

Figure 5—Western blot analysis of potential ‘bait’ fusion proteins. Whole-cell protein extracts were fractionated on a denaturing 12% polyacrylamide gel, transferred to nitrocellulose and detected with anti-LexA monoclonal antibody. Protein was prepared from yeast strains containing pLexA, four independent clones of pLexA-BAR1, pLexA-BBR1.1, or pLexA-SBR13.25. The fusion causes a gel shift when compared to the LexA protein alone. The accumulation of BAR1, SBR1, and BBR1 fusion proteins are shown while accumulation of PBR1 was not detected. pLexA-BAR1.34, -BBR1.1, and -SBR13.25 were used in the yeast two-hybrid analysis.

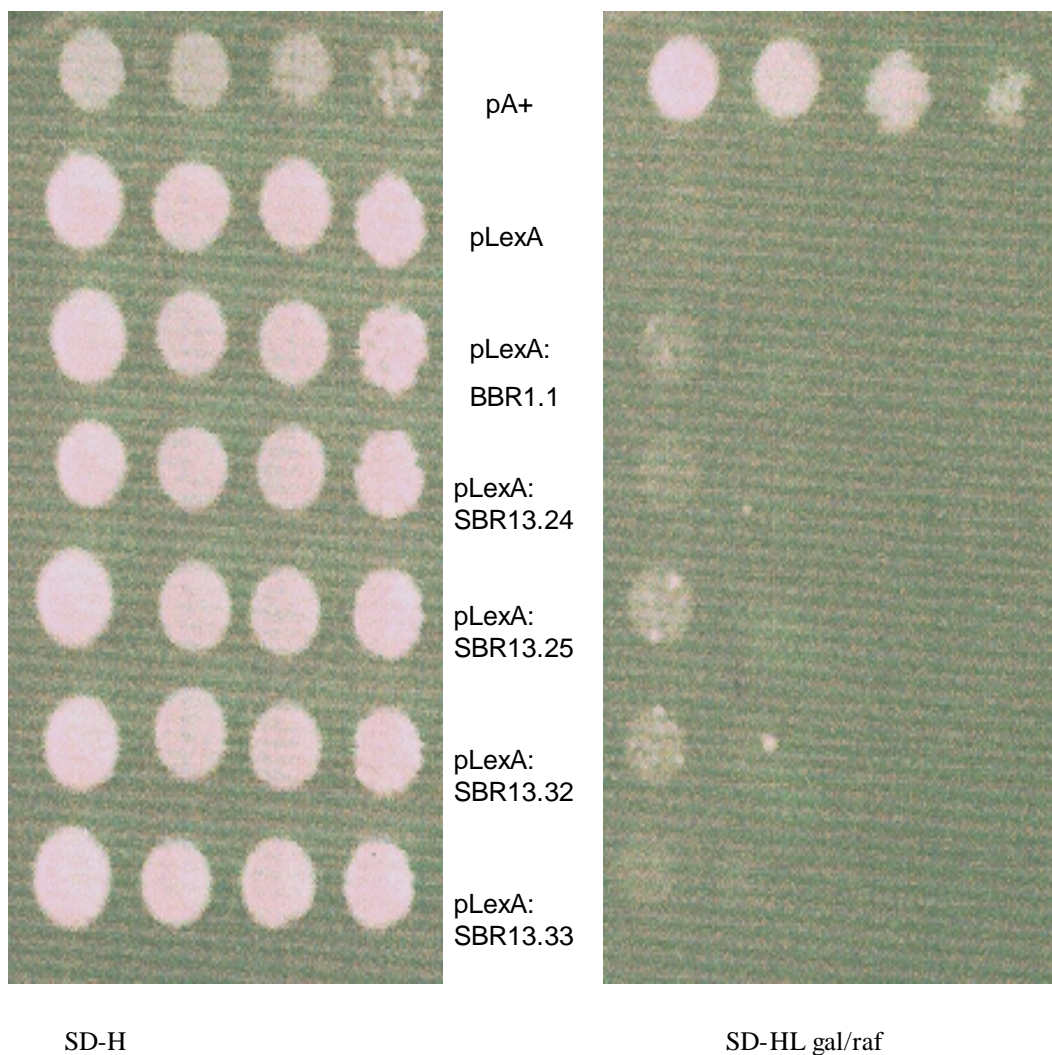


Figure 6—Test of autoactivation by SLCV BR1 ‘bait’ fusions to LexA-DB. Serial dilutions of each yeast strain were spotted onto (left) minimal medium lacking histidine (SD-H) with glucose as the carbon source and (right) minimal medium lacking histidine and leucine with galactose and raffinose as the carbon source (SD-HL gal/raf). The plates were photographed after 5 days of incubation at 30°C. Yeast strains were EGY48 containing pLexA-Pos (pA+), an autoactivation control, pLexA vector, or plasmids expressing LexA-BR1 fusion proteins as indicated.

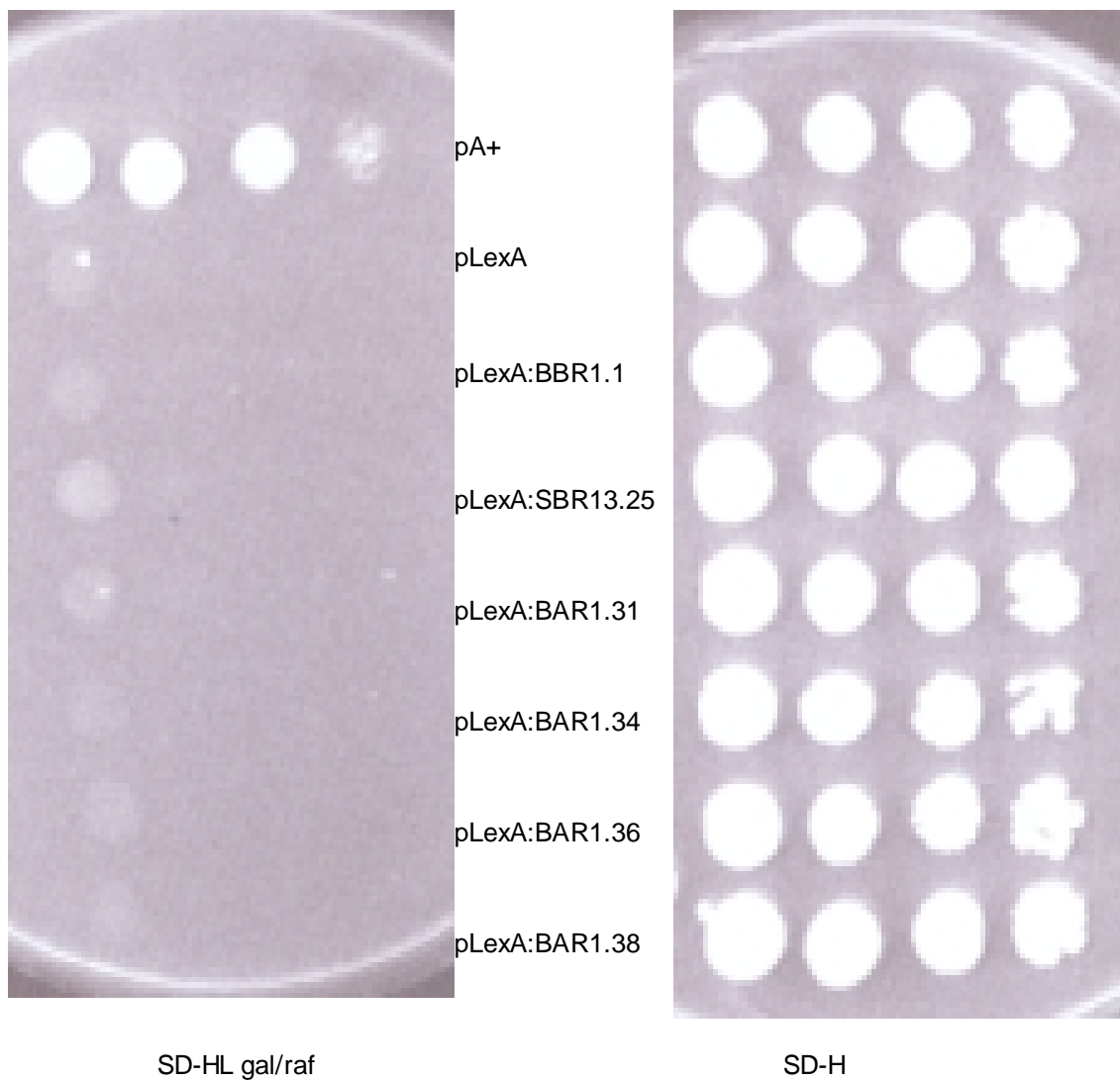


Figure 7— Test of autoactivation by BGMV AR1 ‘bait’ fusions to LexA-DB. Serial dilutions of each yeast strain were plated on SD-H (right) and SD-HL gal/raf (left) media (see legend to figure 6 fo description of media) and incubated at 30°C for 5 days. Yeast strains were EGY48 containing plasmids pLexA-Pos (pA+), pLexA, or its derivatives from which LexA-BBR1, -SBR1, or -BAR1 fusion proteins were expressed.

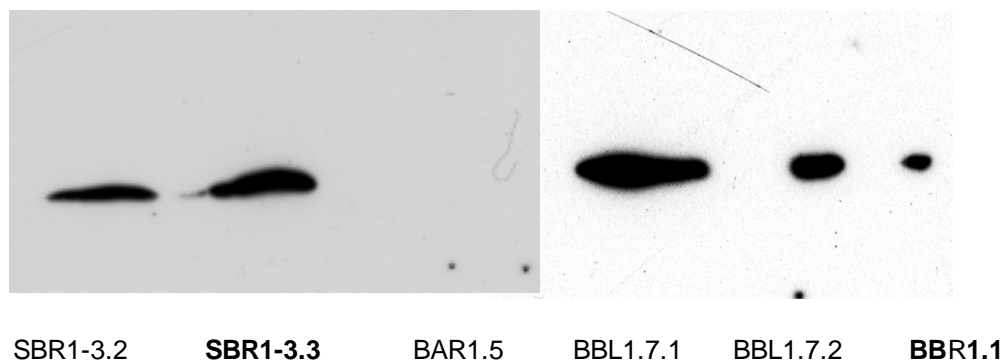


Figure 8—Western blot analysis of potential ‘prey’ fusion proteins. Whole-cell protein extracts were fractionated on a denaturing 12% polyacrylamide gel, transferred to nitrocellulose and detected with anti-HA tag monoclonal antibody. Protein was prepared from yeast strains containing two independent clones of pB42AD-SBR1, and pB42AD-BBL1, pB42AD-BAR1, or pB42AD-BBR1. The accumulation of SBR1, BBL1, and BBR1 fusion proteins are shown, while accumulation of BAR1 was not detected.

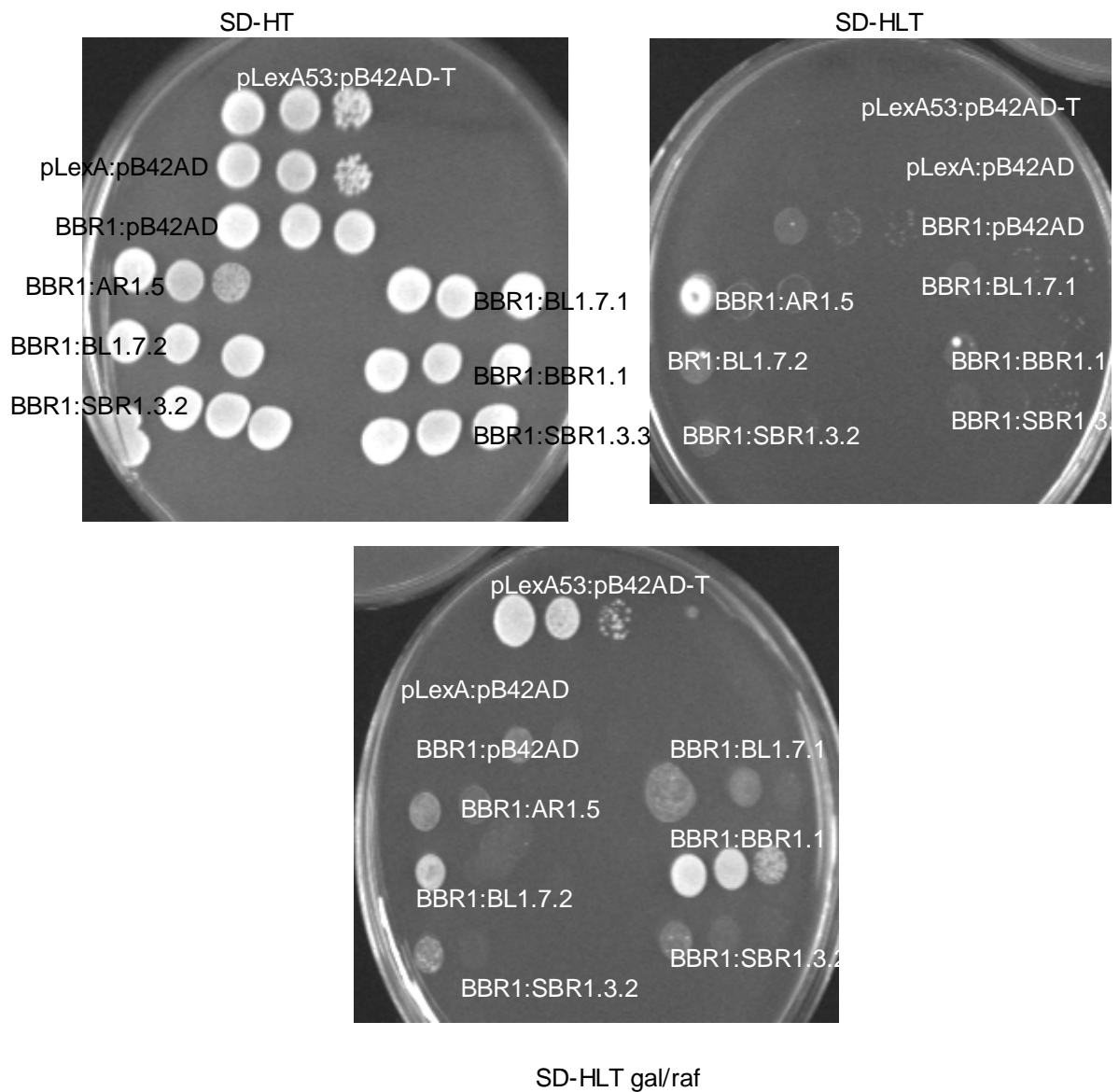


Figure 9—The interactions between BBR1 bait and viral protein preys. Serial dilutions of yeast strains were spotted onto SD-HT, SD-HLT and SD-HLT gal/raf media (see legend to figure 7 for details) and incubated at 30°C for 4 days. Control yeast strains were EGY48 containing pLexA-53+pB42AD-T (Positive control), and pLexA+pB42AD or pLexA-BBR1+pB42AD (positive control). Other strains contained pLexA-BBR1 bait + pB42AD-BAR1, -BBR1, -BBL1, or -SBR1 preys.

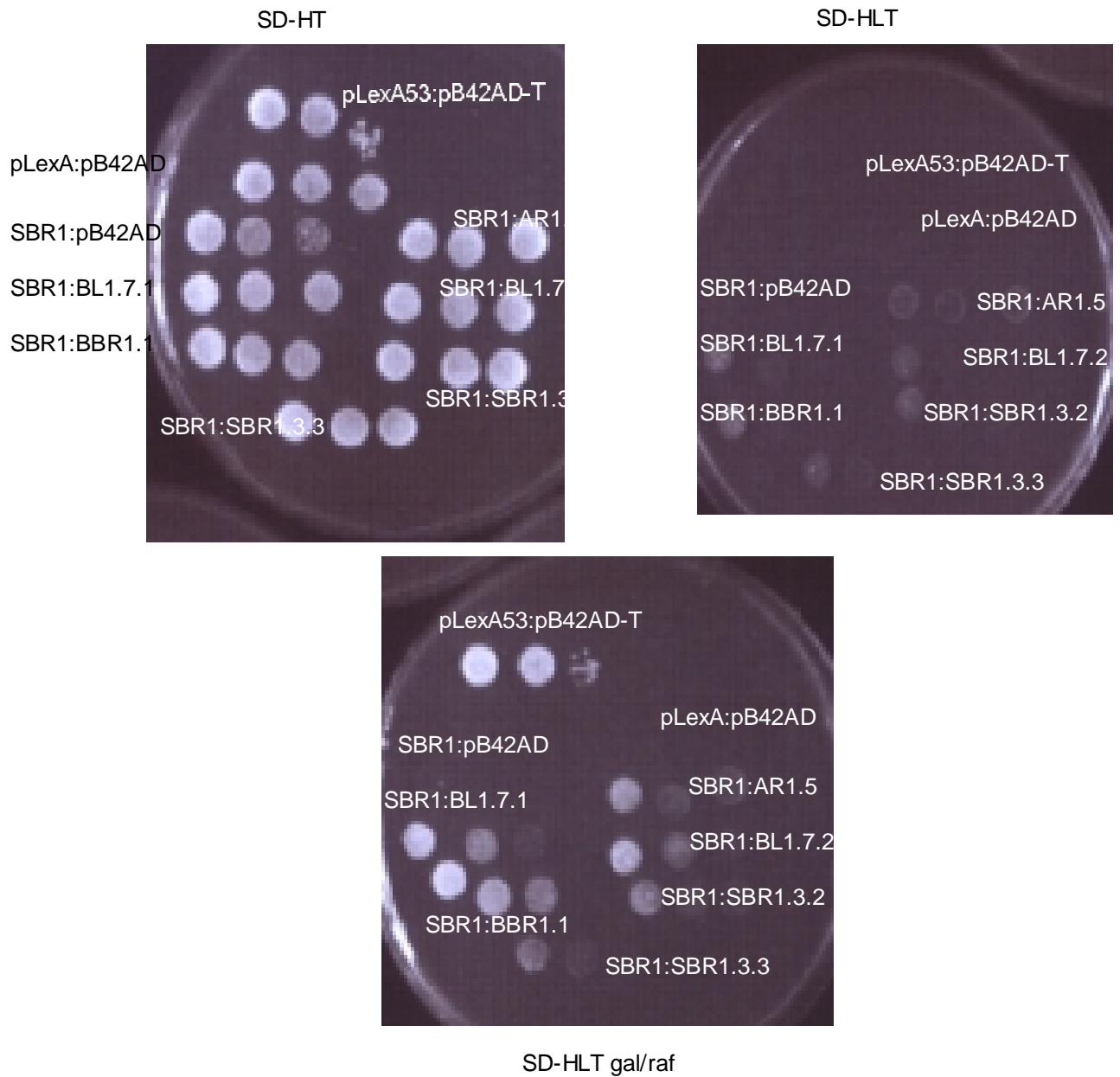


Figure 10—The interaction between the SBR1 bait and the viral protein preys. Serial dilutions of yeast strains were spotted onto SD-HT, SD-HLT and SD-HLT gal/raf media (see legend to figure 7 for details) and incubated at 30°C for 4 days. Control yeast strains were EGY48 containing pLexA-53+pB42AD-T (Positive control), and pLexA+pB42AD or pLexA-SBR1+pB42AD (positive control). Other strains contained pLexA-SBR1 bait + pB42AD-BAR1, -BBR1, -BBL1, or -SBR1 preys.

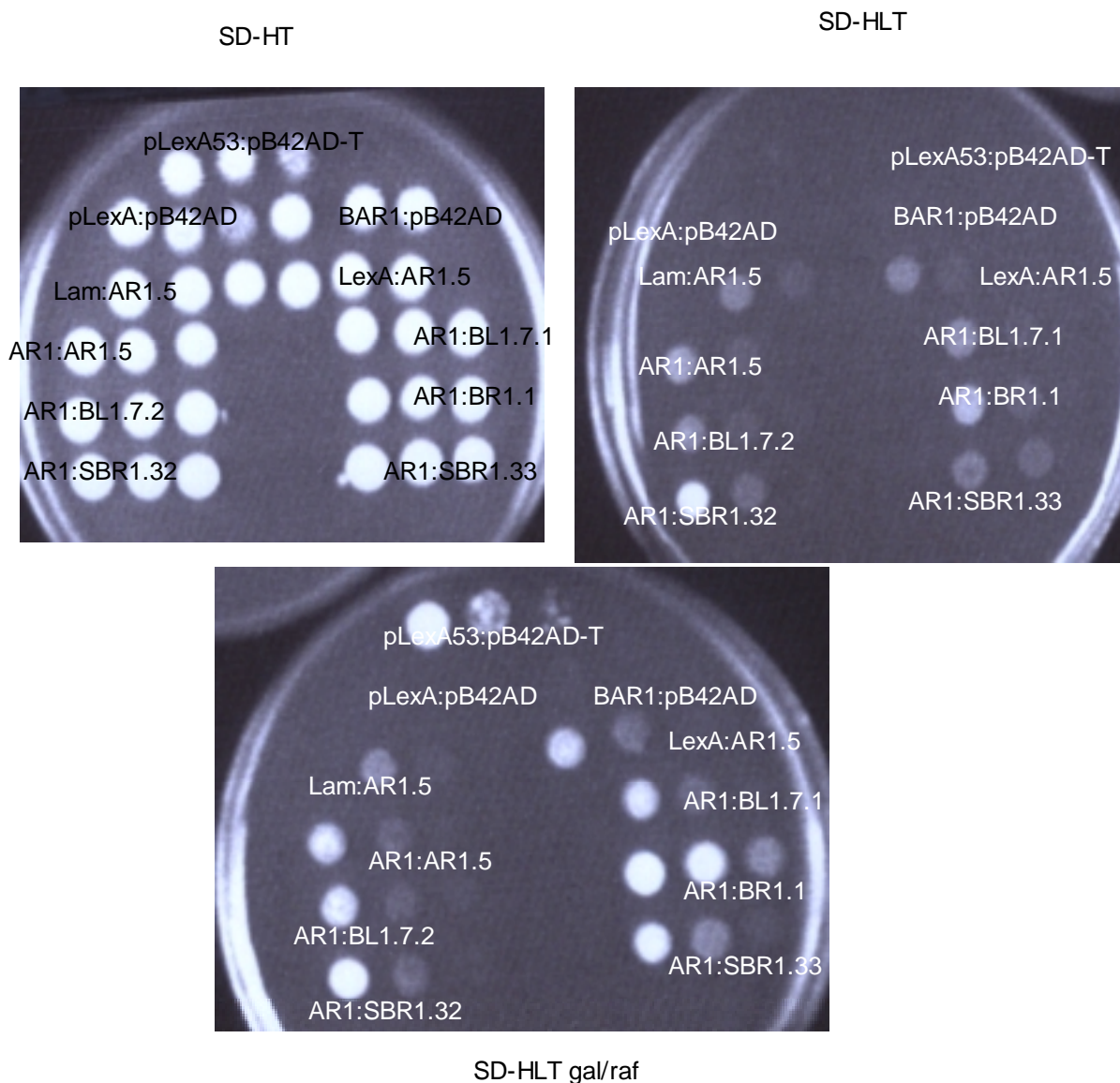


Figure 11—The interaction between the BAR1 bait and the viral protein preys. Serial dilutions of yeast strains were spotted onto SD-HT, SD-HLT and SD-HLT gal/raf media (see legend to figure 7 for details) and incubated at 30°C for 5 days. Control yeast strains were EGY48 containing pLexA-53+pB42AD-T (Positive control), and pLexA+pB42AD or pLexA-BAR1+pB42AD (positive control). Other strains contained pLexA-BAR1 bait + pB42AD-BAR1, -BBR1, -BBL1, or -SBR1 preys.

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1  MYSS--PRGRENVQRVSHIRRTYFKRPYYQTRSDEKRRPTAVWKTHDDIKMSLCR
1  MYISKYKRGSSNYQRRGYSRSOGFFRTSIVKRHDGKRRQHSSKSNEDEKLLVCC

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239 ESKASTEVSFDLEYVG 254
241 MSKASTEVSFDLDYVG 256

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SLCV BR1
BGMV BR1

Figure 12—Amino acid sequence comparison between the BR1 proteins of Squash leaf curl virus (SLCV) and Bean golden mosaic virus (BGMV). Identical amino acids are in black while amino acids with chemical similarity are shown in gray. There is also a 75% similarity between the nucleotide sequences of the respective ORFs.