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Division of Dockets Management (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Rm. 1061
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Re: Request for comments concerning (Feb 2005) FDA Guidance for Industry Considerations for Plasmid DNA Vaccines for Infectious Disease Indications

Dear Dr. Smith:

I would like to call your attention to some recent work from my laboratory involving the ubiquitous translocation of mobile DNA elements known as IS sequences normally found within the chromosome of *E. coli* into isolated plasmid DNA. This is of particular relevance in light of recent proposals to use plasmid DNA produced in laboratory *E. coli* as human therapeutics. Hopefully this Request for Comment is an appropriate venue for sharing these observations.

Enclosed is a report from the May 19, 2006 issue of Science that documents our experiments showing transfer of Insertion Sequences (IS) elements normally found in the *E. coli* chromosome into plasmids produced under a number of different conditions. Our results indicate this is a very common event and in fact, the only completely IS free plasmid preparations we have encountered are those produced from *E. coli* host strains specifically engineered to lack all endogenous IS elements. Other academic groups are currently finishing manuscripts that confirm and extend much of the work discussed in our report and, if appropriate, I will forward these to you as they become available.

You or the appropriate member of your staff may find this material relevant in light of its significance to the purity and homogeneity of plasmid DNA produced in *E. coli* strains containing IS elements. In addition, you might want to consider the hypothetical implications of inadvertently introducing functional transposable sequences such as IS elements into human subjects.

IS Elements as a potential problem for DNA therapies

Fig 1 shows a hypothetical potential route by which IS transposable elements from the *E. coli* genome (red arrow) could make their way into plasmid DNA and thence into a human via injection of a DNA therapeutic agent, followed by transposition into a human chromosome.

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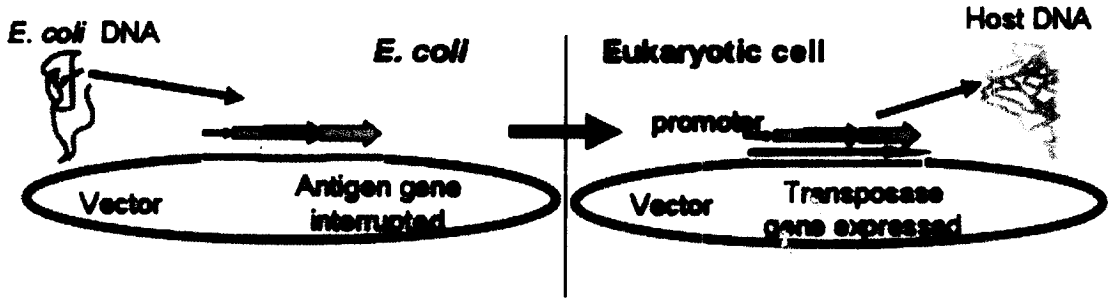

Hypothetical Scheme

The step of activation of a bacterial transposase in a human cell has never been demonstrated experimentally, but it is theoretically possible. Many cases of bacterial marker gene expression (eg GFP, lac Z, neomycin resistance) are well known.

Numerous instances of transposable elements (mostly IS186, IS2 and IS5) have been reported in earlier drafts of the human genome sequence;but this is most likely due to transposition from the *E. coli* production host to the bacterial artificial chromosome (BAC) clones used for sequencing. Many more non human examples are listed in the GenBank database of eukaryotic genes.

The presense of transposases in normal *E. coli* raises concerns about the true identity and purity of plasmid material produced *in E. coli*, not to mention the cost incurred to meet quality assurance and regulatory guidelines in the case of industrial production. Since this possibility is completely avoidable by the use of IS free *E. coli* we suggest this be evaluated experimentally as a part of the FDA process.

I believe these concerns speak directly to the current recommendations within the Guidance for Industry document that Master Cell Banks and Working Cell Banks be free from bacteriophage and other adventitious agent contamination, and that the genetic stability of the MCB and WCB are well established. However there is no particular specific recommendation to control IS element contamination of plasmids contained within the cells, which is very relevant to the purity and possibly even the safety of such materials.

If you would like to discuss this matter in further detail please feel free to contact me at the address below.

With best regards,

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enc: Blattner et al, 2006
Science