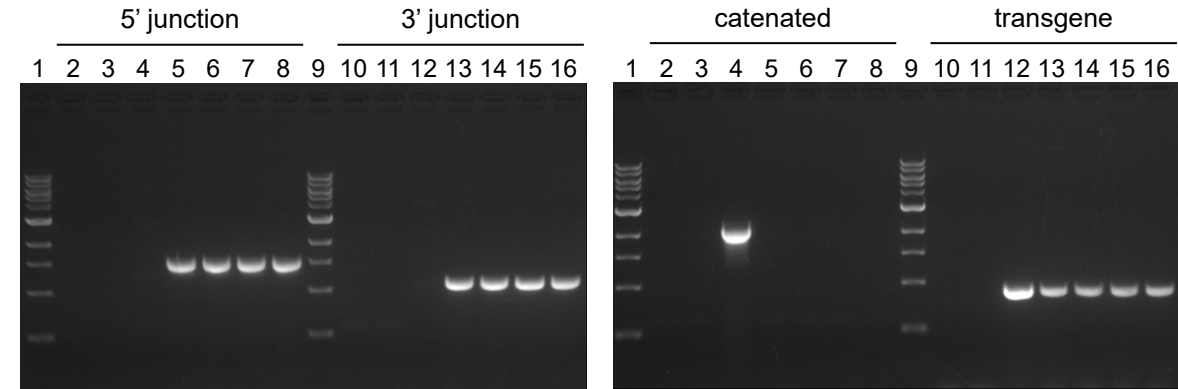
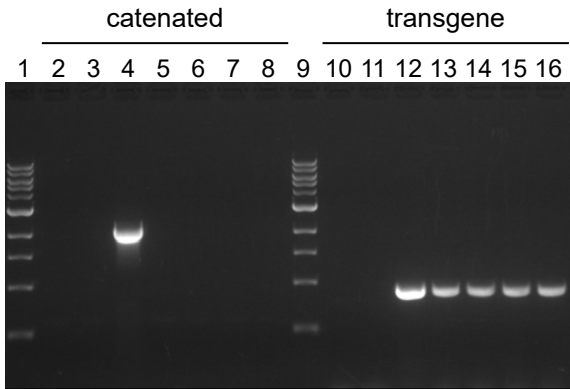


► PCR validations of *C. neoformans* $MF_{\alpha_{Cd}}$ transformants



(File: MFalpha_Cdep_PC Rs_01.jpg)

Note: the 5' junction and 3' junction PCRs detect the junctions between pAP1 and the safe haven insertion site.

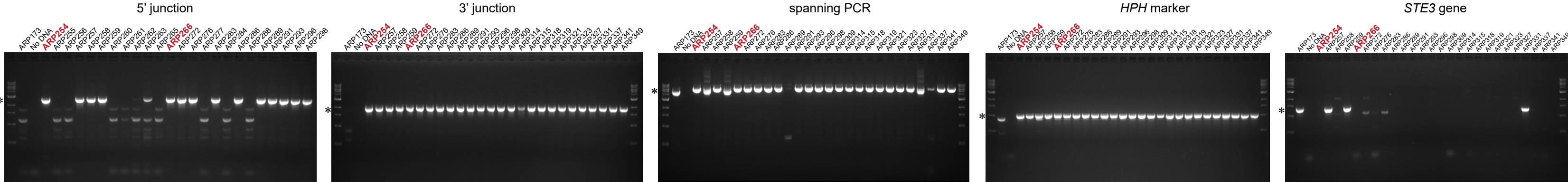


(File: MFalpha_Cdep_PC Rs_02.jpg)

Note: The catenated plasmid PCR uses the 5' junction reverse primer paired with the 3' junction forward primer to detect tandem insertions. Only the plasmid (still circular) yielded a product. The pheromone transgene PCR is specific for $MF_{\alpha_{Cd}}$.

- 1 - Ladder
- 2 - YPH570 (KN99a *crg1* Δ ::*NAT*; parent of transformants ARP173 and ARP174)
- 3 - H99 *crg1* (F99 α *crg1* Δ ::*URA5*; parent of transformants ARP242 and ARP243)
- 4 - pAP1 (plasmid to express $MF_{\alpha_{Cd}}$ integrated at the safe haven locus)
- 5 - ARP173 (KN99a *crg1* Δ ::*NAT* $MF_{\alpha_{Cd}}$ NEO #1)
- 6 - ARP174 (KN99a *crg1* Δ ::*NAT* $MF_{\alpha_{Cd}}$ NEO #2)
- 7 - ARP242 (F99 *crg1* Δ ::*URA5* $MF_{\alpha_{Cd}}$ # 1)
- 8 - ARP243 (F99 *crg1* Δ ::*URA5* $MF_{\alpha_{Cd}}$ # 2)
- 9-16 - same order as before

► PCR to identify which transformants have *STE3* deleted and which ones have ectopic insertion of the *HPH* (*HYG*) marker gene.

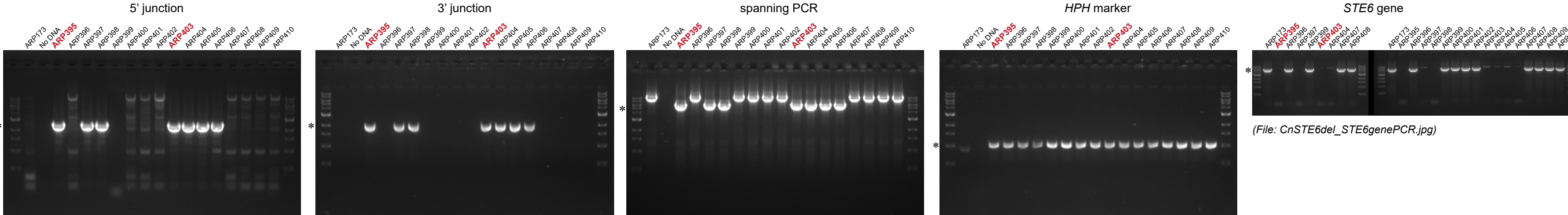


(File: CnSTE3del_5junction.jpg)

Note: correct *ste3* Δ transformants selected for further experiments (ARP254 and ARP266) are marked in red; expected band sizes in each gel are marked with an asterisk

ARP254 - KN99a *crg1* Δ ::*NAT* $MF_{\alpha_{Cd}}$ NEO *ste3* Δ ::*HYG* 1.1
ARP266 - KN99a *crg1* Δ ::*NAT* $MF_{\alpha_{Cd}}$ NEO *ste3* Δ ::*HYG* 2.6

► PCR to identify which transformants have *STE6* deleted and which ones have ectopic insertion of the *HPH* (*HYG*) marker gene.



(File: CnSTE6del_5junction.jpg)

Note: correct *ste6* Δ transformants selected for further experiments (ARP395 and ARP403) are marked in red; expected band sizes in each gel are marked with an asterisk

ARP395 - KN99a *crg1* Δ ::*NAT* $MF_{\alpha_{Cd}}$ NEO *ste6* Δ ::*HYG* 1.1
ARP403 - KN99a *crg1* Δ ::*NAT* $MF_{\alpha_{Cd}}$ NEO *ste6* Δ ::*HYG* 2.1