Evolving a Plant Pattern Recognition Receptor to Gain Resistance to a Pathogen-Derived Effector by Using a Novel Reverse Yeast Two-Hybrid System

Summary of yeast strains used in this study

Name	Description	Resource/construction
OVY216	Used for both forward and reverse yeast two-hybrid. Genotype: *MATa ade2-101, his3-Δ200, leu2-3,112, trp1-901, gal4Δ, gal80Δ, LYS2:(lexAop)4-HIS3, SPAL10::URA3, GAL1-lacZ ADE2::LexA-TSG101*	Vincent et al., 2020
OVY216+pGAL1-ymUKG1	Fluorescent protein reporter strain used to quantify the protein-protein interaction strength using fluorescence output.	This work
FRY70		Azizoglu et al., 2021
FRY70+FRP795	FRY70 with FRP795 integrated	This work
FRY1537	Used for reverse split-ubiquitin yeast two-hybrid *MATa met15-Δ0 his3-Δ1 leu2-Δ0 ura3-Δ0,* TADH1 ::FRP235, PURA3::HygMX -insul-(lexA-box)4-PminCYC 1	Ottoz et al., 2014

Summary of plasmids used in this study.

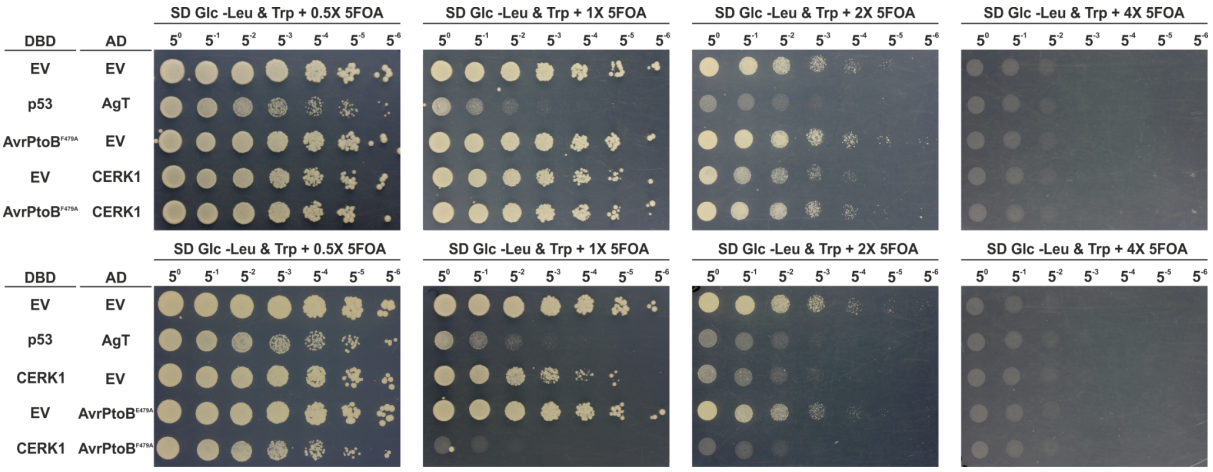
Name	Description	Source and construction procedures
FRP795	insul-(lexA-box)8-PminCYC1-CitrineA206K-TCYC1	From Ottoz et al., 2014
pGAL1-ymUKG1	pGAL1-ymUKG1-tTDH3	Goldengate
pGBKT7		Matchmaker® Gold Yeast Two-Hybrid System (Takara)
pACT2		Matchmaker® Gold Yeast Two-Hybrid System (Takara)
pGBKT7-p53		Matchmaker® Gold Yeast Two-Hybrid System (Takara)
pGADT7-AgT		Matchmaker® Gold Yeast Two-Hybrid System (Takara)
pACT2-AvrPtoB_F479A		
pGBKT7-CERK1cyto		
A series of CERK1 SDM		
pGBKT7-CERK1cyto-$(GS)_3$-BleoR		
pGBKT7-CERK1cyto-GSG-E2A-BleoR		
pGBKT7-CERK1cyto-GSG-O2A-BleoR		
pGBKT7-CERK1cyto-GSG-P2A-BleoR		
Plasmids for plants		
p35S-CERK1(E+T)-[LacZ]-tNOS	An empty vector Lacking CERK1 cytoplasmic domain to construct 35S	Goldengate

	promoter-driven CERK1. CERK1 cytoplasmic domain variants obtained from the reverse yeast two-hybrid assay can be inserted into this empty vector using goldengate cloning. Positive colonies can be screened using Blue-white screening.	
pAtCERK1-CERK1(E+T)-[LacZ]-tNOS		In-fusion.
XVE-OlexA-CERK1(E+T)-[LacZ]-tNOS		Golden gate cloning
pTRV1		Liu et al., 2002
pTRV2-GFP		Lab stock
pTRV2-NbCERK1(fwd)		Classic restriction enzyme cloning.
pTRV2-NbCERK1(rev)		Classic restriction enzyme cloning
XVE-OlexA-AvrPtoB-tNOS		

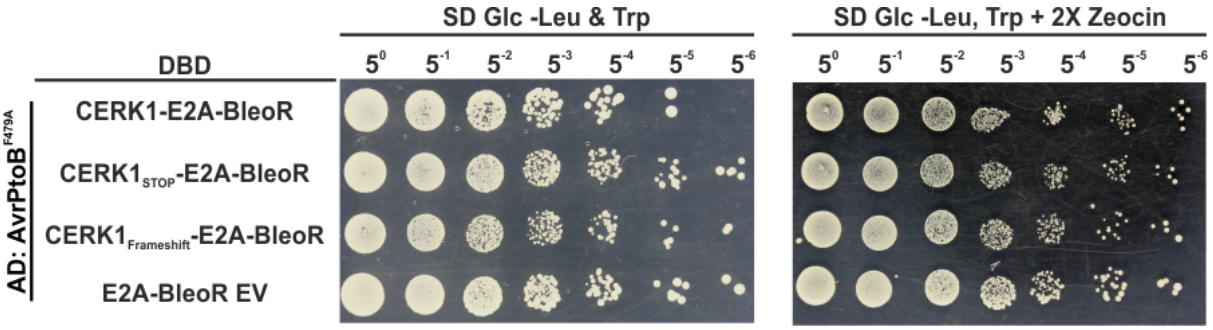
Plasmid Sequences

Plasmid sequences are stored in FASTA format and can be found at https://github.com/melkonyan/trust-y2h-plasmids

Supplementary Figures



Supplementary Figure 1. The effect of differing 5-FOA concentrations on the Y2H plate assay.
5FOA concentration of 1X corresponds to 1 mg mL^{-1}.



Supplementary Figure 2. The BleoR anti-truncation system exhibits a leaky behaviour if not plate immediately post-transformation.
Zeocin concentration of 2X corresponds to 2 mg mL^{-1}.