

Interplay between Mobile Genetic Elements and Nucleoid Associated proteins

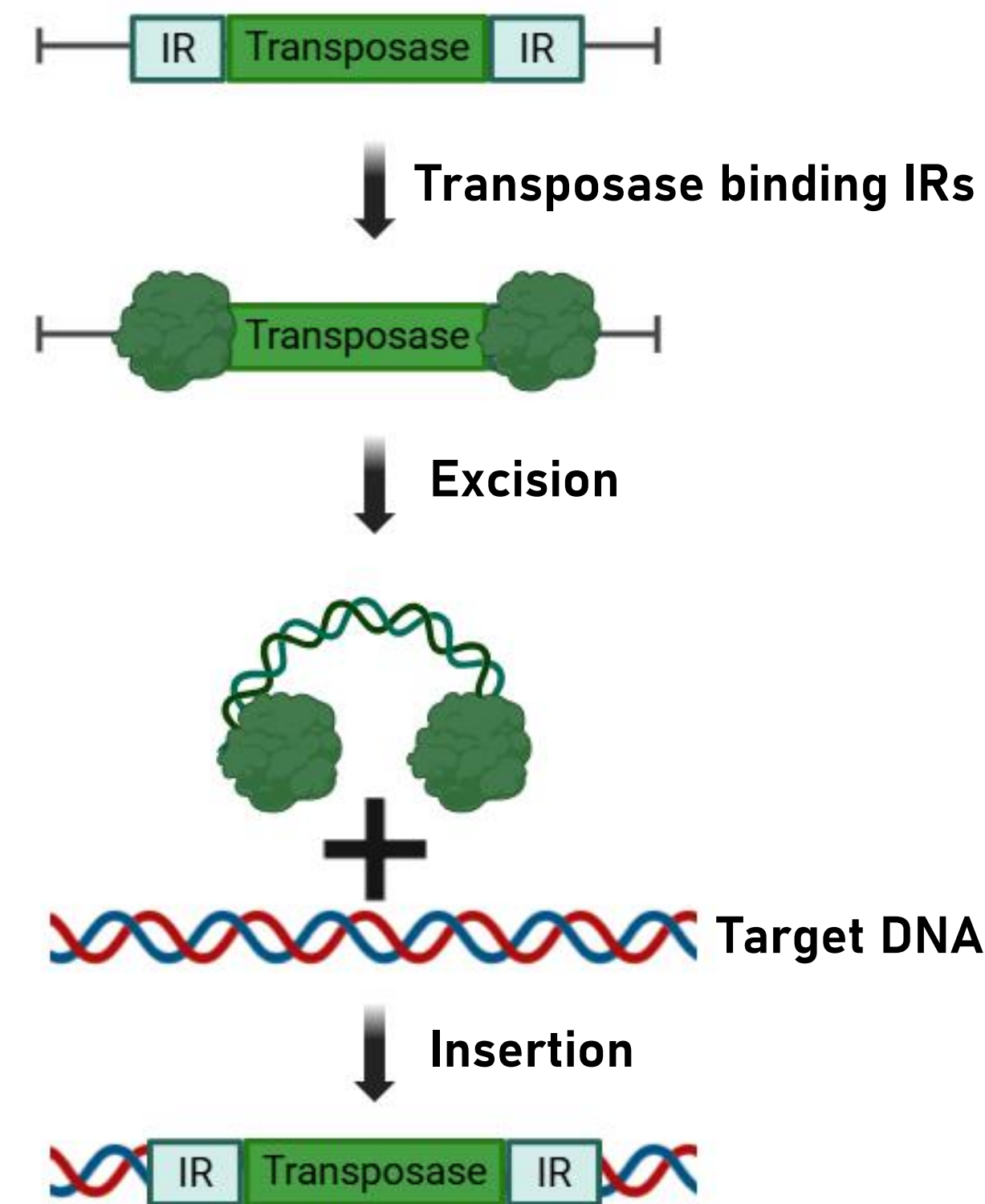
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Abstract :

Mobile genetic elements (MGEs) such as transposons and insertion sequences are major drivers of bacterial evolution but also represent an important source of genome instability causing DNA damage, genomic rearrangements or even cell death [1]. Recent studies suggest that nucleoid-associated proteins (NAPs) in addition to compact DNA inside the cells can act as transcriptional regulators forming heterochromatin-like domains over the bacterial chromosome [2]. Interestingly, MGEs are enriched among heterochromatin structures, preventing their toxic expression [3]. In addition to being solely silencer of foreign DNA, we propose that bacterial heterochromatin/NAPs actively shapes MGEs genomic distribution in the bacterial chromosome by directing MGEs insertion.



In vivo transposition

H-NS increases Tn5 transposition rate in *E. coli*

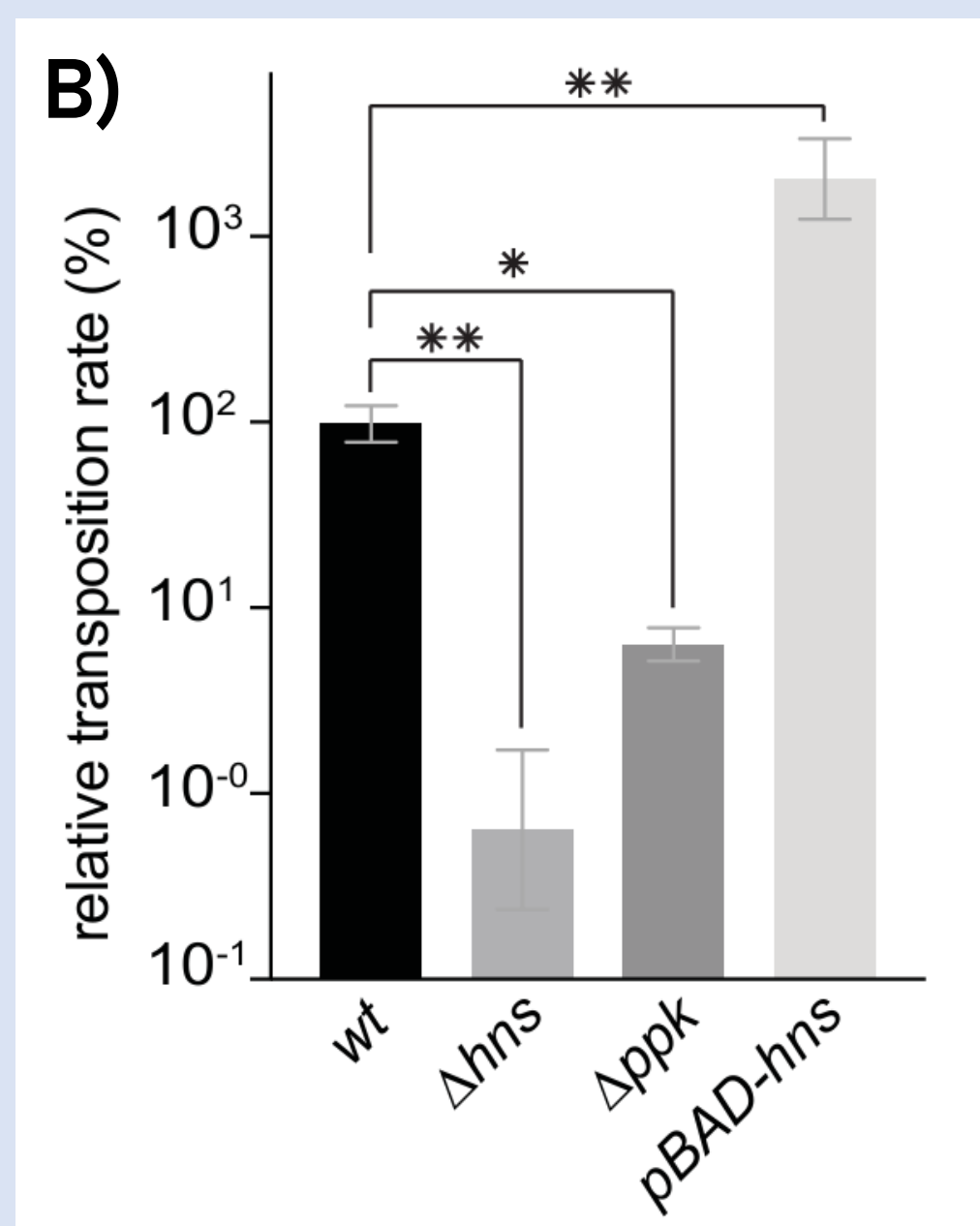
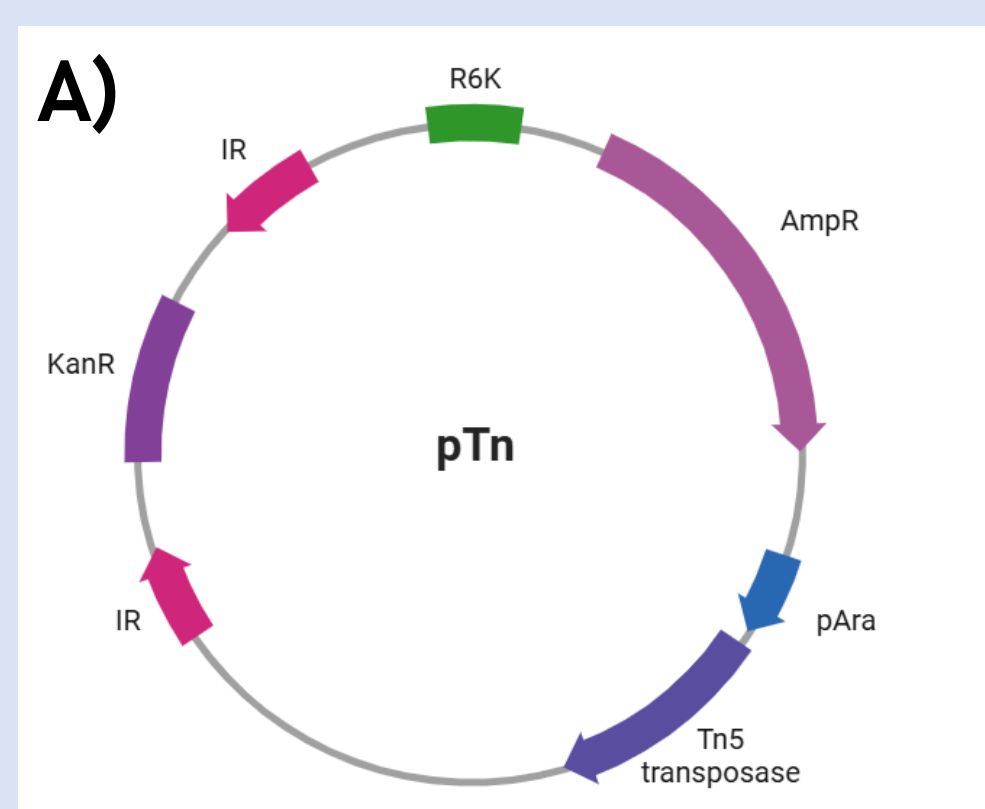


Fig.1 : A) Plasmid (pTn) containing the mini-Tn5-Kan construct, the Tn5 transposase under the control of an arabinose inducible promoter and a non-replicative R6K origin of replication in our *E. coli* strain. B) Transposition events from pTn to the genome were assessed on LB Kan plates and transposition rates were extracted by normalizing this number by the number of transformants with a pBAD-kan transformation control. The wt transposition rate was set to 100%. The transposition rate was assessed in a Δhns , Δppk and a *hns* overexpression genetic background (n=3).

Next step

Perform this assay on all Δ NAPs genetic backgrounds to assess how they impact Tn5 transposition *in vivo*

In vitro transposition

In vitro Tn5 transposition on a plasmid

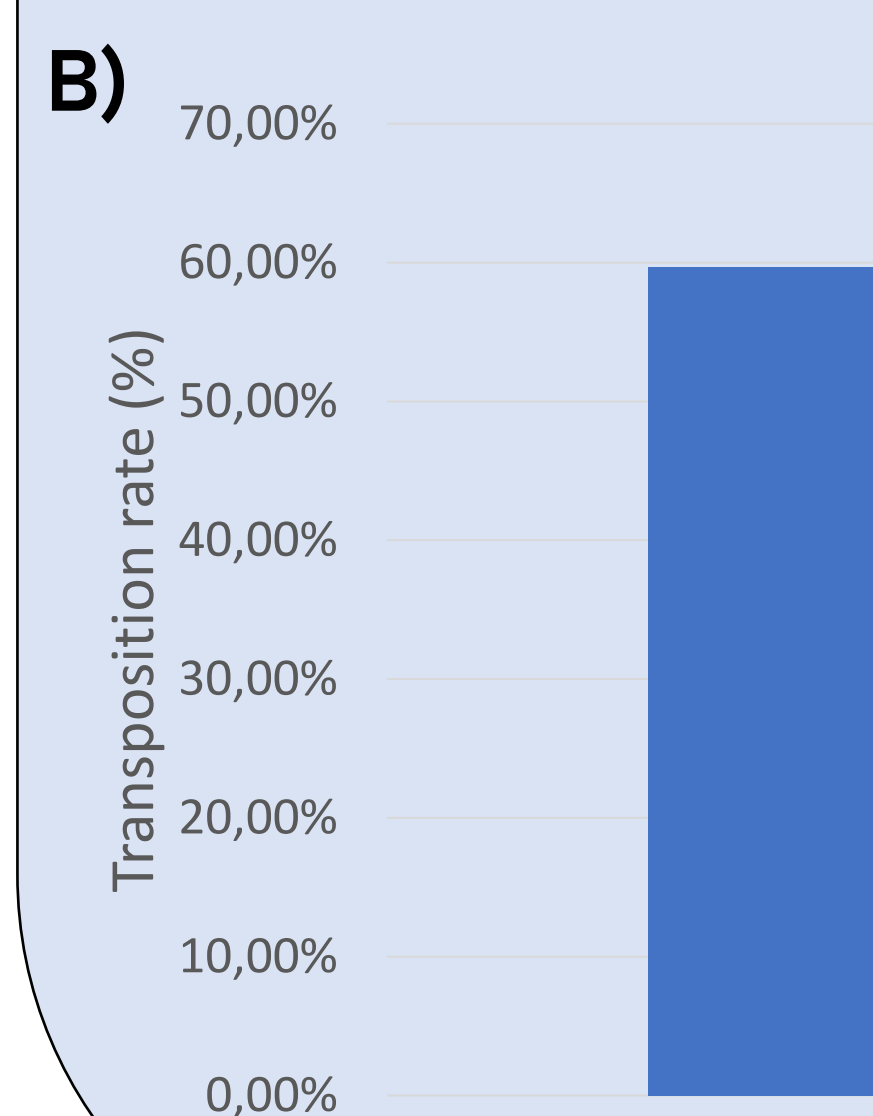
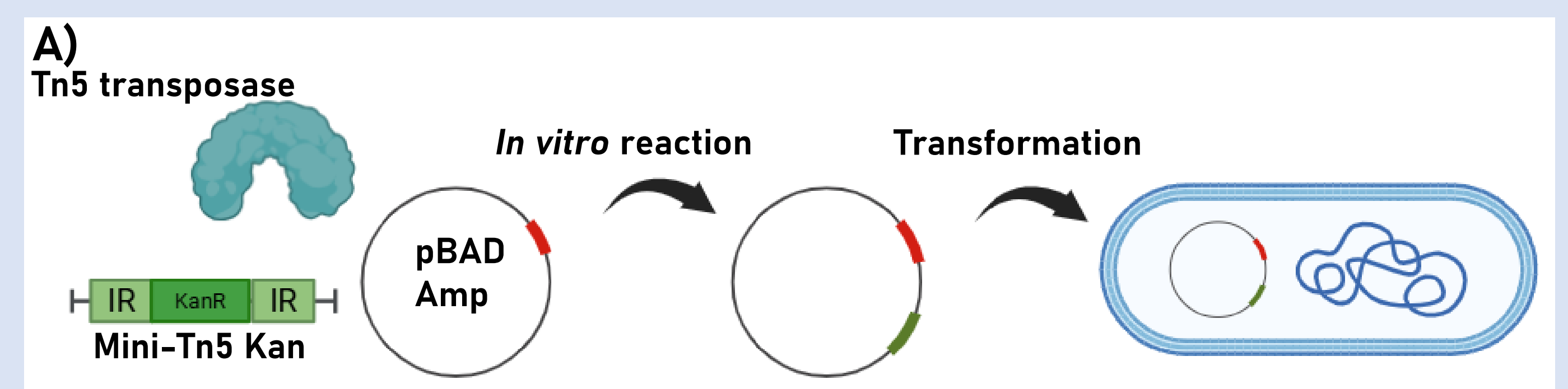


Fig.2 : A) *In vitro* reaction was set up by mixing purified Tn5 transposase, a mini-Tn5 transposon and a target DNA, pBAD-Amp. The reaction was performed at 37 °C for 2h and stopped with 1% SDS together with heat at 70 °C for 10min. Next, the mixture was dialyzed and transformed into competent *E. coli* cells. B) Transposition events in the plasmid were assessed on LB Kan plates and normalized with the number of transformants on LB Amp plates. The transposition rate was then plotted on a graph.

Next step

Perform this assay with purified NAPs and catalytic mutants version of the transposase

Bar-coded Tn-Seq

Insertion sites and their level of expression of Tn5 transposon insertions, whole genome scale

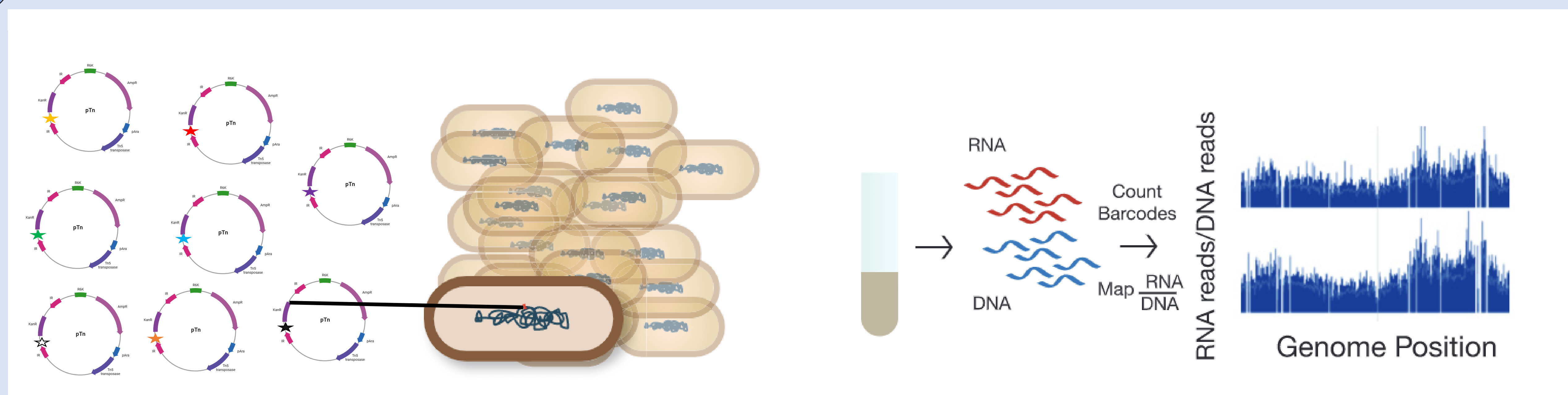


Fig.3 : To generate the barcoded library, random 15bp barcodes are inserted between Tn5's IRs into pTn. Constructs are electroporated into cells and barcoded Tn5 randomly integrated into the *E. coli* genome. Classic Tn-seq methodology identifies insertion frequency for each unique Tn insertion or our library, genes or defined base pair stretch across the genome. In addition, to measure transcriptional levels from each site, total RNA and DNA are extracted and the RNA:DNA ratio for each barcode is mapped to their corresponding genomic locations (Adapted from [4])

Next step

Perform this assay on NAPs deletions candidates (H-NS) and in Δppk genetic backgrounds

Conclusion :

- H-NS NAP seems to promote Tn5 transposition *in vivo*, other NAPs are currently being assessed
- The *In vitro* transposition assay is working well, NAPs such as H-NS are under purification process
- Barcoded Tn5 transposon library on the making