



Overhang set report



EcoFlex level 1->2

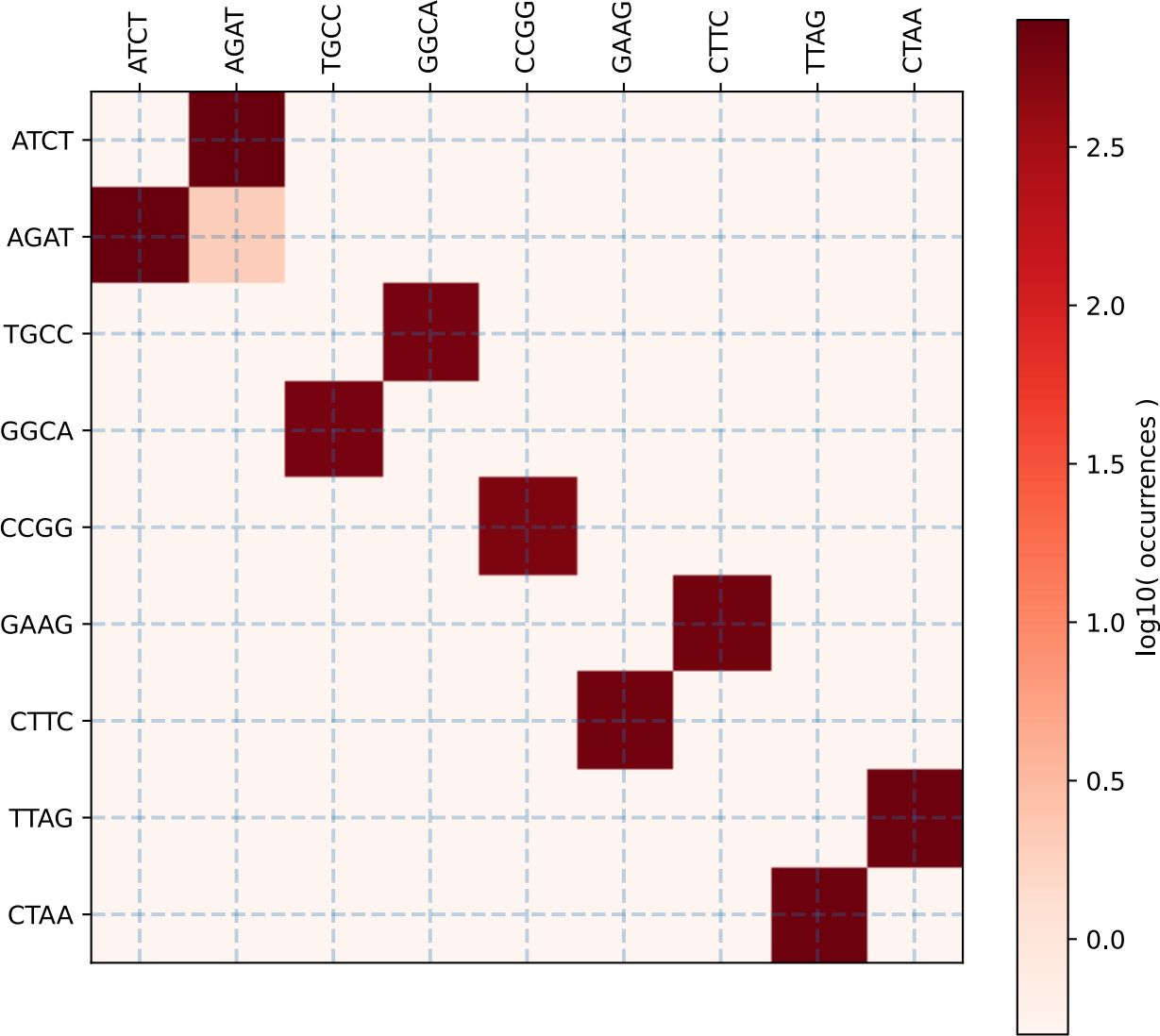
There are **6** overhangs in this set. The restriction enzyme used for this set is **Esp3I**.

Overhangs in this set: ATCT, TGCC, CCGG, GAAG, CTTC, TTAG.

Error! Palindromic overhang(s): CCGG

Error! Nonpalindromic overhang(s) with reverse complement: GAAG; CTTC

Please see the Appendix on the last page for an explanation of details.





AGAT

ATCT

GC content: **25 %**.

Can form the following amino acids in 6 translation frames:

R[SFW*CLY]

[IESAV*KQGPLRT]D

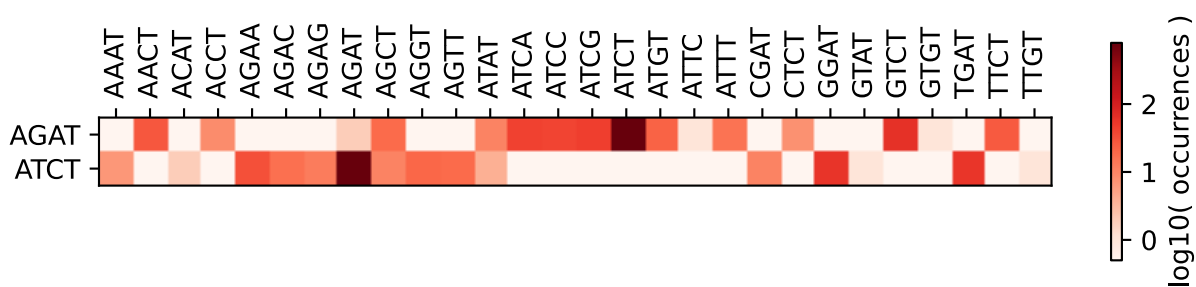
[*EKQ][MI]

I[SFW*CLY]

[IESAV*KQGPLRT]S

[NDYH][L]

Misannealing overhangs:





GGCA

TGCC

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

G[INSMRT]

[GESWAV*KQMPLRT]A

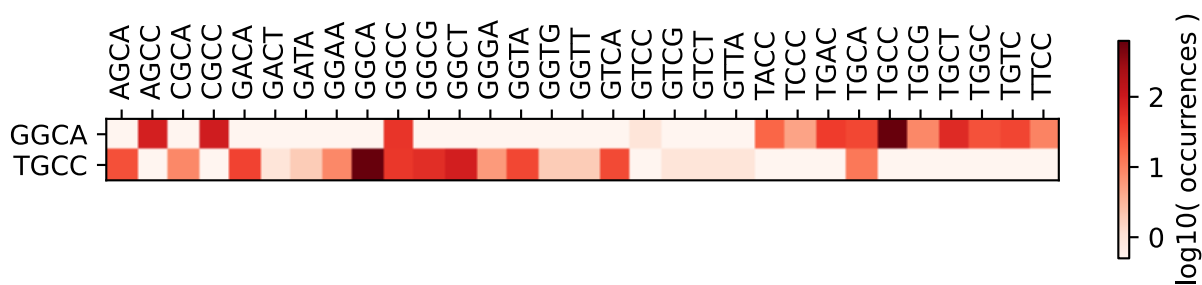
[WGR][HQ]

C[PLRHQ]

[DINSFAVRCGPLYHT]A

[MVL][P]

Misannealing overhangs:





CCGG

CCGG

Extreme GC content: 100 %.

The overhang is palindromic, cannot be used for DNA assembly.

Can form the following amino acids in 6 translation frames:

P[EAVGD]

[DINSFAVRCGPLYHT]R

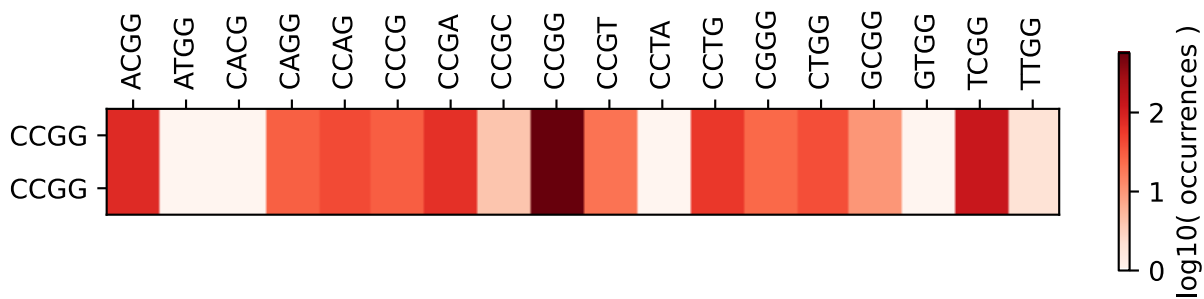
[APST][G]

P[EAVGD]

[DINSFAVRCGPLYHT]R

[APST][G]

Misannealing overhangs:





CTTC

GAAG

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

L[PLRHQ]

[DINSFAVRCGPLYHT]F

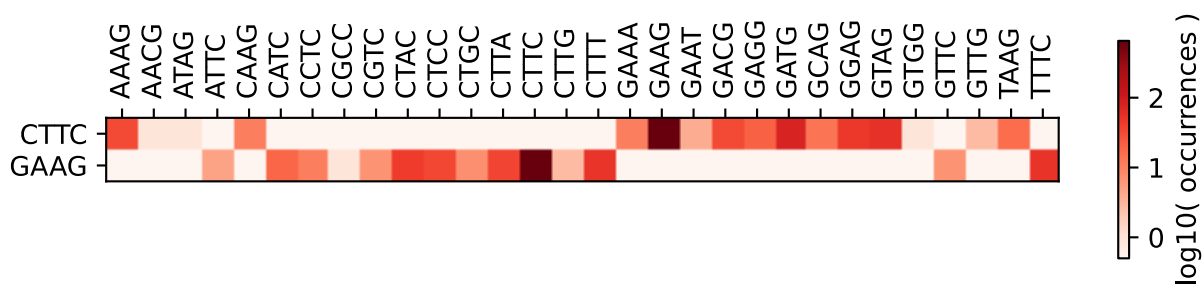
[APST][S]

E[EAVGD]

[GESWAV*KQMPLRT]K

[G*R][RS]

Misannealing overhangs:





CTTC

GAAG

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

L[PLRHQ]

[DINSFAVRCGPLYHT]F

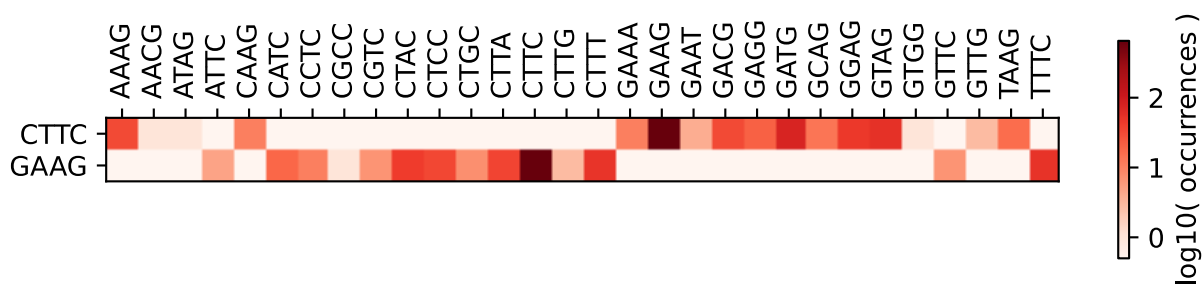
[APST][S]

E[EAVGD]

[GESWAV*KQMPLRT]K

[G*R][RS]

Misannealing overhangs:





CTAA

TTAG

GC content: **25 %**.

The overhang contains a stop codon (TAA, TAG or TGA).

Can form the following amino acids in 6 translation frames:

L[INSKMRT]

[DINSFAVRRCGPLYHT]*

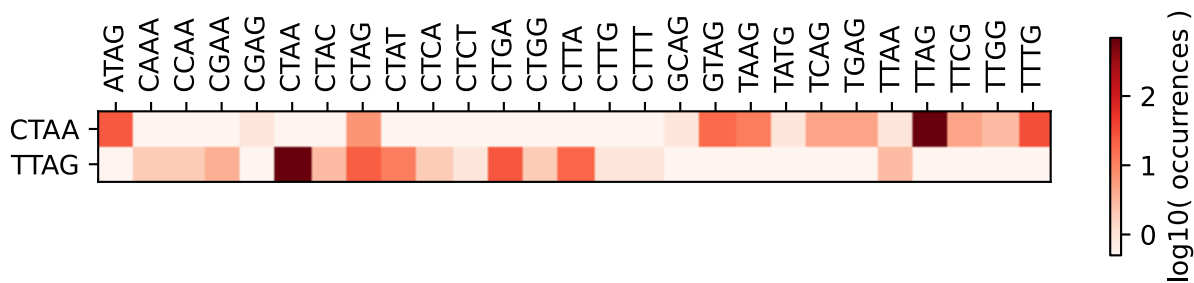
[APST][NK]

L[EAVGD]

[DINSFAVRRCGPLYHT]*

[FVIL][RS]

Misannealing overhangs:



Appendix

The report consists of 3 sections: results, overhangs, appendix.

Result page(s)

The first page describes the overhang set. The result is also summarised with a symbol:

☑ : good overhang set

⚠ : warning; there are ways to significantly improve the set

☒ : error: the set cannot be used for DNA assembly

Overhang pages

Each overhang is also analysed separately. The result is summarised with a symbol:

☑ : good overhang

☒ : unusable palindromic sequence

Overhangs are unpaired nucleotides at the end of a double-stranded linear DNA molecule. These overhangs create "sticky" (non-blunt) DNA ends. Overhangs can be on either strand; 5' or 3' overhangs. During DNA assembly, these overhangs are created by type IIS restriction enzymes, and ligated with another DNA with a complementary overhang, by ligases. The type of the restriction enzyme and the ligase influences the misannealing rate, which is displayed in a summary plot. For more details, see the [Tatapov](#) package and Pryor et al. ([PLoS ONE \(2020\) 15\(9\): e0238592](#)).

After the assembly, these overhangs remain in the sequence as fusion sites ("scars"). If this is in a coding sequence (CDS), then addition of nucleotides can ensure that the two joining parts remain in the same translation frame. By carefully choosing which nucleotides we add, we can select suitable amino acids, or start/stop codons. For each overhang, a list of codons is also displayed that shows translation options. The [GeneDom](#) package can be used for automating the addition of these nucleotides, the overhangs and the enzyme sites.

Overhang sets

Use the [GoldenHinges](#) Python package to generate a set of mutually compatible overhangs that can be used for DNA assembly.