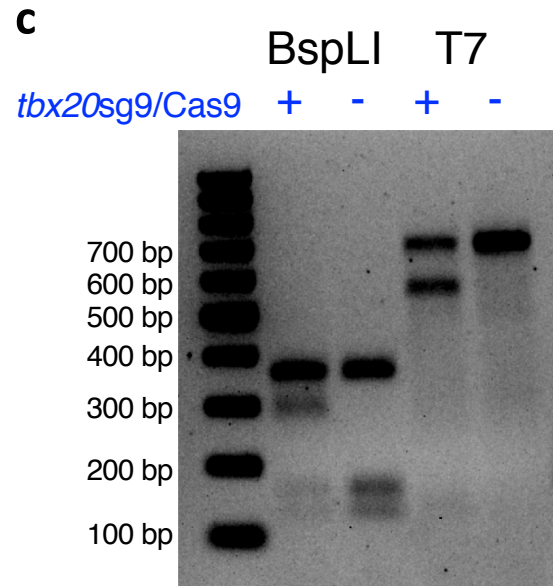


b

GGnnCC BspLI site
 ...CATTTGCATGCTATGGCCCCACAGGTTTTTTTTTTTCTA...
 ↑



Supplementary Figure 1. Assessment of guide RNA activity by T7 endonuclease assay and by loss of a restriction enzyme site. **a.** Diagram of the *tbx20* locus around *tbx20sgRNA9* cut site. Underneath, expected fragment sizes after digestion of T7 endonuclease or BspLI restriction endonuclease. **b.** The BspLI restriction enzyme site overlaps the expected double strand break site, indicating that almost all indels will result in loss of BspLI site. **c.** Gel electrophoresis of PCR fragments digested with T7 Endonuclease or BspLI restriction endonuclease.