



Figure S5. Effects of cofactors, pH and salt concentration on R.Gva14018I-4 activity. **(A)** Plasmid pUC57 was digested with purified R.Gva14018I-4 in the presence of increasing amounts of Mg²⁺ and Ca²⁺ as described in Methods. R.Gva14018I-4 was incubated with pUC57 in reactions supplemented with 1 mM Mg²⁺ at increasing amounts of Ca²⁺. **(B)** pUC57 cleavage in the presence of increasing amounts of Mg²⁺, Mn²⁺, Ni²⁺ and Ca²⁺. **(C)** pUC57 was digested with purified R.Gva14018I-4 in the presence of increasing amounts of Mg²⁺ at pH 7.2 and pH 5.0 as described in Methods. The reactions were supplemented with 1 mM Mg²⁺ to test the effects of salt concentration at pH 7.2. Lane pUC57 in A, B indicates the plasmid with no enzyme added. Lane M: Gene Ruler™ Ladder Mix (Thermo Fisher Scientific).