Application Note

Genomics

Agilent

Trusted Answers

Detection and Analysis of Restriction-Digested Plasmid Fragments

Authors

Chava Pocernich, Jolita Uthe, and Kyle Luttgeharm, Agilent Technologies, Inc.

Abstract

Molecular biology research, including synthetic biology, agrobiology, and systems biology applications often use cloning vectors in their workflows. Quality control analysis of the cloning vector containing the DNA insert of interest is an essential initial step ensuring downstream success. The DNA insert and cloning vector are typically evaluated by restriction digestion and separated by manual gel electrophoresis. An alternative to manual gel electrophoresis is the Agilent ZAG DNA Analyzer system (ZAG system), which was specifically designed for efficient and accurate automated fragment analysis. The ZAG system is ideal for high-throughput fragment analysis with user-specified flagging criteria to easily identify the presence of the DNA insert, eliminating manual analysis errors. To demonstrate the efficiency and accuracy of DNA insert assessment in cloning vectors, the ZAG system was utilized to identify the presence or absence of an insert in two different plasmids. The ZAG system provided essential quality control analysis of the DNA insert with accurate sizing and automated detection.

Introduction

Plasmids or vectors are utilized as a means of transportation for delivering and manipulating foreign DNA inside a host cell. The uses and applications of plasmids continue to expand into all areas of scientific research and are continuously undergoing advancements. Industries that routinely make use of plasmid-based research span from molecular biology, synthetic biology, and agrobiology to medicine, including a wide range of applications such as biofuels and fine chemical production, biosensors, bioremediation, and biomedical therapies[1].

Analysis and validation of DNA plasmids to confirm that they contain the sequence of interest are vital quality control steps that can provide insight into the production process. Fragment analysis will determine if the plasmid was properly constructed by detecting the presence or absence of the insert. A truncated insert or an insert that is longer than expected may suggest an issue in the cloning process. Quality assessment of the plasmid and insert can provide vital insight into the workflow, saving time by pointing the user towards the probable issue.

Screening large sample sets of plasmids is an extremely time-consuming and labor-intensive process typically involving manual agarose gel electrophoresis. This application note demonstrates high-throughput analysis of restriction digested plasmids with the ZAG DNA Analyzer system. Two commercially available plasmids, Cebpb (NM_009883) mouse tagged ORF clone (ORF clone) and pCMV6-Entry tagged cloning vector (cloning vector) were subjected to double restriction digestion and analyzed for the presence or absence of the insert.

Experimental

Plasmid samples

Cebpb (NM_009883) mouse tagged ORF clone (Origene, p/n MR227563) and pCMV6-entry tagged cloning vector (Origene, p/n PS100001) were diluted to 200 ng/µL. The plasmids were double digested with FastDigest SfaAI (Thermo Fisher Scientific, p/n FD2094) and MluI (Thermo Fisher Scientific, p/n FD0564) restriction enzymes, according to the manufacturer's instructions.

Fragment size analysis

The digested samples were diluted with 1x TE to approximately 1 ng/µL and aliquoted to a 96-well plate for analysis with the Agilent ZAG 130 dsDNA kit (75-20000 bp) (ZAG 130 kit) (p/n ZAG-130-5000) on the Agilent ZAG DNA Analyzer system (p/n M5320AA). For comparison, the same samples were analyzed on the Agilent 5200 and 5300 Fragment Analyzer systems (p/n M5310AA and M5311AA, respectively) using the dsDNA 930 Reagent kit (75-20000 bp) (p/n DNF-930).

Results and Discussion

Analysis with the ZAG DNA Analyzer system

Many applications utilizing cloning plasmids require a quality control checkpoint to ensure that the plasmids have the proper DNA inserts. Conventionally, plasmids with the insert of interest are linearized by digestion and screened by gel electrophoresis. Typical agarose gel electrophoresis requires significant monitoring and manual data annotation using external software analysis of the gel image. An alternative to traditional gel electrophoresis workflows is the ZAG system. High-throughput needs are met by analyzing 96 samples simultaneously, in as little as 30 minutes. The ZAG system has the added advantage of the Agilent ProSize data analysis software (ProSize software), which provides automatic sizing and flag analysis with optional Boolean operators for simplified identification of the presence of a fragment[2], eliminating additional manual annotations required by traditional gel electrophoresis protocols. In addition, the ZAG system provides reproducible separation of DNA fragments with a 3 bp resolution[3] allowing for the detection of closely sized fragments. The ZAG system is an indispensable tool for high-throughput applications and ensures quick screening for desired DNA inserts in cloning plasmids with automatic software analysis.

To demonstrate the efficiency of the ZAG system for quality control of cloning plasmids, two plasmids with and without inserts were analyzed. The ORF clone contained a DNA insert while the cloning vector did not. Both plasmids underwent double restriction digestion and were directly analyzed on the ZAG system with the ZAG 130 kit. Restriction enzymes cut the circular plasmid at specific cloning sites separating the plasmid from the DNA insert. In general, analysis by electrophoresis separates the insert from the plasmid displaying two bands if the insert was present in the plasmid. Those plasmids not containing an insert produce a single fragment representing the empty plasmid backbone. Analysis with the ZAG system of the double-digested ORF clone showed the insert fragment at 838 bp and the plasmid backbone at 4,598 bp, while the cloning vector resulted in a single fragment at 4,544 bp representing the plasmid backbone. ProSize software displays the results as both an electropherogram and a digital gel image allowing for easy visualization of the insert (Figure 1). The results matched the expected number of fragments from each plasmid.

Analysis of the double-digested plasmids also provides quality control information on the DNA insert. The complete and correct DNA insert needs to be introduced into the plasmid to ensure successful downstream outcomes. A quick assessment of the size of the insert will give insight as to whether the correct and complete insert has been included into the plasmid. A truncated or longer than expected insert will alert the investigator to the fact that there is a problem in the cloning process.

Comparison of the expected insert size of 888 bp and reported size of 838 bp demonstrated a very close concordance. The ZAG system reported very high accuracy with less than 7.5% error and high precision with ≤ 1.3% CV for both the insert and plasmid backbone (Table 1). Analysis by the ZAG system of the DNA insert and plasmid backbone provided very accurate and precise sizing and confirmed that the correct insert was present.

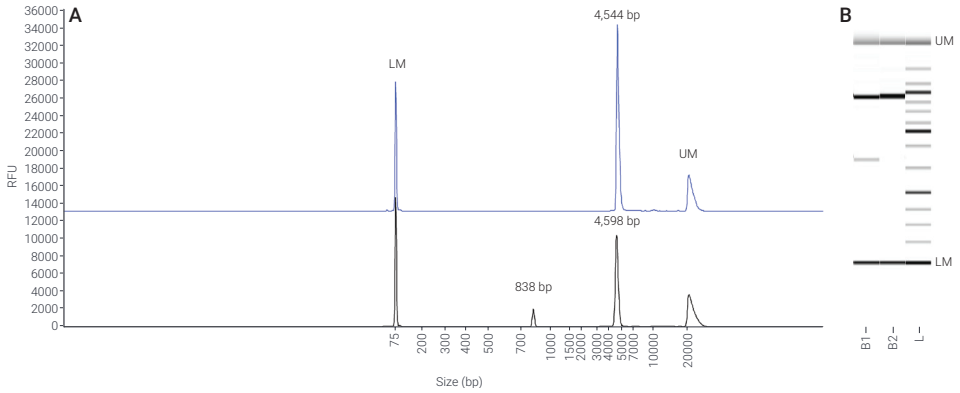


Figure 1. Overlay of restriction digested plasmids with a DNA insert (B1, black) and without (B2, blue) analyzed on the Agilent ZAG DNA Analyzer system with the ZAG 130 dsDNA kit (75-20000 bp). (A) Electropherogram; (B) Corresponding digital gel image. LM = lower marker; UM = upper marker; L = ladder.

Table 1. Sizing accuracy and precision of the restriction digested plasmid fragments using the Agilent ZAG DNA Analyzer system and ZAG 130 dsDNA kit (75-20000 bp).

Fragment Sizing with the ZAG DNA Analyzer System				
Sample	**Expected Size**	**Average Size**	**Accuracy**	**Precision**
	(bp)	(bp, n=423)	(%Error)	(%CV)
Cebpb (NM_009883) Mouse Tagged ORF Clone	Insert - 888	838	5.6%	-0.8%
	Plasmid - 4,912	4,598	-6.4%	1.0%
pCMV6-Entry Tagged Cloning Vector	Plasmid - 4,912	4,544	-7.5%	1.3%

Analysis with the 5200 and 5300 Fragment Analyzer system

The ZAG system and associated analysis kits were developed for fast, high-throughput fragment analysis while providing highly accurate sizing results. The 5200 and 5300 Fragment Analyzer systems are also capable of fragment analysis, utilizing the same technology as the ZAG system. Typically, the Fragment Analyzer systems are utilized with their quantitative kits, providing quality assessment, concentration, and sizing of nucleic acid samples. However, the Fragment Analyzer also has qualitative kits that provide fragment analysis and sizing analogous to the ZAG system. Researchers requiring high-throughput DNA fragment analysis utilize a ZAG system, while laboratories with low-to-high throughput requirements that require the flexibility of both DNA and RNA analysis prefer a Fragment Analyzer system.

To demonstrate the congruity of fragment analysis between the ZAG and Fragment Analyzer systems, the digested plasmids were also analyzed with the 5200 and 5300 Fragment Analyzer systems and the dsDNA 930 Reagent kit (75-20000 bp).

The 5200 Fragment Analyzer system analyzes 12 samples at a time, while the 5300 Fragment Analyzer system is analogous to the ZAG system in that they are both capable of simultaneous analysis of 96 samples. The ZAG 130 dsDNA kit and Fragment Analyzer dsDNA 930 Reagent kit have the same analytical specifications, allowing for seamless comparison between instruments (Table 2). The wide concentration range offered by both kits often eliminates a dilution step and enables direct processing of samples with unknown concentrations, saving time in the workflow.

The plasmid and insert sizes from both the 5200 and 5300 Fragment Analyzer systems were similar to the fragment sizes reported by the ZAG system and varied by ≤ 1.5% (Figure 2). Accuracy and precision with both the 5200 and 5300 Fragment Analyzer systems were likewise similar to the ZAG system with accuracy ≤ 6.8% error and precision ≤ 1.3% CV. The results demonstrated an excellent concordance between the ZAG and the 5200 and 5300 Fragment Analyzer systems.

Table 2. Comparison of the analytical specifications of the Agilent ZAG 130 dsDNA kit and the Fragment Analyzer dsDNA 930 Reagent kit.

Analytical Specifications	ZAG System 130 dsDNA Kit	5200/5300 Fragment Analyzer System dsDNA 930 Reagent Kit
Size Range	75 to 20,000 bp	75 to 20,000 bp
Sizing Accuracy	10%	10%
Sizing Precision	2%	2%
Concentration Range	0.5 to 50 ng/µL	0.5 to 50 ng/µL

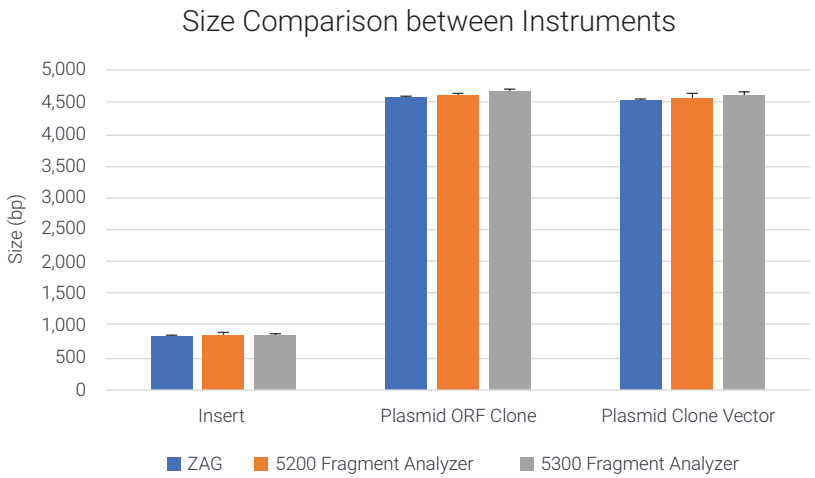


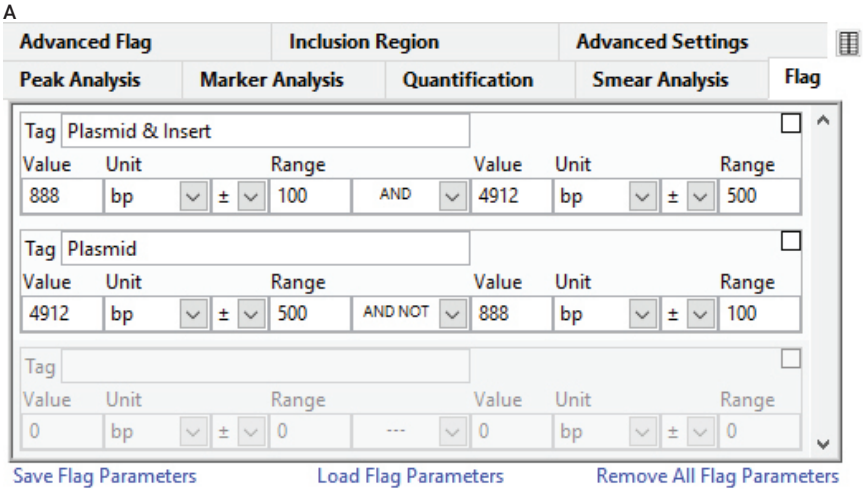
Figure 2. Plasmid and DNA insert sizing comparison between the Agilent ZAG DNA Analyzer system and the Agilent 5200 and 5300 Fragment Analyzer systems.

Flag Analysis by ProSize data analysis software

Automated processing of the large data sets and generation of digital reports allows researchers to save time and avoid errors from tedious manual data processing, especially in high-throughput, fast-pace research environments. Processing of large data sets is supported by the Flag Analysis option in the ProSize software. Flag Analysis allows the user to specify criteria to be met within the data. The numerical range for parameters of interest (i.e., sample size, or concentration, or peak height) can be defined by several Boolean operators such as AND, OR, AND NOT, and NOR. Boolean operators are essential in defining the precise fragment search results, especially in complex scenarios where fast and accurate detection of many different DNA fragments or their combinations is required. The binary outcome where 0 = false and 1 = true specifies if the flag criteria were met.

In this study, presence or absence of the insert fragment in the digested plasmids was reported by utilizing Flag Analysis, where the binary designation of the true/false result offered a quick evaluation of the entire 96-well sample plate. The flag criteria were set with the Boolean operators AND, AND NOT to screen for the DNA insert of a known size (Figure 3). The flag analysis data can be exported in a .CSV format to an excel spreadsheet for processing.

A



B

Peak Table | Flag Analysis

	Sample ID	Plasmid & Insert 888 +/- 100 bp AND 4912 +/- 500 bp	Plasmid 4912 +/- 500 bp AND NOT 888 +/- 100 bp
A1	SampA1	1	0
A2	SampA2	0	1
A3	SampA3	1	0
A4	SampA4	0	1
A5	SampA5	1	0
A6	SampA6	0	1
A7	SampA7	1	0
A8	SampA8	0	1
A9	SampA9	1	0
A10	SampA10	0	1
A11	SampA11	1	0
A12	SampA12	0	1
B1	SampB1	1	0
B2	SampB2	0	1

C

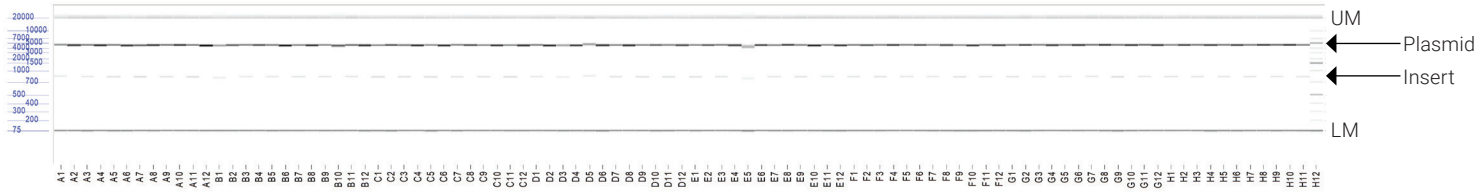


Figure 3. Flag Analysis with the Agilent ProSize data analysis software: (A) table for setting the Flag Analysis criteria using Boolean operators; (B) Flag Analysis results table; (C) digital gel image of the entire 96-well plate. Wells on the plate receive a 1=true or 0=false conveying if they met the criteria of two fragments within the designated size ranges.

Conclusion

Plasmid constructs are commonly used across industries for a multitude of molecular biology applications that are involved in the production of DNA fragments, RNA fragments, proteins, and enzymes. Quality analysis of the plasmid and the DNA insert of interest is a necessary first step to ensure downstream success. Analysis of two plasmids, the Cebpb (NM_009883) mouse tagged ORF clone and pCMV6-entry tagged cloning vector, with the Agilent ZAG DNA Analyzer system identified the presence of a DNA insert in the Cebpb (NM_009883) mouse tagged ORF clone but not in the pCMV6-Entry tagged cloning vector. Analysis with the ZAG system verified the expected results from the plasmid constructs. The ZAG and Fragment Analyzer systems both provided accurate sizing of the plasmid and DNA insert. Sizing of the DNA insert offered essential quality information confirming the insert was not altered during the cloning process.

References

1. Nora, L. C.; Westmann, C. A.; Martins-Santana, L.; de Fátima Alves, L.; Monteiro, L.; Guazzaroni, M.; Silva-Rocha, R. The Art of Vector Engineering: Towards the Construction of Next-Generation Genetic Tools. *Microb. Biotech.* **2019**, *12*(1), 125–147.
2. Genotyping with Agilent ZAG DNA Analyzer System. *Agilent Technologies application note*, publication number 5994-1186EN, **2019**.
3. Highly Resolved Separation of DNA Fragments on the Agilent 5200 Fragment Analyzer system. *Agilent Technologies application note*, publication number 5994-0517EN, **2019**.

www.agilent.com/genomics/zag

PR7000-7626

Printed in the USA, December 15, 2020
5994-2919EN

Agilent
Trusted Answers