Elucidating the molecular basis for I-Pcal toxicity in E.coli

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I-SceI and I-PcaI are homologous homing endonucleases that recognize the same 18 bp target site for cutting DNA. However, expression of I-PcaI shows toxicity in E.coli that is not observed with I-SceI. We hypothesize that the toxicity of I-PcaI expression is due to decreased specificity for its target site, resulting in more genomic cuts compared to I-SceI. Pulse field gel electrophoresis (PFGE) was employed to visualize large genomic DNA fragments created by digestion with I-PcaI and I-SceI. Thus far, results have shown resolution of uncut E.coli genomic DNA as well as the ability to digest a lambda DNA marker with the restriction enzyme XbaI. However, digestion of E.coli genomic DNA and Salmonella ser. Braenderup standards have yet to be visualized using PFGE, prompting the need for troubleshooting of the current methodology. Troubleshooting experiments have shown that a new proteinase K reagent and longer cell lysis incubations may aid in this matter. Once consistent visualization of digested genomic DNA fragments occurs, amplification and sequencing of the fragments can be done for analysis and comparison. The model of I-PcaI’s lower specificity will be verified if it generates more genomic fragments than I-SceI, providing a possible explanation for its toxicity.