a keen eye to the complex scenario, and they make reasonable evaluation while recruiting new faculty. Whereas, in most other places, the candidacy evaluation by concerned authorities seems to be a mosaic in their peering of the number of publications and JIF within a major discipline, e.g. cell biology, neurobiology, ecology, evolution, etc. Perhaps, such mosaic evaluation may (partially) exist even at higher levels while rewarding research excellence within a major discipline[6]. At the outset, the evaluation of science is complex and it begs an eagle's eye-view approach, as suggested by Gadagkar[1] and Balaram[4].

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Recombineering: potential for generation of live attenuated bacterial strains

Infectious diseases remain one of the largest killers of mankind. The development and availability of a number of vaccines against the viral and bacterial pathogens has reduced mortality a great deal. Eradication of smallpox and near eradication of poliomyelitis had only been possible due to the availability of good vaccines and effective immunization. Bacterial diseases such as tetanus and pertussis could be controlled due to active immunization. Among the different types of vaccines, live attenuated remain one of the most effective vaccines due to their ability to elicit both humoral and cellular immune response[1].

Live attenuated bacterial strains have traditionally been generated by chemical mutagenesis or serial passage through non-permissive host. The early approaches lacked specificity and were replaced by techniques of reverse genetics. The method required better understanding of pathogenic mechanisms and genome sequencing; and targeted inactivation or deletion of the virulence-associated genes. We know that the bacterial pathogens owe their disease-causing ability to the presence of virulence traits encoded by the various genes present either on chromosomes or plasmids. Typically, the techniques of reverse genetics or classical allelic exchange system involved engineering recombinant-replication defective plasmids (suicide vectors) to contain long stretches of homologous DNA sequences flanking the genes to be deleted. Construction of such recombinant plasmids requires extensive *in vitro* manipulations. Hence, the method is laborious and time-consuming. However, it had been extremely useful in generating mutants of various bacterial species.

Since the late 1990s, new *in vivo* technologies of homologous recombination have emerged that have greatly simplified the process of target gene deletion. Among these, one of the most promising techniques is 'recombineering', which utilizes the lambda recombination proteins Bet, Exo and Gam, encoded by 'red' genes and linear ds or ss DNA with short homologies to the target gene[2,3]. The Gam protein prevents degradation of the linear ds DNA from recBCD or SbcCD of host bacteria, whereas Exo and Bet help in the homologous recombination of the linear substrate. The linear ds DNA can easily be generated by PCR and requires as little as 50 nt homology to the target gene. The red genes of bacteriophage lambda can be cloned on the plasmid under control of heterologous, inducible promoter and their expression can be regulated by temperature or arabinose. Recombineering has several advantages over the classical allelic exchange system, e.g. greater ease of the technique, increased recombination efficiency, smaller region of homology and most importantly, the technique requires less time.

Recombineering has been used to modify the genomes of *Escherichia coli*, *Shigella*, *Yersinia*, *Salmonella*, and enteropathogenic and enterohaemorrhagic *E. coli*[3–6]. These studies have used homologies of 35–500 nt to engineer the target gene deletion and generate a candidate prototypic live, attenuated vaccine strain. In our experience, we have found that homologies of about 300–400 nt were required to make a *phoP–phoQ* null mutant of *Yersinia pseudotuberculosis*[7]. The mutant with defined genetic lesions can be made targeting either the virulence or the regulator gene(s). Many of the live, attenuated prototypic vaccine strains generated by recombineering look to be promising for their vaccine potential[4,5]. With the option of gene manipulation even in plasmids, as may be applicable in *Yersinia pestis*, recombineering can be a promising technology for producing the prototypic live attenuated bacterial vaccine strains in future.

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