



中国农业科学院植物保护研究所

Institute of Plant Protection(IPP), Chinese Academy of Agricultural Sciences(CAAS)

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求实 创新



利用双生病毒Rep蛋白防治禾谷镰孢菌

The control of *Fusarium graminearum* with the Rep of geminivirus

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利用双生病毒的Rep对禾谷镰孢菌的控制

The control of *Fusarium graminearum* with the Rep of geminivirus

Outline

课题简述

背景

生信分析

小组工作

意义



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一、课题简述



课题简述

←

大前提：环状 ssDNA 病毒编码同源复制相关蛋白(Rep)已在大多数真核生物超组中被发现—— circular ssDNA viruses encoding a homologous replication-associated protein (Rep) have been identified in the majority of eukaryotic supergroups, generating interest in the ecological effects and evolutionary history of circular Rep-encoding ssDNA viruses (CRESS DNA) viruses. (<https://doi.org/10.1016/bs.aivir.2018.10.001>) ←

←

←

双生病毒符合这种 circular ssDNA viruses 模式 ←

←

←

那么双生病毒的 REP 有同源性/保守性 ←

←

本实验室在禾谷镰刀菌里发现鉴定出一个新的双生病毒(W)，找到其他双生病毒的 REP(X) ←

←

←

基于 X 的 Blastp 找到与 X 的互作蛋白后查文献验证得互作蛋白 Y ←

←

←

在本实验主要研究的禾谷镰刀菌里对 Y 进行 blastp 找到在禾谷镰刀菌里与 Y 相似的蛋白 Y' ←

←

←

进行蛋白互作验证实验 (H2B、CO-IP) 验证 Y' 与 W 的 REP 互作 ←

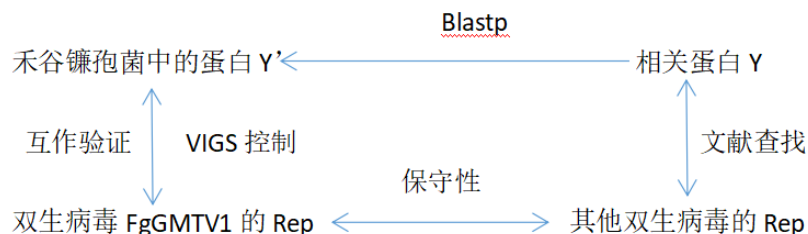
←

←

利用以 FgGMTV1 构建的 VIGS (virus induced gene silencing) 系统插入 Y' 的相关序列，达到对禾谷镰孢菌功能的控制 ←

←

双生病毒的 Rep 具有保守性





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二、背景



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背景

禾谷镰孢菌 (*Fusarium graminearum*) 引起的小麦赤霉病是小麦生产上一种极具毁灭真菌病害，我国是世界上受害面积最大的国家，该病害严重威胁我国粮食生产和食品安全，通过双生病毒Rep的保守性照猫画虎在禾谷镰孢菌中找到与FgGMTV1 Rep互作的蛋白，在同过酵母双杂、免疫共沉淀等方法验证互作之后建立候选蛋白家族，后续通过VIGS (病毒诱导的基因沉默) 载体进行相关功能控制。



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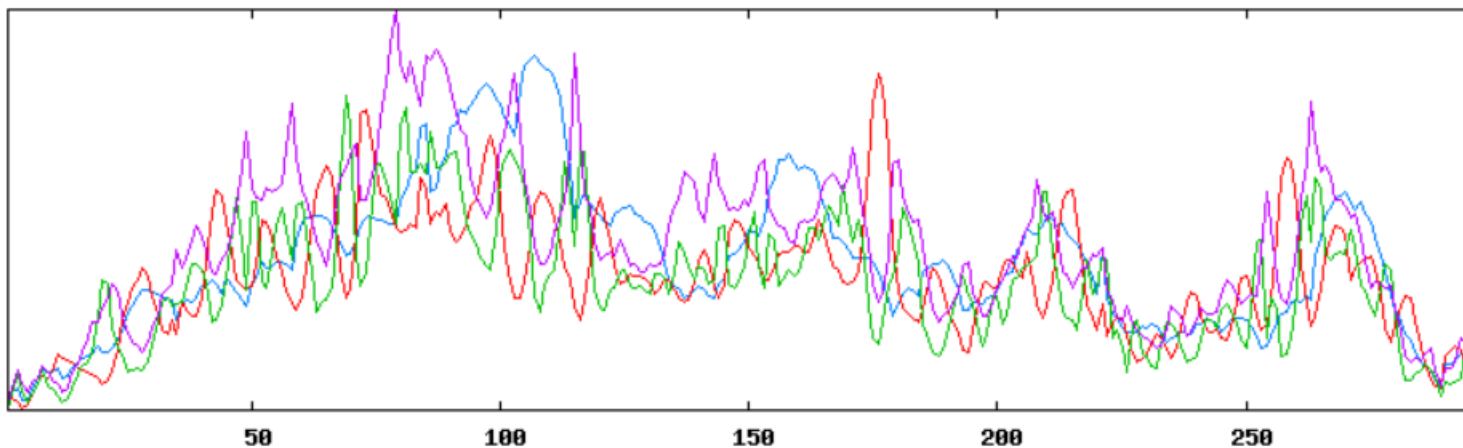
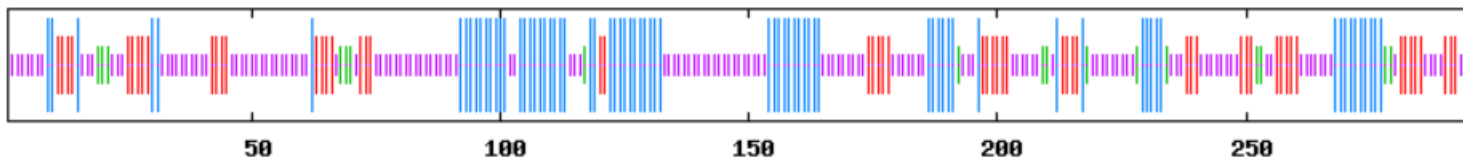
三、对Rep[FgGMTV1]蛋白分析



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α 螺旋(Hh): 75 25.34%

延伸链(Ee): 57 19.26%

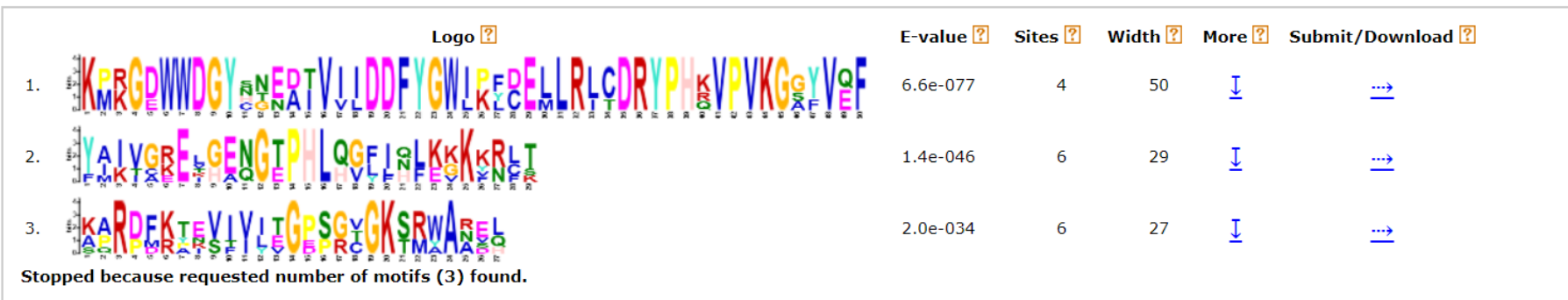
β 转角(Tt): 17 5.74%

无规则卷曲(Cc): 147 49.66%

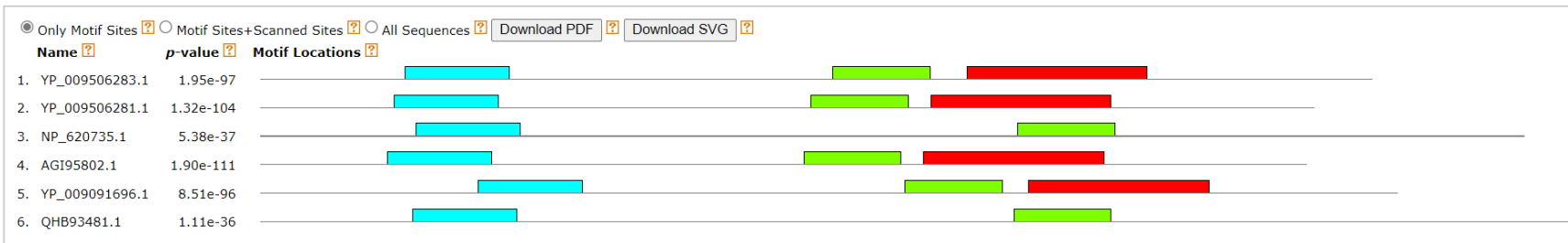


Motif预测

DISCOVERED MOTIFS



MOTIF LOCATIONS





Rep (FgGMTV1) 亲疏水性预测——利用 ExPASy

亲疏水性预测结果
(红框为亲水性较强的区域)

Position (10-30) :

VYLVISKEQHK
DGGFHHHVYW

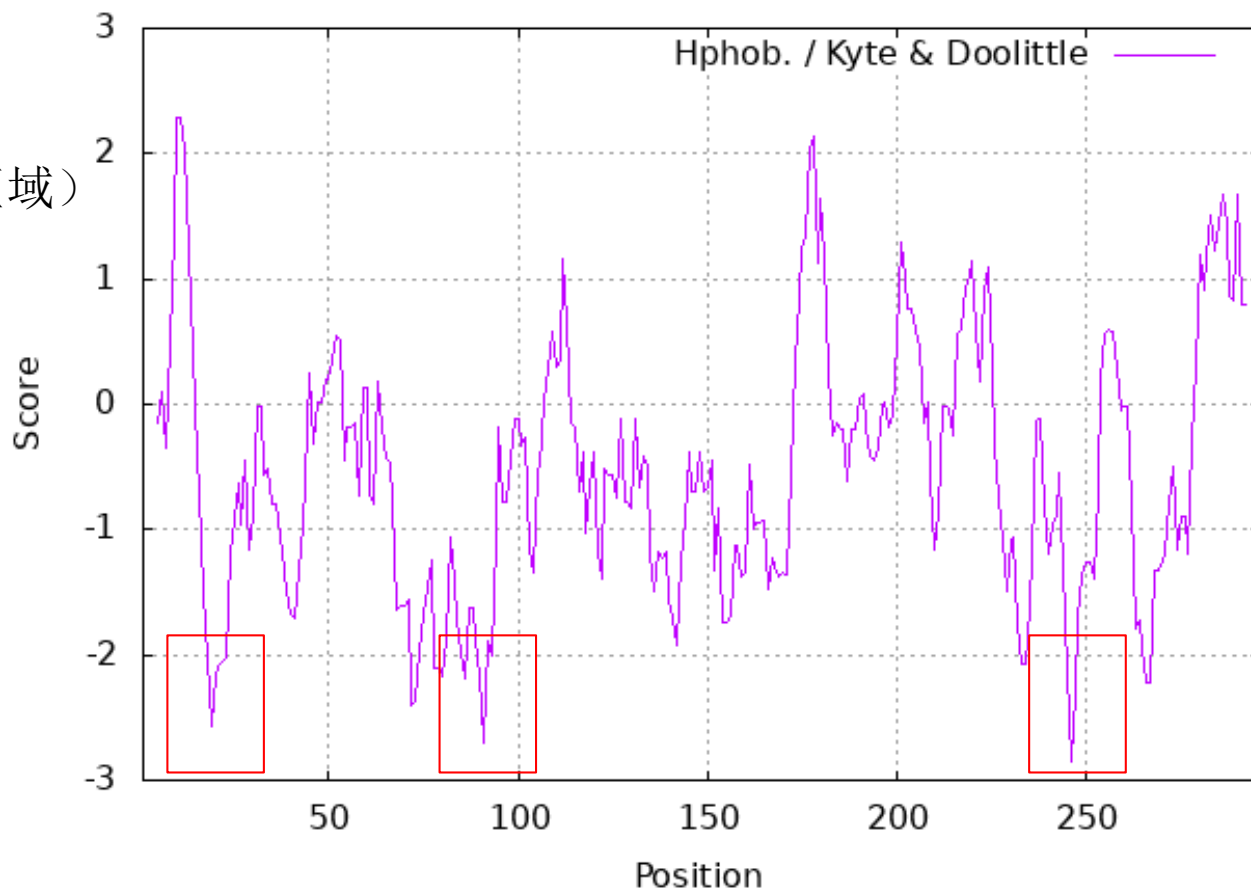
Position (70-100) :

DNDIQWEEGTP
PHQPSLNQKN
MDKEWLRIID

Position (240-250) :

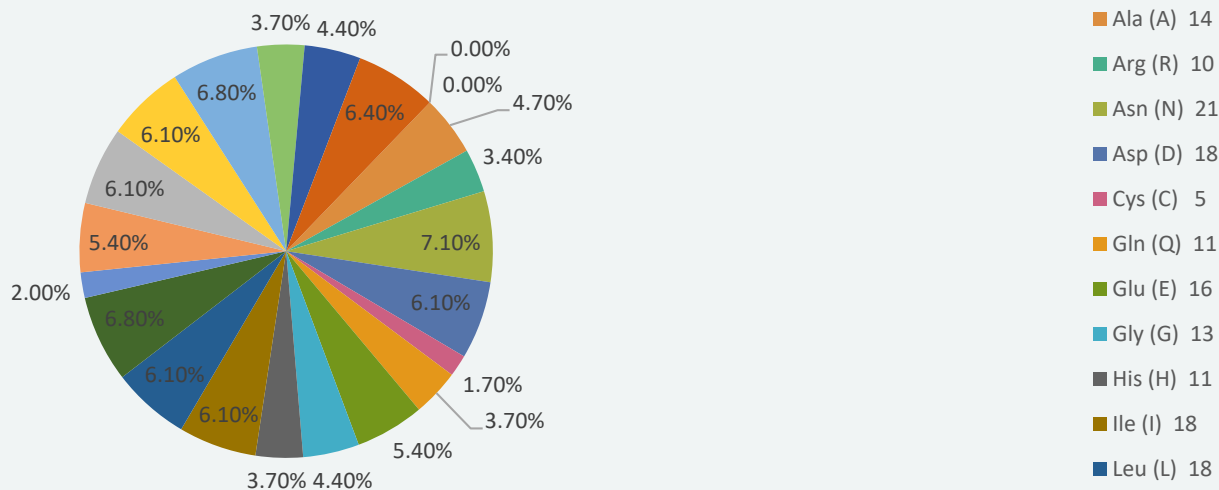
VATDKYK GKQR

ProtScale output for user_sequence





氨基酸分布



分子量: **34453.94**

分子式: **C₁₅₆₇H₂₃₃₇N₄₁₁O₄₄₈S₁₁**

等电点: **6.26**

脂肪族氨基酸指数: **70.78**

亲水性平均值: **-0.525**

氨基酸含量前三: **Asn (N) 21 7.10% (↑3.04%)**

Lys (K) 20 6.80% (↑5.42%)

Thr (T) 20 6.80% (↑1.45%)



5、在uniprot里找到与Rep (FgGMTV1) 相似的50个蛋白，使用MEGA7建立系统发育树，探明发育分支，

Species/Abbrv	Group Name	*
1. QIA59410.1_replication_initiation_protein_Fusarium_graminearum_gemytrip	M	M
2. sp O39521 REPA_BEYDV_Replication-associated_protein_A_OS_Bean_yelli	M	M
3. sp P03567 REP_TGMVY_Replication-associated_protein_OS_Tomato_golde	M	M
4. sp P27260 REP_TYCSV_Replication-associated_protein_OS_Tomato_yellow	M	M
5. sp O39522 REP_BEYDV_Replication-associated_protein_OS_Bean_yellow	M	M
6. sp Q67622 REP_WDVS_Replication-associated_protein_OS_Wheat_dwarf	M	M
7. sp Q9YPS2 REP_MYMVV_Replication-associated_protein_OS_Mungbean_ye	M	M
8. sp P14991 REP_BCTVC_Replication-associated_protein_OS_Beet_curly_to	M	M
9. sp P14978 REP_MSVMN_Replication-associated_protein_OS_Maize_streak	M	M
10. sp P36279 REP_TLCVA_Replication-associated_protein_OS_Tomato_leaf	M	M
11. sp P0CK40 REP_BGYMJ_Replication-associated_protein_OS_Bean_golde	M	M
12. sp P18919 REP_CSMV_Replication-associated_protein_OS_Chloris_striati	M	M
13. sp P31618 REP_TYDVA_Replication-associated_protein_OS_Tobacco_yell	M	M
14. sp Q67590 REP_MISV9_Replication-associated_protein_OS_Miscanthus_s	M	M
15. sp O40986 REP_MSVMSE_Replication-associated_protein_OS_Maize_strea	M	M
16. sp P14989 REP_MSVMSE_Replication-associated_protein_OS_Maize_streak	M	M
17. sp P14988 REP_MSVMSE_Replication-associated_protein_OS_Maize_streak	M	M
18. sp Q80GM6 REP_SSVN_Replication-associated_protein_OS_Sugarcane_s	M	M
19. sp P06847 REPA_WDVS_Replication-associated_protein_A_OS_Wheat_dv	M	M
20. sp P14990 REPA_MSVMSE_Replication-associated_protein_A_OS_Maize_stre	M	M
21. sp P0C647 REP_PASVK_Replication-associated_protein_OS_Panicum_str	M	M
22. sp Q91MG2 REP_MSVMSE_Replication-associated_protein_OS_Maize_strea	M	M
23. sp Q91MF8 REP_MSVMSE_Replication-associated_protein_OS_Maize_strea	M	M
24. sp P27259 REP_TYLCI_Replication-associated_protein_OS_Tomato_yellov	M	M
25. sp Q91GY7 REP_MSVMSE_Replication-associated_protein_OS_Maize_streak	M	M
26. sp Q67620 REP_TYCS2_Replication-associated_protein_OS_Tomato_yellc	M	M
27. sp P29048 REP_SLCV_Replication-associated_protein_OS_Squash_leaf	M	M
28. sp Q00338 REPA_PASVK_Replication-associated_protein_A_OS_Panicum	M	M
29. sp P60470 REPE_ONYPE_Geminivirus-like_replication_protein_OS_Onion	M	M
30. sp Q88888 REP_TPCTV_Replication-associated_protein_OS_Tomato_pse	M	M
31. sp P03568 REPA_MSVMSE_Replication-associated_protein_A_OS_Maize_stre	M	M
32. sp P21947 REP_ABMWV_Replication-associated_protein_OS_Abutilon_mo	M	M
33. sp P38609 REP_TYCS1_Replication-associated_protein_OS_Tomato_yellc	M	M
34. sp P31617 REPA_TYDVA_Replication-associated_protein_A_OS_Tobacco	M	M
35. sp P14982 REP_CLVK_Replication-associated_protein_OS_African_cassa	M	M
36. sp Q91MF7 REPA_MSVMSE_Replication-associated_protein_A_OS_Maize_s	M	M
37. sp Q89822 REPA_SSVN_Replication-associated_protein_A_OS_Sugarcane	M	M
38. sp P0CK39 REP_BGYMVV_Replication-associated_protein_OS_Bean_golde	M	M
39. sp Q82676 REP_ICMV_Replication-associated_protein_OS_Indian_cassav	M	M
40. sp P14972 REP_CLVN_Replication-associated_protein_OS_African_cassa	M	M
41. sp Q06923 REP_PHUV_Replication-associated_protein_OS_Pepper_huasi	M	M
42. sp Q9DXE5 REP_TYLCI_Replication-associated_protein_OS_Tomato_yell	M	M
43. sp Q91GY6 REPA_MSVMSE_Replication-associated_protein_A_OS_Maize_str	M	M
44. sp Q67591 REPA_MISV9_Replication-associated_protein_A_OS_Miscanthu	M	M
45. sp P14980 REPA_MSVMSE_Replication-associated_protein_A_OS_Maize_stre	M	M
46. sp Q91MG1 REPA_MSVMSE_Replication-associated_protein_A_OS_Maize_s	M	M
47. sp P18921 REPA_CSMV_Replication-associated_protein_A_OS_Chloris_st	M	M
48. sp Q06657 REP_TMOV_Replication-associated_protein_OS_Tomato_mottl	M	M
49. sp O40987 REPA_MSVMSE_Replication-associated_protein_A_OS_Maize_st	M	M
50. sp P27258 REP_PYMVV_Replication-associated_protein_OS_Potato_yellov	M	M
51. sp Q96704 REP_CALCV_Replication-associated_protein_OS_Cabbage_gei	M	M

Species/Abbrv	Group Name	*
17. sp P14988 REP_MSVMSE_Replication-associated_protein_OS_Maize_streak	M	M
18. sp Q80GM6 REP_SSVN_Replication-associated_protein_OS_Sugarcane_s	M	M
19. sp P06847 REPA_WDVS_Replication-associated_protein_A_OS_Wheat_dv	M	M
20. sp P14990 REPA_MSVMSE_Replication-associated_protein_A_OS_Maize_stre	M	M
21. sp P0C647 REP_PASVK_Replication-associated_protein_OS_Panicum_str	M	M
22. sp Q91MG2 REP_MSVMSE_Replication-associated_protein_OS_Maize_strea	M	M
23. sp Q91MF8 REP_MSVMSE_Replication-associated_protein_OS_Maize_strea	M	M
24. sp P27259 REP_TYLCI_Replication-associated_protein_OS_Tomato_yellov	M	M
25. sp Q91GY7 REP_MSVMSE_Replication-associated_protein_OS_Maize_streak	M	M
26. sp Q67620 REP_TYCS2_Replication-associated_protein_OS_Tomato_yellc	M	M
27. sp P29048 REP_SLCV_Replication-associated_protein_OS_Squash_leaf	M	M
28. sp Q00338 REPA_PASVK_Replication-associated_protein_A_OS_Panicum	M	M
29. sp P60470 REPE_ONYPE_Geminivirus-like_replication_protein_OS_Onion	M	M
30. sp Q88888 REP_TPCTV_Replication-associated_protein_OS_Tomato_pse	M	M
31. sp P03568 REPA_MSVMSE_Replication-associated_protein_A_OS_Maize_stre	M	M
32. sp P21947 REP_ABMWV_Replication-associated_protein_OS_Abutilon_mo	M	M
33. sp P38609 REP_TYCS1_Replication-associated_protein_OS_Tomato_yellc	M	M
34. sp P31617 REPA_TYDVA_Replication-associated_protein_A_OS_Tobacco	M	M
35. sp P14982 REP_CLVK_Replication-associated_protein_OS_African_cassa	M	M
36. sp Q91MF7 REPA_MSVMSE_Replication-associated_protein_A_OS_Maize_s	M	M
37. sp Q89822 REPA_SSVN_Replication-associated_protein_A_OS_Sugarcane	M	M
38. sp P0CK39 REP_BGYMVV_Replication-associated_protein_OS_Bean_golde	M	M
39. sp Q82676 REP_ICMV_Replication-associated_protein_OS_Indian_cassav	M	M
40. sp P14972 REP_CLVN_Replication-associated_protein_OS_African_cassa	M	M
41. sp Q06923 REP_PHUV_Replication-associated_protein_OS_Pepper_huasi	M	M
42. sp Q9DXE5 REP_TYLCI_Replication-associated_protein_OS_Tomato_yell	M	M
43. sp Q91GY6 REPA_MSVMSE_Replication-associated_protein_A_OS_Maize_str	M	M
44. sp Q67591 REPA_MISV9_Replication-associated_protein_A_OS_Miscanthu	M	M
45. sp P14980 REPA_MSVMSE_Replication-associated_protein_A_OS_Maize_stre	M	M
46. sp Q91MG1 REPA_MSVMSE_Replication-associated_protein_A_OS_Maize_s	M	M
47. sp P18921 REPA_CSMV_Replication-associated_protein_A_OS_Chloris_st	M	M
48. sp Q06657 REP_TMOV_Replication-associated_protein_OS_Tomato_mottl	M	M
49. sp O40987 REPA_MSVMSE_Replication-associated_protein_A_OS_Maize_st	M	M
50. sp P27258 REP_PYMVV_Replication-associated_protein_OS_Potato_yellov	M	M
51. sp Q96704 REP_CALCV_Replication-associated_protein_OS_Cabbage_gei	M	M



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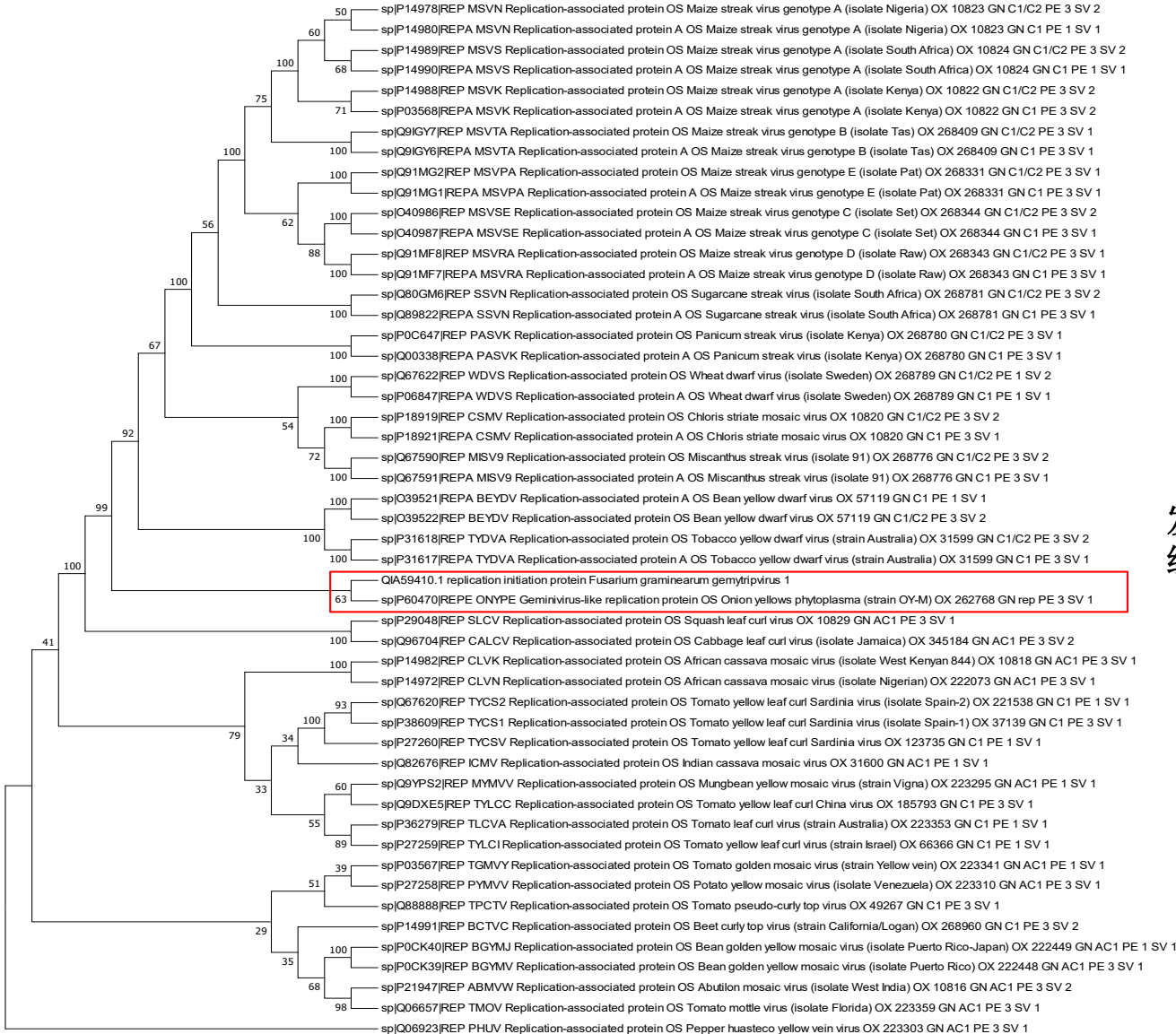
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选择80个（双生病毒科与环病毒科）病毒的
Rep序列与Rep（FgGMTV1）建立系统发育
树



>YP_009506283.1 replication-association protein [Human stool-associated circular virus NG13]			
>YP_009506281.1 replication-association protein [Chimpanzee stool avian-like circovirus Chimpl7]			
>NP_620735.1 replication-associated protein [Tomato pseudo-curly top virus]			
>AGI95802.1 replication-associated protein [Starling circovirus]			
>YP_009091696.1 replication-associated protein [Silurus glanis circovirus]			
>QHB93481.1 replication-associated protein [Potato yellow mosaic virus]			
>NP_065678.1 replication-associated protein [Porcine circovirus 1]			
>YP_009552846.1 replication-associated protein [Paguma larvata circovirus]			
>AXU38623.1 replication-associated protein [Mink circovirus]			
>QGR26085.1 replication-associated protein [Gull circovirus]			
>YP_803549.1 replication-associated protein [Finch circovirus]			
>YP_009552020.1 replication-associated protein [Culex circovirus-like virus]			
>UKF74909.1 replication-associated protein [Bat associated circovirus 12]			
>BBI18987.1 replication-associated protein [Bat associated circovirus 10]			
>YP_009253909.1 replication-associated protein [Avian-like circovirus]			
>BAP81728.1 replication protein [Porcine circovirus 2]			
>QOW03282.1 replication protein [Duck circovirus]			
>YP_009506372.1 replication protein [Abutilon golden mosaic Yucatan virus]			
>ABC75108.1 replication initiation protein [Chino del tomate virus]			
>AMQ09449.1 replication associated protein, partial [Zebra finch circovirus]			
>YP_009508172.1 replication associated protein [Tobacco mottle leaf curl virus]			
>YP_009051691.1 replication associated protein [Rose leaf curl virus]			
>QJF49266.1 Replication associated protein [Chilli leaf curl virus]			
>QNO39334.1 replication associated protein [Canine circovirus]			
>AWV91968.1 replication associated protein [Cabbage leaf curl virus]			
>YP_009666509.1 replication associated protein [Bean leaf crumple virus]			
>UMP08212.1 replication associated protein [Bean golden yellow mosaic virus]			
>YP_010084304.1 replication associated protein [Bat associated circovirus 11]			
>AEB60990.1 replication associated protein [Barbel circovirus]			
>ARQ32250.2 replication associated protein [Alfalfa leaf curl virus]			
>AEI91422.1 replication associated protein [Abutilon mosaic Brazil virus]			
>NP_795340.1 replication associated protein ACI [Cotton leaf crumple virus]			
>CCH63381.1 replication associated protein (Rep) [African cassava mosaic virus]			
>YP_010084329.1 replication associated protein (Rep) [African cassava mosaic Burkina Faso virus]			
>ATG83581.1 replicase-associated protein, partial [Grapevine red blotch virus]			
>YP_009134739.1 replicase [Zebra finch circovirus]			
>YP_010084302.1 replicase [Bamboo rat circovirus]			
>CAR64698.1 RepA [Wheat dwarf virus H07]			
>YP_009551801.1 RepA [Rice latent virus 2]			
>YP_009553476.1 RepA [Rice latent virus 1]			
>QFR15884.1 RepA [Maize striate mosaic virus]			

>QGX90142.1 rep, partial [Porcine circovirus 3]			
>QCZ25039.1 rep [Wheat dwarf virus]			
>AFK64739.1 Rep [Turnip curly top virus]			
>CDF47271.1 Rep [Tobacco curly shoot virus]			
>ACY79444.1 Rep [Sweet potato leaf curl virus]			
>YP_010084724.1 Rep [Rodent associated circovirus 6]			
>YP_010084720.1 Rep [Rodent associated circovirus 5]			
>YP_010084722.1 Rep [Rodent associated circovirus 4]			
>YP_010084725.1 Rep [Rodent associated circovirus 3]			
>YP_010084726.1 Rep [Rodent associated circovirus 2]			
>YP_010084723.1 Rep [Rodent associated circovirus 1]			
>YP_009551800.1 Rep [Rice latent virus 2]			
>YP_009553475.1 Rep [Rice latent virus 1]			
>AFK85001.1 Rep [Rhinolophus ferrumequinum circovirus 1]			
>AKV94652.1 Rep [Mungbean yellow mosaic India virus]			
>QFR15885.1 Rep [Maize striate mosaic virus]			
>QJD25914.1 Rep [Maize streak virus]			
>ALZ45069.1 rep [Goose circovirus]			
>YP_009508415.1 Rep [Common bean mottle virus]			
>YP_009508415.1 Rep [Common bean mottle virus]			
>AAZ68048.2 Rep [Columbid circovirus]			
>YP_009507981.1 Rep [Cabbage leaf curl Jamaica virus]			
>AAS86154.1 Rep [Beet curly top virus]			
>YP_004429235.1 Rep [Bean yellow mosaic Mexico virus]			
>QNG62393.1 Rep [Bean golden mosaic virus]			
>ADN80874.1 Rep [Beak and feather disease virus]			
>YP_009508628.1 Rep [Bat associated circovirus 9]			
>YP_009506280.1 Rep [Bat associated circovirus 8]			
>YP_009506279.1 Rep [Bat associated circovirus 7]			
>YP_009506278.1 Rep [Bat associated circovirus 6]			
>YP_009506277.1 Rep [Bat associated circovirus 5]			
>CAH18979.1 rep protein [Tomato leaf curl China virus]			
>AR077518.1 rep protein [Tick circovirus]			
>YP_764455.1 rep protein [Raven circovirus]			
>YP_009091698.1 putative replication-associated protein [Swan circovirus]			
>QFR15883.1 CP [Maize striate mosaic virus]			
>YP_003778175.1 coat protein [Turnip curly top virus]			
>YP_004376206.1 coat protein [Cleome golden mosaic virus]			
>ASA49173.1 capsid protein [Rice latent virus 2]			
>YP_009553474.1 capsid protein [Rice latent virus 1]			



发现QIA59410.1与sp|P60470|的亲缘关系较近



- 小组工作以及结果

通过查找大量文献和Blast，小组找出9个候选蛋白（从双生病毒科和环病毒科中的

80个病毒的Rep中查找大量文献找出相关蛋

白Y，后将Y进行禾谷镰孢菌物种内的

Blastp) 找到Y'，并明确Y的功能和Y'可供后续利用

VIGS载体对F. g进行相关功能的沉默以达到控制



双生病毒复制相关蛋白=A	文献序号	与A互作的蛋白=B	function	F. g中与B相似的蛋白	文献号
IMYMV (geminiviridae) Rep	1	PCNA (tobacco) Q209W0	PCNA下调 REP	PCNA (Fg. PH-1) Sequence ID: CZS84581.1和 EYB33954.1	DOI:10.1128/JVI.78.21.11890-11903.2004
TGMV AL1	2	histone H3 [Saccharomyces cerevisiae S288C] NP_009564	可能对双病毒的复制和转录有直接影响	histone H3 (Fg. PH-1) Sequence ID: XP_011321327.1、 XP_011318547.1、XP_011327332.1	DOI: 10.1105/tpc.003681
TGMV AL1	2	geminivirus rep interacting kinase 1 [Arabidopsis thaliana] AY100691	下调病毒侵染能力	hypothetical protein [Fusarium graminearum PH-1] Sequence ID: XP_011326610.1、 XP_011318803.1、XP_011328493.1、 XP_011317466.1、XP_011326636.1	DOI: 10.1105/tpc.003681
Mungbean yellow mosaic virus (MYMV) 的CP	4、5	importin alpha 2 [Capsicum annuum]	提供NLS结合位点, 完成对NPC(核孔复合物)的转运	importin subunit alpha [Fusarium graminearum PH-1] Sequence ID: XP_011326851.1	DOI: 10.1099/vir.0.80920-0
Mungbean yellow mosaic virus (MYMV) 的CP	4、5	importin alpha 2 [Capsicum annuum]	提供NLS结合位点, 完成对NPC(核孔复合物)的转运	vacuolar protein 8 [Fusarium graminearum PH-1] Sequence ID: XP_011328805.1	DOI: 10.1099/vir.0.80920-0



双生病毒复制相关蛋白=A	文献序号	与A互作的蛋白=B	function	F. g中与B相似的蛋白	文献号
Porcine circovirus 1 (PCV1) 猪圆环病毒1 REP	7	锌指蛋白 265 (ZNF265)、胸腺嘧啶 DNA 糖基化酶 (TDG)和血管生成因子 VG5Q	Rep和ZNF265的相互作用可能会影响转录和可变剪接, VG5Q和TDG可能		DOI: 10.1046/j .1364 - 3703.200
CCH63381.1 African cassava mosaic virus (Rep)	8	XP_020948293.1	未知	XP_011324787.1、XP_011316555.1、XP_011328252.1、PCD22389.1、XP_011317805.1、PCD38961.1、XP_011320098.1、CAF3481845.1、PCD38961.1	Nature volume 301, pages260 -262
Identification of mungbean yellow mosaic India virus (MYMIV) Rep	9	Q9FL33.1	沉默效果	XP_011317771.1、XP_011318568.1、PCD36408.1、XP_011326807.1、XP_011326427.1、XP_011318396.1、XP_011316178.1	DOI: 10.1080/ 07391102 .2021.193 5319



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