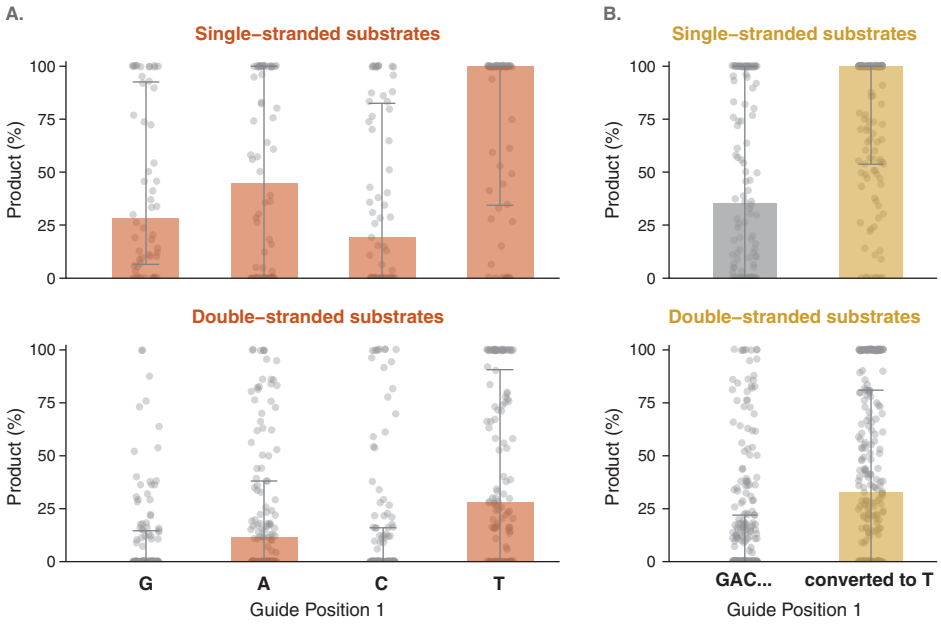
# GUIDElines for optimization of *Tth* Argonaute (TtAgo) reactions

NEB scientists have screened a large collection of single-stranded DNA guides targeting randomized single- and double-stranded DNA sequences in order to provide to following useful tips to help with designing guides for your own unique applications. The underlying mechanisms of *in situ* prokaryotic argonaute guide production are an area of ongoing research, and there are a variety of factors that can affect the activity of guide/argonaute combinations that have yet to be elucidated. As such, the following recommendations represent a "best foot forward" approach for guide design, however they do not guarantee that a guide designed in accordance will be functional.

## GUIDEline #1: Begin guides for TtAgo with a thymidine

### The identity of the first position in the guide nucleotide sequence can affect TtAgo activity



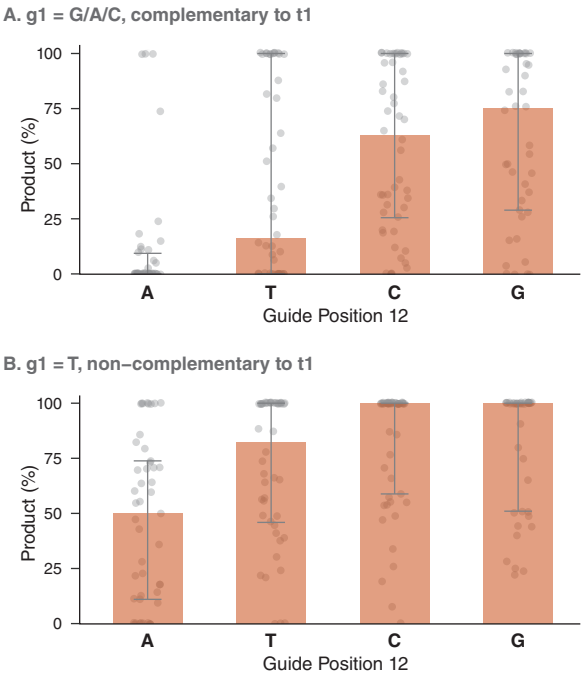

*(A) When screening guides that are fully-complementary to their target sequence a T in guide position 1 showed the highest level of activity.*

*(B) The first base of the guide is not critical for base-pairing to the target sequence, thus changing guide position 1 to a T even though it is not complementary to target position 1 can improve overall reaction performance as observed for a population of randomized guides.*

*Percent product is measured relative to substrate remaining after a 30 minute reaction in ThermoPol® Reaction Buffer (NEB #B9004) at 75°C where 17-nucleotide guide oligo is kept in 5X molar excess over TtAgo and substrate. Bar plots represent the population median with error bars corresponding to the upper and lower quartiles of the population of several reactions utilizing randomized guides and substrates.*

# GUIDEline #2: Avoid adenosine in the twelfth position of guides for TtAgo

## The identity of the twelfth position in the guide nucleotide sequence can affect TtAgo activity



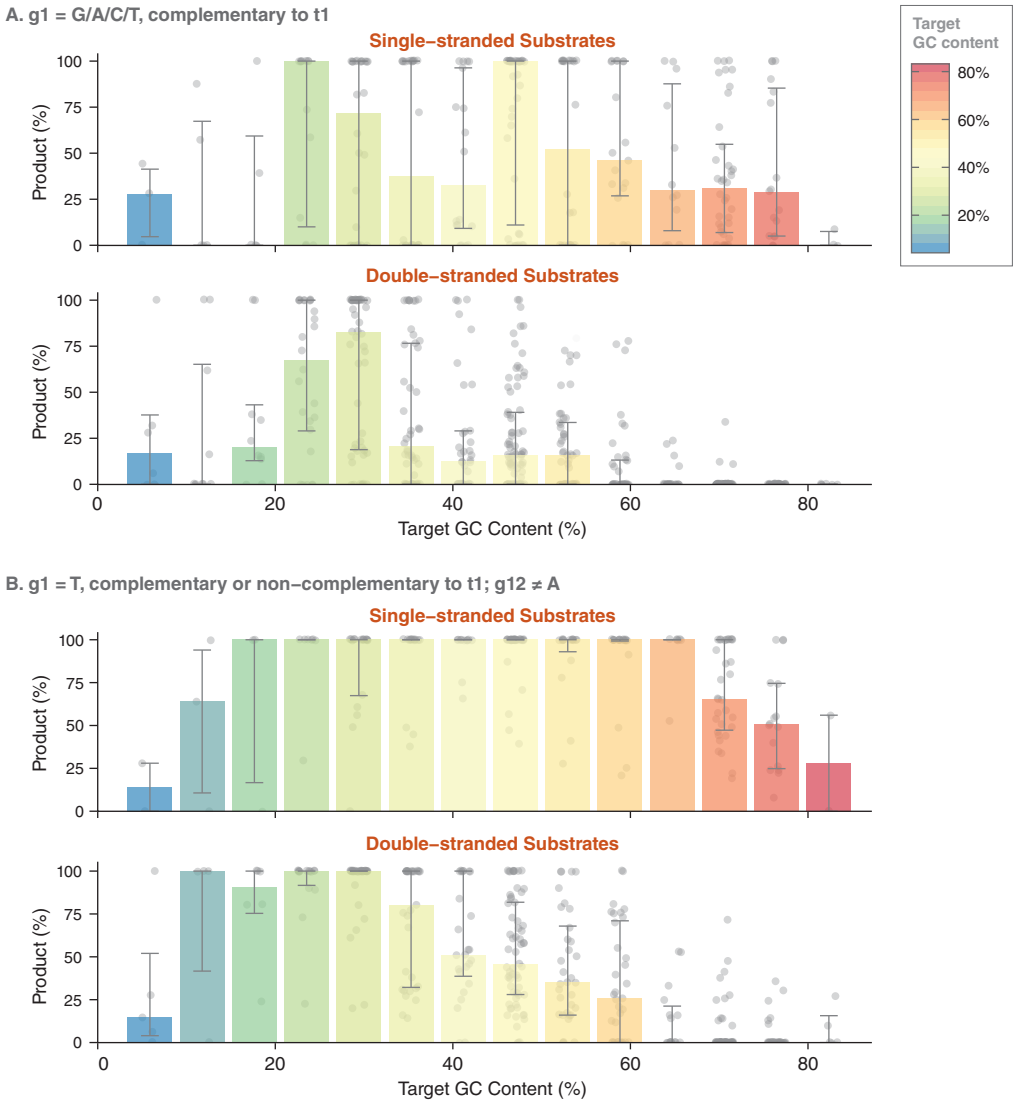

*(A) Observing the reaction efficiency for a large population of randomized guides, reactions proceeded poorly when the twelfth position of the guide was adenosine.*

*(B) This trend continued even when adhering to GUIDEline #1 and the guide first position base was always a thymidine.*

*Percent product is measured relative to substrate remaining after a 30 minute reaction in ThermoPol Reaction Buffer (NEB #B9004) at 75°C where 17-nucleotide guide oligo is kept in 5X molar excess over TtAgo and substrate. Bar plots represent the population median with error bars corresponding to the upper and lower quartiles of the population of several reactions utilizing randomized guides and substrates.*

# GUIDEline #3: Select an optimal target sequence GC content for TtAgo

## GC content preference in the TtAgo target sequence

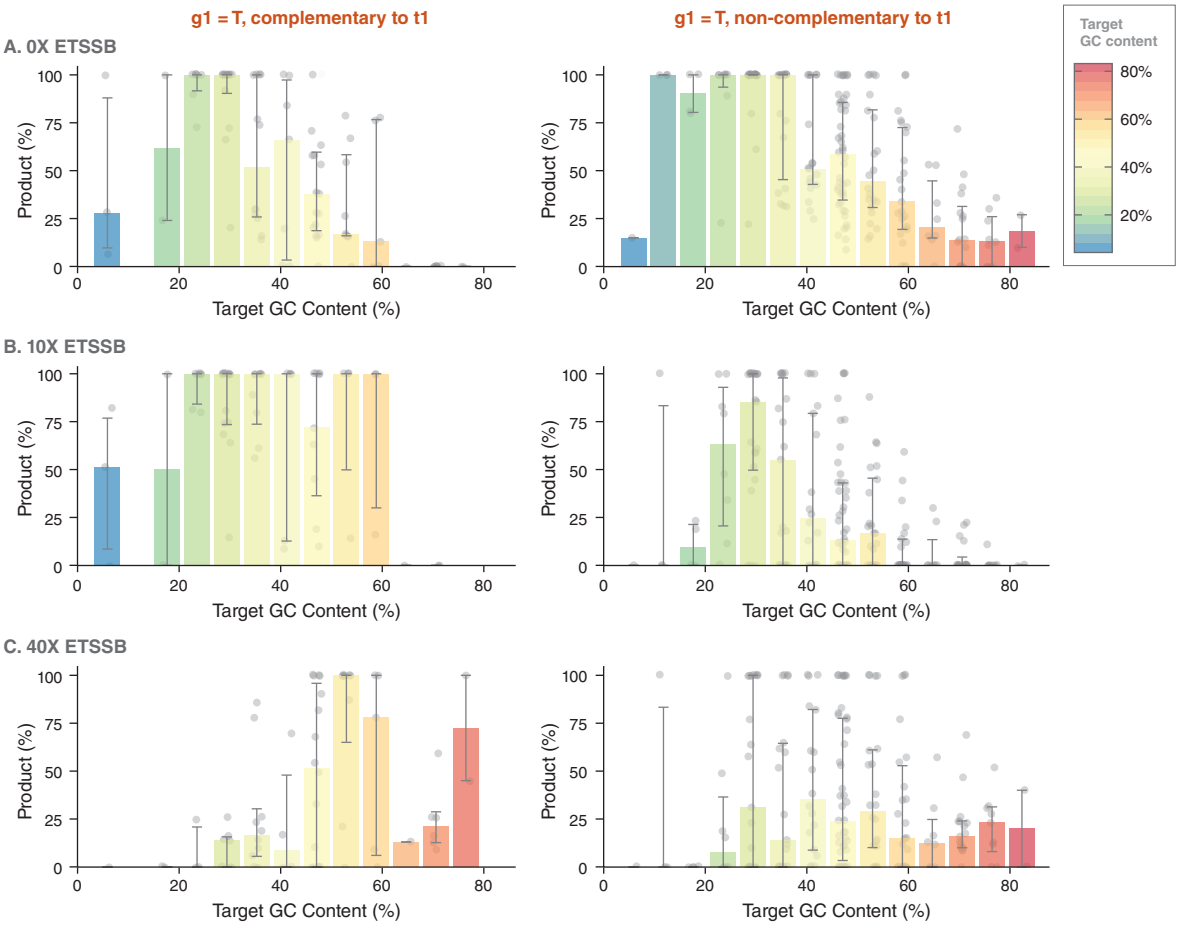



*(A) TtAgo shows a preference for lower GC content substrates, however (B) by following GUIDElines #1 and #2 (e.g., g1=T, g≠12A) the overall success of cleaving ssDNA and dsDNA substrates is greatly improved.*

*Percent product is measured relative to substrate remaining after a 30 minute reaction in ThermoPol Reaction Buffer (NEB #B9004) at 75°C where 17-nucleotide guide oligo is kept in 5X molar excess over TtAgo and substrate. Bar plots represent the population median with error bars corresponding to the upper and lower quartiles of the population of several reactions utilizing randomized guides and substrates.*

# GUIDEline #4: ET SSB may aid in cutting higher GC content substrates under specific conditions

## Including ET SSB in TtAgo reactions can improve cleavage of higher GC content substrates

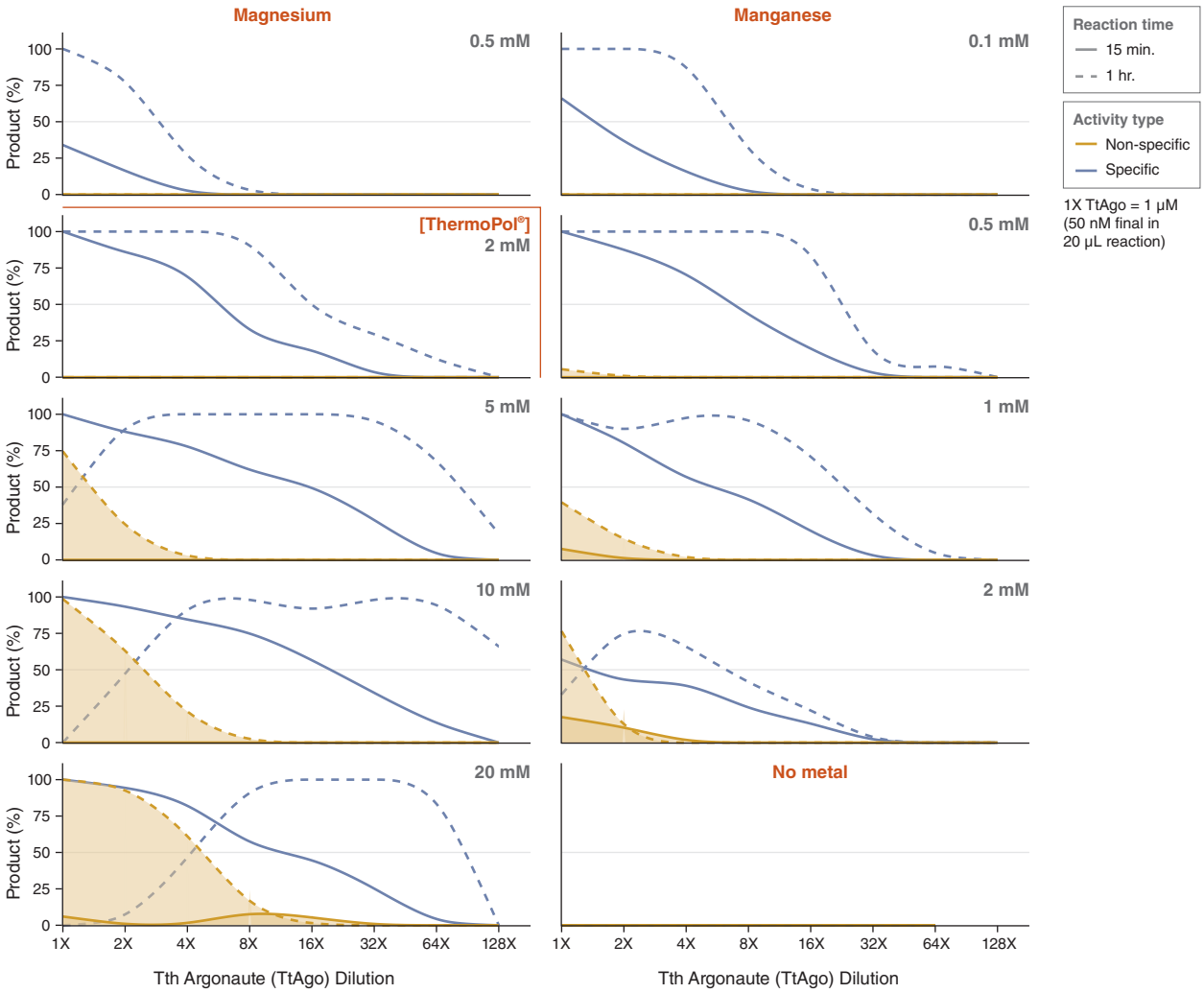



*In some cases the addition of ET SSB (NEB #M2401) can aid in the cleavage of higher GC content dsDNA substrates. ET SSB must be titrated to find an optimal concentration for the application, and is only found to be beneficial when guides are fully complementary to the target sequence (i.e., g1 is complementary to t1). (A) no ET SSB (B) ET SSB included at 10X molar excess over substrate (C) ET SSB included at 40X molar excess over substrate.*

*Percent product is measured relative to substrate remaining after a 30 minute reaction in ThermoPol Reaction Buffer (NEB #B9004) at 75°C where 17-nucleotide guide oligo is kept in 5X molar excess over TtAgo and substrate. Bar plots represent the population median with error bars corresponding to the upper and lower quartiles of the population of several reactions utilizing randomized guides and substrates.*

# GUIDEline #5: ThermoPol® Reaction Buffer with 2 mM magnesium(II) is optimal for most reactions

## Activity of TtAgo with magnesium(II) or manganese(II) present in reaction




*TtAgo can utilize both magnesium(II) and manganese(II) in its active site. Manganese(II) must be used at approximately 10-fold lower concentrations than magnesium(II) and can be inhibitory to the reaction, leading to rapid degradation of substrate at higher concentrations especially under the heating conditions required for TtAgo activity. Magnesium(II) gives more predictable activity and prolonged incubations can be performed without introducing non-specific degradation. Higher concentrations of magnesium(II) and manganese(II) can lead to non-specific degradation of substrate. ThermoPol Reaction Buffer provides broadly applicable reaction conditions, however, titration of the metal used can be performed using ThermoPol II (Mg-free) Reaction Buffer (NEB #B9005) and varying the amount of TtAgo depending on the nature of the application.*

*Percent product is measured relative to substrate remaining after a 15 minute or 1 hour reaction at 80°C where 17-nucleotide guide oligo is kept in 5X molar excess (at 1X enzyme) over TtAgo and substrate.*



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