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稻曲病菌 UV-2 菌株细菌人工染色体文库构建及分析

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摘 要: 【目的】稻曲病(Rice false smut)是由稻曲病菌[*Villosiclava virens* (Cooke) Tak.]引起的严重危害水稻的真菌病害。构建稻曲病菌 UV-2 的大片段 DNA 细菌人工染色体(Bacterial artificial chromosome, BAC)文库，为致病相关基因的鉴定及在图位克隆、比较基因组学等方面的研究奠定基础。【方法】以幼嫩菌丝为材料制备大分子基因组 DNA 包埋块，用 *Hin*d III 部分酶解后经脉冲凝胶电泳筛选，回收大片段 DNA 并与 pIndigoBAC536-S 载体连接，连接产物转化大肠杆菌菌株 DH10B T1 Phage-Resistant 细胞后进行蓝白斑筛选，白色菌落捡入 384 孔板置于−80 °C 低温保存。【结果】成功构建 UV-2 菌株的高质量、高覆盖度的 BAC 文库，该文库共含 10 368 个克隆，平均插入片段为 124.4 kb，空载率小于 1%，约覆盖该菌基因组的 36.8 倍。【结论】克服了真菌大分子基因组 DNA 制备难控制的技术难题，建立了首个稻曲病菌的 BAC 文库。该文库已作为一种公共基因组资源向研究者开放(http://GResource.hzau.edu.cn)。

关键词: 大分子 DNA，细菌人工染色体，水稻，稻曲病，稻曲病菌

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Construction of a bacterial artificial chromosome library of *Villosiclava virens* UV-2 genome

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Abstract: [Objective] Rice false smut caused by *Villosiclava virens* (Cooke) Tak. is a serious fungal disease of rice. This study aimed to construct a high molecular weight bacterial artificial chromosome (BAC) library of UV-2 genome. The BAC library will help identify pathogenicity-associated genes and provide genome resource for map-based cloning and comparative genomic analysis. **[Methods]** High molecular weight genomic DNA was isolated from young mycelia of UV-2 strain, digested with restriction enzyme *Hin*d III and size selected by PFGE. Large genomic DNA fragments were recovered and ligated to the pIndigoBAC536-S vector. The ligation product was used to transform the *Escherichia coli* strain DH10B T1 Phage-Resistant cells and the transformants were selected on a blue-white selection medium. White colonies were individually picked into 384-well microtiter plates and stored at −80 °C. **[Results]** We constructed a high quality deep coverage BAC library of UV-2 strain. The BAC library consisted of 10 368 clones with an average insert size of 124.4 kb and an empty clone rate of lower than 1%, covering 36.8-fold of the UV-2 genome. **[Conclusion]** We established a method for preparation of fungal high molecular weight genomic DNA, and constructed the first BAC library of *V. virens* genome successfully. The BAC library has been opened to researchers as a public genomic resource (http://GResource.hzau.edu.cn).

Keywords: High molecular weight DNA, Bacterial artificial chromosome, Rice, Rice false smut, *Villosiclava virens*

[*Villosiclava virens* (Cooke) Tak.] (Rice false smut)

, ,

Villosiclava , *Ustilaginoidea* [2] 70 ,

virens (Cooke) Tak., ,

[1]

,

, BAC
, , BAC
,
DNA ,
DNA
,
DNA
,
BAC , ,
UV-2 ,
BAC
,

1 材料与方法

1.1 稻曲病菌的培养

UV-2
UV-2
(PSA)
4 d, PSA
, 28 °C 3 d,
100 mL [16−17] ,
180 r/min 28 °C 2 d,
DNA

1.2 UV-2 菌株大分子基因组 DNA 包埋块的制备

BAC [14−15,18]
1.1 50 g ,
2 , 0.7 mmol/L NaCl 2 ,
50 mL 0.7 mmol/L NaCl ,
(Driselase, Sigma) 10 g/L, 100 r/min
31 °C 4 h,

Miracloth (Calbiochem) , ,
1 500×*g* 15 min 40 mL
0.7 mmol/L NaCl , 1 mL
1.2 mol/L , 1%
(1.2 mol/L) ,
, 30 min; (Plug) ,
40 mL 1 g/L K (Sigma) (1%
N-lauroylsarcosine) 50 mL , 50 °C
2×24 h (K 1)
Plug, 1 mmol/L PMSF (
) $T_{10}E_{10}$ (10 mmol/L Tris-HCl
10 mmol/L EDTA, pH 8.0) 2 , TE
(10 mmol/L Tris-HCl 1 mmol/L EDTA, pH 8.0)
2 , 1 h, ; ,
TE , 4 °C

1.3 部分酶解及大片段 DNA 的第一次筛选

DNA *Hin*d

, ,
DNA ,
, UV-2
,
, DNA ,

,
DNA , ,
: 0.2
0.3 U/18 min 0.4 0.6 U/15 min
25 μL H_2O 10 μL 10×Buffer
10 μL 40 mmol/L Spermidine (Sigma) 5 μL
*Hin*d 1/2 Plug, 37 °C
,
0.5×TBE
14 °C 1−50 s 120° 6 V/cm 18 h
,
18
, λ ladder PFG
marker (NEB), ,
, 0.5 cm
λ ladder PFG marker
(0.5 mg/L) , ,

1.4 大片段 DNA 的第 2 次筛选和洗脱回收

,
1
110−220 kb 220−300 kb
2 0.5×TBE 14 °C 4−4 s
120° 6 V/cm 18 h 1
,
,
a1 a2 (a2>a1), b1 b2 (b2>b1),
DNA, DNA,

1.5 克隆载体的制备

,
pIndigoBAC536-S
pIndigoBAC536[18] ,
pHZAUBAC1[15],
pHZAUBAC1 Qiagen
, pHZAUBAC1 *Hin*d
FastAP (Fermentas)
, pIndigoBAC536-S,
[14−15] DNA
λDNA ,
6 mg/L 30%−40% , −80 °C ,
λDNA/*Hin*d III ,
UV-2 BAC

1.6 BAC 文库构建

2 DNA
84 μL DNA
4 μL 2 μL 5 U/μL T4 Ligase (Fermentas)
10 μL T4 Buffer, 16 °C 65 °C
(1% +2%) 1 h,
4 °C 2 μL
20 μL DH10B T1 Phage-Resistant
(Invitrogen) , 325 V
, SOC 1 h ,
12.5 mg/L , 80 mg/L X-gal,
100 mg/L IPTG LB , 37 °C
,

4 °C ,
384 ,
−80 °C

1.7 文库质量分析

384 4−5 ,
, -*Sce* (NEB) ,
:
0.5×TBE 14 °C 5−15 s 120° 6 V/cm 17 h
,
,

2 结果与分析

2.1 基因组 DNA 包埋块(Plug)的制备及部分酶解

Plug ,
UV-2 ,
, DNA Plug
, Plug DNA ,
,
, DNA

DNA 1A UV-2
DNA *Hin*d ,
, UV-2 DNA Plug DNA
, ,
, DNA
, DNA ,
,
DNA , 0.3 U/18 min
100−150 kb DNA ,
0.6 U/15 min ,
0.4 U/15 min (37 °C)
DNA

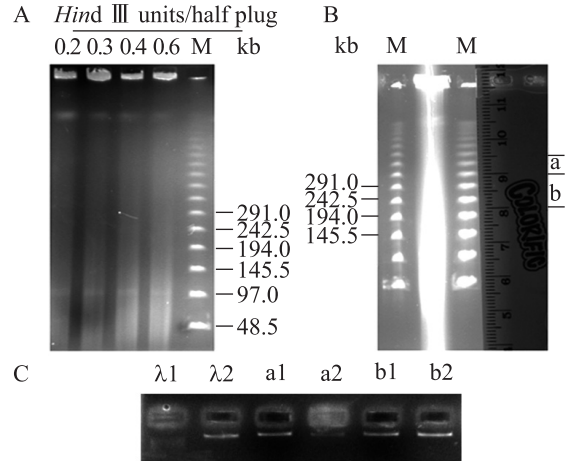

图 1 稻曲病菌 UV-2 基因组 DNA 部分酶解条件比较(A)、大片段 DNA 第 1 次筛选(B)和第 2 次筛选后回收 DNA 浓度检测(C)

Fig. 1 Comparison of partial digestion conditions of the *Villosiclava virens* UV-2 genomic DNA (A), the first screening of large fragment DNA (B) and concentration of recovered large fragment DNA after the second screening (C)

: 0.2 U 0.3 U: *Hin*d III 37 °C 18 min; 0.4 U 0.6 U: *Hin*d III 37 °C 15 min; M: λ ladder PFG (NEB). λ1 λ2 1 2 ng DNA ; a1 a2 b1 b2 1 μL DNA.

Note: 0.2 U, 0.3 U: *Hin*d III digest at 37 °C for 18 min; 0.4 U, 0.6 U: *Hin*d III digest at 37 °C for 15 min; M: λ ladder PFG DNA marker (NEB); λ1, λ2 are the DNA standards of 1 and 2 ng respectively; a1, a2, b1, b2: 1 μL DNA recovered after the second screening of large fragment DNA.

2.2 大片段 DNA 的两次筛选和回收

*Hin*d 37 °C 15 min
, 18 Plug ,
DNA 1
1 (1B),
, Plug DNA *Hin*d

110−220 kb (a) 220−300 kb (b)
2 , DNA
, a b ,
a1 a2 b1 b2
DNA ,
a1 a2 b1 b2 ,
λDNA (1C), a1 a2
b1 b2 1−2 mg/L , a2
, 1 mg/L , DNA

2.3 载体制备

,
,
λDNA
pIndigoBAC536-S 10 mg/L (2),
6 mg/L,
30%−40% , −80 °C
,
pIndigoBAC536-S (*Hin*d)

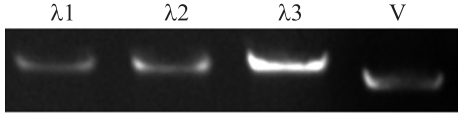

图 2 载体 pIndigoBAC536-S 浓度检测

Fig. 2 Concentration estimation for the vector pIndigoBAC536-S

: λ1 λ2 λ3 8 16 32 ng DNA ; V: 1 μL pIndigoBAC536-S .

Note: λ1, λ2, λ3 are the DNA standards of 8, 16 and 32 ng respectively; V: 1 μL pIndigoBAC536-S vector.

λDNA (30 mg/L) ,
λDNA ,
100 μL ,
, 5 ; λDNA
, 100 μL , λDNA
30 ng:60 ng ,
, 100 μL 800 ,
,
, BAC

2.4 BAC 文库构建和质量检测

UV-2 *Hin*d III DNA a1 a2
b1 b2 16 °C ,
4 °C , a1, a2

(a1>a2>1 200), ,
20 ,
, a1 100 kb ,
a2 130 kb b1
b2
(b2<b1<400), b1 b2
, , ,
a2
8 , 1 500
384
, 27 384 10 368 ,
2 −80 °C
,
168 , -*Sce*
, 168
, 1% 3
, 3
, 4
168 ,
120−130 kb ,

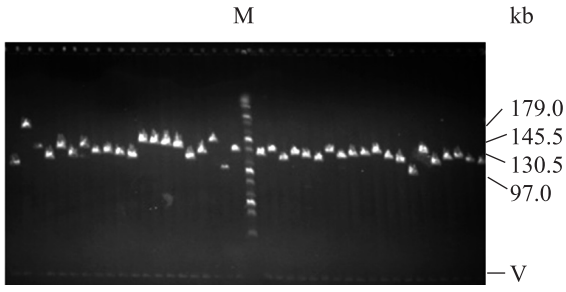

图 3 UV-2 BAC 文库随机 BAC 克隆的插入片段检测

Fig. 3 Insert size estimation of randomly selected BAC clones of the UV-2 BAC library

Note: M: Middle range PFG marker; V: pIndigoBAC536-S vector (−7 kb) .

,

168 124.4 kb,

UV-2 (35 Mb) 36.8

3 讨论

,

,

,

,

,

UV-2

BAC BAC

DNA

[14−15,18], ,

DNA

DNA , BAC

DNA , DNA

,

DNA , 17−78 kb [19],

DNA

, , DNA ,

DNA ,

,

DNA

, , DNA

,

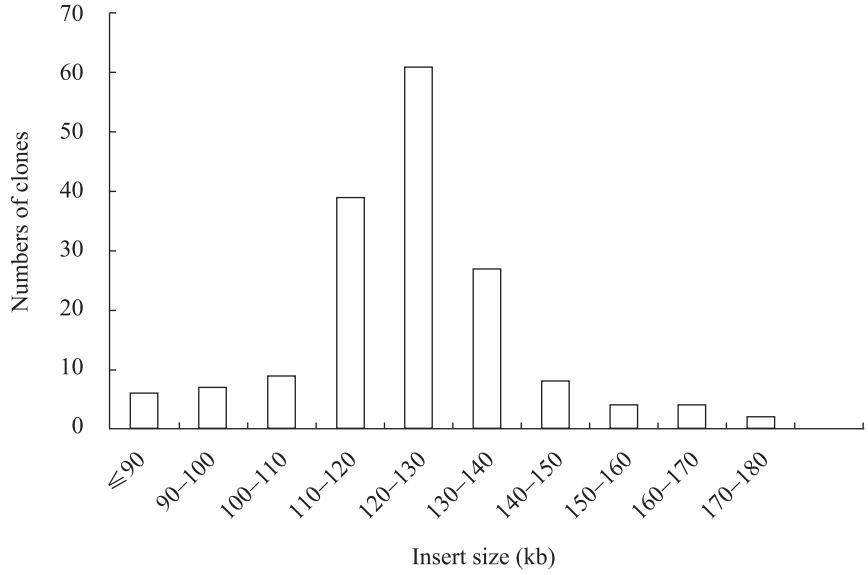

图 4 UV-2 BAC 文库插入片段分析(共 168 克隆)

Fig. 4 Insert size analysis of the UV-2 BAC library (168 clones)

,

DNA

,

(BAC) ,

,

UV-2 BAC

(124.4 kb), 36.8 ,

,

,

,

BAC ,

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