To properly assess the size of the xpC pool, we found it was necessary to plate on selective media both pre-and post-Car selection. We suspect the MegaX E. coli cells are transformed with multiple plasmids and do not segregate them properly; thus the need to distinguish between cells that carry Tmp^R and Car^R on a single plasmid (as desired), and those that carry it on separate plasmids. Because of the possibility the strain carries multiple plasmids, co-selection with Tmp and Car may overestimate the true number of mutagenized expression plasmids in the pool (as counted from Iso+Tmp+Car plates). Theoretically, by performing the Car and Tmp selection sequentially, we minimize the selective pressure for MegaX cells to maintain multiple plasmids.

First, to assess the transformation efficiency, recovered cells were plated (prior to Car selection overnight) and found to contain 5x10^6 Car^R colonies. This represents the number of independent expression vectors (mutagenized or not) in the xpC pool. Second, to determine how many of these Car^R colonies are also Tmp^R, we took a portion of the cells from the xpC pool (post-Car selection) onto LB+Car plates, and then replica plated to various selective media to determine the precise drug-resistance of each colony (Table S5).

In detail: cells from the xpC frozen stock were resuspended in PBS, diluted, and plated to get roughly 200 colonies per LB+Car plate; 4 LB+Car plates were used. These plates were grown overnight at 37°C. Each LB+Car plate was then replica plated (in order) to LB+Kan, Iso+Tmp+Kan, Iso+Tmp, Iso+Tmp+Car, and LB+Car plates, and grown at 37°C overnight. The total number of colonies were counted on each plate. Additionally the Iso+Tmp+Car and Iso+Tmp+Kan were compared to identify any colonies resistant to Tmp, Car, and Kan. This would suggest a single cell contains two plasmid populations, indicating sub-optimal plasmid segregation in MegaX cells.

Calculations for drug resistance of colonies were performed as follows:

\[ \text{Tmp}^\text{R} \text{Kan}^\text{R} \text{Car}^\text{R} \text{colonies} = \# \text{colonies on Iso+Tmp+Kan plate (is Car}^\text{R}, \text{based on growth on source plate).} \]

\[ \text{Tmp}^\text{R} \text{Kan}^\text{R} \text{colonies} = \text{(colonies on Iso+Tmp+Kan plates)} - \text{( \# of Tmp}^\text{R} \text{Kan}^\text{R} \text{Car}^\text{R} \text{colonies)} \]

\[ \text{Tmp}^\text{R} \text{Car}^\text{R} \text{colonies} = \text{(colonies on Iso+Tmp+Car plates)} - \text{( \# of Tmp}^\text{R} \text{Kan}^\text{R} \text{Car}^\text{R} \text{colonies)} \]

\[ \text{Kan}^\text{R} \text{Car}^\text{R} \text{colonies} = \text{( \# colonies on LB+Kan plate)} - \text{( \# of Tmp}^\text{R} \text{Kan}^\text{R} \text{Car}^\text{R} \text{colonies)} - \text{( \# of Tmp}^\text{R} \text{Kan}^\text{R} \text{colonies)} \]

\[ \text{Car}^\text{R} \text{only colonies} = \text{( \# colonies on LB+Car plate)} - \text{( \# of Tmp}^\text{R} \text{Kan}^\text{R} \text{Car}^\text{R} \text{colonies)} - \text{( \# of Tmp}^\text{R} \text{Car}^\text{R} \text{colonies)} - \text{( \# of Kan}^\text{R} \text{Car}^\text{R} \text{colonies)} \]
See results of calculations in Table S5. The correction factor was calculated as the Tmp\(^{\text{r}}\)Car\(^{\text{r}}\)/ (total Car\(^{\text{r}}\)), as we selected for only Car\(^{\text{r}}\) in the culture and we can assume we counted the LB + Car plates accurately. “total Car\(^{\text{r}}\)” would be the sum of (Tmp\(^{\text{r}}\)Kan\(^{\text{r}}\)Car\(^{\text{r}}\)), (Tmp\(^{\text{r}}\)Car\(^{\text{r}}\)), (Kan\(^{\text{r}}\)Car\(^{\text{r}}\)) and (Car\(^{\text{r}}\) only) colonies. We found that an average 5% of the Car\(^{\text{r}}\) colonies in the xpC culture were actually Tmp\(^{\text{r}}\)Car\(^{\text{r}}\). Thus, 5% of the 5x10\(^6\) Car\(^{\text{r}}\) transformants counted would estimate our Tmp\(^{\text{r}}\)Car\(^{\text{r}}\) population to be 2.5x10\(^5\) Tmp\(^{\text{r}}\)Car\(^{\text{r}}\) colonies in the xpC pool, as shown in Table 1.