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Screening of bacmids for recombination

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Abstract
In molecular biology, we most often handle with bacmid when working with baculovirus expression system. The cloning involves a two-step process and the recombinant bacmids are screened by blue-white lac operon system, white colonies are thought to be positive for transposition. But in our study with PCR based approach, we found that not all the white colonies are recombinants. Hence, it is necessary to be cautious while screening for recombinant bacmids and may be it is good too screen by two-way approach, by blue-white lac operon system followed by PCR approach using one bacmid specific and one gene specific primers.

Keywords: bacmid, PCR, transposition

1. Introduction
In molecular biology, the use of bacuclovirus as a vector for expression was increasing day-by-day. The cloning into bacmid was a two-step process, which involves cloning into a transfer vector and then transferred to bacmid by site-specific transposition [1]. The most common method to screen for recombinants is by blue-white lac operon system, white colonies are thought to be positive for transposition [2]. However, in our study we found that more often there would be mixed colonies and not all the white colonies are positive for transposition. In our study, we used the PCR approach for an alternate way to screen [3].

2. Methods
2.1. Transformation of recombinant baculovirus transfer plasmid into DH10Bac competent cells and blue-white screening
The frozen aliquot of DH10Bac competent cells were thawed on ice. 1µl (25ng) of transfer plasmid DNA was added and mixed by flicking the tube 4-5 times. The mixture was incubated on ice for 30min. After incubation, the cells were given heat shock by keeping the cells in a water bath maintained at 42ºC for 50sec and snap cooled on ice for 5min. 700µl of SOC was added to the cells and kept in shaking incubator (220rpm) at 37ºC for 4hours. The cells were centrifuged and resuspended in 500µl of the fresh LB media. 80µl of the resuspended cells were spread on LB agar plate containing IPTG (40µg/ml), Bluo-gal (100µg/ml), kanamycin (50µg/ml), gentamicin (7µg/ml) and tetracycline (10µg/ml) using a spreader and incubated at 37ºC for 16hours. Plates were kept at 4ºC and colonies were observed for blue-white selection. The blue colonies were considered as negative and white colonies as positive.

2.2. Screening of recombinant DH10Bac cells by colony PCR
Subsequent to transformation the white colonies which appeared on LB plate where screened further by colony PCR. The single white colony resuspended in 10µl of sterile PBS was used as DNA template. PCR was carried out in 50µl of reaction volume using vector specific M13 forward primer (5' CCCAGTCACGACGTTGTAAAACG 3') and gene specific VP reverse primer (5' AGTCAGTCAGGCTGCCGTAGACACC 3').

Reagents	Volume to be added
Cells	1µl
20 µM Forward primer	1µl
20 µM Reverse primer	1µl
dNTP	1µl
Enzyme	0.25µl (1U)
Buffer	5µl
FQW	40.75µl
Total reaction volume	50µl

Thermocycling conditions for exponential amplification as follows:

Step	Temperature	Time	No. of cycles
Initial denaturation	95ºC	3min	1
Exponential amplification			
Denaturation	96ºC	20sec	30
Annealing	55ºC	30sec	
Extension	72ºC	2min 30sec	
Final extension	72ºC	3min	1
Hold	4ºC	10min	1

The PCR product was analyzed in 0.8% agarose gel alongside with a DNA ladder.

3. Results

Fig. 1 show the plates with blue-white colonies after transposition. We could get 106 white colonies /175 colonies on plate 1, 98 white colonies /136 colonies on plate 2, 69 white colonies /112 colonies on plate 3, 88 white colonies /128 colonies on plate 4, 72 white colonies /105 colonies on plate 5, 63 white colonies /94 colonies on plate 6.

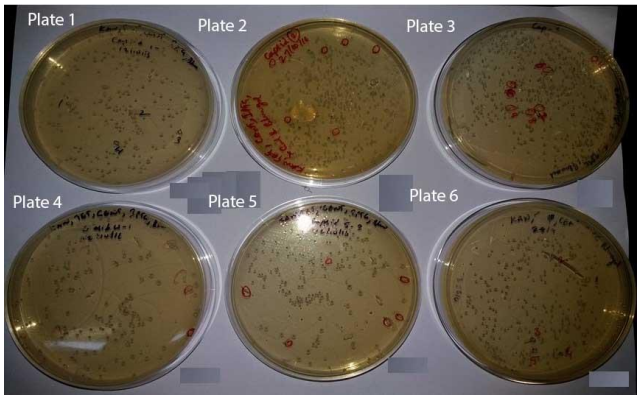

Fig 1: Blue-white colonies of DH10Bac cells containing recombinant bacmid (LB agar with kanamycin, tetracycline, gentamicin, IPTG, Bluo-Gal)

We selected 10 white colonies randomly for screening by PCR approach. Fig. 2 show the positive colonies of recombination has amplified a fragment of 2.2Kb whereas there is no amplification in negative case of recombination. We could also notice that the amplified fragment in Lane 5 show a faint band.

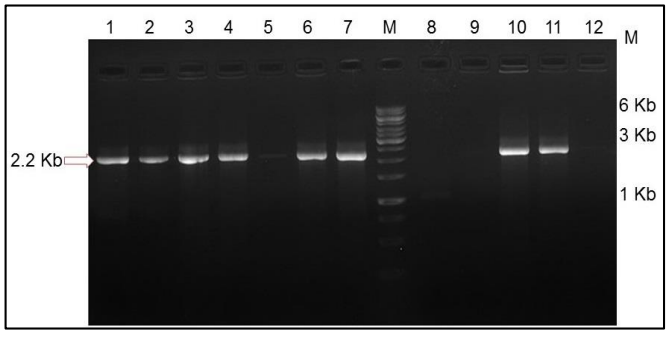

Fig 2: Colony PCR of recombinant DH10Bac cells using M13F and VP2R primers (0.8% agarose gel)

Lane M: GeneRuler 1Kb DNA ladder (Fermantas), Lane 11: Positive control for amplification, Lane 12: Negative control for amplification Lanes 1, 2, 3, 4, 5, 6, 7, 10 showing amplification of 2.2Kb are positive for recombination, Lanes 8, 9 showing no amplification are negative for recombination.

4. Discussion

The combination of blue-white screening and PCR based screening would be the best choice for getting the positive recombinants. The most important point to remember while screening the bacmids by PCR approach is the primers should be designed with the regions complimentary with the 3' end of bacmid and 5' end of the transposed region or vice-versa or may be one primer complimentary to bacmid and other to transposed region to avoid false positive results. As the transfer plasmid may be within the cell but may not be transposed in which conditions use of gene specific primers will give false positive results. In our study by PCR approach of screening we could also detect the mixed colonies present after recombination. The probable explanation for mixed colonies may be because the bacmid rejects the transposed fragment to revert to wild-type condition. Hence, it is necessary to be cautious while screening for recombinant bacmids as not all the white colonies are positive for recombination.

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