Site-nonspecific mutation

- Chemical, physical, and insertional mutagen
  - ✓ How do those mutagens create mutations?
  - ✓ What are characters of the induced mutations
  - ✓ How to use the induced mutations to study gene's function
    - Reverse genetics
    - Forward genetics
  - ✓ How to create gene-index catalogue for induced mutants (locate mutation sites for all mutants)

Gene-indexed catalogue of physical and chemical mutagens induced mutants

- Whole genome sequencing
- Exome capture sequencing
  - Exome is all exons of protein coding genes in a genome
  - Exome capture sequencing is a technique for sequencing all exons of the protein coding genes in a genome

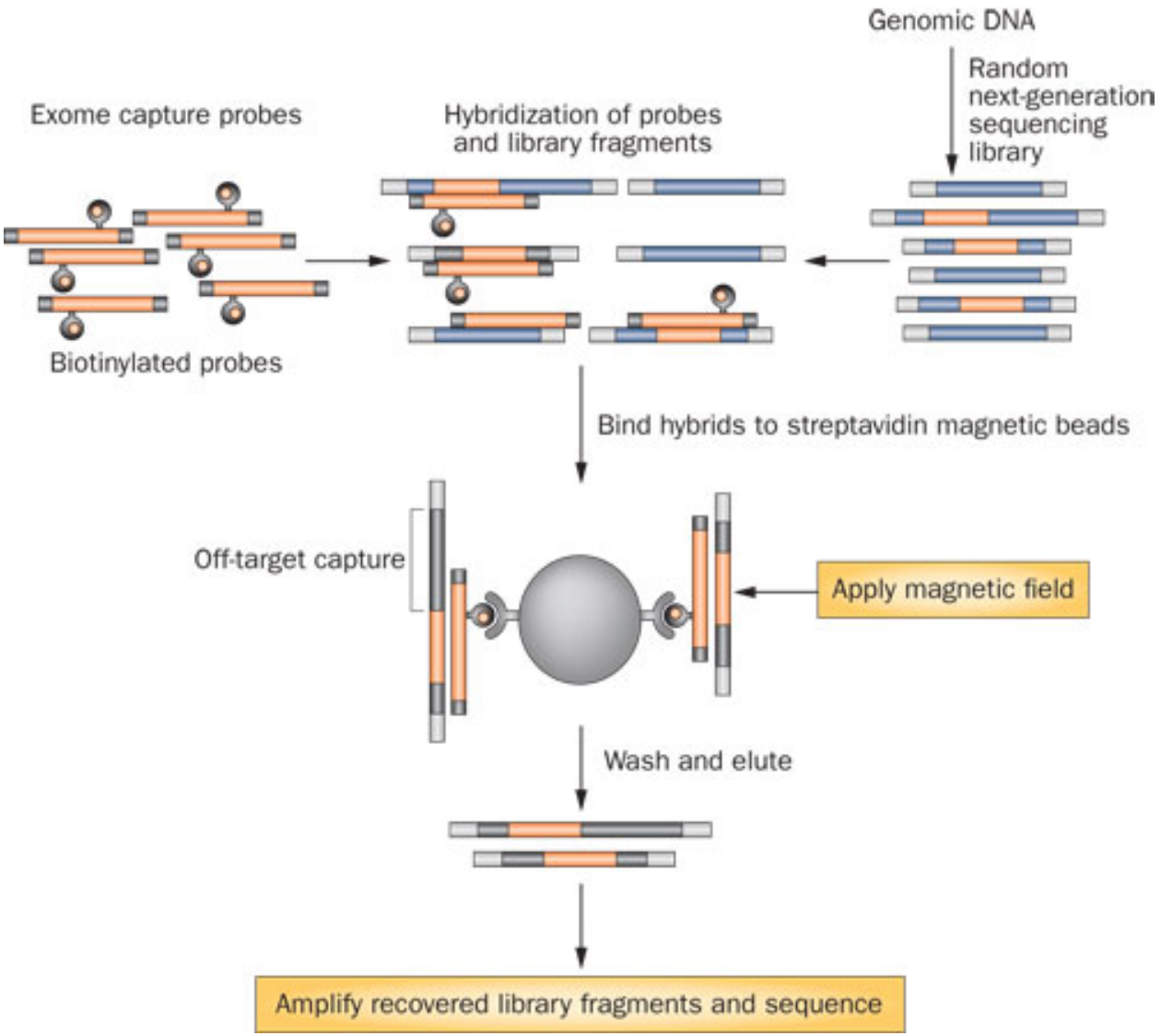
Whole-genome sequencing (WGS) or exome capture sequencing (ECS)

$2000/lane of Illumina HiSeq2000, 200 million reads of SE-100

Species	Analysis	Size of Target (Mb)	Reads for 20× Coverage (Million)	Capture Cost ($)	Total Cost ($)
Most plant species	**ECS**	**40**	**20**	**40**	**307**
Arabidopsis	WGS	120	12	0	180
Rice	WGS	380	38	0	507
Tomato	WGS	900	90	0	1,200
Maize	WGS	~2,300	230	0	3,067
wheat	WGS	~15,960	1,596	0	21,280

Henry et al., The Plant Cell, 2014

Exome capturing



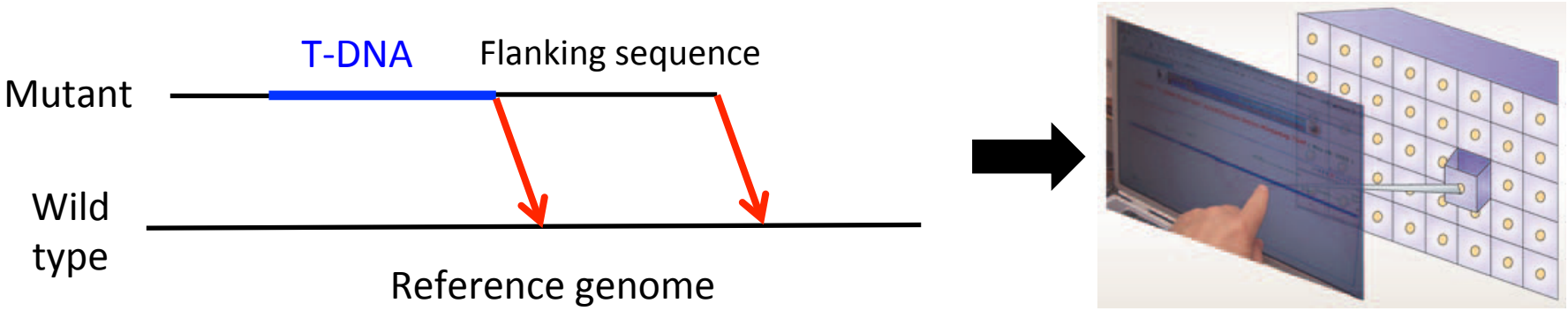
Exome capture sequencing of wheat EMS-induced mutants

- A wheat exome capture platform was developed and used to sequence the coding regions of 2,735 wheat mutants
- The targeted exon space in wheat was selected
  - An 84-Mb exome capture assay including overlapping probes covering 82,511 transcripts
- Over 10 million mutations were identified from the 2,735 mutants
- On average, 2,705-5,351 mutations per mutant, 35–40 mutations per kb

Krasileva et al., PNAS, 2017

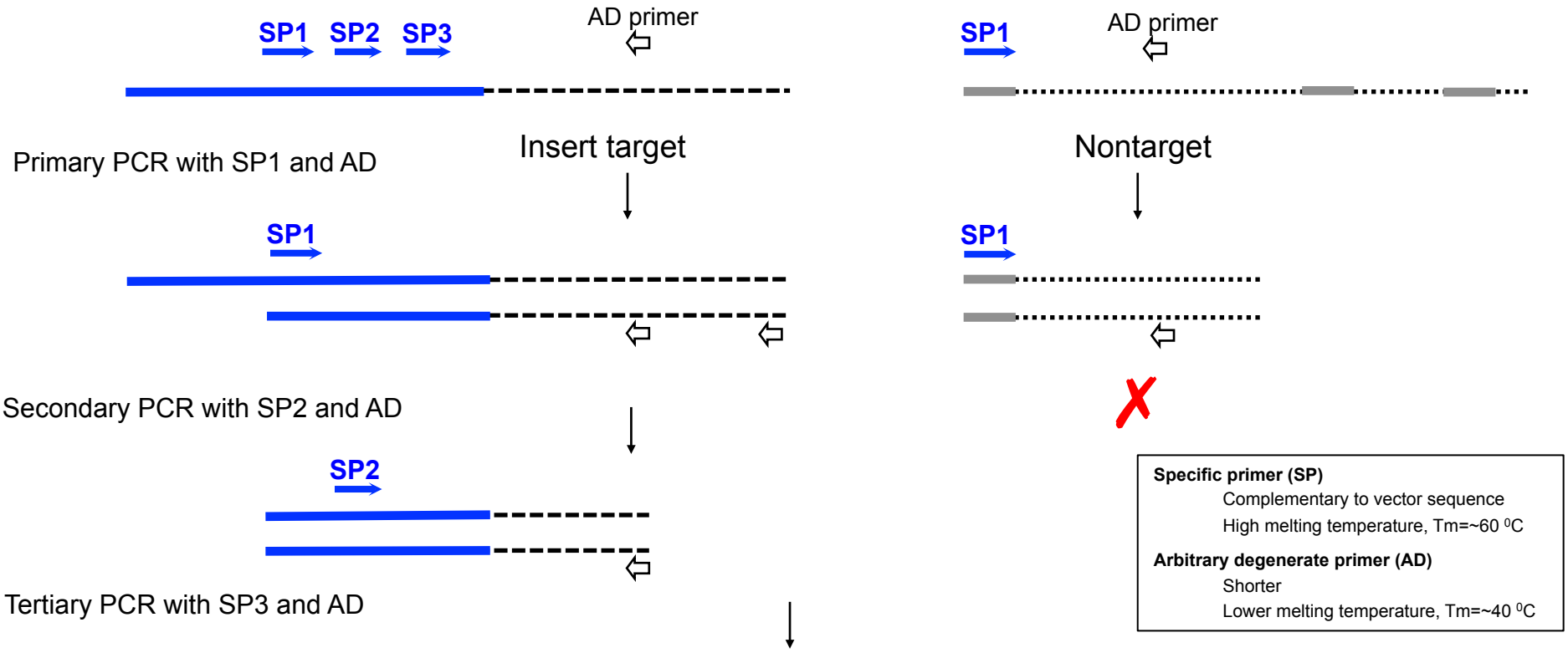
Gene-index catalogue for T-DNA insertional mutants

- Hundreds of thousands of T-DNA insertional mutants available in Arabidopsis
- A method to locate T-DNA inserts in all mutants is to get flanking sequences of the insertion site and then align the flanking sequences to reference genome



Thermal asymmetric interlaced PCR (TAIL-PCR) to obtain flanking sequences

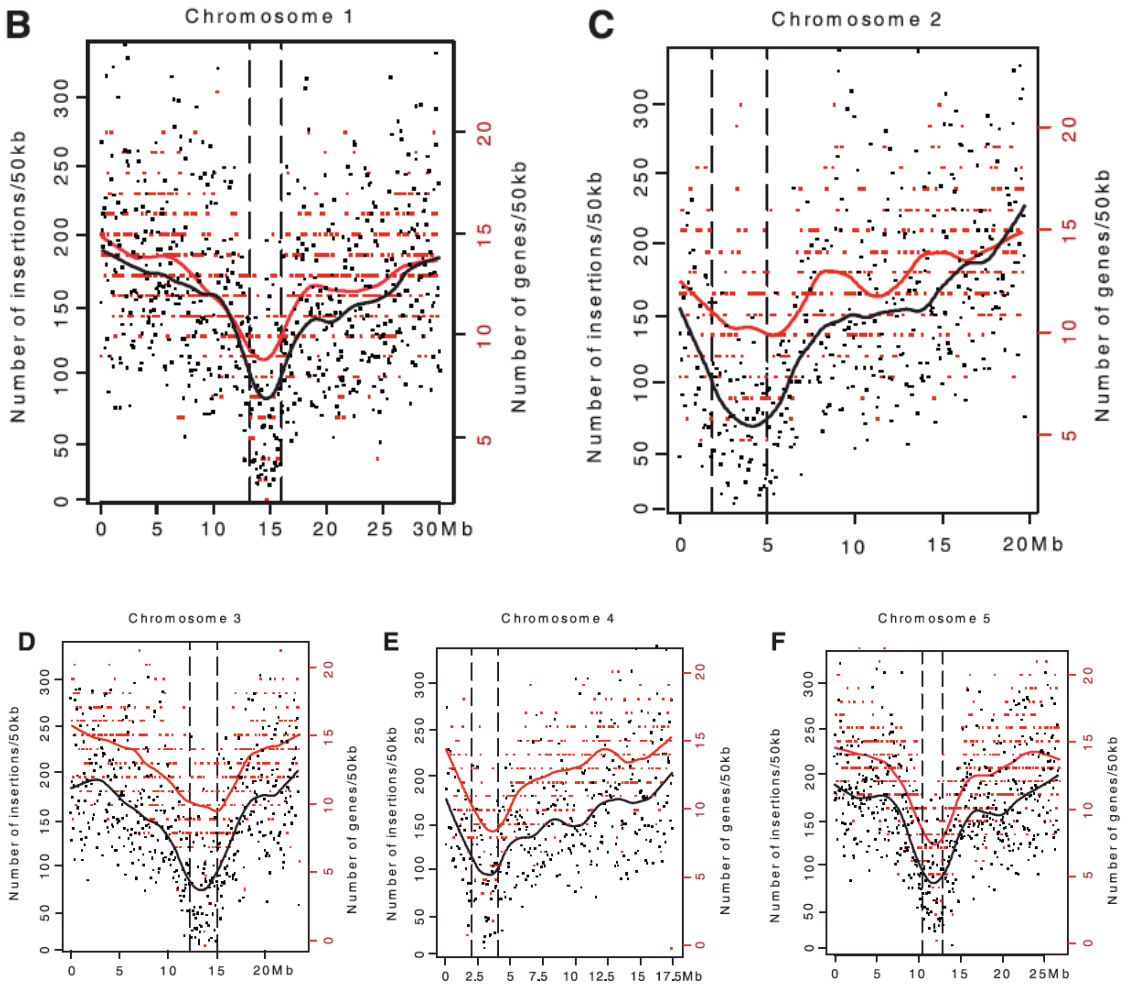
TAIL-PCR: Three PCR reactions are carried out sequentially to amplify target sequences using nested T-DNA-specific primers (SP1-3) on one side and an AD primer on the other side



Liu et al., Plant Journal 1995

Example: A gene-indexed catalogue of insertion mutants in Arabidopsis

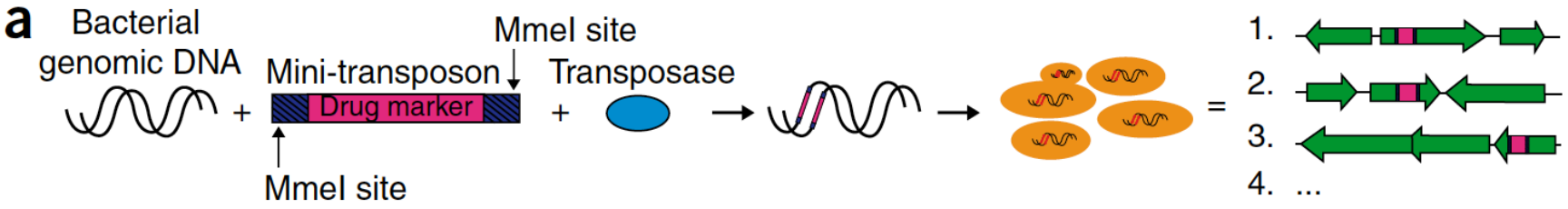
- Screened 127,706 mutants using TAIL-PCR
- 88,122 insertion sites identified in 21,799 genes
- Fewer T-DNA integration events were consistently observed in regions surrounding each of the five centromeres



Alonso et al., Science 2003

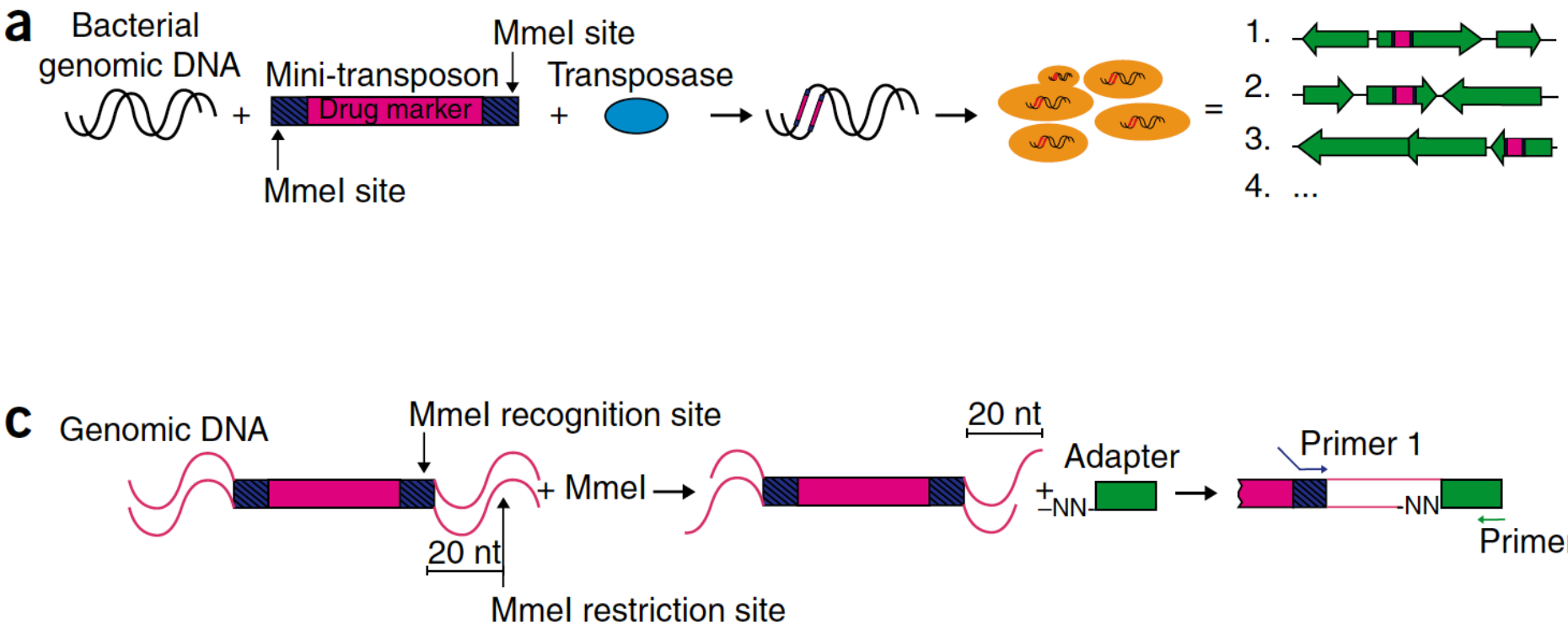
Create an insertional mutation library of *S. pneumoniae* using mini-transposon

- A gene disruption library is constructed by first transposing the mini-transposon into bacterial genomic DNA **in vitro** and then transforming a bacterial population with the transposed DNA
- The transpose inserts randomly in the genome, requiring only a TA dinucleotide at the insertion site



van Opijnen et al., Nature methods 2009

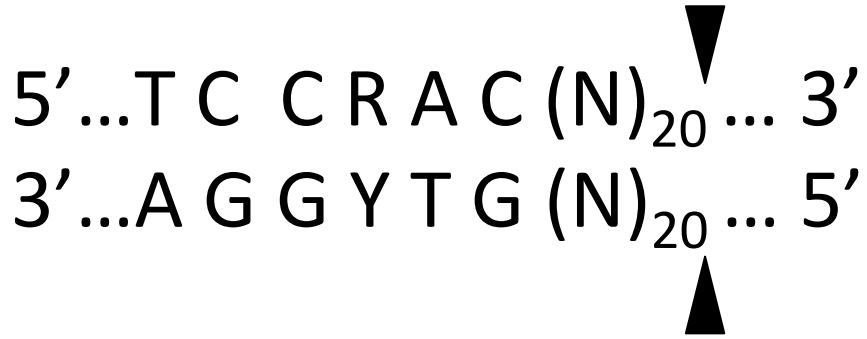
Locate insertional sites in an insertional mutation library of *S. pneumoniae*



van Opijnen et al., Nature methods 2009

Restriction enzymes recognize specific sequence

- Restriction enzymes is a protein that can cleave DNA or RNA into fragments
- Restriction enzymes recognize a specific sequence and cut the DNA within, near or remote from the specific site
- For example, *MmeI* recognizes a specific sequence of 6 bp



What need to know for final exam

- Can describe exome capture sequencing
- Can describe how to use TAIL-PCR to obtain flanking sequences of inserts and locate T-DNA insertional sites for a large number of insertional mutants

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