Quantification of Plasmid Copy Number of Escherichia coli (E. coli) Master Cell Bank by Digital PCR (dPCR)

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ABSTRACT

Accurate measurement of plasmid copy number (PCN) is highly desirable for *E. coli* master cell banks to understand the distribution of plasmids in daughter cells, and thus is crucial for plasmid DNA (pDNA) production by upstream processes. Here, we developed a fast and robust method utilizing dual channel dPCR to monitor the activity of designed promotors in DH5α cells. To prevent any bias toward genomic DNA and plasmid DNA generated by DNA isolation, no extra step of DNA purification was required for this assay. Simply boiling the bacterial cultures after a serial dilution, samples are then spun down to separate the supernatant (containing the genomic information), from the cell debris pellet. Primer-probe sets designed to target Kanamycin Resistance and *cysG* genes represent the plasmid DNA and the chromosomal DNA respectively. A restriction digestion is included prior to dPCR analysis to facilitate the partitioning of template DNA leading to precise quantification. Our results showed that fragmentation of the template DNA increased the plasmid/chromosome ratio at least 5-fold compared to reactions without enzyme treatment, thus accurately detecting the PCN. In conclusion, we present a simple and efficient method optimized for the accurate quantification of PCN by dPCR in *E.coli* master cell banks.

RESULTS

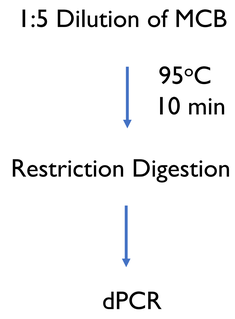

Figure 1. Simplified method for determination of plasmid copy number in a master cell bank by looking at the ratio of a plasmid DNA marker (*kanR*) to that of an *E. coli* chromosomal marker (*cysG*).

Target	*kanR* (copies/µL)	*cysG* (copies/µL)
Run 1	282.45	0
Run 2	295.25	0
Run 3	0.941	26585.55
Run 4	0	7970.8

Table 1. Copies/µL of *kanR* and *cysG* amplicons in two genetic backgrounds demonstrating specificity of the primer/probe sets used for each element. Run 1 and 2: plasmid DNA with *kanR* insert / Run 3 and 4: *E.coli* genomic DNA

Titer	Results (Copies/uL	
	Run # 1	Run #2
kanR MCB vial 1	5.19E+10	4.68E+10
cysG MCB vial 1	6.14E+08	6.24E+08
kanR MCB vial 2	8.62E+10	8.93E+10
cysG MCB vial 2	9.15E+08	8.42E+08

Table 2. Copies/µL of *kanR* and *cysG* elements in 2 vials of MCB

	Average	SD	%CV
PCN	89.76	13.38	14.91%

Table 3. Plasmid copy number across four runs (Fig. 1) of one vial of MCB demonstrating excellent reproducibility.

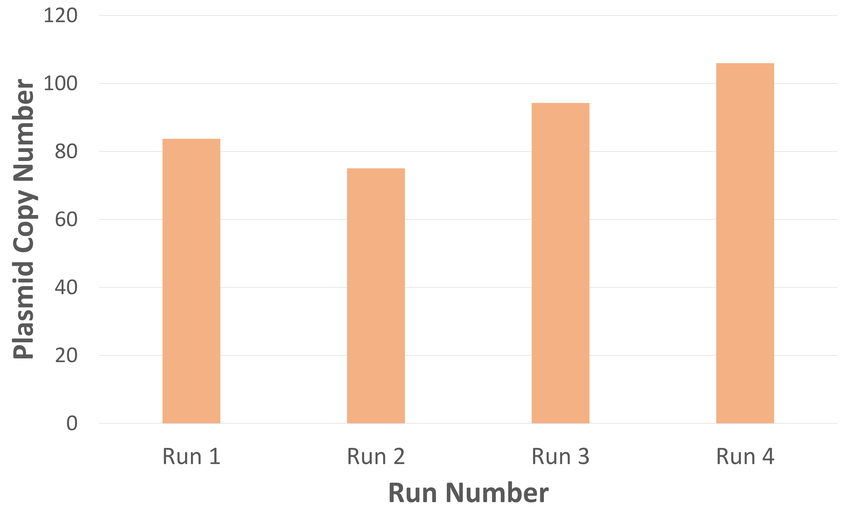

Figure 2. Plasmid copy number (*kanR* element) across 4 runs of MCB

SUMMARY

- We have developed a simplified workflow for PCN determination which requires two steps and dPCR absolute quantitation of MCB.
- We have shown the specificity of the primers and probes used in the assay to discriminate plasmid from E.coli chromosomal DNA
- The assay is robust and shows excellent reproducibility across runs with a CV of 14.91%