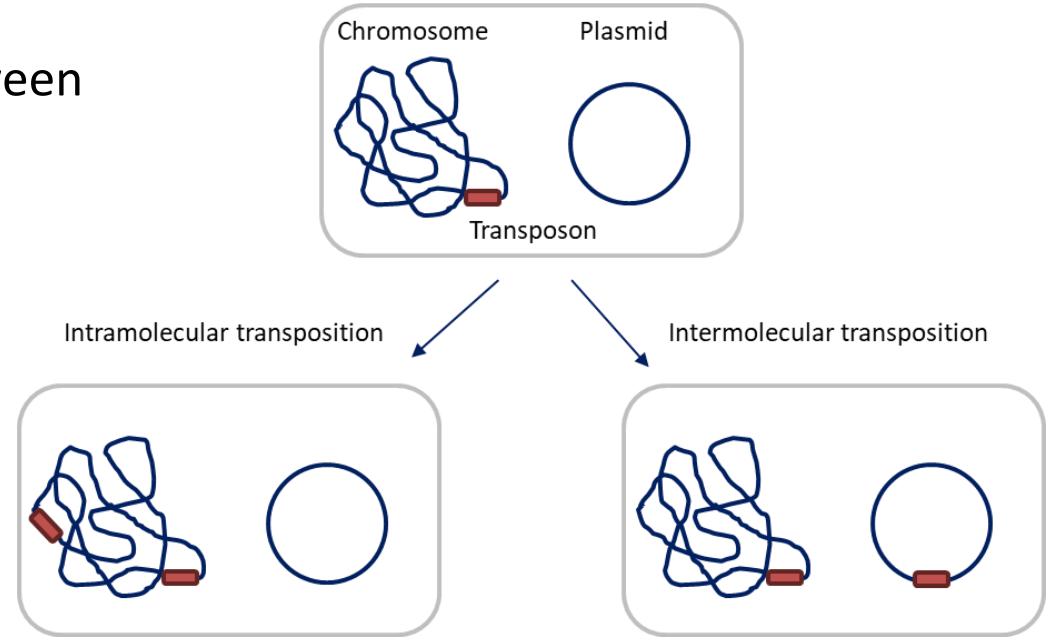
Transposons: How do they move?

The enzyme called transposase mediates transposon movement:

- By replicative or "cut-and-paste" mechanisms.



- Within or between genomes.



Transposons: history and importance

Transposons were originally discovered as “controlling elements” in maize by Barbara McClintock in the mid-1940s.

Trends in Biochemical Sciences
Volume 26, Issue 7, 1 July 2001, Pages 454-457

CellPress

Forum

From controlling elements to transposons: Barbara McClintock and the Nobel Prize

Why did it take so long for Barbara McClintock (Fig. 1) to win the Nobel Prize? In the mid-1940s, McClintock discovered genetic transposition in maize. She published her results over several years and, in 1951, gave a famous presentation at the Cold Spring Harbor Symposium, yet it took until 1983 for her to win a Nobel Prize. The delay is widely attributed to a combination of gender bias and gendered science. McClintock's results were not accepted, the story goes, because women in science are marginalized, because the idea of transposition was too far-fetched and because her scientific style was too intuitive, too holistic and too feminine to be believed.

Transposons history in bacteria

In the 1960s and 1970s, transposable elements were isolated in bacteria whose amenability to genetic manipulation facilitated both detailed molecular studies of the transposition process as well as the development of transposons as molecular tools.

"Transposons can be used as tools to manipulate the genes of bacteria, phage or plasmids in ways which are otherwise difficult or impossible"
Kleckner et al., 1977

jmb
Journal of Molecular Biology
Volume 97, Issue 4, 5 October 1975, Pages 561-564, IN15, 565-575

Mutagenesis by insertion of a drug-resistance element carrying an inverted repetition *

Nancy Kleckner, Russell K. Chan †, Bik-Kwoon Tye ‡, David Botstein

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https://doi.org/10.1016/S0022-2836(75)80059-3

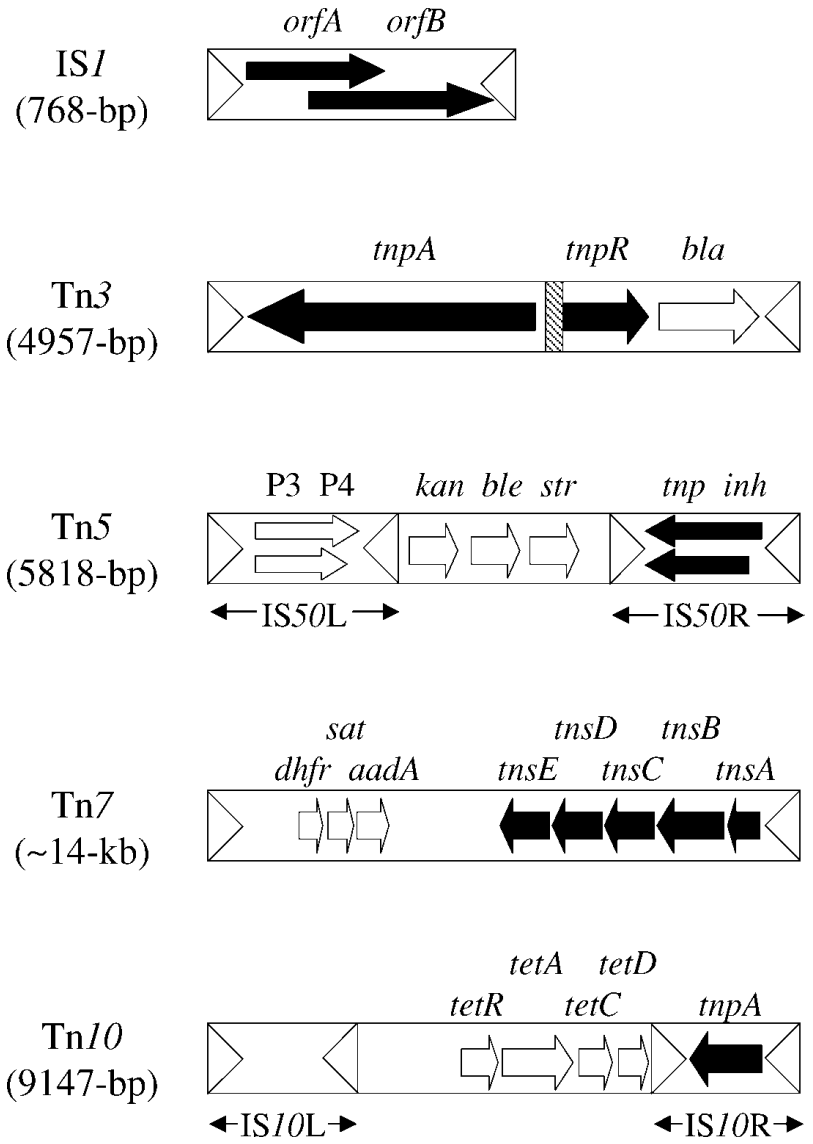
A novel genetic element, which carries genes conferring tetracycline resistance (flanked by a 1400 base-pair inverted repetition), is capable of translocation as a unit from one DNA molecule to another. The tet^R element, which is found in nature on a variety of R-factors, was acquired by bacteriophage P22 (producing P22Tc-10 and P22Tc-106) and has now been observed to insert into a large number of different sites on the *Salmonella* chromosome. Insertion of the tet^R element is mutagenic when it occurs within a structural gene, and polar when it occurs within an operon. Insertion of the element is usually precise, occurring without loss of information on the recipient DNA molecule. Excision, on the other hand, is usually *not* precise, although excisions precise enough to restore a gene function can always be detected at low frequencies. Both insertion and excision processes are independent of the *recA* function.

Transposons importance in bacteria

One of the major goals in bacterial genetics is to understand the genetic mechanisms underlying the phenotypes of interest.

Transposons were widely employed as random insertion mutagens both at a genetic and genomic level and have contributed significantly to gene discovery in bacteria mainly through loss-of-function screening.

The transposon insertion tool in bacterial genomes has been utilized extensively for the study of bacterial pathogenesis and biology.



Transposon mutagenesis in bacteria

Phenotype of interest

genetic mechanism?

Making **transposon jump in vitro**
in order to loss the function of the gene(s)
responsible for that phenotype

Which is the gene disrupted by transposon insertion?

Localize the transposon position

Making transposons jump in vitro

In vitro transposition reaction requires:

- Transposon terminal inverted repeats
- Purified transposase
- DNA target substrate
- Reaction buffer

The in vitro transposition reaction, that can proceed with high efficiency, have been used to generate genomewide insertion mutations in a diversity of bacteria.

TABLE 1 Microbial genomes mutagenized using in vitro transposition reactions

Microorganism	Significance	Transposon	Reference
Campylobacter jejuni	Food-borne pathogen	Tn*552*	26, 66
Erwinia carotovora	Plant pathogen	Mu	87
Escherichia coli	Model bacterium for genetic analysis	Tn*5* Mu	47 87
Haemophilus influenzae	Pulmonary infectious agent	*mariner* Tn*7*	2, 3 56
Helicobacter pylori	Gastric infections and ulcers	*mariner*	55
Mycobacterium spp.	Opportunistic pathogen	Tn*552*	13, 79
Neisseria meningitidis	Meningitis agent	Tn*10*	145
Proteus vulgaris	Opportunistic pathogen	Tn*5*	47, 70
Pseudomonas sp.	Opportunistic pathogen	Tn*5*	70
Rhodococcus sp.	Opportunistic pathogen	Tn*5*	37
Saccharomyces cerevisiae	Model lower eukaryotic for genetic analysis	Tn*5*	47
Salmonella typhimirium	Food-borne pathogen	Tn*5* Mu	47, 70 87
Streptococcus pneumoniae	Pneumonia agent	*mariner*	2
Streptomyces coelicolor	Antibiotic producer	Tn*5*, *mariner*	44
Synechocystis sp.	Photosynthetic cyanobacterium	ND[a]	12
Xylella fastidiosa	Plant pathogen	Tn*5*	54
Yersinia enterocolitica	Systemic infectious agent	Mu	87

[a]ND, not described.

Hayes, 2003

Example of classical transposons mutagenesis application

Ferric Uptake Regulator Fur Is Conditionally Essential in *Pseudomonas aeruginosa*

Martina Pasqua,[a] Daniela Visaggio,[b] Alessandra Lo Sciuto,[a] Shirley Genah,[a] Ehud Banin,[c] Paolo Visca,[b] Francesco Imperi[a]

Background

Ferric Uptake Regulator (Fur) depletion makes *Pseudomonas aeruginosa* cells severely defective in colony growth on solid media.

Aim

Investigate the mechanism(s) underlying the inhibitory or toxic effect of the lack of Fur-mediated repression on colony development.

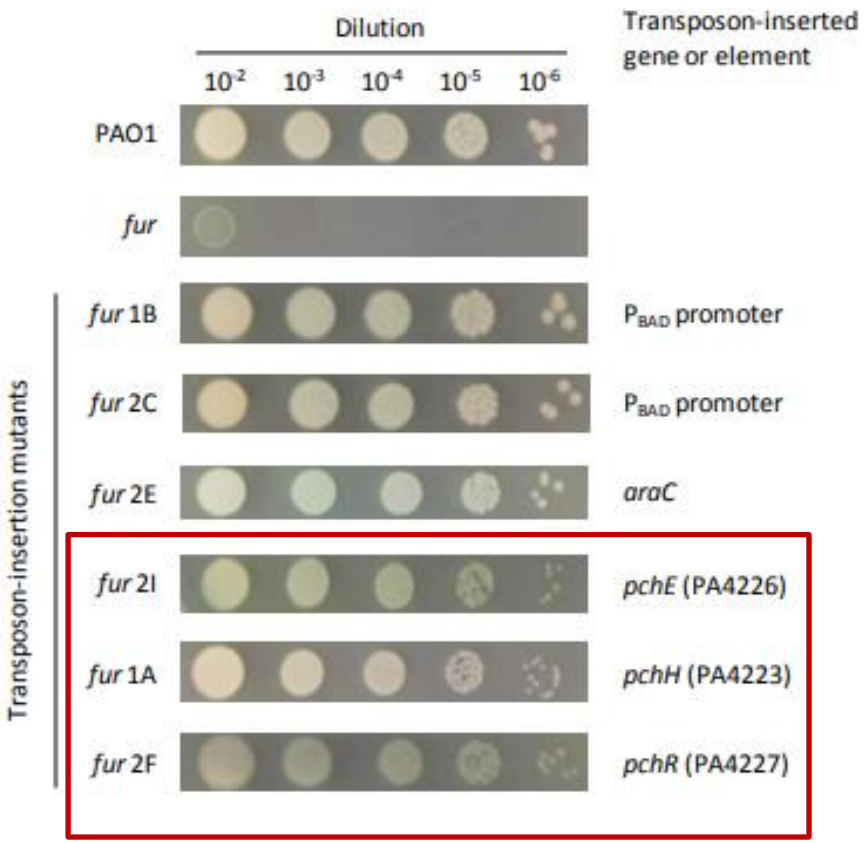
Method

by performing transposon mutagenesis screening in order to select transposon insertion derivatives of the fur mutant able to grow on MH agar plates.

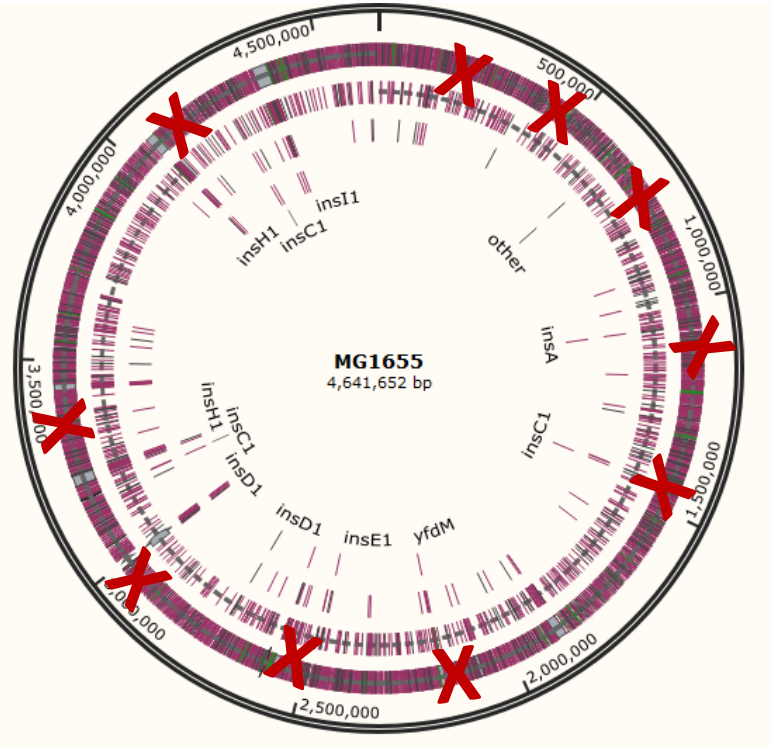
Example of classical transposons mutagenesis application

Result

the screening of almost 30,000 transposon insertion mutants led to identify 3 clones whose colony growth phenotype resembled that of the wild-type strain .



Limitits of a classical transposon mutagenesis approach



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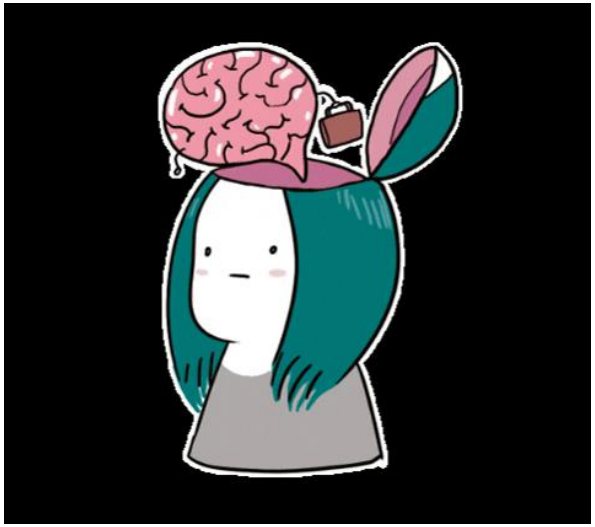

The necessity to assess the phenotype of each mutant individually requires considerable amount of labor and time thus limiting the total number of mutants that could be screened.

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Present: applications of transposon mutagenesis

With the advent of high-throughput molecular biology approaches such as rapid nucleotide sequencing, an enormous advance in the use of transposon mutagenesis as powerful genetic instruments was made.

It is now possible to sequence many transposon mutants simultaneously allowing genome-wide analyses.

Transposon Insertion Sequencing

Is the most recent incarnation of transposon-based genomic analyses.

Is a group of similar techniques that combine transposon mutagenesis with massively parallel sequencing (MPS)

Transposon insertion sequencing (**TIS**)

It requires:

1. The construction of a transposon insertion library
2. Growth of the library in defined in vitro or in vivo conditions
3. MPS of the transposon junctions of the population at the start and at the end of the experiment
4. Define the frequency of each mutant in the population in order to quantify the fitness of each gene in each condition.

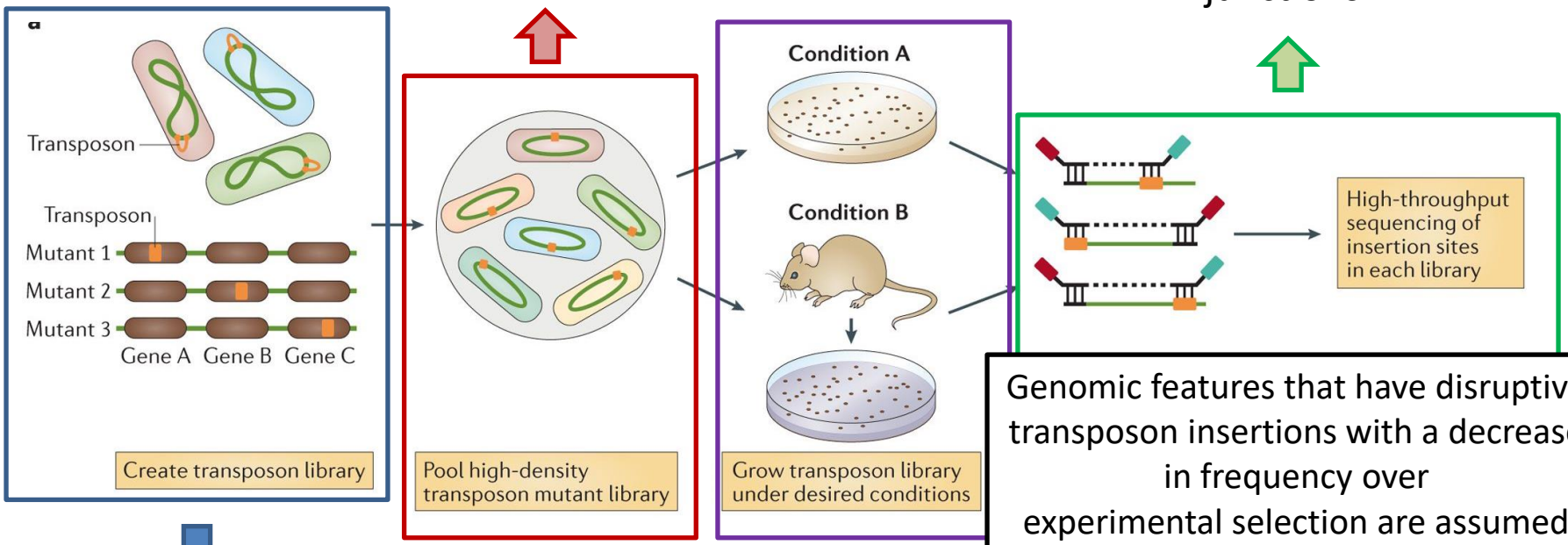
Strenghts of Transposon Insertion Sequencing

- Experiments are performed with pooled transposon libraries
- Critical tool to help interpret the mounting levels of genome sequencing data being generated
- Sensitive enough to detect even minor changes in mutant fitness
- Precise enough to be able to assay not only genes but also intergenic regions, promoter regions and essential protein domains within coding regions

Transposon insertion sequencing workflow

High-density transposon insertion library containing multiple insertions in every non-essential genomic locus is created

High-throughput sequencing is used to quantify all transposon junctions.



Transposon mutagenesis is used to create a pool of insertion mutants in which ideally all genomic loci have been disrupted at multiple sites.

This library of transposon insertion mutants can then be grown under selective conditions.

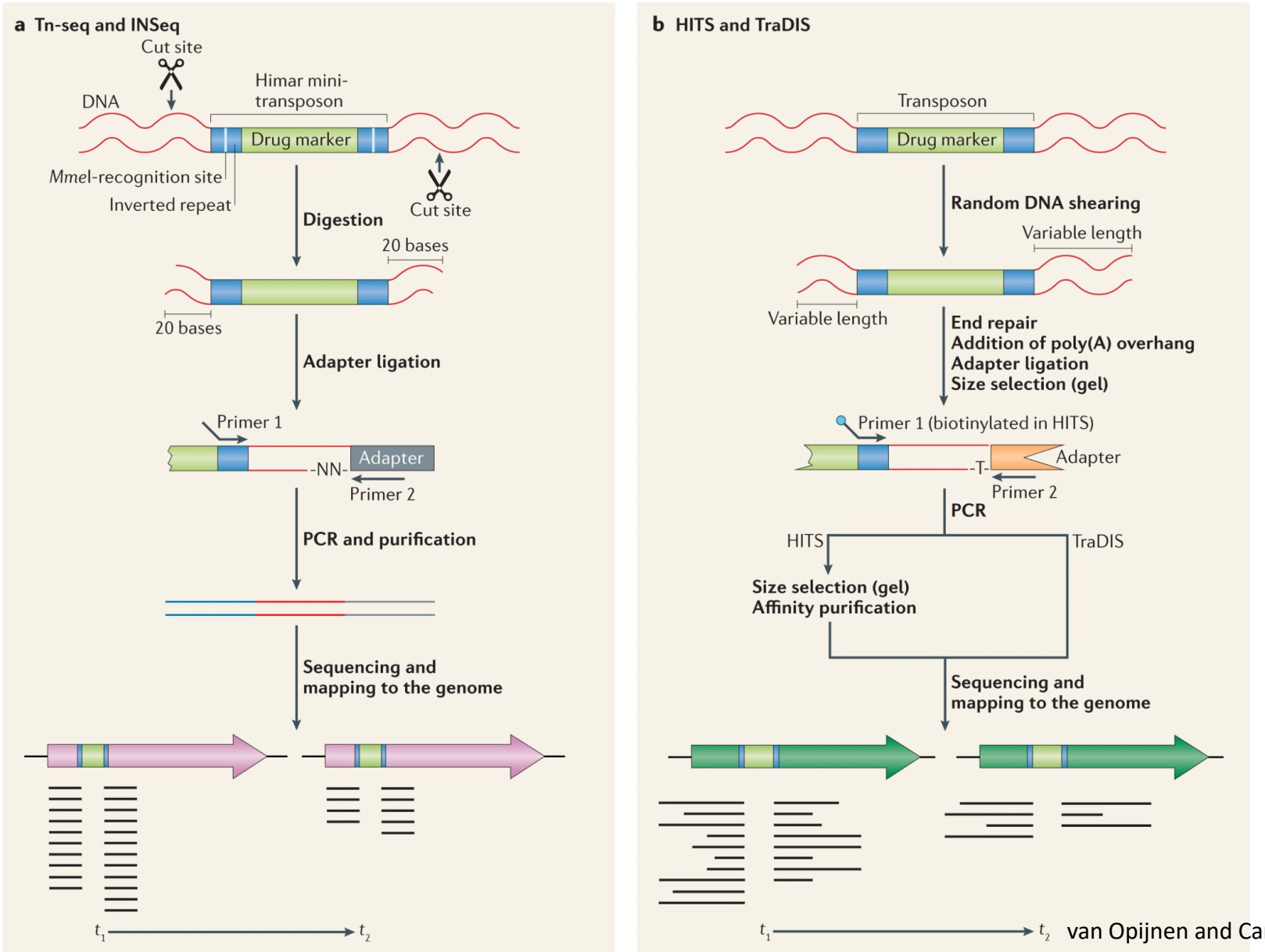
Genomic features that have disruptive transposon insertions with a decrease in frequency over experimental selection are assumed to be important for fitness in the test conditions

Variations of the TIS approach

Experimental parameters that vary among TIS studies:

- selected transposon;
- complexity of the transposon libraries generated (number of independent mutants per library);
- the constraints imposed by the experimental conditions chosen;
- reliability with which representative DNA libraries are created and sequenced;
- downstream data normalization and statistical methods involved in TIS analysis.

Transposon sequencing methods



van Opijnen and Camilli, 2013

Transposon sequencing methods

Tn-seq
Transposon sequencing

Similar methods
Advantage: shorter sample preparation protocol

INSeq
Insertion sequencing

Common features:

- Purification of gDNA from a poooled population of mutants
- Cleavage of DNA
- Attachment of one or more adaptors to the DNA to facilitate PCR amplification
- MPS of the amplified fragments
- Determine the location of the transposon and the relative abundance of mutants containing a transposon at this site

HITS
High-throughput insertion tracking by deep sequencing

Similar methods
Advantage: applicable to any transposon

TraDIS
Transposon-directed insertion site sequencing

TraDIS approach

Systems biology

The TraDIS toolkit: sequencing and analysis for dense transposon mutant libraries

Lars Barquist[1,2], Matthew Mayho[1], Carla Cummins[1], Amy K. Cain[1], Christine J. Boinett[1], Andrew J. Page[1], Gemma C. Langridge[1], Michael A. Quail[1], Jacqueline A. Keane[1] and Julian Parkhill[1,*]

[1]Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK and [2]Institute for Molecular Infection Biology, University of Würzburg, Würzburg D-97080, Germany

Transposon-insertion sequencing screens unveil requirements for EHEC growth and intestinal colonization

Alyson R. Warr[1,2☯], Troy P. Hubbard[1,2☯], Diana Munera[1,2☯¤a], Carlos J. Blondel[1,2☯¤b], Pia Abel zur Wiesch[1,2¤c], Sören Abel[1,2¤c], Xiaoxue Wang[1,2¤d], Brigid M. Davis[1,2], Matthew K. Waldor[1,2,3]*

RESEARCH ARTICLE Open Access

Combining *Shigella* Tn-seq data with gold-standard *E. coli* gene deletion data suggests rare transitions between essential and non-essential gene functionality

Nikki E. Freed[1,2], Dirk Bumann[2] and Olin K. Silander[1,2*]

Simultaneous assay of every *Salmonella* Typhi gene using one million transposon mutants

Gemma C. Langridge,[1,6] Minh-Duy Phan,[1,6] Daniel J. Turner,[1,6] Timothy T. Perkins,[1] Leopold Parts,[1] Jana Haase,[2] Ian Charles,[3] Duncan J. Maskell,[4] Sarah E. Peters,[4] Gordon Dougan,[1] John Wain,[5] Julian Parkhill,[1,7] and A. Keith Turne

Genome-Wide Identification by Transposon Insertion Sequencing of *Escherichia coli* K1 Genes Essential for *In Vitro* Growth, Gastrointestinal Colonizing Capacity, and Survival in Serum

Alex J. McCarthy,[a]* Richard A. Stabler,[b] Peter W. Taylor[a]

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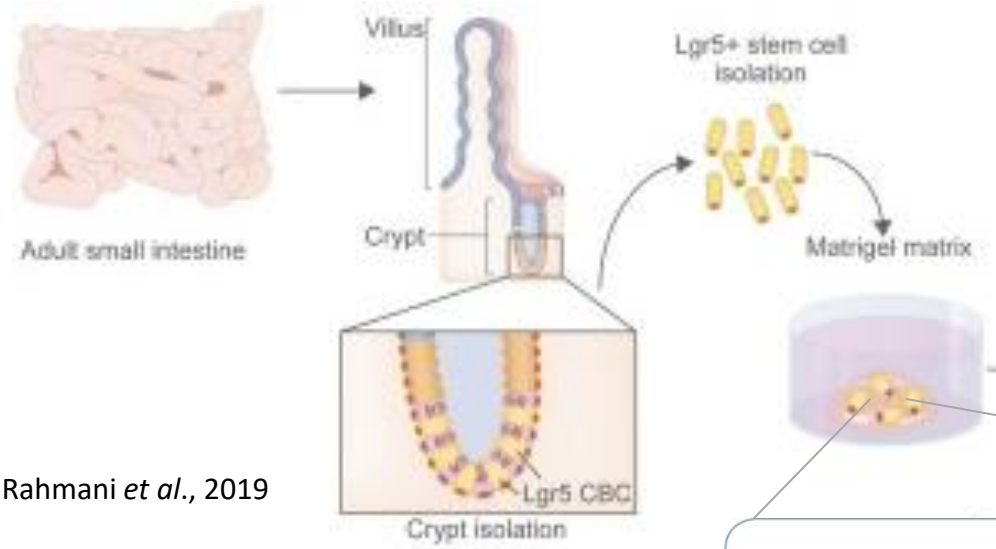
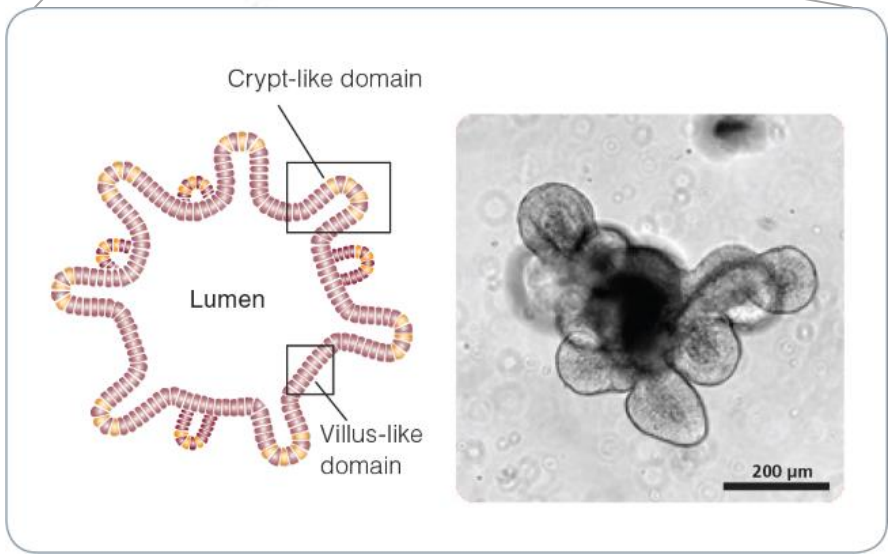
Intestinal organoids: promising and unprecedented new tool



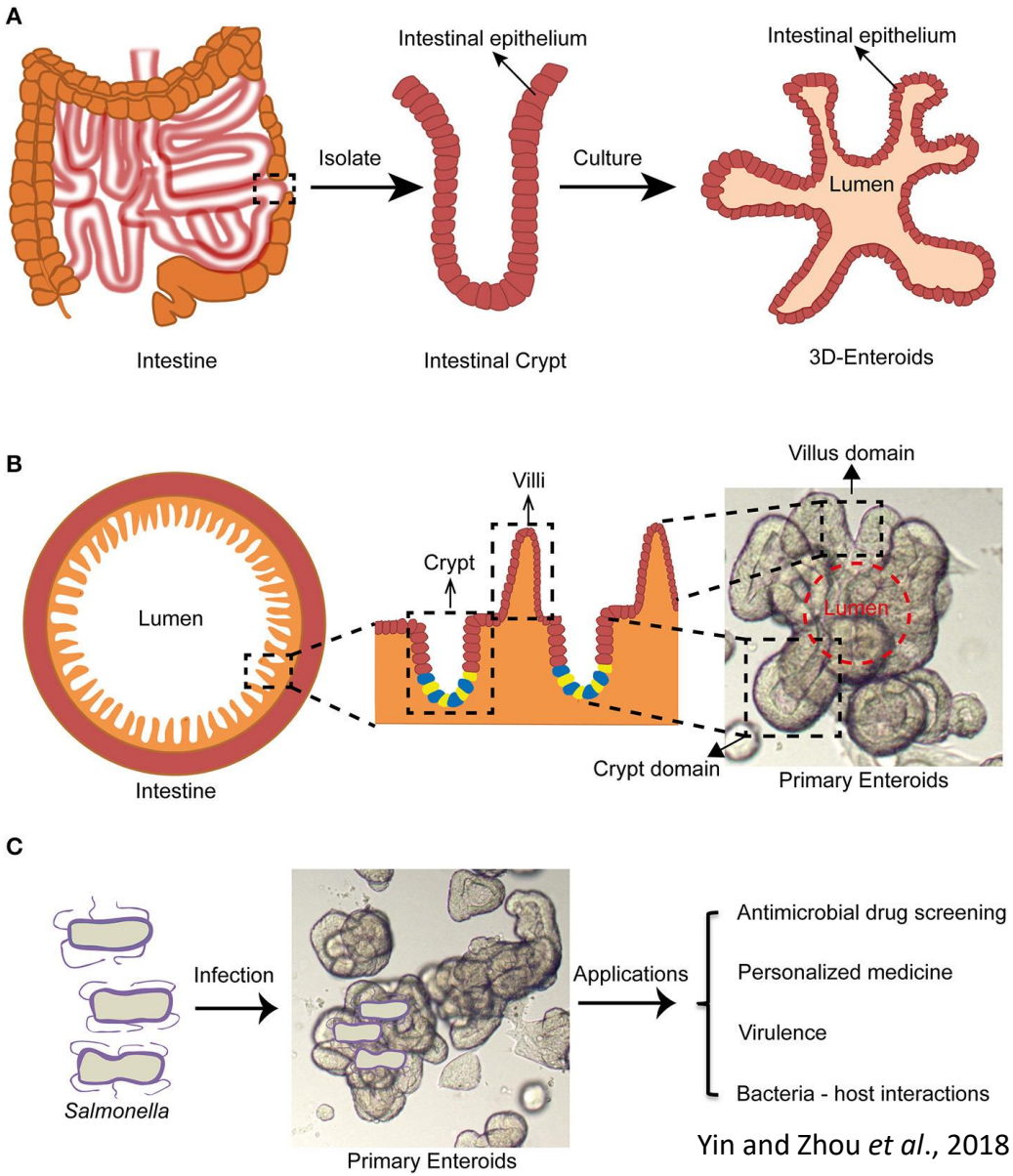
Rahmani *et al*., 2019

Organoids are stem cell-originated and self-organized 3D clusters of organ-specific cells capable of maintaining the functionality, molecular and cellular heterogeneity of the originating organ.

Intestinal organoids or mini-gut derive from *ex vivo* culture of intestinal stem cells and are capable of closely replicating the structure and cellular composition of a functional native intestinal epithelium.



Enteroid model and its potential application in studying host-pathogen interaction



Yin and Zhou *et al*., 2018

Enteroids are emerging as effective infection models due to their closeness in mimicking the infected tissues/organs.
They have been used successfully to explore bacterial pathogeneses

For instance, it has been shown that *Salmonella* quickly attaches and invades the enteroids causing the typical morphologic changes of the host cells during *Salmonella* invasion as well as the disruption of epithelial tight junctions.

nature genetics

Article

https://doi.org/10.1038/s41588-025-02218-x

A scalable gut epithelial organoid model reveals the genome-wide colonization landscape of a human-adapted pathogen

Received: 24 July 2024

Accepted: 1 May 2025

Published online: 12 June 2025

Check for updates

Maria Letizia Di Martino[1], Laura Jenniches[2], Anjeela Bhetwal[1], Jens Eriksson[1], Ana C. C. Lopes[1], Angelika Ntokaki[1], Martina Pasqua[1,11], Magnus Sundbom[3], Martin Skogar[3], Wilhelm Graf[3], Dominic-Luc Webb[4], Per M. Hellström[4], André Mateus[5,6], Lars Barquist[2,7,8,9] & Mikael E. Sellin[1,10]

Studying the pathogenesis of human-adapted microorganisms is challenging, since small animal models often fail to recapitulate human physiology. Hence, the comprehensive genetic and regulatory circuits driving the infection process of principal human pathogens such as *Shigella flexneri* remain to be defined. We combined large-scale *Shigella* infections of enteroids and colonoids with transposon-directed insertion sequencing and Bayesian statistical modeling to address infection bottlenecks, thereby establishing the comprehensive genome-wide map of *Shigella* genes required to infect human intestinal epithelium. This revealed the *Shigella* virulence effectors essential for epithelial cell colonization across geometries and intestinal segments, identified over 100 chromosomal genes involved in the process and uncovered a post-transcriptional mechanism whereby tRNA-modification enzymes and differential codon usage exert global control of a bacterial virulence program. Our findings provide a broadly applicable framework for combining advanced organotypic tissue

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