

# SB265 BLAST

**orf19.3066** ENGI CGDID:CAL0001461 Contig19-10163 (264153, 267590) (3438 nucleotides)  
Endo-1,3-beta-glucanase required for cell separation after division,  
orthologous to *S. cerevisiae* Dse4p; caspofungin repressed; fungal-specific  
(no human or murine homolog)  
Length = 3438

[\[Retrieve Sequence / CGD GBrowse / CGD Locus page\]](#)

Minus Strand HSPs:

Score = 3027 (460.2 bits), Expect = 3.8e-133, P = 3.8e-133

**Identities = 631/654 (96%)** Positives = 631/654 (96%), Strand = Minus / Plus

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Query:  697 TTAAGTAAANATGCTTCGA-TCCTTCTGC-GATGATACT-ATTNCNTGTTTCAAGAATG 641
      |||  | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 2785 TTAGTGAGAGACGCTTCGAATCCTTCTGCCGATGATACTTATTTCCTGTTTCAAGAATG 2844

Query:  640 TTTGACTGGT-CTCTGCCCATTCATGG-CAACTGGTTTGTTCGTCACTTCAAGACCATT 583
      || | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 2845 TTTGACTGGTCTCTGGTCATTCATGGCAACTGGTTTGTTCGTCACTTCAAGAACATT 2904

Query:  582 GAATCAAGTTCNGAATCATTACATTTTGTCTGCTATATAAAATTATGGGGTAAAGTAG-C 524
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Sbjct: 2905 GAATCAAGTTCNGAATCATTACATTTTGTCTGCTATATAAAATTATGGGGTAAAGTAGTC 2964

Query:  523 GGTGATCAATCGATGGAAGCAAGAGGTGGTTTGTATGATTCCATTATGGCACGTTCTTTT 464
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Sbjct: 2965 GGTGATCAATCGATGGAAGCAAGAGGTGGTTTGTATGATTCCATTATGGCACGTTCTTTT 3024

Query:  463 AACATG-ATTTCTACTATAAAATCAGATAATACTGTGGA-CCAAANCAAATTTTACCAAAC 406
      || | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 3025 AACATGATTTCTACTATAAAATCAGATAATACTGTGGAACCAAANCAAATTTTACCAAAC 3084

Query:  405 AAAAGTCAGTGGTATATTTTTCGAGAATAAAAGTTGATTACACTACATTCTTTGGAACACCA 346
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Query:  345 GCTGATCATCCAGAATATGTCCATGGTATCCATATGCTTCCAATTACACCTTCTCATCG 286
      || | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 3145 GCTGATCATCCAGAATATGTCCATGGTATCCATATGCTTCCAATTACACCTTCTCATCG 3204

Query:  285 TTGGTTAGAAAAGACTTCTTACGTTCAAGAAGAAATGGAAAAGATCAAATGCTGGTTTATT 226
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Sbjct: 3205 TTGGTTAGAAAAGACTTCTTACGTTCAAGAAGAAATGGAAAAGATCAAATGCTGGTTTATT 3264

Query:  225 GATAATGTTGATAGTGGCTGGACTTGTATTTTGTAGATTAACCAAGCTTTATTTGATCCT 166
      || | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 3265 GATAATGTTGATAGTGGCTGGACTTGTATTTTGTAGATTAACCAAGCTTTATTTGATCCT 3324

Query:  165 AAATCATCATATGAATTTTTCATCAAAATAACTGGGATGACAAAGTGTTAGATAATGGT 106
      || | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 3325 AAATCATCATATGAATTTTTCATCAAAATAACTGGGATGACAAAGTGTTAGATAATGGT 3384

Query:  105 CAAAGTAGAACTTGGAGTTTGGCTTTTGTCTGCTGCTCAATGCTAGTTAG 52
      || | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 3385 CAAAGTAGAACTTGGAGTTTGGCTTTTGTCTGCTGCTCAATGCTAGTTAG 3438
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## 圖一A、序列SB265比對結果

以上結果是將序列SB265和CGD資料(<http://www.candidagenome.org/>)進行核苷酸序列比對之結果，發現序列SB265和白色念株菌中的orf19.3066序列相似度高，約有96%的相似度，和orf19.10584互為對偶基因，可轉譯成endo-1,3-beta glucanase水解酶。

# SB265 BLAST

[orf19\\_10584](#) [FMG1\\_CGDID:CAL0001461 Contig19-20163 \(263792, 267232\)](#) (3441 nucleotides)  
[Endo-1,3-beta-glucanase](#) required for cell separation after division,  
orthologous to *S. cerevisiae* Dse4p; caspofungin repressed; fungal-specific  
(no human or murine homolog)  
Length = 3441

[\[Retrieve Sequence / CGD GBrowse / CGD Locus page\]](#)

Minus Strand HSPs:

Score = 3027 (460.2 bits), Expect = 3.8e-133, P = 3.8e-133

[Identities = 631/654 \(96%\)](#), Positives = 631/654 (96%), Strand = Minus / Plus

```
Query: 697 TTAAGTAAANATGCTTCGA-TCCTTCTGC-GATGATACT-ATTMCCNTGTTTCAAAGAATG 641
      ||| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 2788 TTAGTGAGAGACGCTTCGAATCCTTCTGCGCATGATACTTATTCCCTGTTTCAAAGAATG 2847

Query: 640 TTTGACTGGT-CTCTGCCCATTCATGG-CAACTGGTTTGTTTCGTCACCTTNCAGACCATT 583
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 2848 TTTGACTGGTTCTCTGGTCAATTCATGGGCAACTGGTTTGTTTCGTCACCTTACAAGAACATT 2907

Query: 582 GAATCAAAGTTCNGAATCATTACATTTTTGCTGCTGTATATAAAAATTATGGGGTAAAAGTAG-C 524
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 2908 GAATCAAAGTTCGGAATCATTACATTTTTGCTGCTGTATATAAAAATTATGGGGTAAAAGTAGTC 2967

Query: 523 GGTGATCAATCGATGGAAGCAAGAGGTGGTTTGATGATTCCATTATGGCACGTTCTTTTT 464
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 2968 GGTGATCAATCGATGGAAGCAAGAGGTGGTTTGATGATTCCATTATGGCACGTTCTTTTT 3027

Query: 463 AACATG-ATTTCTACTATAAAATCAGATAATACTGTGGA-CCAAANCAAATTTTACCAAAC 406
      ||| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 3028 AACATGATTTCTACTATAAAATCAGATAATACTGTGGAACCAAAACAATTTTACCAAAC 3087

Query: 405 AAAGTCAGTGGTATATTTTTTCGAGAATAAAAGTTGATTACACTACATCTTTGGAACACCA 346
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Sbjct: 3088 AAAGTCAGTGGTATATTTTTTCGAGAATAAAAGTTGATTACACTACATCTTTGGAACACCA 3147

Query: 345 GCTGATCATCCAGAAATATGTCCATGGTATCCATATGCTTCCAATTACACCTTCCTCATCG 286
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Sbjct: 3148 GCTGATCATCCAGAAATATGTCCATGGTATCCATATGCTTCCAATTACACCTTCCTCATCG 3207

Query: 285 TTGGTTAGAAAAGACTTCTTACGTTCAAGAAGAATGGAAAAGATCAAATTGCTGGTTTTATT 226
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Sbjct: 3208 TTGGTTAGAAAAGACTTCTTACGTTCAAGAAGAATGGAAAAGATCAAATTGCTGGTTTTATT 3267

Query: 225 GATAATGTTGATAGTGGCTGGACTTGTATTTTGAGATTAACCAAGCTTTATTTGATCCT 166
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Query: 165 AAATCATCATATGAAATTTTTTGCATCAAAATAACTGGGATGACAAGTGGTTAGATAATGGT 106
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Sbjct: 3328 AAATCATCATATGAAATTTTTTGCATCAAAATAACTGGGATGACAAGTGGTTAGATAATGGT 3387

Query: 105 CAAAGTAGAACTTGGAGTTTGGCTTTTGTCTGCTGCTCAATGCTAGTTAG 52
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Sbjct: 3388 CAAAGTAGAACTTGGAGTTTGGCTTTTGTCTGCTGCTGCTCAATGCTAGTTAG 3441
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圖一B、序列SB265比對結果

以上結果是將序列SB265和CGD資料(<http://www.candidagenome.org/>)  
進行核苷酸序列比對之結果，發現序列SB265和白色念株菌中的  
orf19.10584序列相似度高，約有96%的相似度，和orf19.3066互  
為對偶基因，可轉譯成endo-1,3-beta glucanase水解酶。

# SB240 BLAST

[orf19.7304](#) orf19.7304 CGDID:CAL0004507 Contig19-2511 (40544, 39066), reverse complemented (1479 nucleotides)  
Predicted ORF from Assembly 19  
Length = 1479  
[\[Retrieve Sequence / CGD GBrowse / CGD Locus page\]](#)

Minus Strand HSPs:

Score = 3346 (508.1 bits), Expect = 3.5e-147, P = 3.5e-147  
Identities = 698/723 (96%), Positives = 698/723 (96%), Strand = Minus / Plus

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Query: 754 ATTATTTGNATTTTCNACA-ATNCCA-GNAT-ATTTTATGGCCA-TTAT-ANGG-AGTT-C 702
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Sbjct: 719 ATTTTGTAT-TTCCTACAGATGCCATGTATTATTTTATTGTCAATTATTATGGTAGTTTC 777

Query: 701 CTCCAAGT-GTATTAAGTATGGGTTAAGTTATATCCCATATCAGTTTATTTGGATCA 643
      || ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct: 778 CTACAAGTTGTATTAAGTATTGGTTAAGTTATATCACATTATCAGTTTATTTGGATCA 837

Query: 642 GGATATGCTAATTTACCAATTAATCCACCTCAATTTTGTGGAAATTATACGTTGGTATA 583
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Sbjct: 838 GGATATGCTAATTTACCAATTAATCCACCTCAATTTTGTGGAAATTATACGTTGGTATA 897

Query: 582 AATATTTTAGCCACTTTTATTTCAATTTGAAAAGATGCTTTAGGTGTCGAAGAA 523
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Sbjct: 898 AATATTTTAGCCACTTTTATTTCAATTTGAAAAGATGCTTTAGGTGTCGAAGAA 957

Query: 522 ATCATTGTGAATTCAGAAAAGTTATCAAATCATTGATAAATCGATTTTGTCAATAAAGAA 463
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Sbjct: 958 ATCATTGTGAATTCAGAAAAGTTATCAAATCATTGATAAATCGATTTTGTCAATAAAGAA 1017

Query: 462 TATAAAACAATGGTTACTACTTACATTTTGTATTGGGAAAAAATTGTCGTTACGGTTTTA 403
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Query: 402 GCATATACAAAATTGACTATTAGTGCTTTAGTTCATTTGGCTAGTTTTGGATATGGATTG 343
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Sbjct: 1078 GCATATACAAAATTGACTATTAGTGCTTTAGTTCATTTGGCTAGTTTTGGATATGGATTG 1137

Query: 342 AAACTTTATTGGAATTTGAAAAAGAGTAACAACAATAGAGTTACCAGACCATTATTACAA 283
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Sbjct: 1138 AAACTTTATTGGAATTTGAAAAAGAGTAACAACAATAGAGTTACCAGACCATTATTACAA 1197

Query: 282 TCAATCGTGTTACATTTGGTTTCTTGGCGTATATTTGGTAGGATTATCATTGCTAATTTTC 223
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Sbjct: 1198 TCAATCGTGTTACATTTGGTTTCTTGGCGTATATTTGGTAGGATTATCATTGCTAATTTTC 1257

Query: 222 TATAGTGATATAAATTTGACGGGAGTGTTTGACGTTGGAGAAAATGTTAAGTGTAATAGGT 163
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Sbjct: 1258 TATAGTGATATAAATTTGACGGGAGTGTTTGACGTTGGAGAAAATGTTAAGTGTAATAGGT 1317

Query: 162 AATTTGATTGAATTCAGGATTGAAAATTTACGGCATTACGTTTGTGAAAGTCTTATTG 103
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Query: 102 CTTTGGTTTATCTGGAGTTTCAATAAACCCTTGATTTCAAAAAGACTAAAAGAGATGAC 43
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
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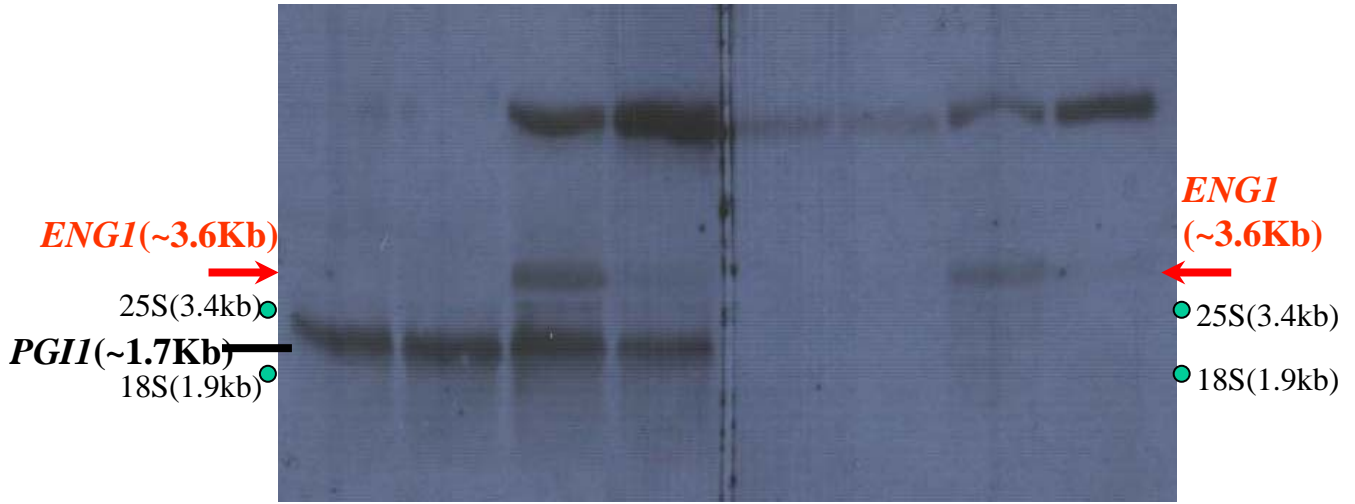
Query: 42 AAG 40
      |||
Sbjct: 1438 AAG 1440
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圖一C、序列SB240比對結果

以上結果是將序列SB240和CGD資(<http://www.candidagenome.org/>)進行核苷酸序列比對之結果，發現序列SB240只和白色念株菌中的orf.7304相似度較高，約有96%的相似度，並沒有發現對偶基因的存在。

1. JKC19 (EFG1/EFG1 *cph1/cph1*)    2. SC5314 (EFG1/EFG1 CPH1/CPH1)    3. HLC54 (*efg1/efg1 cph1/cph1*)    4. HCL52 (*efg11/efg1 CPH1/CPH1*)    1. JKC19 (EFG1/EFG1 *cph1/cph1*)    2. SC5314 (EFG1/EFG1 CPH1/CPH1)    3. HLC54 (*efg1/efg1 cph1/cph1*)    4. HCL52 (*efg11/efg1 CPH1/CPH1*)

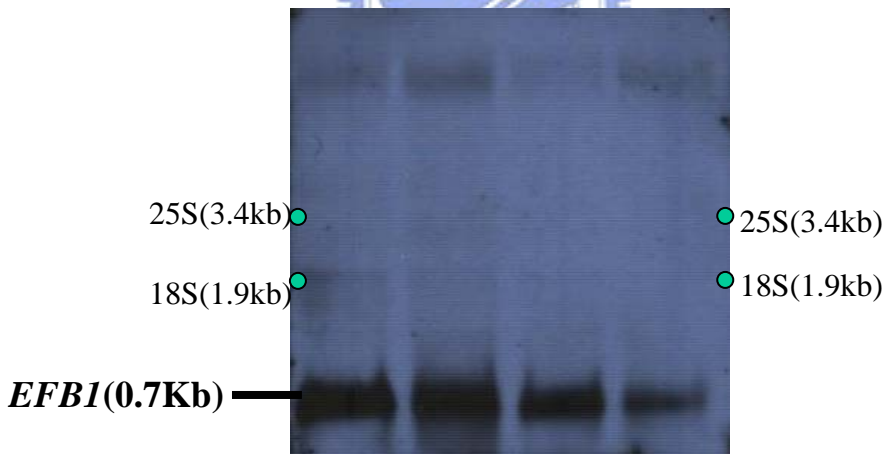
1    2    3    4    1    2    3    4



**Probe:ENG1+PGII**

**Probe:ENG1**

SC5314 (EFG1/EFG1 CPH1/CPH1)    HCL52 (*efg11/efg1 CPH1/CPH1*)    JKC19 (EFG1/EFG1 *cph1/cph1*)    HLC54 (*efg1/efg1 cph1/cph1*)

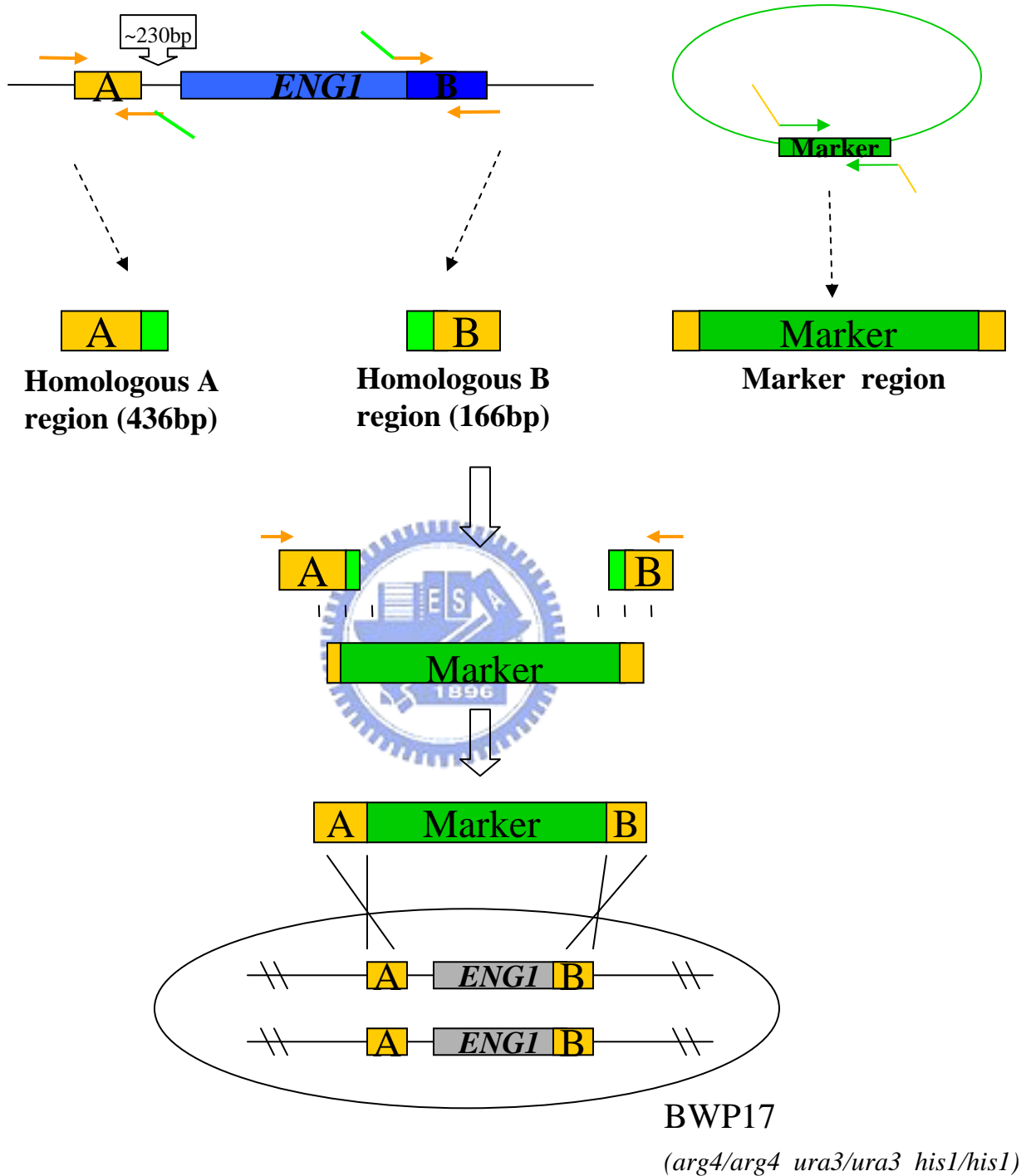


**Probe:GS+EFB1**

圖二、ENG1和GS北方墨點法之結果

圖上方表示不同的基因破壞株，野生株SC5314 (EFG1/EFG1 CPH1/CPH1)、突變株為JKC19 (EFG1/EFG1 *cph1/cph1*)、HLC54 (*cph1/cph1 efg1/efg1*)、HCL52 (*efg1/efg1 CPH1/CPH1*)北方墨點法之結果。分別以PGII和EFG1作為internal control，大小約為1.7 Kb和0.7 Kb。由圖預測ENG1大小約為3.6 Kb左右;GS則沒有明顯的表現或表現量很低。

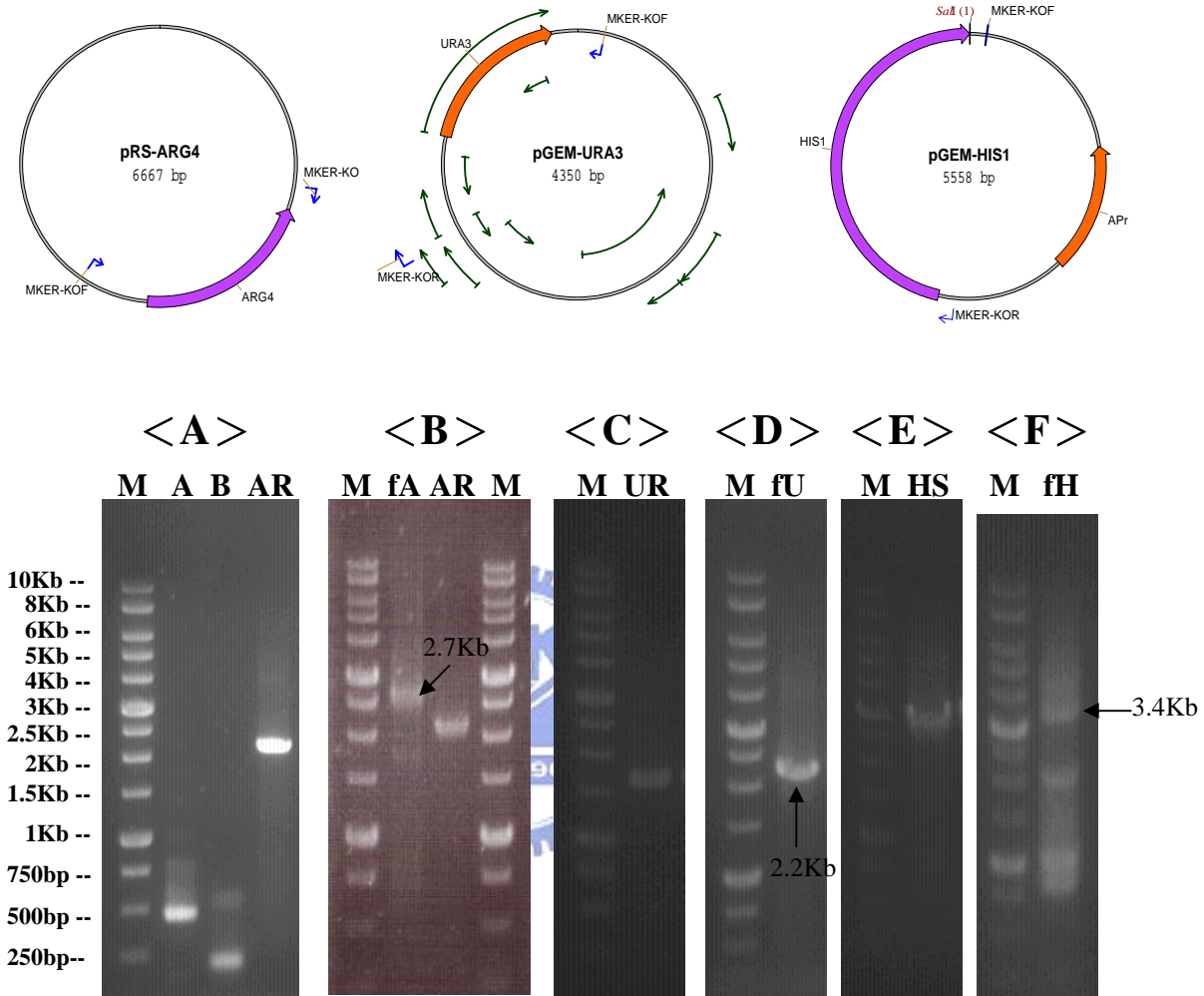
# 以Fusion PCR 取得DNA進行 knock out 示意圖



圖三、Fusion PCR流程示意圖

上方圖示的Marker region為篩選標記，本實驗共用到三種篩選標記，分別為*ARG4*、*URA3*、*HIS1*，皆用此法得到特定之DNA片斷，以供轉形至菌株BWP17之用。

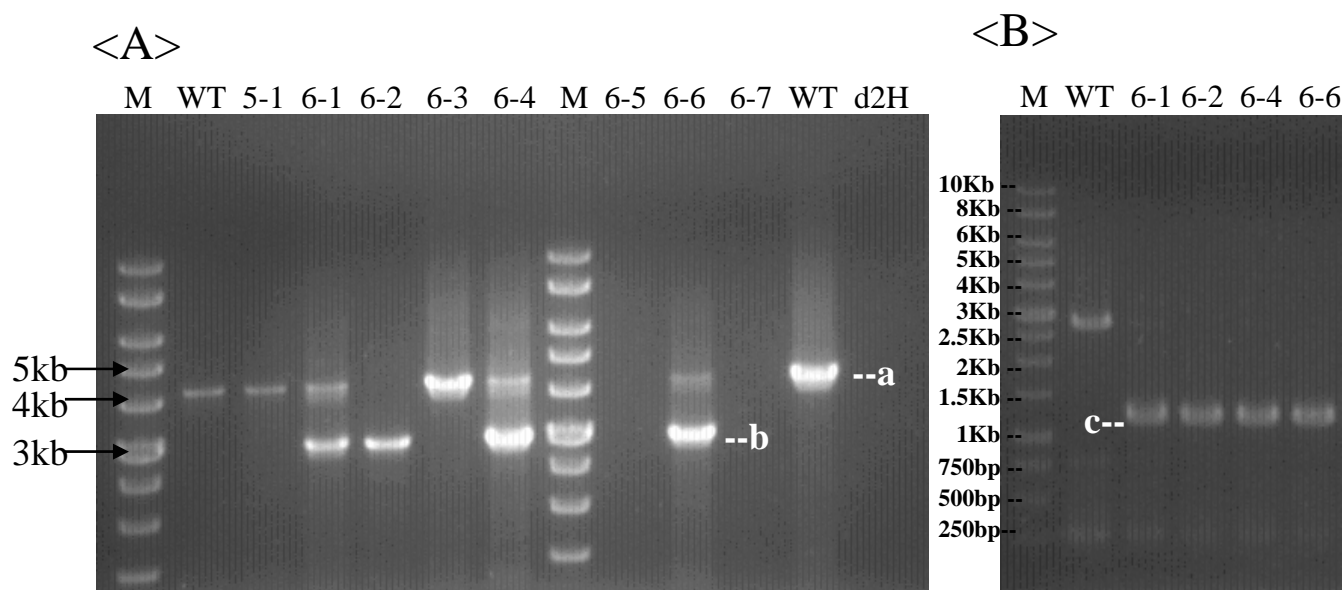
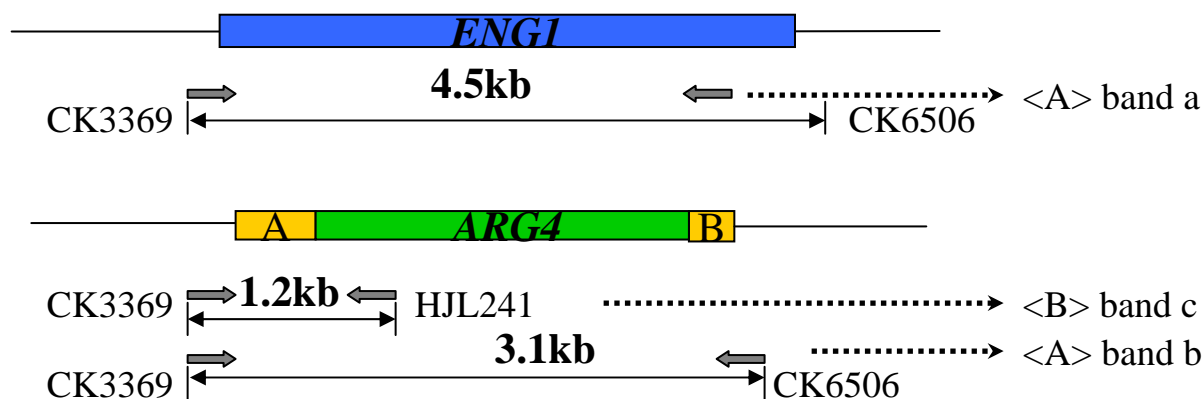
# Result of fusion PCR



圖四、Fusion PCR產物

分別利用引子MKER-KOF和MKER-KOR將質體pRS-ARG4、pGEM-URA3和pGEM-HIS1上的篩選序列ARG4 (圖A-lane AR, 約2.1Kb)、URA3 (圖C-lane UR, 約1.6Kb) 和HIS1 (圖E-lane HS, 約2.8Kb)利用PCR的方式產生, 將同源重組區域region A (圖A-lane A)、region B (圖A-lane B)亦由PCR的方式產生, 利用fusion PCR的設計原理連接成一DNA片段(如箭頭所指, 圖B-lane fA約2.7Kb、圖D-lane fU 2.2Kb、圖F-fH約3.4 Kb), 以供轉形至白色念珠菌中做knock out之用。Lane M為marker。

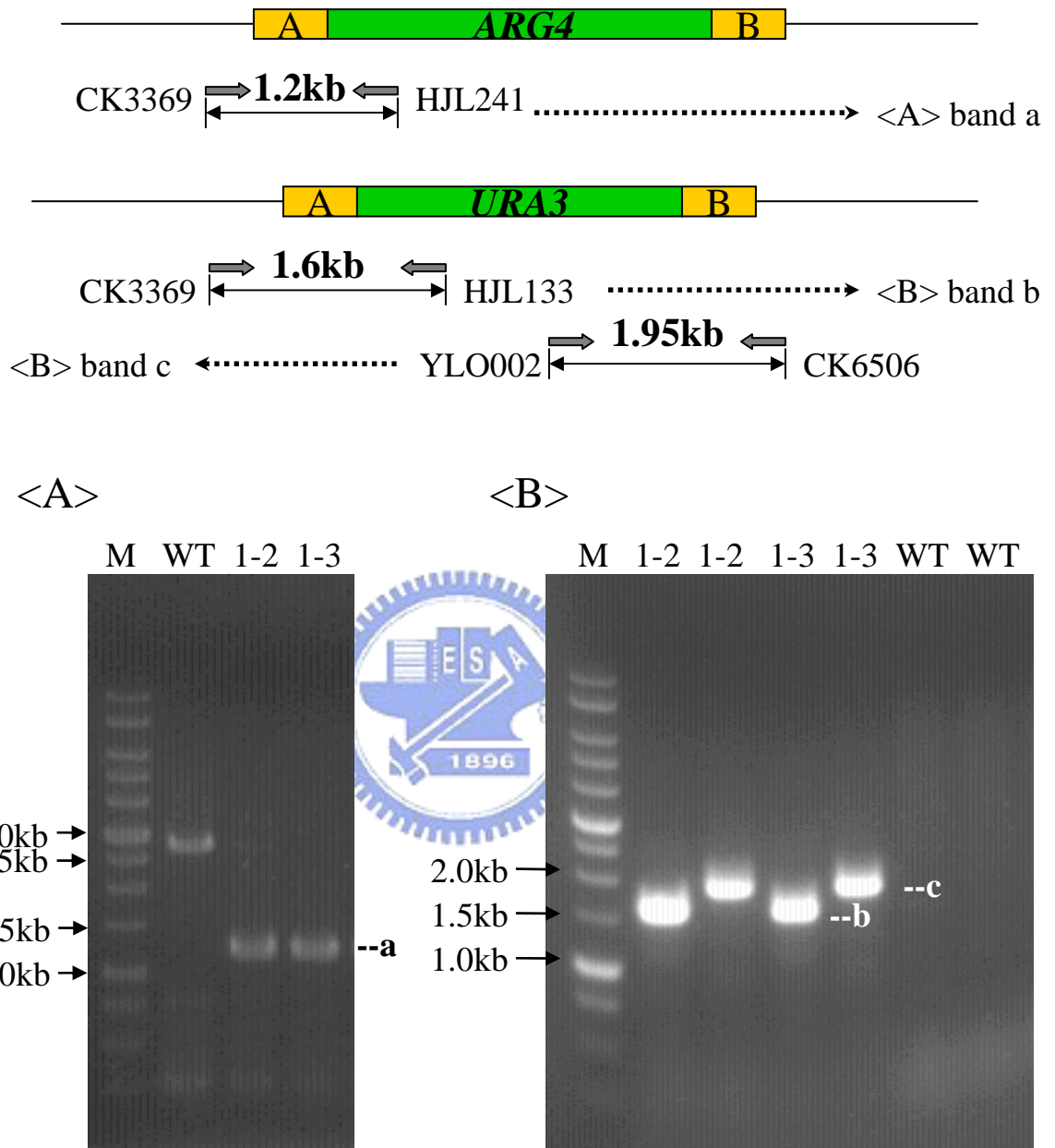
# PCR result of *ENG1* heterozygous knockout



圖五、PCR確認*ENG1*單套基因之破壞

<A> Lane WT為SC5314 (WT)的染色體DNA，在此做為negative control，WT genome 只可得4.5 Kb的DNA片段(band a)，若有轉形成功，可得到DNA片段約3.0 kb (band b)，取此符合預期的四株菌株，進行圖<B>之PCR反應，Lane WT為SC5314 (WT)的染色體DNA，做為negative control，應沒有band若有轉形成功可得到約1.2 Kb DNA片段 (band c)。Lane M表示marker。

# PCR result of *ENG1* homozygous knockout

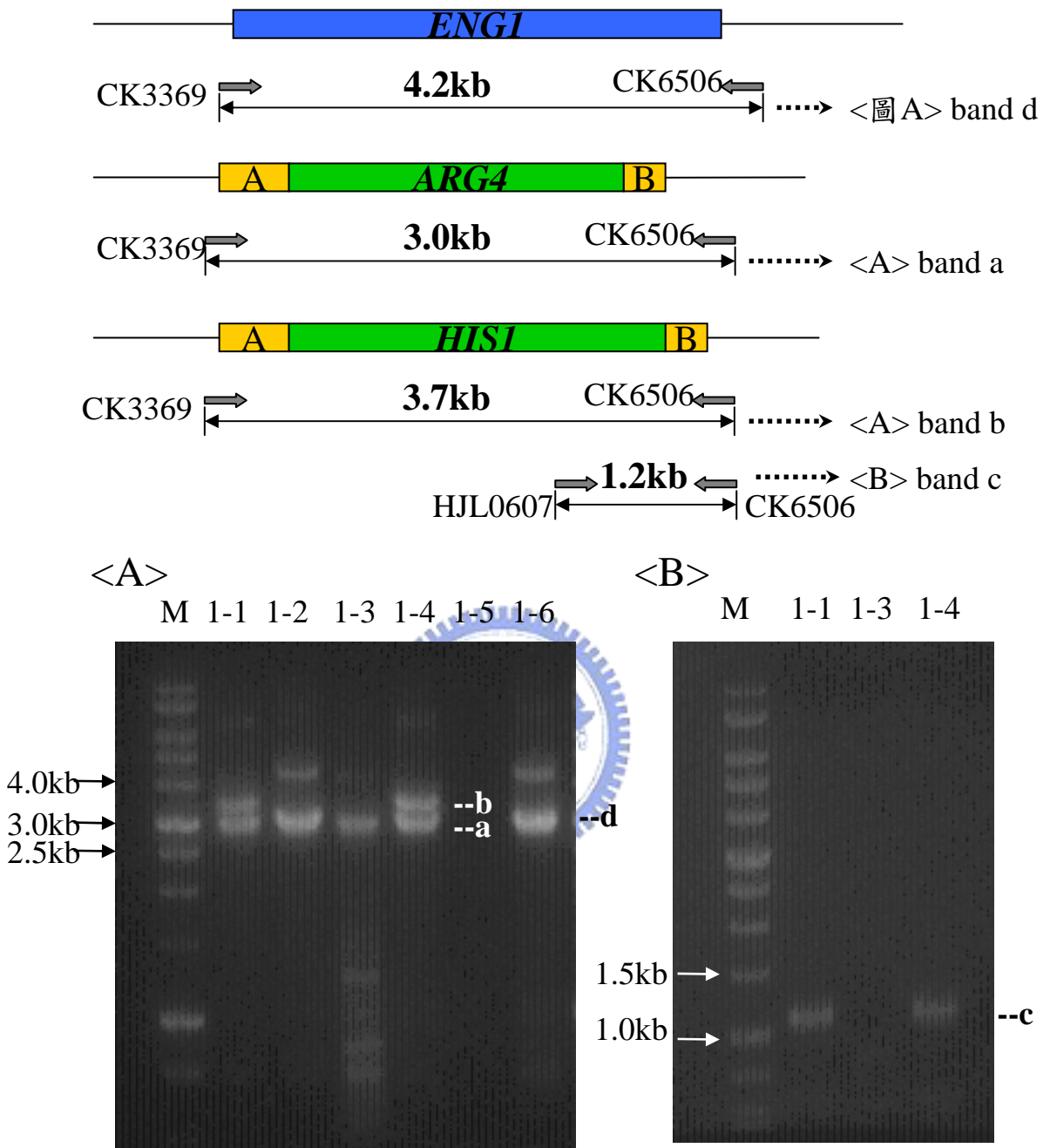


圖六、PCR確認*ENG1*雙套基因以被篩選標記*ARG4*、*URA3*破壞

<A> Lane WT為SC5314 (WT)的染色體DNA，在此做為negative control，若有置換成功應會出現一大小為1.2 Kb的DNA片段(band a)  
 <B> Lane W為SC5314(WT)的染色體DNA，在此做為negative control，若有置換成功應會出現一大小為1.6 Kb (band b)和1.95 Kb (band c)的DNA片段。Lane M表示marker。

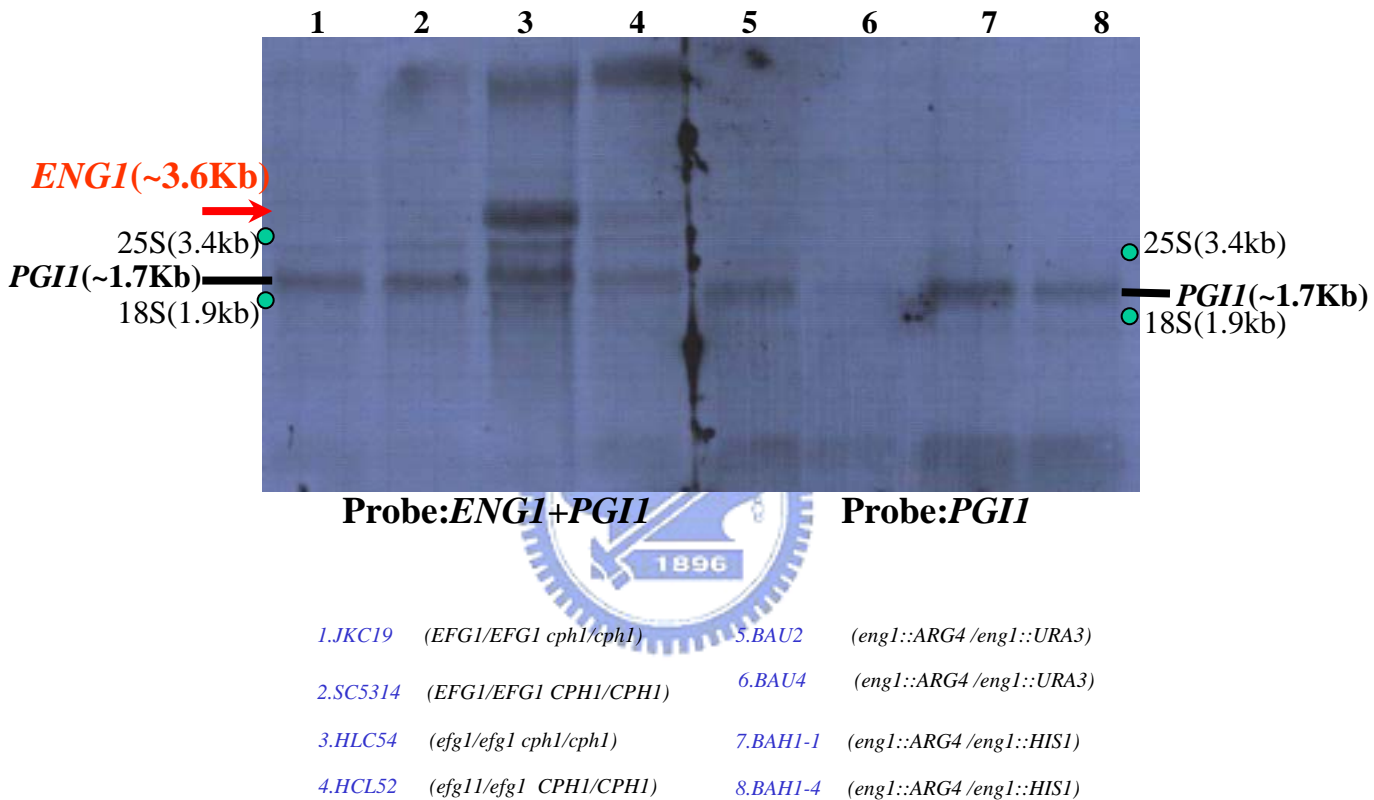


# PCR result of *ENG1* homozygous knockout



圖七、PCR確認*ENG1*雙套基因以被篩選標記*ARG4*、*HIS1*破壞

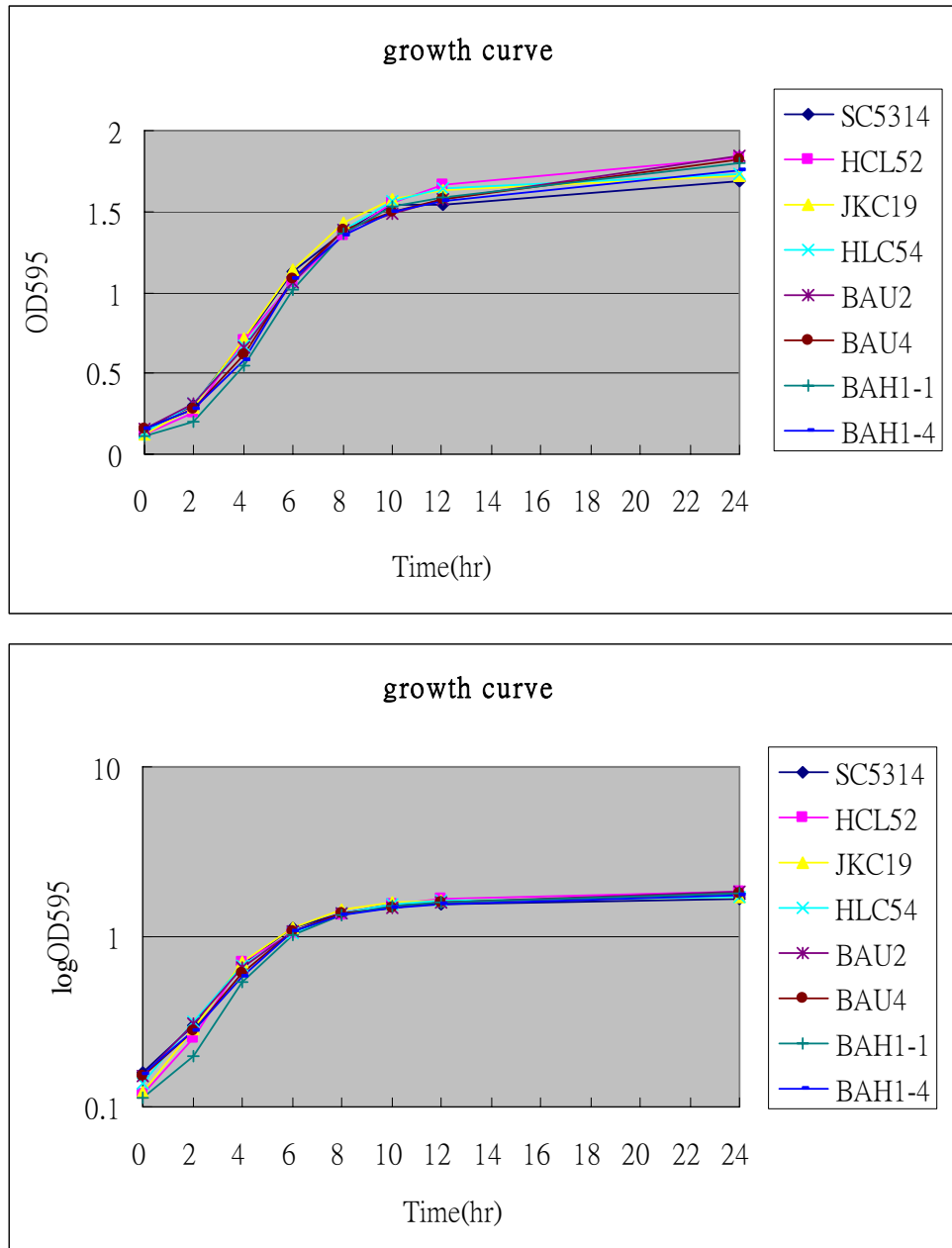
編號1-1~1-6為轉形後所得之六個不同菌株，若轉形成功，經過圖<A>的PCR反應可得兩段分別為3.0 Kb (band a)和3.7 Kb (band b)之DNA片段，若未轉形成功則會出現一長為4.2 Kb (band d)之片段。將突變株1-1、1-3、1-4之染色體DNA再由圖<B>PCR反應，若有轉形成功，可得約1.2 Kb的DNA片段 (band c)，由圖<A>和<B>可知突變株1-1和1-4符合預期。Lane M表示marker。



圖八、*ENG1* 雙套突變株北方墨點法之結果

圖上方表示不同的基因破壞株，以突變株JKC19(*EFG1/EFG1 cph1/cph1*)、HLC54 (*cph1/cph1 efg1/efg1*) HCL52(*efg1/efg1 CPH1/CPH1*)、野生株SC5314(*EFG1/EFG1 CPH1/CPH1*) 為正負對照。在37°C有加山羊血清(10%)的情況下比較*ENG1*雙套突變株BAU2、BAU4、BAH1-1、BAH1-4和上述各菌株RNA表現量的差異。以*PGII*作為 internal control，大小約為1.7Kb。由圖預測*ENG1*大小約為3.6 Kb左右。

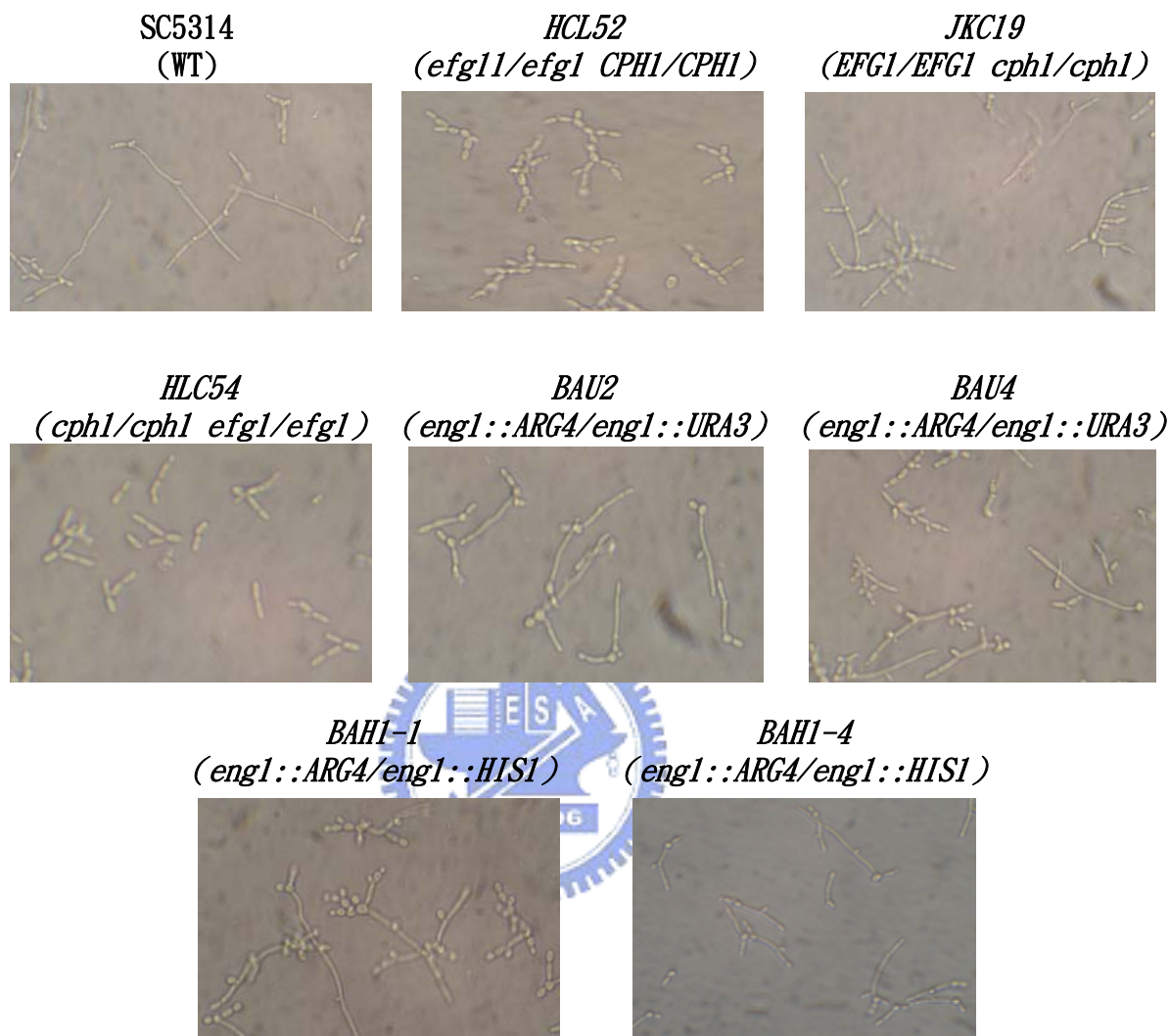
# Growth curve of different strain



圖九、各種突變株在YPD培養液中的生長型態

圖A表示不同的基因破壞株之生長曲線，圖B顯示取對數後的生長趨勢，菌株分別為野生株 SC5314、突變株JKC19 (*EFG1/EFG1 cph1/cph1*)、HCL52 (*efg1/efg1 CPH1/CPH1*)、HLC54 (*cph1/cph1 efg1/efg1*)，突變株BAU2 (*eng1::ARG4/eng1::URA3*)、BAU4 (*eng1::ARG4/eng1::URA3*)、BAH1-1 (*eng1::ARG4/eng1::HIS1*) 和 BAH1-4 (*eng1::ARG4/eng1::HIS1*)。在30°C的YPD培養液中，每兩小時測一次濃度所觀測到菌液濃度變化所得到之結果。

# Germ tube assay

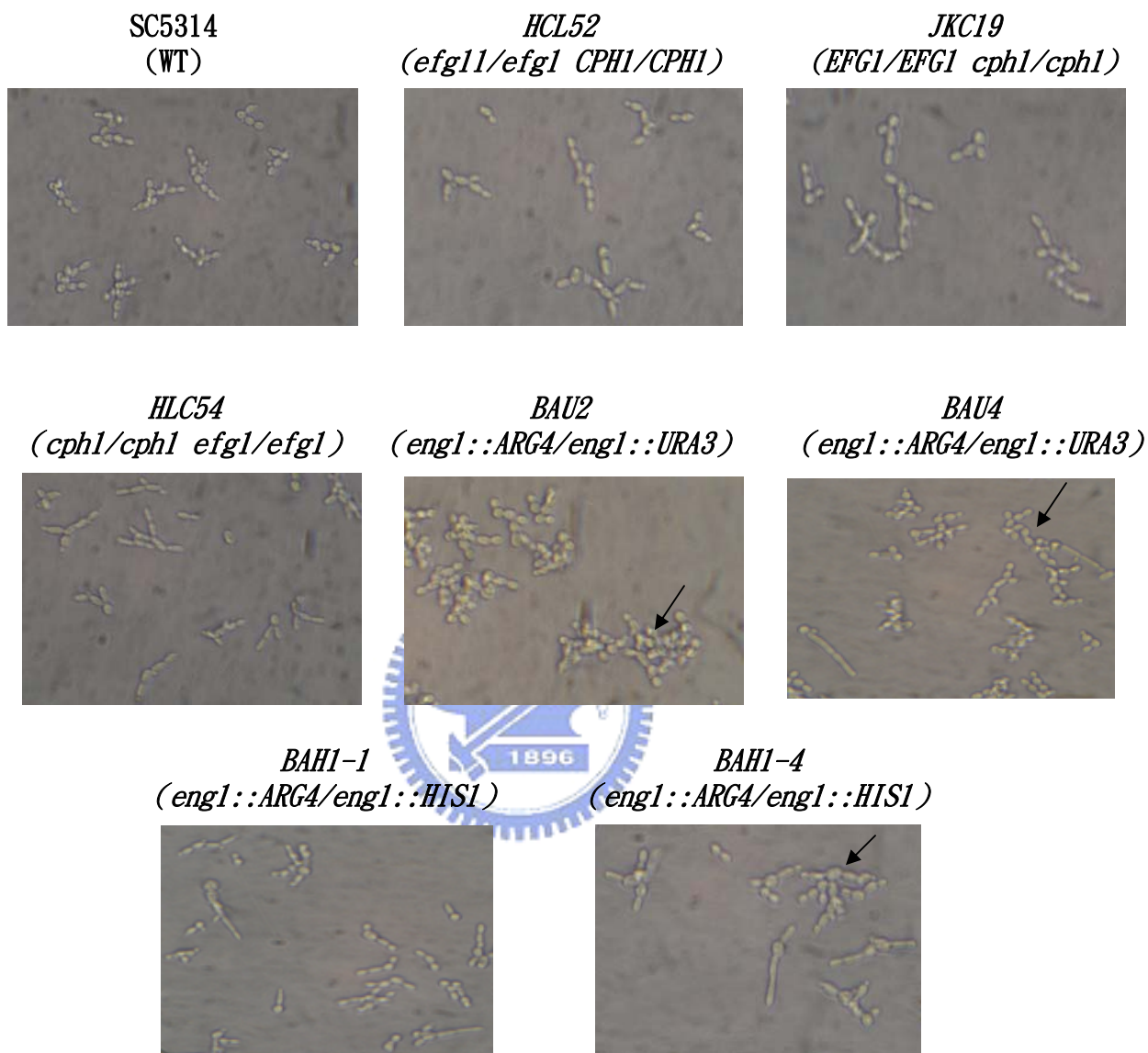


YPD broth with 10% goat serum incubate at 37 °C for 5hr

圖十A、芽管試驗 (germ tube assay)

圖上方表示不同的基因破壞株，以野生株 SC5314、突變株 JKC19 (*EFG1/EFG1 cph1/cph1*)、HCL52 (*efg1/efg1 CPH1/CPH1*)、HLC54 (*cph1/cph1 efg1/efg1*) 作正負對照，在37°C 有加血清的YPD培養液中，培養5小時後，利用倒立式顯微鏡觀測所得到之結果。BAH1-1、BAH1-4有外加uridine。

# Germ tube assay

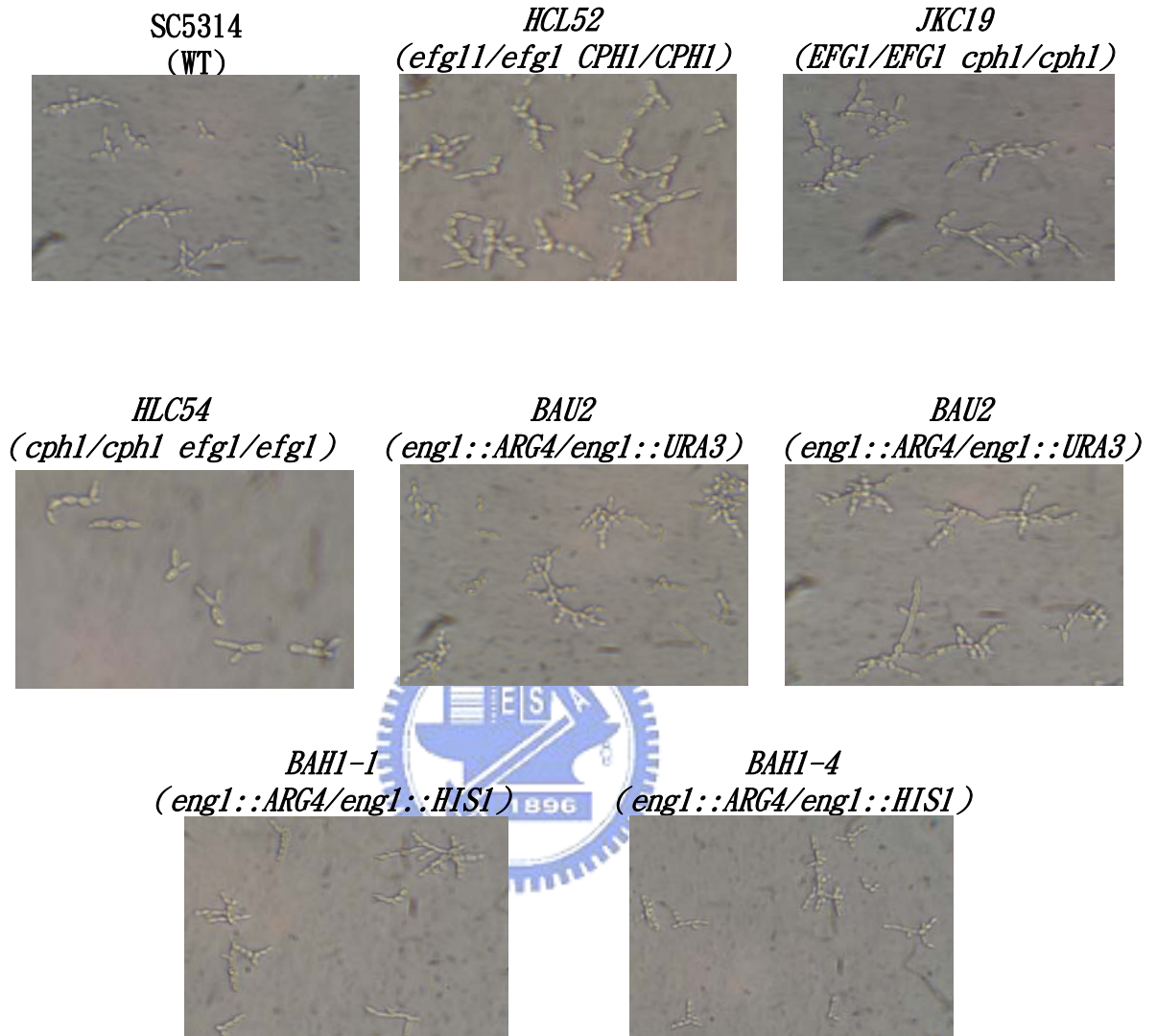


YPD broth with 10% goat serum incubate at 30°C for 5hr

圖十B、芽管試驗 (germ tube assay)

圖上方表示不同的基因破壞株，以野生株 SC5314、突變株 JKC19 (*EFG1/EFG1 cph1/cph1*)、HCL52 (*efg1/efg1 CPH1/CPH1*)、HLC54 (*cph1/cph1 efg1/efg1*) 作正負對照，在30°C有加血清的YPD培養液中，培養5小時後，利用倒立式顯微鏡觀測所得到之結果。BAH1-1、BAH1-4有外加uridine。

# Germ tube assay

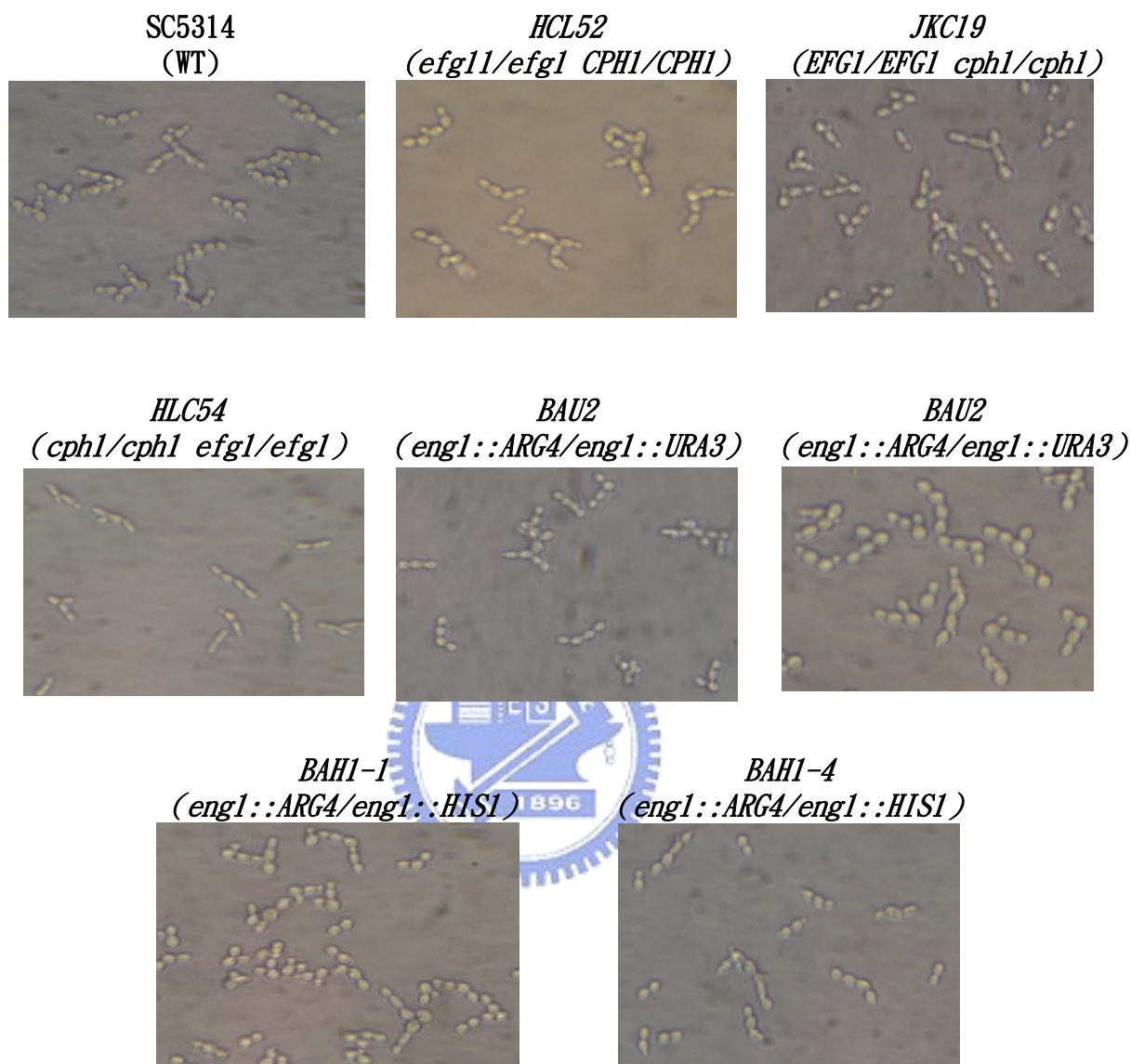


YPD broth incubate at 37 °C  
for 5hr , no serum

圖十C、芽管試驗 (germ tube assay)

圖上方表示不同的基因破壞株，以野生株SC5314、突變株JKC19(*EFG1/EFG1 cph1/cph1*)、HCL52 (*efg1/efg1 CPH1/CPH1*)、HLC54 (*cph1/cph1 efg1/efg1*) 作正負對照，在30°C 有沒有加血清的YPD培養液中，培養5小時後，利用倒立式顯微鏡觀測所得到之結果。BAH1-1、BAH1-4有外加uridine。

# Germ tube assay

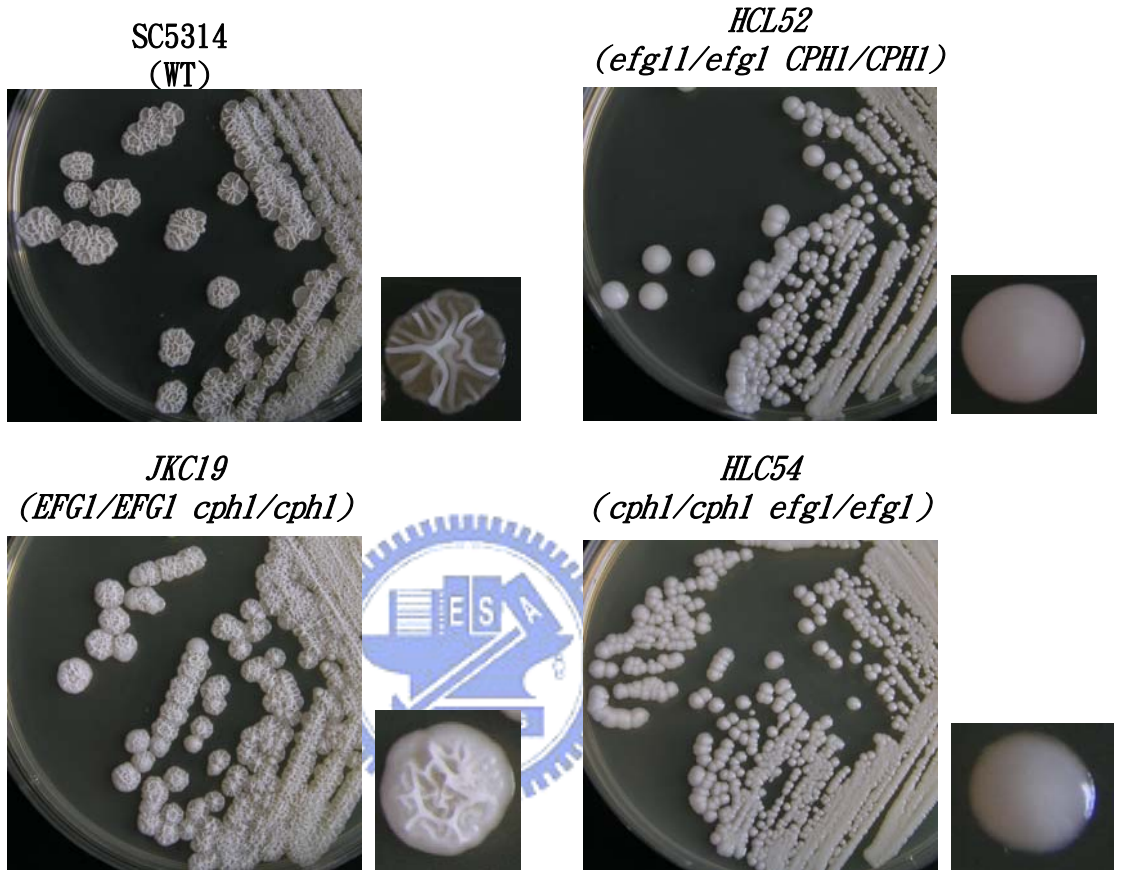


YPD broth incubate at 30 °C  
for 5hr , no serum

圖十D、芽管試驗 (germ tube assay)

圖上方表示不同的基因破壞株，以野生株SC5314、突變株JKC19(*EFG1/EFG1 cph1/cph1*)、HCL52(*efg1/efg1 CPH1/CPH1*)、HLC54 (*cph1/cph1 efg1/efg1*) 作正負對照，在30°C 有沒有加血清的YPD培養液中，培養5小時後，利用倒立式顯微鏡觀測所得到之結果。BAH1-1、BAH1-4有外加uridine。

# Colony on YPD plate with serum

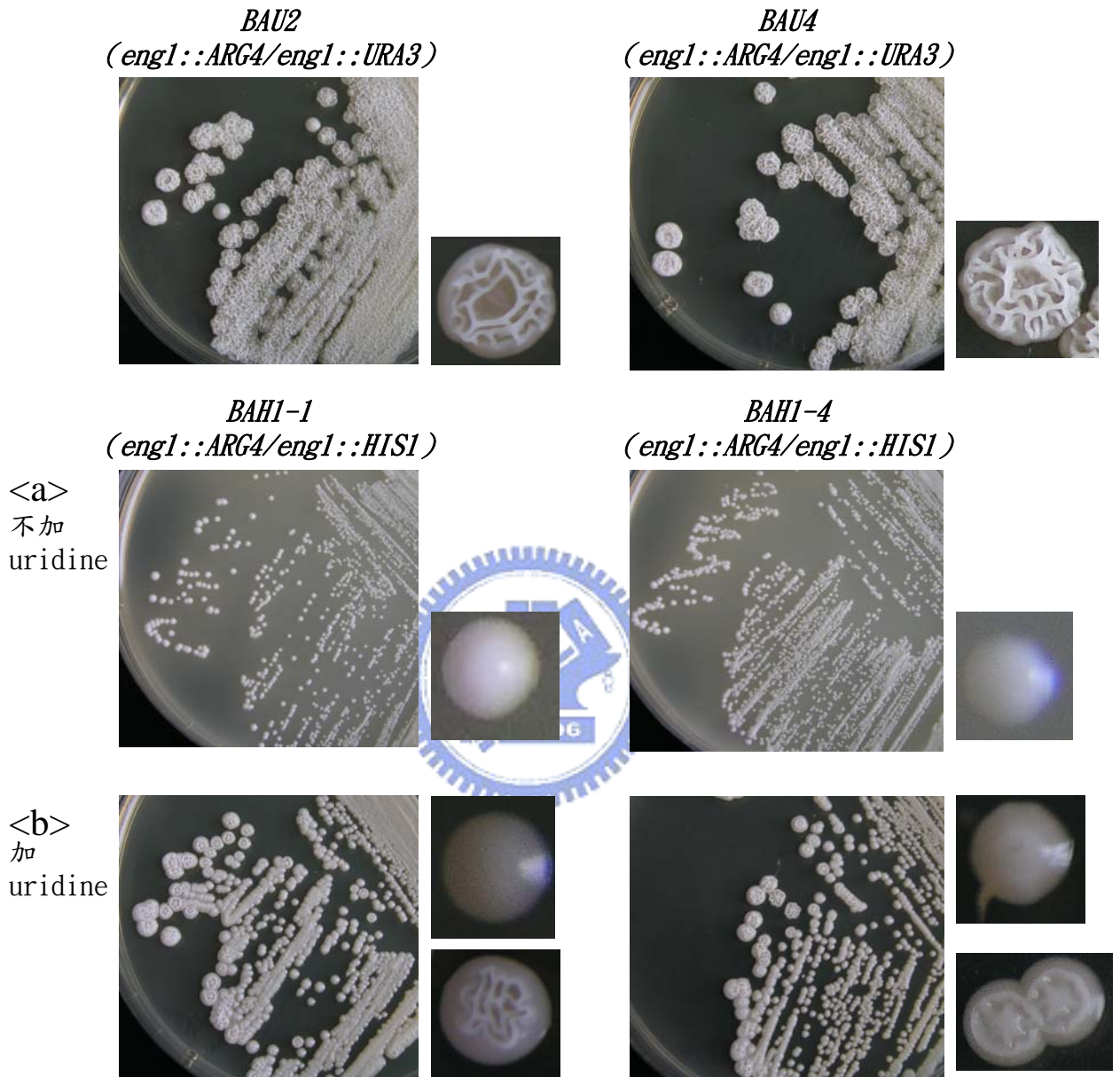


圖十一A、各種突變株在YPD培養基上的生長型態

圖上方表示不同的基因破壞株，分別為野生株 SC5314、突變株 JKC19 (*EFG1/EFG1 cph1/cph1*)、HCL52 (*efg1/efg1 CPH1/CPH1*)、HLC54 (*cph1/cph1 efg1/efg1*)，用來作正負對照。在 37°C 含有 4% 山羊血清的 YPD 培養基中，培養三天後，觀測培養基所得到之結果。大圖為培養基之概觀，右側之小圖為菌落型態之微觀。培養基皆未加入其他營養源。



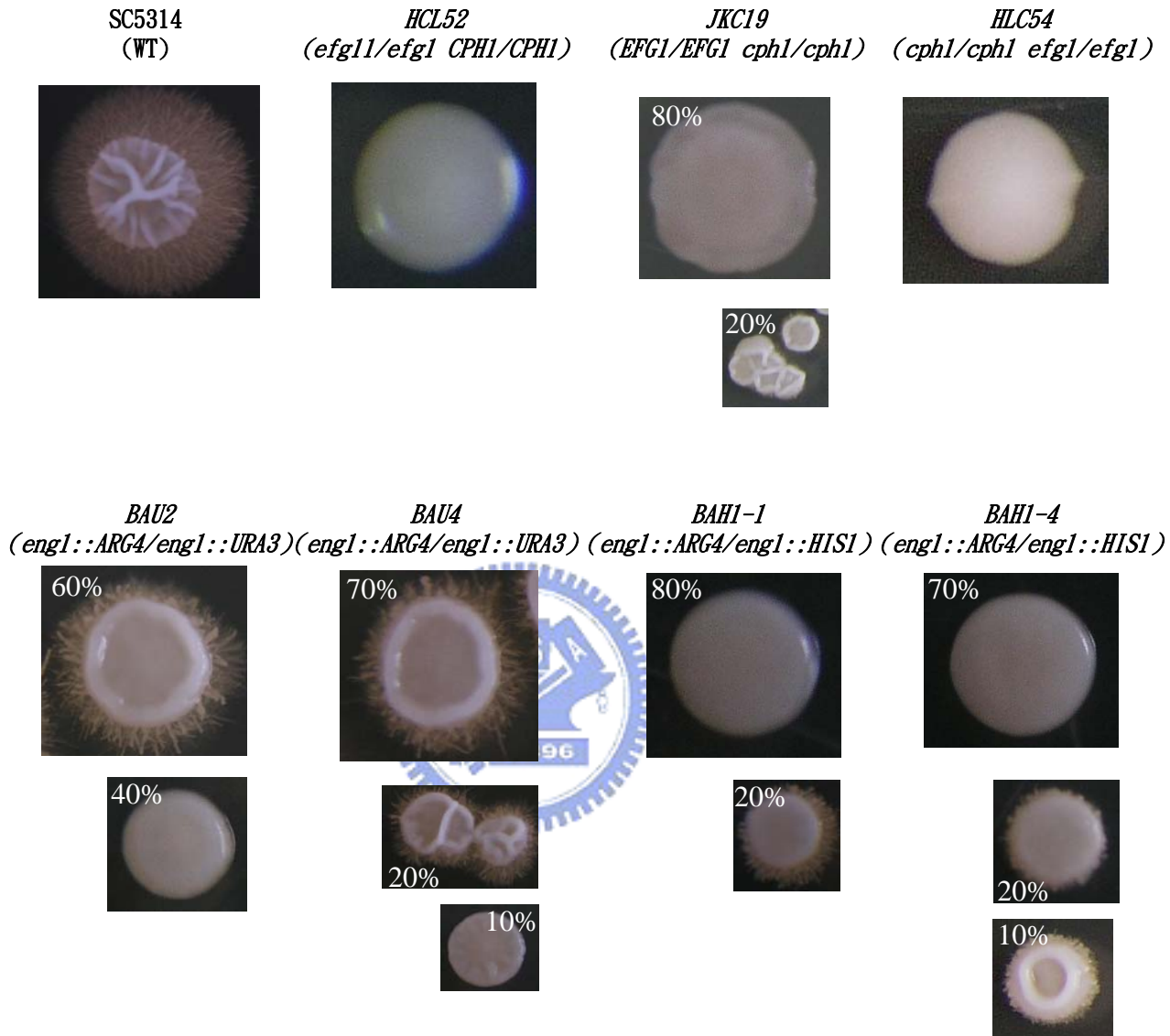
# Colony on YPD plate with serum



圖十一B、各種突變株在YPD培養基上的生長型態

圖上方表示不同的基因破壞株，分別為突變株BAU2、BAU4、BAH1-1和BAH1-4，以前一頁已知的四種菌株作正負對照。在37°C有加4%山羊血清的YPD培養基中，培養三天後，觀測培養基所得之結果。大圖為培養基之概觀，右側之小圖為菌落型態之微觀。BAU2、BAU4培養基未加入其他營養源。  
<a>培養基未加入uridine，<b>培養基有加入uridine。

# Solid spider colony



圖十二、菌株在solid spider培養基上形成的菌落型態

圖上方表示不同的基因破壞株，以野生株 SC5314、突變株 JKC19 (*EFG1/EFG1 cph1/cph1*)、HCL52 (*efg1/efg1 CPH1/CPH1*)、HLC54 (*cph1/cph1 efg1/efg1*) 作正負對照。接種在solid spider培養基中，37°C培養7天所得到之結果。第一列之大圖為主要性狀，第二列或第三列小圖則為次要性狀。百分比顯示此種性狀在培養基上所佔的比例，為估算值。培養基皆未加入其他營養源。