



**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 Mg<sup>2+</sup> and Ca<sup>2+</sup> as described in Methods. R.Gva14018I-4 was incubated with pUC57 in reactions supplemented with 1 mM Mg<sup>2+</sup> at increasing amounts of Ca<sup>2+</sup>. **(B)** pUC57 cleavage in the presence of increasing amounts of Mg<sup>2+</sup>, Mn<sup>2+</sup>, Ni<sup>2+</sup> and Ca<sup>2+</sup>. **(C)** pUC57 was digested with purified R.Gva14018I-4 in the presence of increasing amounts of Mg<sup>2+</sup> at pH 7.2 and pH 5.0 as described in Methods. The reactions were supplemented with 1 mM Mg<sup>2+</sup> 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).