

ARTICLE

Construction of genomic libraries from *Flavobacterium columnare* using Lucigen's BigEasy™ Linear Cloning System

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Since completion of the first *Haemophilus influenzae* genome sequence in 1995 (Fleischmann *et al.* 1995), 395 genomes have been completed and about 1650 genome sequencing projects are underway (<http://www.genomesonline.org>). In addition to advances in sequencing technology, robotics, and computational tools, the development of new vectors for library construction in shotgun sequencing projects has enabled the accelerating pace of genome sequencing. However, despite the development of new cloning systems, AT-rich genomes and genomes containing inverted repeats may still pose problems in construction of stable genomic libraries (Godiska *et al.* 2005).

Recently, we undertook whole genome sequencing of *Flavobacterium columnare*, an important bacterial pathogen of catfish that has an AT-rich genome with estimated AT content of 68% (Bernardet and Grimont 1989). To overcome anticipated stability problems, we used two Lucigen cloning systems for library construction: the new BigEasy Linear Cloning Kit based on the linear pJAZZ™-KA plasmid, and the transcription-free pSMART® cloning system. In this study, we show successful application of the BigEasy Linear Cloning Kit in preparation of one small and two medium insert genomic libraries. Our results indicate that the BigEasy linear cloning system overcomes instability problems caused by cloning large fragments from an AT-rich genome.

Materials and Methods

Small and medium insert genomic libraries were constructed using pJAZZ-KA and pSMART-HCKan blunt vectors. Briefly, genomic DNA was purified, mechanically sheared, end-repaired, and 3-6 kb, 6-10 kb, and 10-12 kb fragments were size-selected by agarose gel electrophoresis. Approximately 400 ng insert DNA were ligated into pJAZZ-KA and pSMART-HC Kan blunt vectors, and ligations were transformed into BigEasy-pTEL™ and *E. coli*® 10G ELITE electrocompetent cells, respectively. 25 µl of transformants were plated in duplicate on YT agar plates containing appropriate antibiotics. IPTG and X-gal were included for background determination in pJAZZ-KA transformants. Remaining transformants were frozen in 20% glycerol. Recombinant colony numbers were determined from plate counts, and the percentage of plasmids with inserts, insert size, and empty vector background were calculated by visualizing NotI (pJAZZ-KA) and EcoRI (pSMART-HC) digested plasmid mini-preps by agarose gel electrophoresis. For shotgun sequencing, template DNAs were prepared using a commercial kit in 96-well format (Millipore Montage®). Big Dye® v3.1 was used in sequencing reactions containing 60-180 ng and 80-240 ng template in forward and reverse directions, respectively. Cycling conditions were 96°C

(continued, next page)

In This Issue**ARTICLE 1**Construction of genomic libraries from *Flavobacterium columnare* using Lucigen's BigEasy™ Linear Cloning System**NEW PRODUCT 4**

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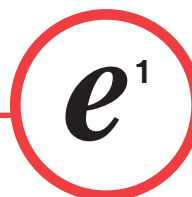
TECH TIP 11

The importance of RNase I treatment in DNA cloning

Table 1.
Comparison of pJAZZ-KA and pSMART libraries.

Library name	CFU/Ligation	Efficiency
Fco-pJAZZ-KA 3-6 kb	174,400	20/20
Fco-pJAZZ-KA 6-10 kb	98,400	10/10
Fco-pJAZZ-KA 10-12 kb	59,200	10/10
Fco-SMART-HC 3-6 kb	800,000	14/20
Fco-SMART-HC 6-10 kb	624,000	6/10
Fco-SMART-HC 10-12 kb	200,000	7/20

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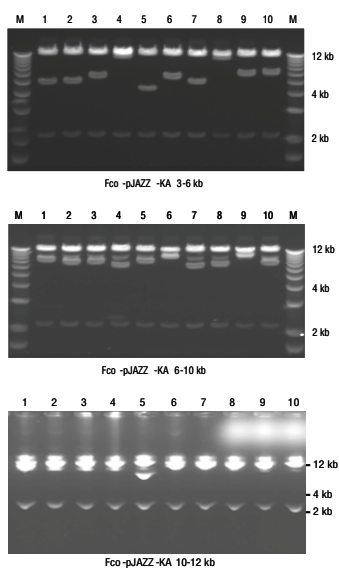


Figure 1. *F. columnare* genomic libraries in pJAZZ-KA vector. Plasmids were prepared from 2 ml overnight cultures by alkaline lysis and digested with NotI. M indicates 1 kb Plus DNA ladder (Invitrogen). The 2.2 kb and 11.8 kb bands are the vector arms of pJAZZ-KA.

for 4 min, followed by 25 cycles of 95°C for 15 sec, 55°C for 5 sec, and 60°C for 2 min. Sequencing reactions were cleaned by ethanol precipitation, and data were collected using an ABI 3730xl DNA Analyzer (Applied Biosystems).

Results and Discussion

Results indicated that genomic libraries with the desired insert length were constructed with the BigEasy™ linear cloning system (Figure 1). Although pSMART®-HC Kan vector produced more recombinant colonies, the cloning efficiency was lower (Table 1, previous page). In addition, insert sizes in pSMART-HC Kan were smaller than those obtained in pJAZZ-KA vector, indicating instability of AT-rich inserts in this vector. Inclusion of IPTG and X-gal on plates revealed about 2.25% blue colonies produced from the undigested vector in pJAZZ™-KA transformants. Template DNA with a concentration range of 20-60 ng/μl was obtained without induction.

In our hands, sequencing with the BigEasy linear vector required some optimization such as primer design, adjustment of template amount, and cycling conditions. Forward primer was replaced by a custom forward primer, which produced high quality sequences (Figure 2, next page). Reverse sequencing reactions were optimized by increasing the template amount.

We have now reached 3.8 X coverage in our genome sequencing project, and the current assembly has 296 contigs with a Phrap average length of 927 bp. Sequences consisted of approximately 69% AT, confirming the estimated GC content of the bacterial genome. Low vector background and low levels of clones containing short inserts were in agreement with our pre-sequencing library characterization.

Conclusions

The BigEasy Linear Cloning System from Lucigen resulted in successful construction of 3-6 kb, 6-10 kb, and 10-12 kb genomic libraries using genomic DNA from an AT-rich bacterial genome, *Flavobacterium columnare*. Prepared libraries produced clean readable sequences. BigEasy outperformed pSMART in terms of stability and desired insert size for *F. columnare*. Considering our success with the system, it is expected that BigEasy Linear Cloning Kit can be successfully used in constructing libraries from organisms that have poor cloning efficiency with other vectors.

References

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