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# Compendium of overhangs

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There are **136** overhangs in this report. The restriction enzyme used in this compendium is **Bsal**.

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



# AAAG

# CTTT

GC content: 25 %.

Has 3 identical bases in a row. However, this has not shown to be very important.

Can form the following amino acids in 6 translation frames:

K[EAVGD]

[IESAV\*KQGPLRT]K

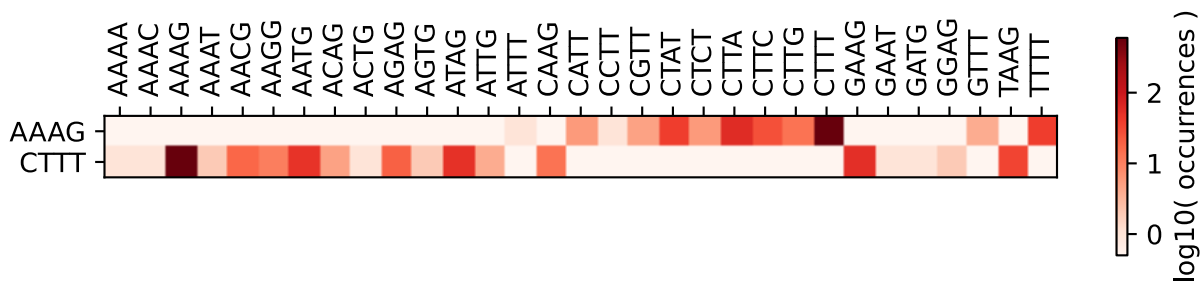
[\*EKQ][RS]

L[SFW\*CLY]

[DINSFAVRRCGPLYHT]F

[APST][FL]

Misannealing overhangs:





# AAAT

# ATTT

Extreme GC content: 0 %.

Has 3 identical bases in a row. However, this has not shown to be very important.

Can form the following amino acids in 6 translation frames:

K[SFW\*CLY]

[IESAV\*KQGPLRT]N

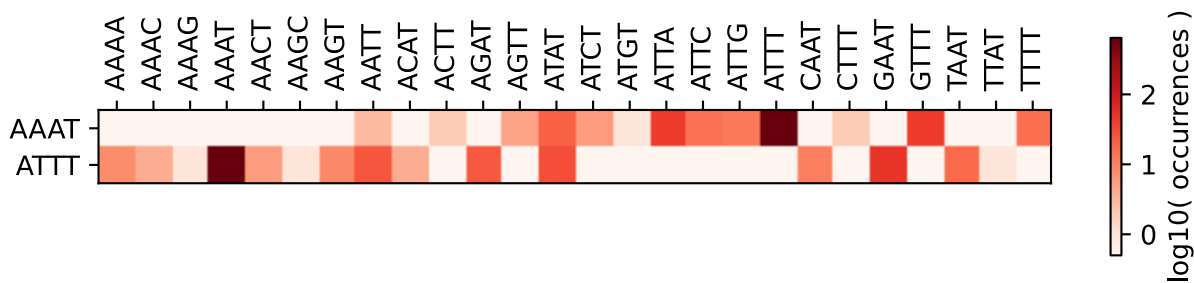
[\*EKQ][MI]

I[SFW\*CLY]

[IESAV\*KQGPLRT]F

[NDYH][FL]

Misannealing overhangs:





# AACC

# GGTT

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

N[PLRHQ]

[IESAV\*KQGPLRT]T

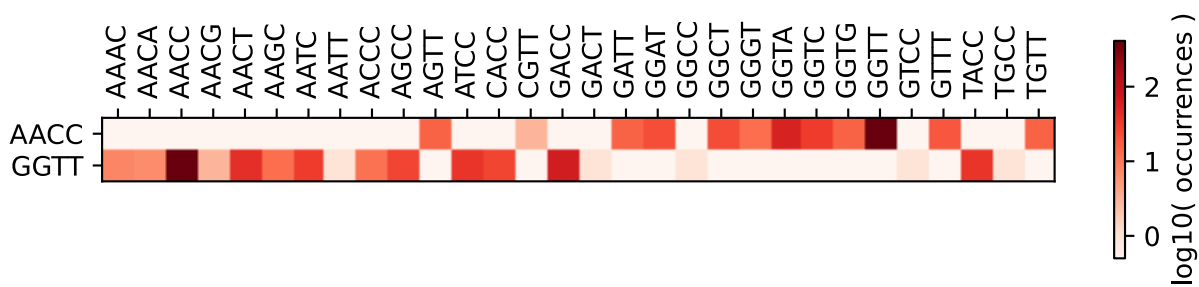
[\*EKQ][P]

G[SFW\*CLY]

[GESWAV\*KQMPLRT]V

[WGR][FL]

Misannealing overhangs:





# AACG

# CGTT

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

N[EAVGD]

[IESAV\*KQGPLRT]T

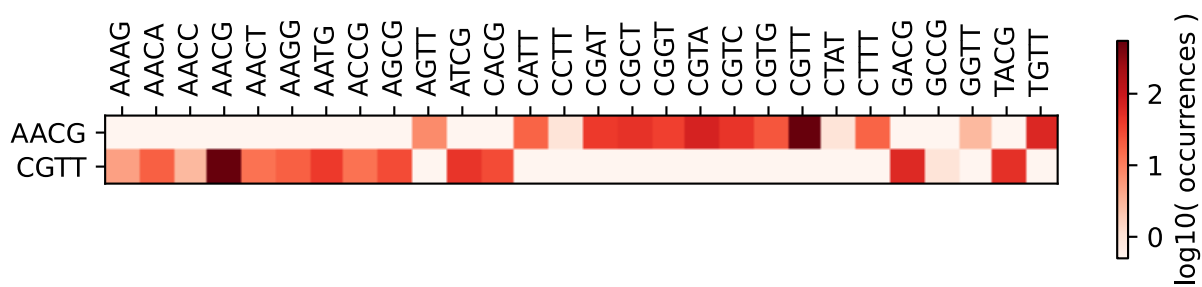
[\*EKQ][R]

R[SFW\*CLY]

[DINSFAVRCGPLYHT]V

[APST][FL]

Misannealing overhangs:





# AAGC

# GCTT

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

K[PLRHQ]

[IESAV\*KQGPLRT]S

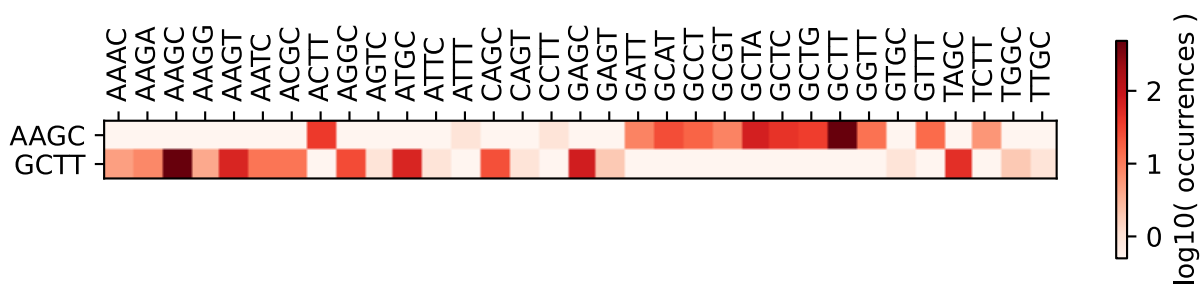
[\*EKQ][A]

A[SFW\*CLY]

[GESWAV\*KQMPLRT]L

[GRCS][FL]

Misannealing overhangs:





# AATG

# CATT

GC content: 25 %.

The overhang contains the start codon ATG.

Can form the following amino acids in 6 translation frames:

N[EAVGD]

[IESAV\*KQGPLRT]M

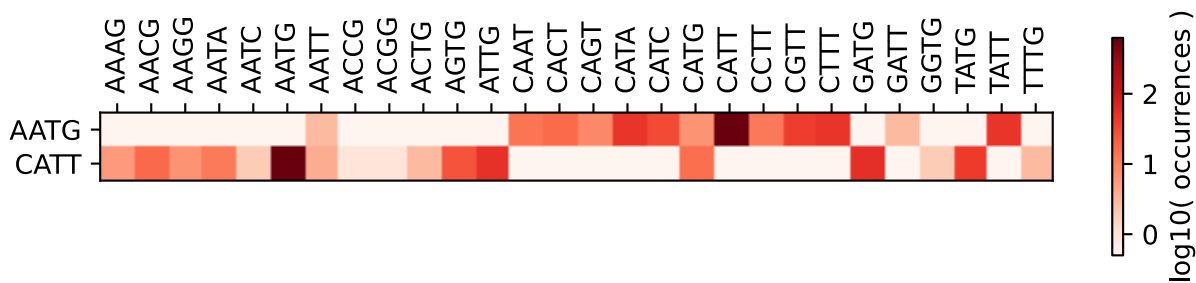
[\*EKQ][W\*C]

H[SFW\*CLY]

[DINSFAVRRCGPLYHT]I

[APST][FL]

Misannealing overhangs:





# AATT

# AATT

Extreme GC content: 0 %.

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

Can form the following amino acids in 6 translation frames:

N[SFW\*CLY]

[IESAV\*KQGPLRT]I

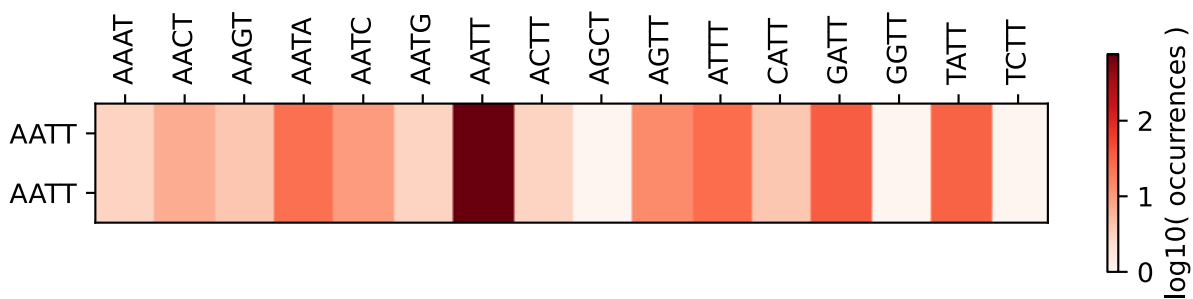
[\*EKQ][FL]

N[SFW\*CLY]

[IESAV\*KQGPLRT]I

[\*EKQ][FL]

Misannealing overhangs:







# ACAA

# TTGT

GC content: 25 %.

Can form the following amino acids in 6 translation frames:

T[INSMRT]

[IESAV\*KQGRLRT]Q

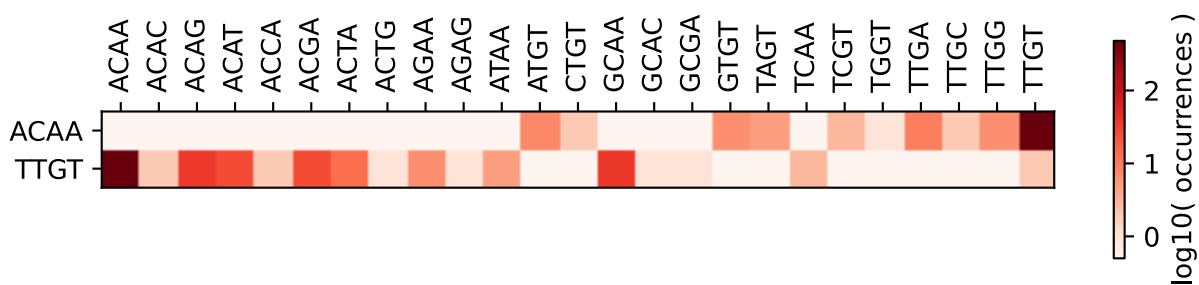
[NDYH][NK]

L[SFV\*CLY]

[DINSFAVRCLPLYHT]C

[FVIL][V]

Misannealing overhangs:





# ACAT

# ATGT

GC content: 25 %.

The overhang contains the start codon ATG.

Can form the following amino acids in 6 translation frames:

T[SFW\*CLY]

[IESAV\*KQGPLRT]H

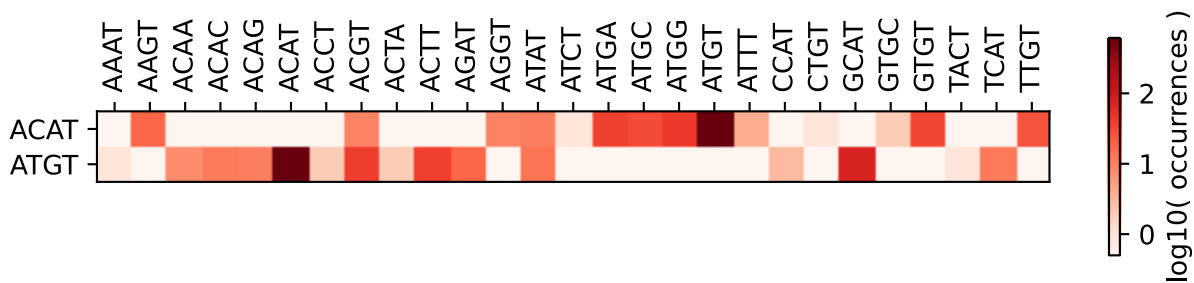
[NDYH][MI]

M[SFW\*CLY]

[IESAV\*KQGPLRT]C

[NDYH][V]

Misannealing overhangs:





# ACCA

# TGGT

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

T[INSMRT]

[IESAV\*KQGPLRT]P

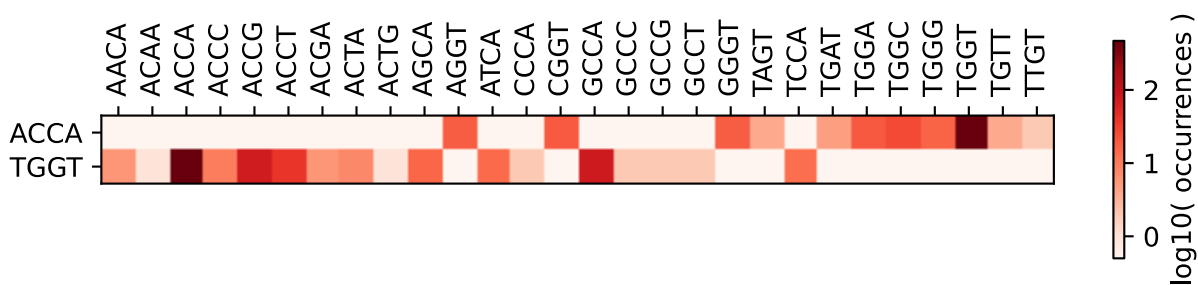
[NDYH][HQ]

W[SFW\*CLY]

[DINSFAVRCGPLYHT]G

[MVL][V]

Misannealing overhangs:





# ACCC

# GGGT

GC content: **75 %**.

Has 3 identical bases in a row. However, this has not shown to be very important.

Can form the following amino acids in 6 translation frames:

T[PLRHQ]

[IESAV\*KQGPLRT]P

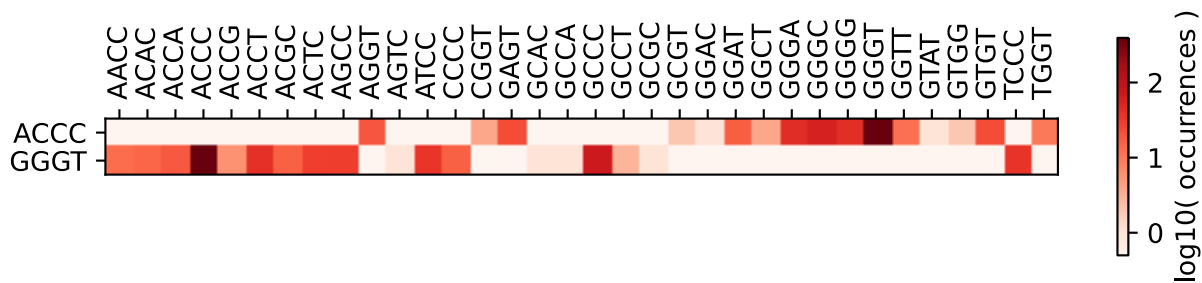
[NDYH][P]

G[SFW\*CLY]

[GESWAV\*KQMPLRT]G

[WGR][V]

Misannealing overhangs:





# ACGA

# TCGT

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

T[INSMRT]

[IESAV\*KQGPLRT]R

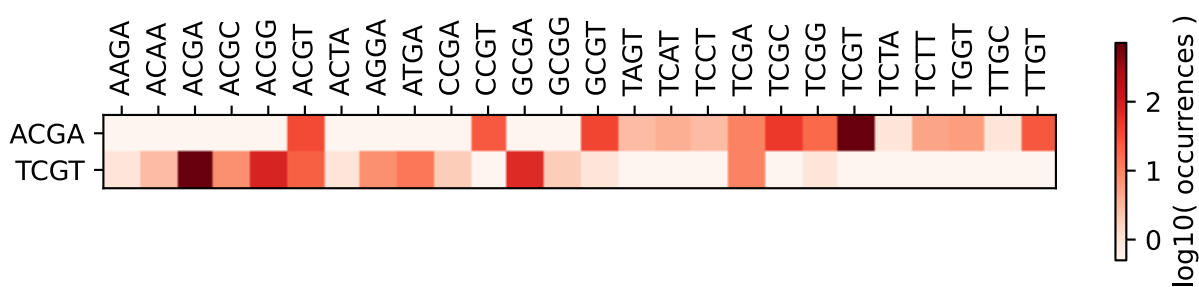
[NDYH][ED]

S[\$FW\*CLY]

[DINSFAVRCGPLYHT]R

[FVIL][V]

Misannealing overhangs:





# ACGC

# GCGT

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

T[PLRHQ]

[IESAV\*KQGPLRT]R

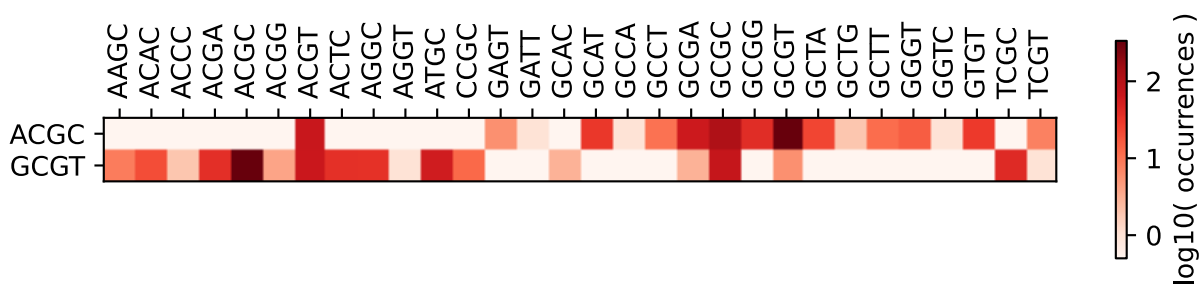
[NDYH][A]

A[SFW\*CLY]

[GESWAV\*KQMPLRT]R

[GRCS][V]

Misannealing overhangs:





# ACGT

# ACGT

GC content: 50 %.

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

Can form the following amino acids in 6 translation frames:

T[SFW\*CLY]

[IESAV\*KQGPLRT]R

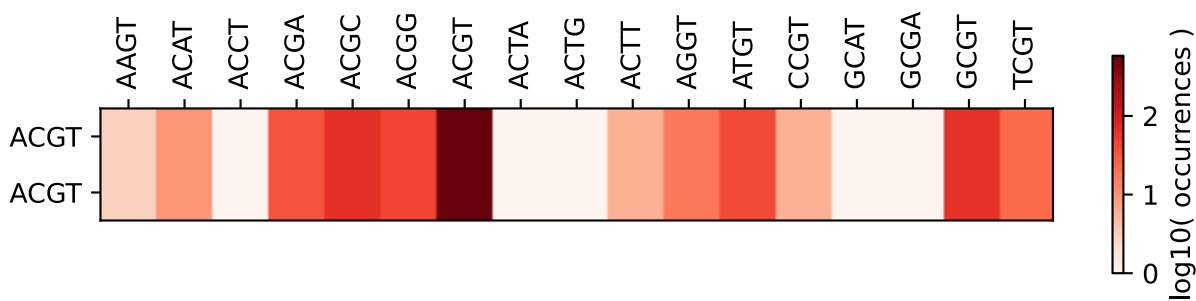
[NDYH][V]

T[SFW\*CLY]

[IESAV\*KQGPLRT]R

[NDYH][V]

Misannealing overhangs:





# ACTA

# TAGT

GC content: 25 %.

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

Can form the following amino acids in 6 translation frames:

T[INSMRT]

[IESAV\*KQGRLRT]L

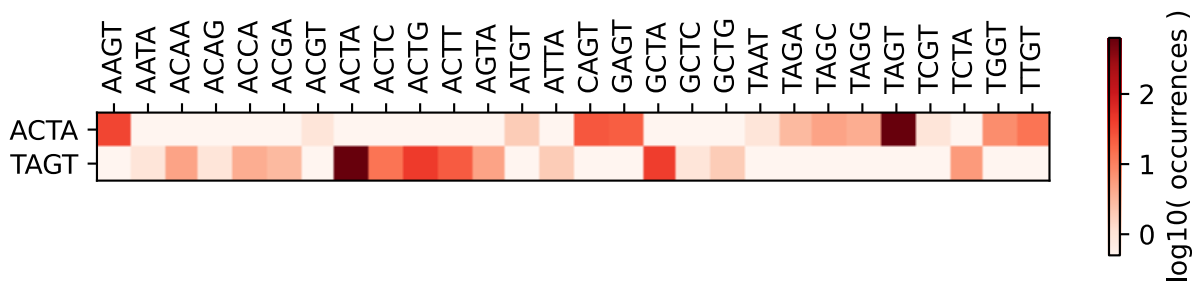
[NDYH][Y\*]

\*[SFW\*CLY]

[DINSFAVRRCGPLYHT]S

[VIL][V]

Misannealing overhangs:







# ACTC

# GAGT

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

T[PLRHQ]

[IESAV\*KQGPLRT]L

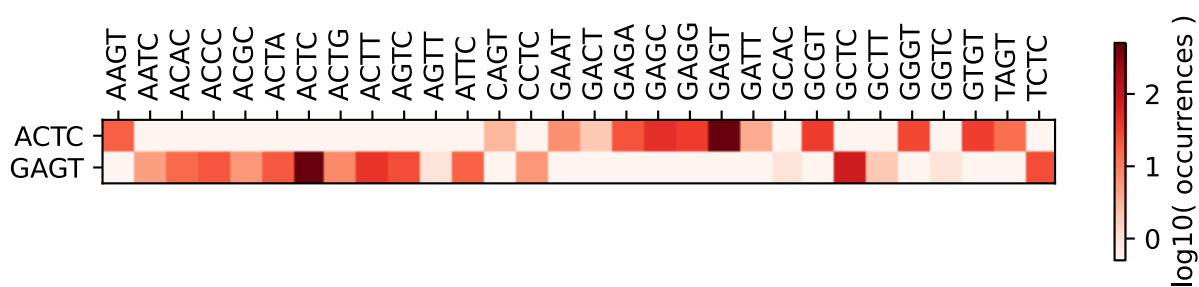
[NDYH][S]

E[SFW\*CLY]

[GESWAV\*KQMPLRT]S

[G\*R][V]

Misannealing overhangs:





# ACTG

# CAGT

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

T[EAVGD]

[IESAV\*KQGPLRT]L

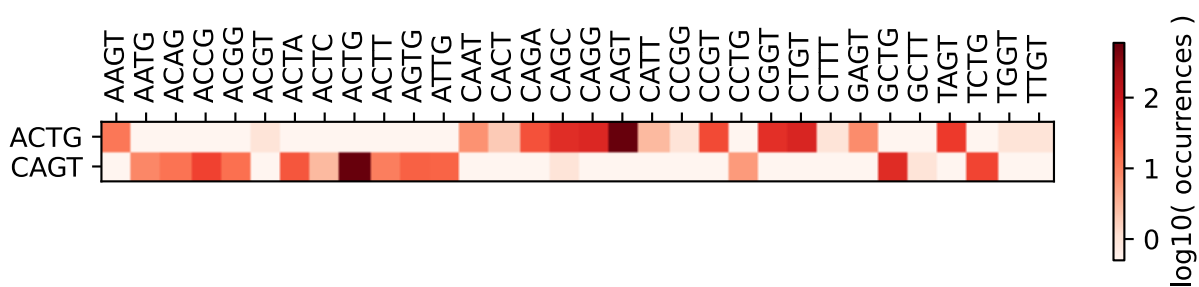
[NDYH][W\*C]

Q[SFW\*CLY]

[DINSFAVRRCGPLYHT]S

[APST][V]

Misannealing overhangs:





# AAGT

# ACTT

GC content: **25 %**.

Can form the following amino acids in 6 translation frames:

K[SFW\*CLY]

[IESAV\*KQGPLRT]S

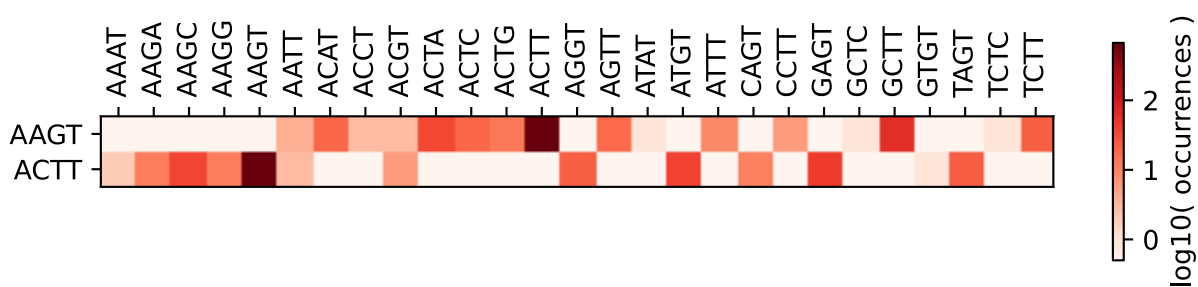
[\*EKQ][V]

T[SFW\*CLY]

[IESAV\*KQGPLRT]L

[NDYH][FL]

Misannealing overhangs:





# AGAA

# TTCT

GC content: **25 %**.

Can form the following amino acids in 6 translation frames:

R[INSMRT]

[IESAV\*KQGRLRT]E

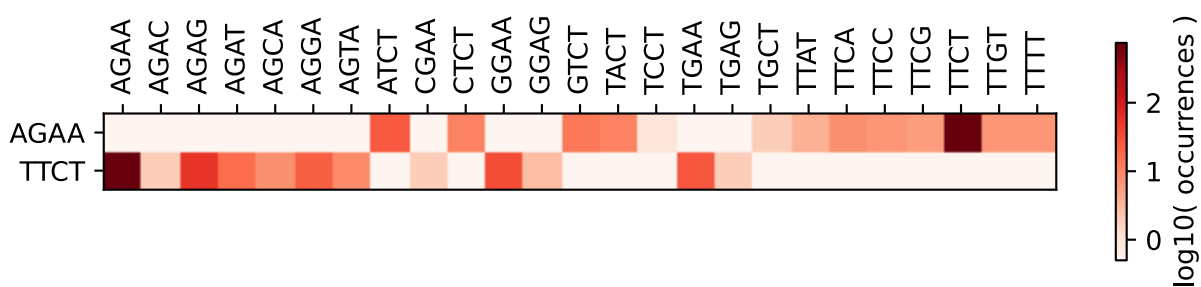
[\*EKQ][NK]

F[SFV\*CLY]

[DINSFAVRRCGPLYHT]S

[FVIL][L]

Misannealing overhangs:





# AGCA

# TGCT

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

S[INSMRT]

[IESAV\*KQGRLRT]A

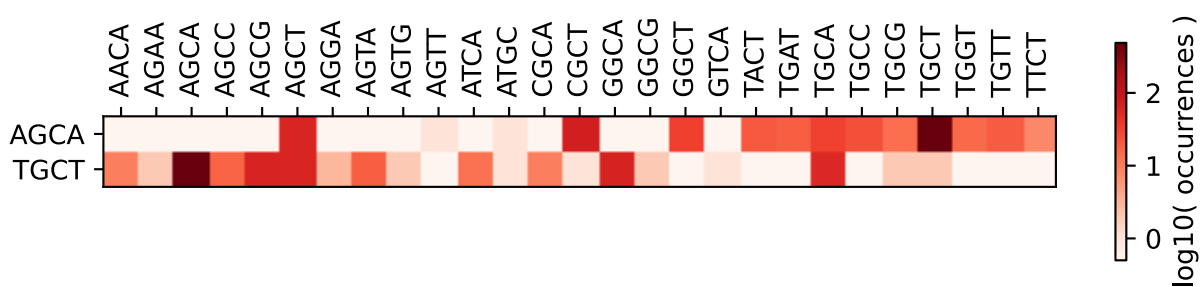
[\*EKQ][HQ]

C[SFW\*CLY]

[DINSFAVRGPLYHT]A

[MVL][L]

Misannealing overhangs:





# AGCC

# GGCT

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

S[PLRHQ]

[IESAV\*KQGPLRT]A

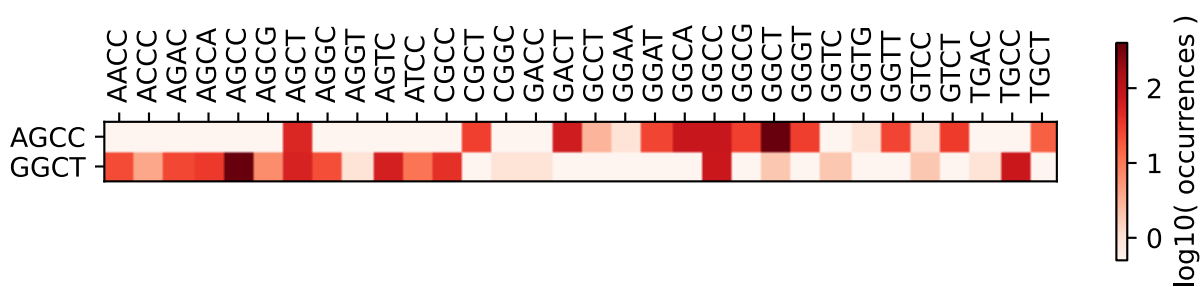
[\*EKQ][P]

G[SFW\*CLY]

[GESWAV\*KQMPLRT]A

[WGR][L]

Misannealing overhangs:





# AGCG

# CGCT

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

S[EAVGD]

[IESAV\*KQGPLRT]A

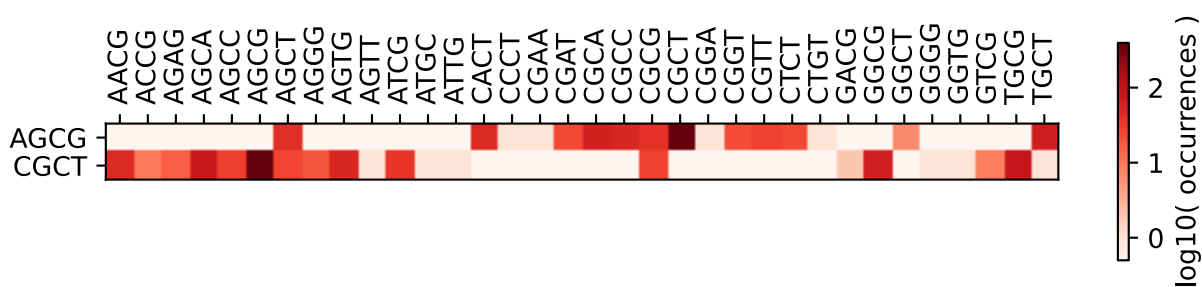
[\*EKQ][R]

R[SFW\*CLY]

[DINSFAVRCGPLYHT]A

[APST][L]

Misannealing overhangs:





# AGCT

# AGCT

GC content: 50 %.

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

Can form the following amino acids in 6 translation frames:

S[FW\*CLY]

[IESAV\*KQGRLRT]A

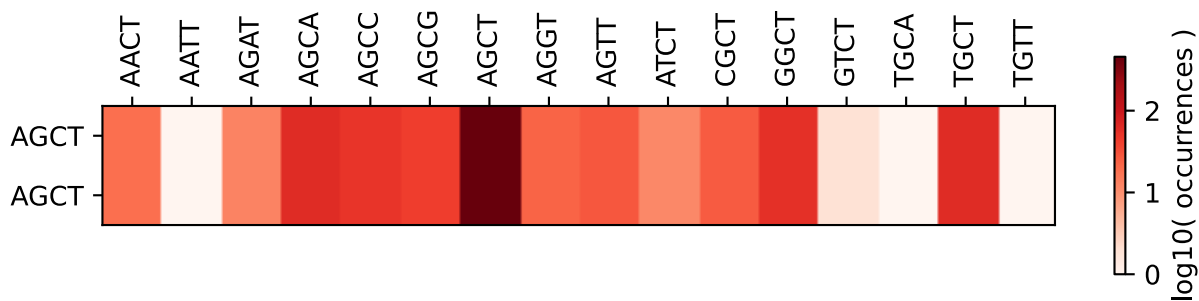
[\*EKQ][L]

S[FW\*CLY]

[IESAV\*KQGRLRT]A

[\*EKQ][L]

Misannealing overhangs:







# AGGC

# GCCT

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

R[PLRHQ]

[IESAV\*KQGPLRT]G

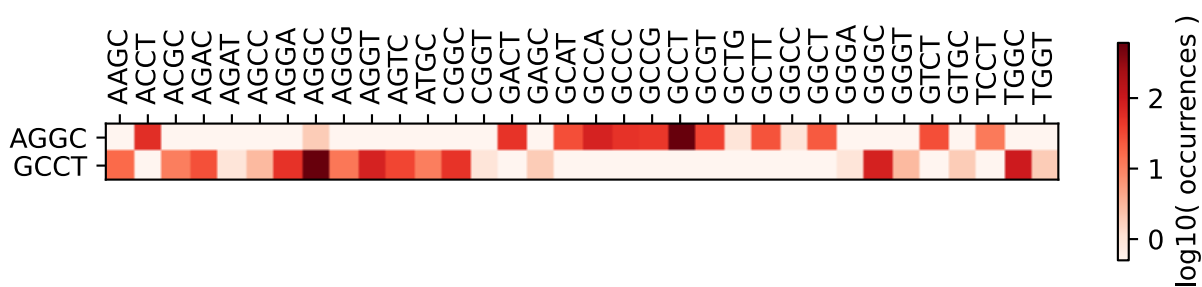
[\*EKQ][A]

A[SFW\*CLY]

[GESWAV\*KQMPLRT]P

[GRCS][L]

Misannealing overhangs:





# ACCT

# AGGT

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

T[SFW\*CLY]

[IESAV\*KQGPLRT]P

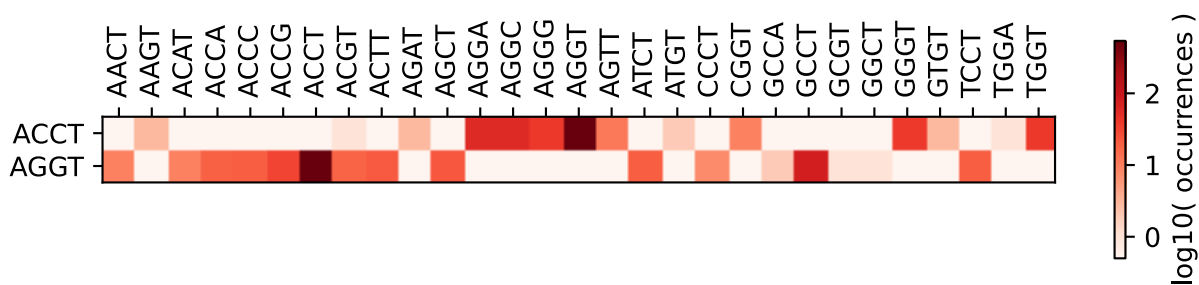
[NDYH][L]

R[SFW\*CLY]

[IESAV\*KQGPLRT]G

[\*EKQ][V]

Misannealing overhangs:





# AGTA

# TACT

GC content: 25 %.

Can form the following amino acids in 6 translation frames:

S[INSMRT]

[IESAV\*KQGRLRT]V

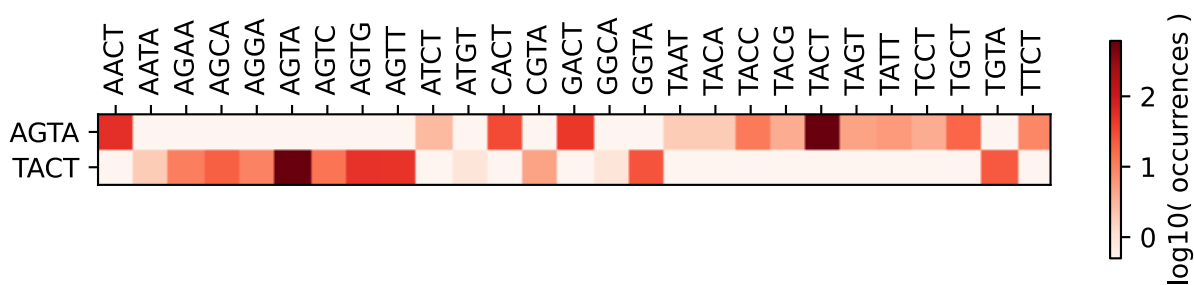
[\*EKQ][Y\*]

Y[SFW\*CLY]

[DINSFAVRRCGLYHT]T

[VIL][L]

Misannealing overhangs:





# AACT

# AGTT

GC content: **25 %**.

Can form the following amino acids in 6 translation frames:

N[SFW\*CLY]

[IESAV\*KQGPLRT]T

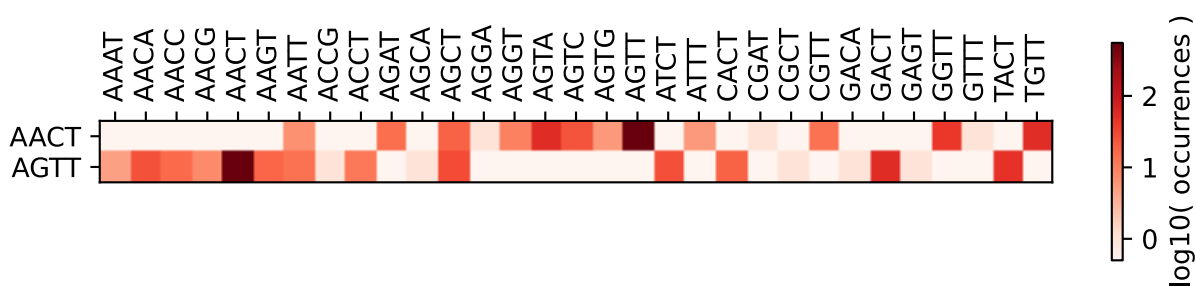
[\*EKQ][L]

S[SFW\*CLY]

[IESAV\*KQGPLRT]V

[\*EKQ][FL]

Misannealing overhangs:





# ATAC

# GTAT

GC content: 25 %.

Can form the following amino acids in 6 translation frames:

I[PLRHQ]

[IESAV\*KQGPLRT]Y

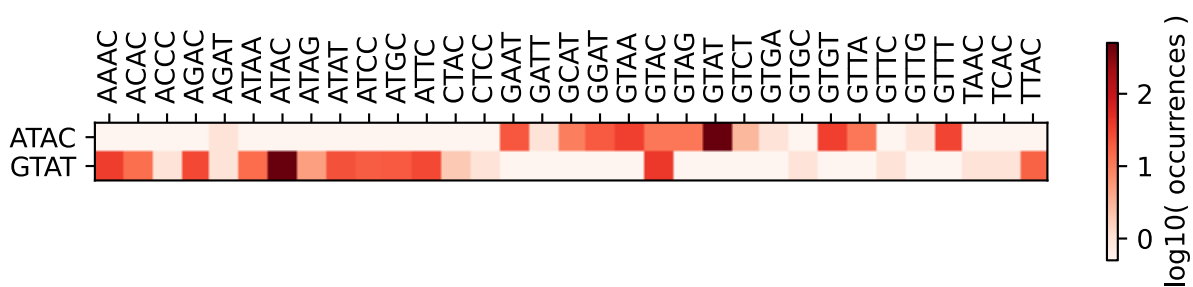
[NDYH][T]

V[SFW\*CLY]

[GESWAV\*KQMPLRT]Y

[GRCS][MI]

Misannealing overhangs:





# ATAT

# ATAT

Extreme GC content: 0 %.

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

Can form the following amino acids in 6 translation frames:

I[SW\*CLY]

[IESAV\*KQGRLRT]Y

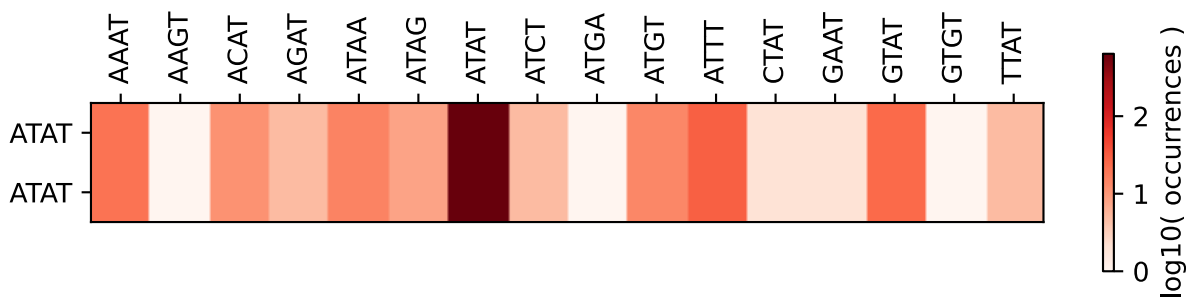
[NDYH][MI]

I[SW\*CLY]

[IESAV\*KQGRLRT]Y

[NDYH][MI]

Misannealing overhangs:





# ATCA

# TGAT

GC content: 25 %.

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

Can form the following amino acids in 6 translation frames:

I[INSKMRT]

[IESAV\*KQGPLRT]S

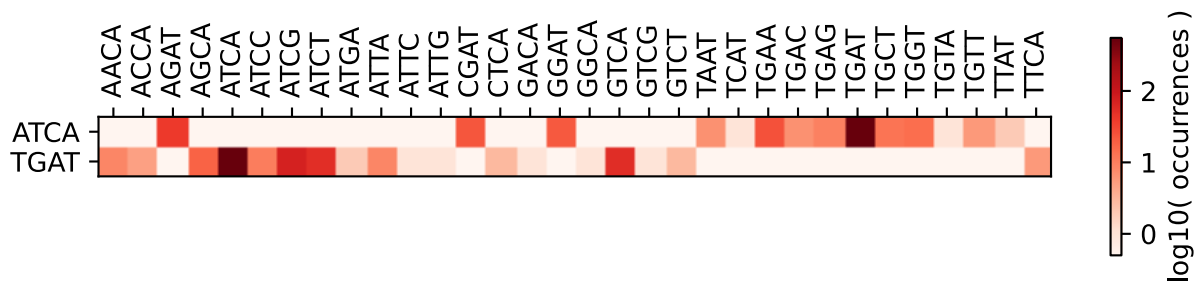
[NDYH][HQ]

\*[SFW\*CLY]

[DINSFAVRRCGPLYHT]D

[MVL][MI]

Misannealing overhangs:





# ATCC

# GGAT

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

I[PLRHQ]

[IESAV\*KQGPLRT]S

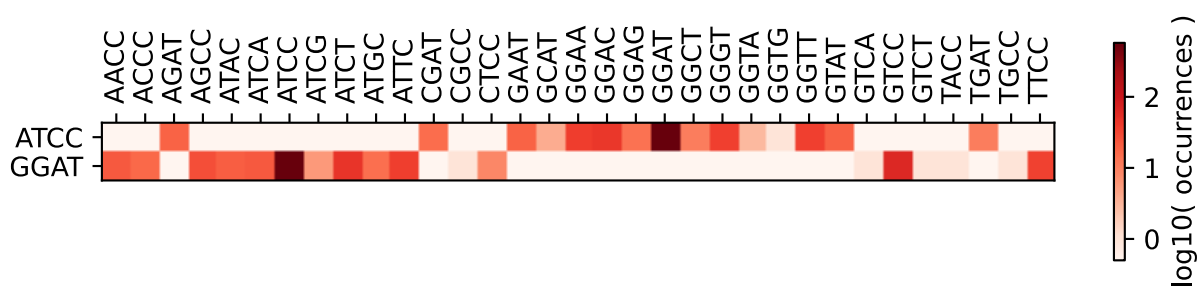
[NDYH][P]

G[SFW\*CLY]

[GESWAV\*KQMPLRT]D

[WGR][MI]

Misannealing overhangs:







# AGAT

# ATCT

GC content: **25 %**.

Can form the following amino acids in 6 translation frames:

R[SFW\*CLY]

[IESAV\*KQGPLRT]D

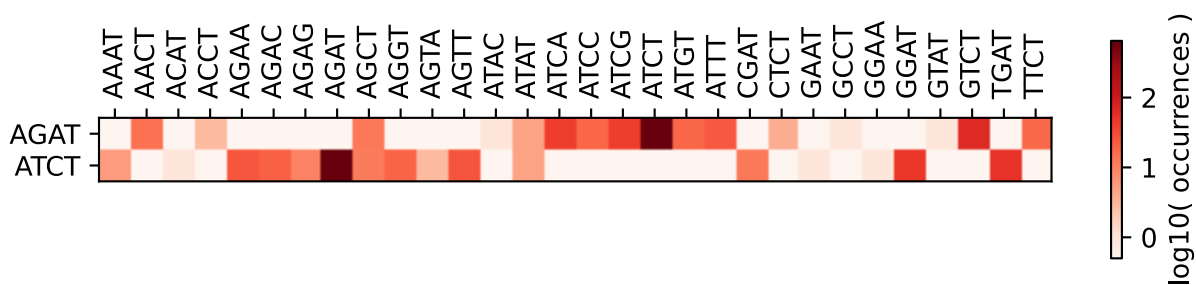
[\*EKQ][MI]

I[SFW\*CLY]

[IESAV\*KQGPLRT]S

[NDYH][L]

Misannealing overhangs:





# ATGG

# CCAT

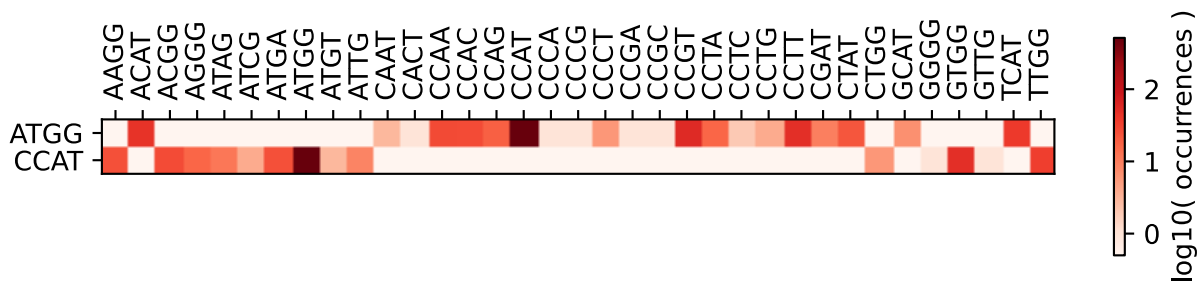
GC content: **50 %**.

The overhang contains the start codon ATG.

Can form the following amino acids in 6 translation frames:

M[EAVGD]  
[IESAV\*KQGPLRT]W  
[NDYH][G]  
P[SFW\*CLY]  
[DINSFAVRRCGPLYHT]H  
[APST][MI]

Misannealing overhangs:





# ATTC

# GAAT

GC content: **25 %**.

Can form the following amino acids in 6 translation frames:

I[PLRHQ]

[IESAV\*KQGPLRT]F

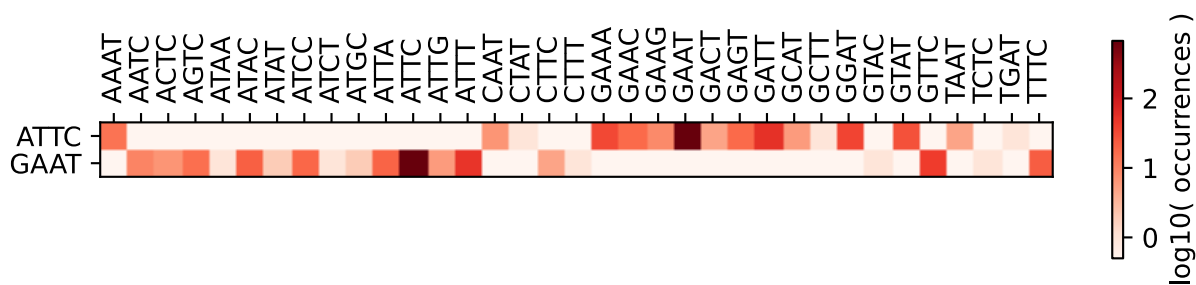
[NDYH][S]

E[SFW\*CLY]

[GESWAV\*KQMPLRT]N

[G\*R][MI]

Misannealing overhangs:





# ATTG

# CAAT

GC content: **25 %**.

Can form the following amino acids in 6 translation frames:

I[EAVGD]

[IESAV\*KQGPLRT]L

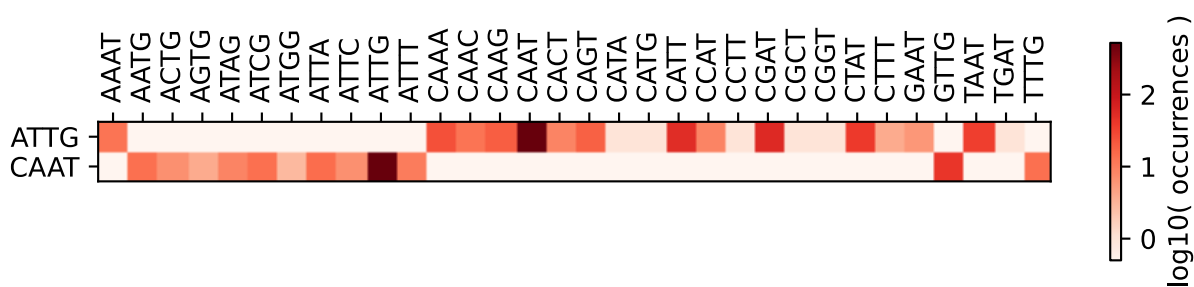
[NDYH][W\*C]

Q[SFW\*CLY]

[DINSFAVRCGPLYHT]N

[APST][MI]

Misannealing overhangs:





# CAAA

# TTTG

GC content: **25 %**.

Has 3 identical bases in a row. However, this has not shown to be very important.

Can form the following amino acids in 6 translation frames:

Q[INSKMRT]

[DINSFAVRRCGPLYHT]K

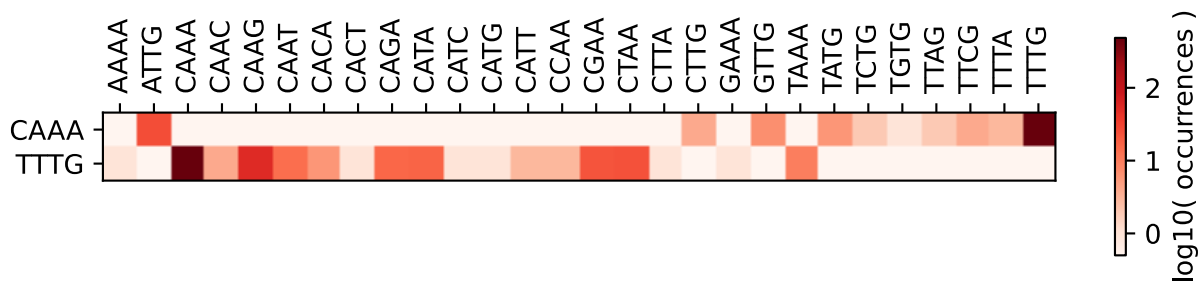
[APST][NK]

F[EAVGD]

[DINSFAVRRCGPLYHT]L

[FVIL][W\*C]

Misannealing overhangs:





# CAAC

# GTTG

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

Q[PLRHQ]

[DINSFAVRCGPLYHT]N

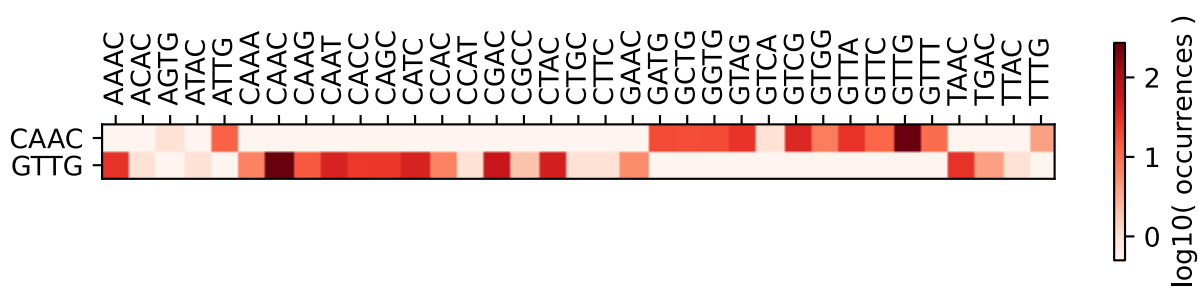
[APST][T]

V[EAVGD]

[GESWAV\*KQMPLRT]L

[GRCS][W\*C]

Misannealing overhangs:





# CACA

# TGTG

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

H[INSMRT]

[DINSFAVRCGPLYHT]T

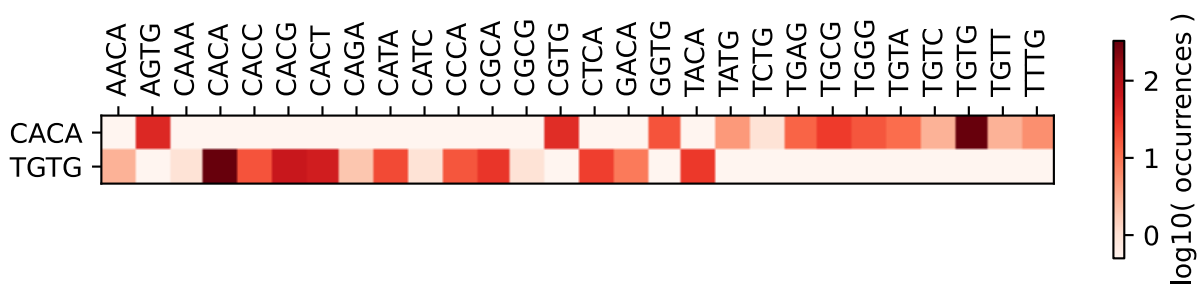
[APST][HQ]

C[EAVGD]

[DINSFAVRCGPLYHT]V

[MVL][W\*C]

Misannealing overhangs:





# CACC

# GGTG

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

H[PLRHQ]

[DINSFAVRCGPLYHT]T

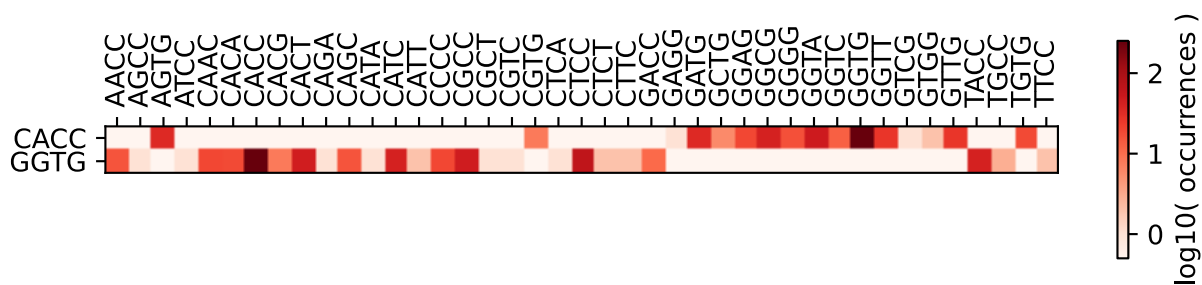
[APST][P]

G[EAVGD]

[GESWAV\*KQMPLRT]V

[WGR][W\*C]

Misannealing overhangs:







# CACG

# CGTG

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

H[EAVGD]

[DINSFAVRCGPLYHT]T

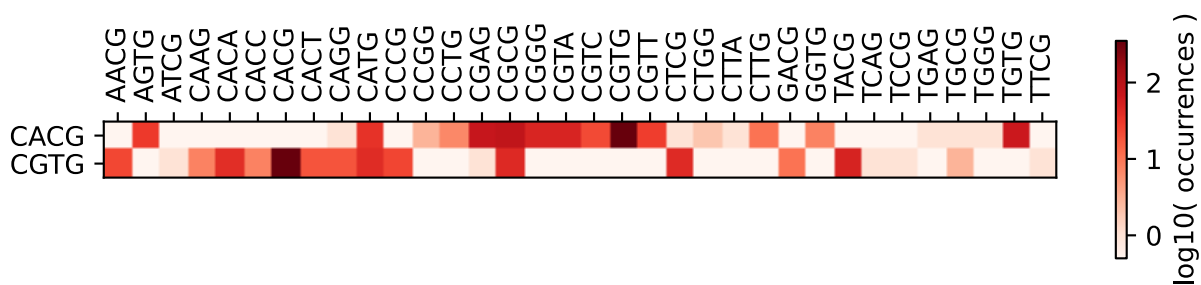
[APST][R]

R[EAVGD]

[DINSFAVRCGPLYHT]V

[APST][W\*C]

Misannealing overhangs:





# AGTG

# CACT

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

S[EAVGD]

[IESAV\*KQGPLRT]V

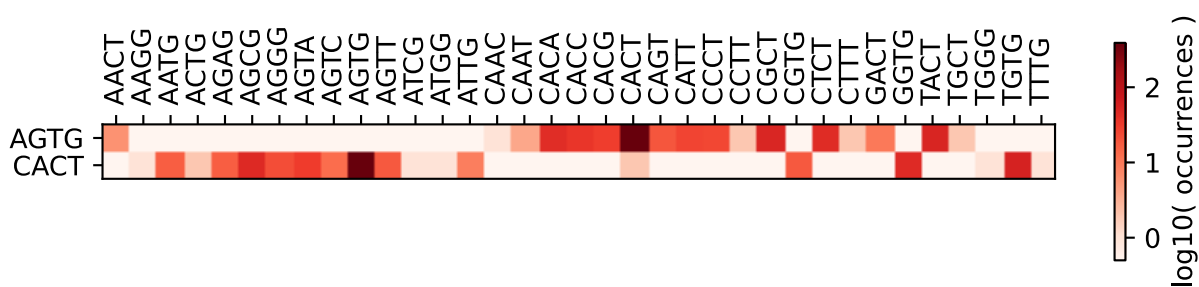
[\*EKQ][W\*C]

H[SFW\*CLY]

[DINSFAVRCGPLYHT]T

[APST][L]

Misannealing overhangs:





# CAGA

# TCTG

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

Q[INSKMRT]

[DINSFAVRCGPLYHT]R

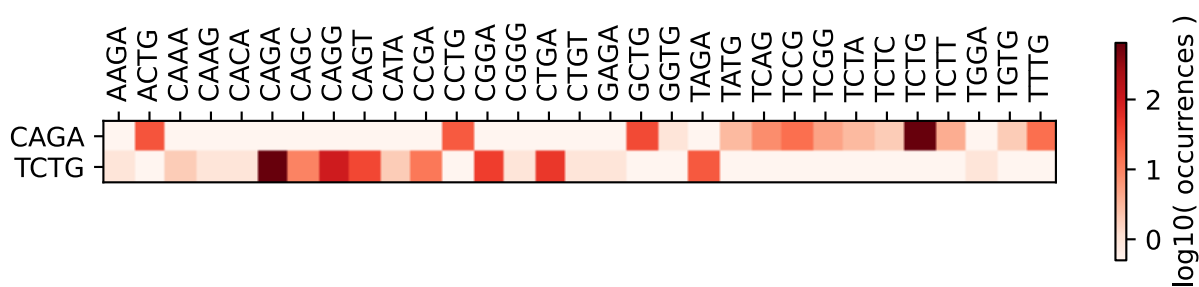
[APST][ED]

S[EAVGD]

[DINSFAVRCGPLYHT]L

[FVIL][W\*C]

Misannealing overhangs:





# CATG

# CATG

GC content: 50 %.

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

The overhang contains the start codon ATG.

Can form the following amino acids in 6 translation frames:

H[EAVGD]

[DINSFAVRCGPLYHT]M

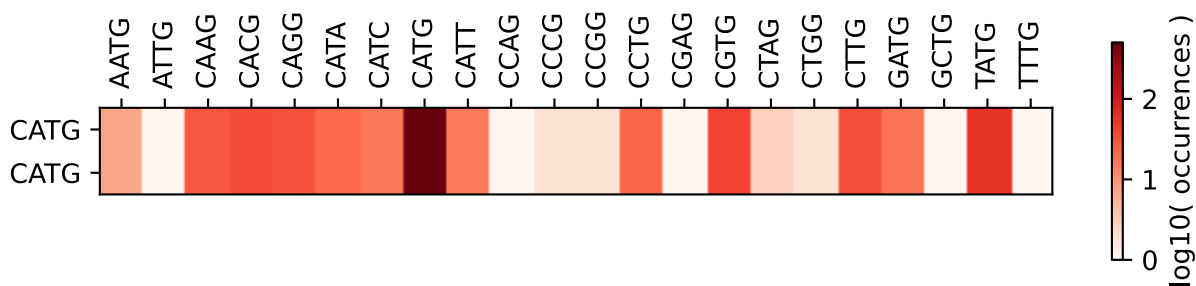
[APST][W\*C]

H[EAVGD]

[DINSFAVRCGPLYHT]M

[APST][W\*C]

Misannealing overhangs:





# CCAA

# TTGG

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

P[INSKMRT]

[DINSFAVRCGPLYHT]Q

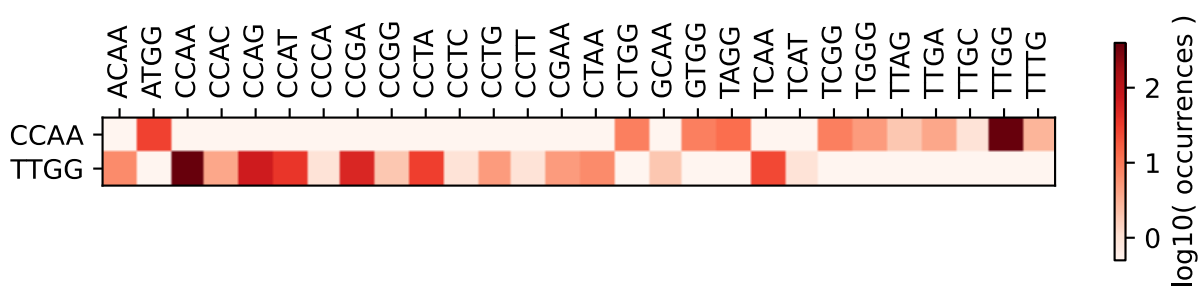
[APST][NK]

L[EAVGD]

[DINSFAVRCGPLYHT]W

[FVIL][G]

Misannealing overhangs:





# CCCA

# TGGG

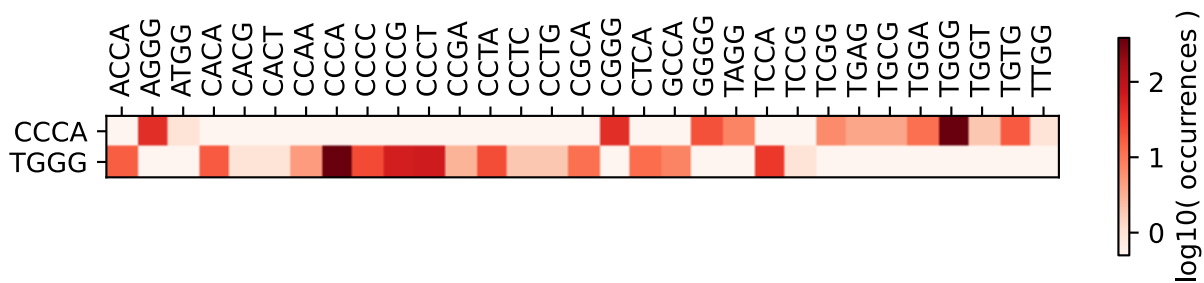
GC content: **75 %**.

Has 3 identical bases in a row. However, this has not shown to be very important.

Can form the following amino acids in 6 translation frames:

P[INSKMRT]  
[DINSFAVRCGPLYHT]P  
[APST][HQ]  
W[EAVGD]  
[DINSFAVRCGPLYHT]G  
[MVL][G]

Misannealing overhangs:





# AGGG

# CCCT

GC content: **75 %**.

Has 3 identical bases in a row. However, this has not shown to be very important.

Can form the following amino acids in 6 translation frames:

R[EAVGD]

[IESAV\*KQGPLRT]G

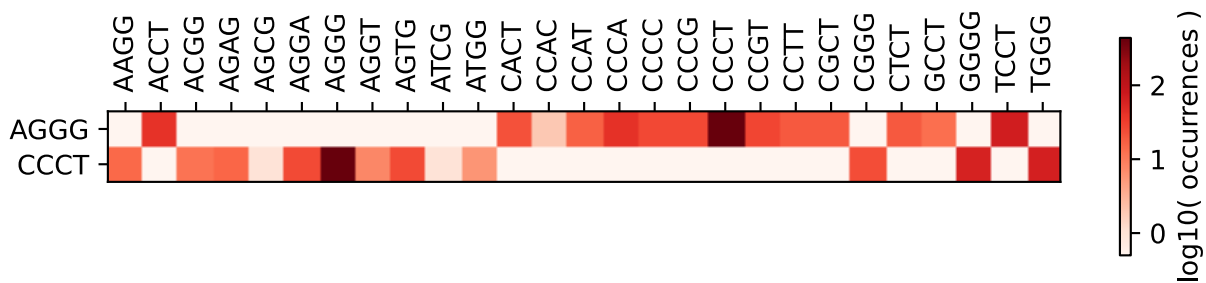
[\*EKQ][G]

P[SFW\*CLY]

[DINSFAVRRCGPLYHT]P

[APST][L]

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

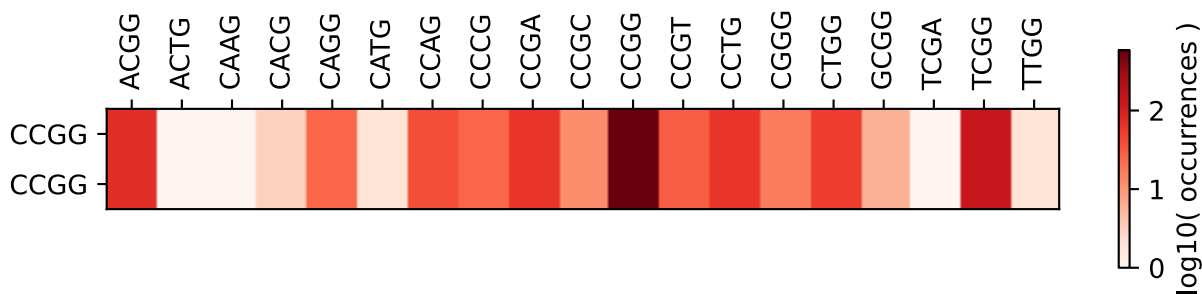
[APST][G]

P[EAVGD]

[DINSFAVRCGPLYHT]R

[APST][G]

Misannealing overhangs:







# ACGG

# CCGT

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

T[EAVGD]

[IESAV\*KQGPLRT]R

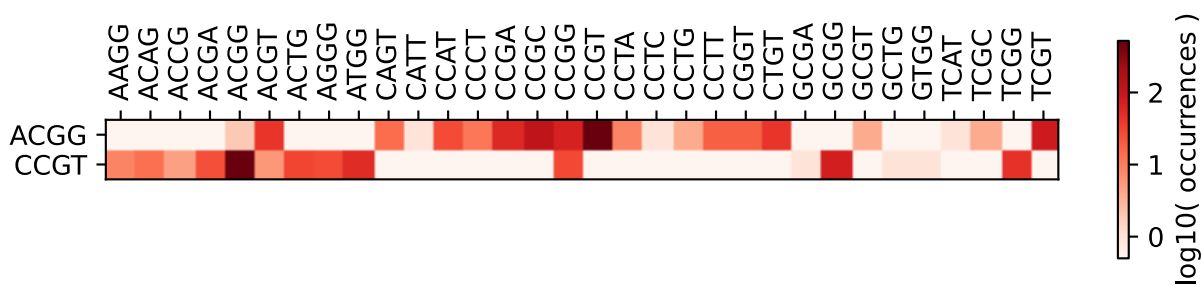
[NDYH][G]

P[SFW\*CLY]

[DINSFAVRCGPLYHT]R

[APST][V]

Misannealing overhangs:





# CCTA

# TAGG

GC content: **50 %**.

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

Can form the following amino acids in 6 translation frames:

P[INSKMRT]

[DINSFAVRRCGPLYHT]L

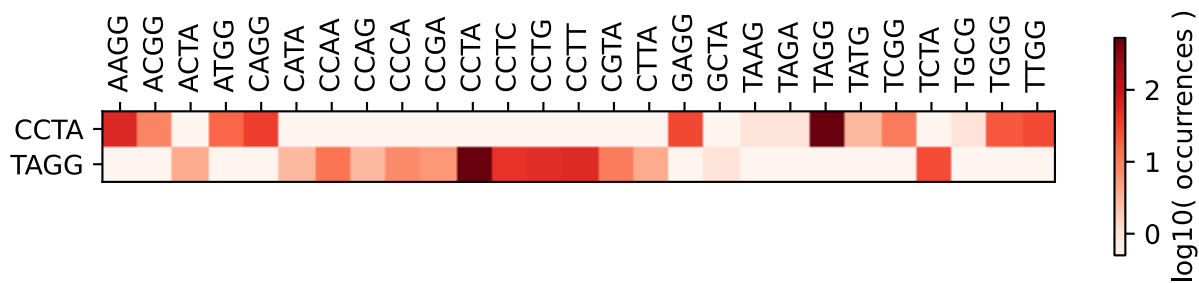
[APST][Y\*]

\*[EAVGD]

[DINSFAVRRCGPLYHT]R

[VIL][G]

Misannealing overhangs:





# CCTC

# GAGG

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

P[PLRHQ]

[DINSFAVRCGPLYHT]L

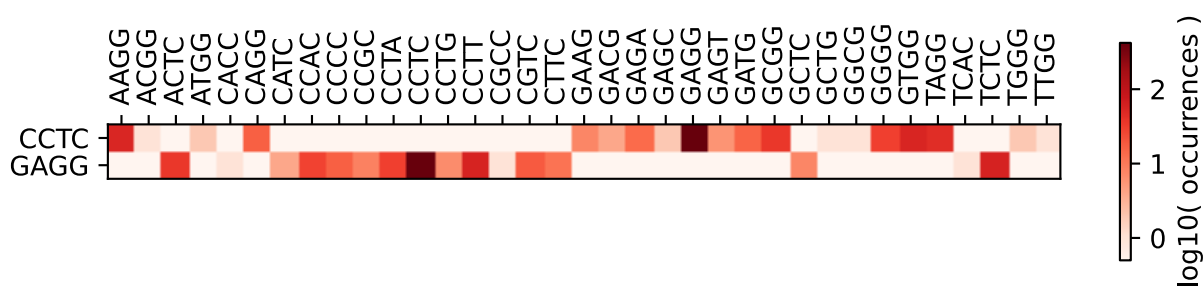
[APST][S]

E[EAVGD]

[GESWAV\*KQMPLRT]R

[G\*R][G]

Misannealing overhangs:





# CAGG

# CCTG

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

Q[EAVGD]

[DINSFAVRCGPLYHT]R

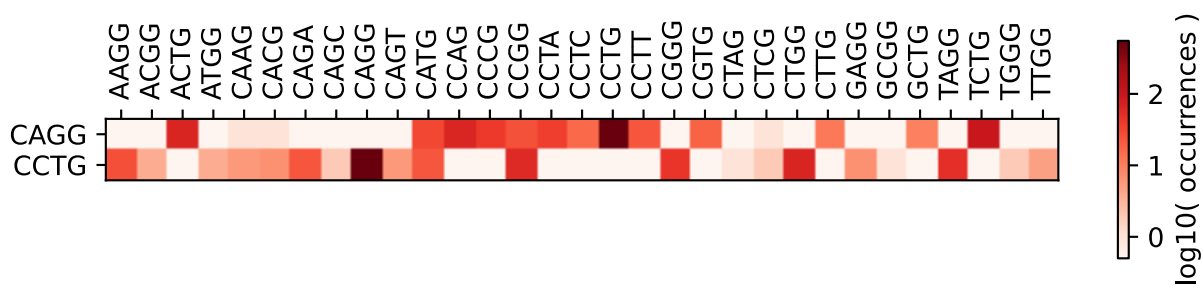
[APST][G]

P[EAVGD]

[DINSFAVRCGPLYHT]L

[APST][W\*C]

Misannealing overhangs:





# AAGG

# CCTT

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

K[EAVGD]

[IESAV\*KQGPLRT]R

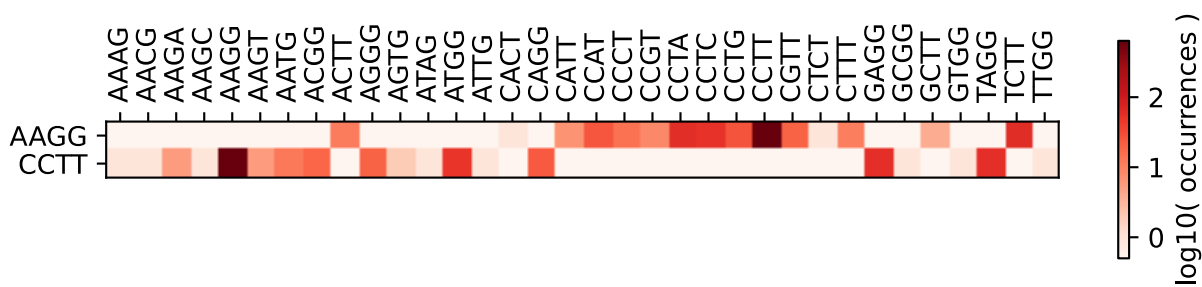
[\*EKQ][G]

P[SFW\*CLY]

[DINSFAVRCGPLYHT]L

[APST][FL]

Misannealing overhangs:





# CGAG

# CTCG

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

R[EAVGD]

[DINSFAVRCGPLYHT]E

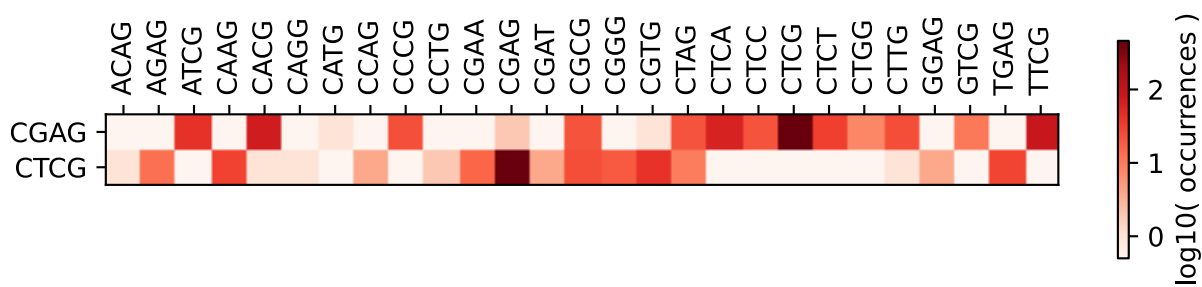
[APST][RS]

L[EAVGD]

[DINSFAVRCGPLYHT]S

[APST][R]

Misannealing overhangs:





# ATCG

# CGAT

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

I[EAVGD]

[IESAV\*KQGPLRT]S

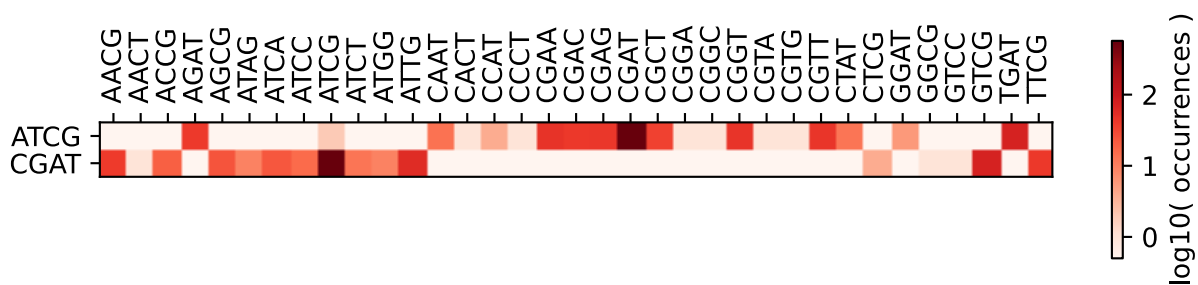
[NDYH][R]

R[SFW\*CLY]

[DINSFAVRCGPLYHT]D

[APST][MI]

Misannealing overhangs:





# CGCG

# CGCG

Extreme GC content: 100 %.

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

Can form the following amino acids in 6 translation frames:

R[EAVGD]

[DINSFAVRCGPLYHT]A

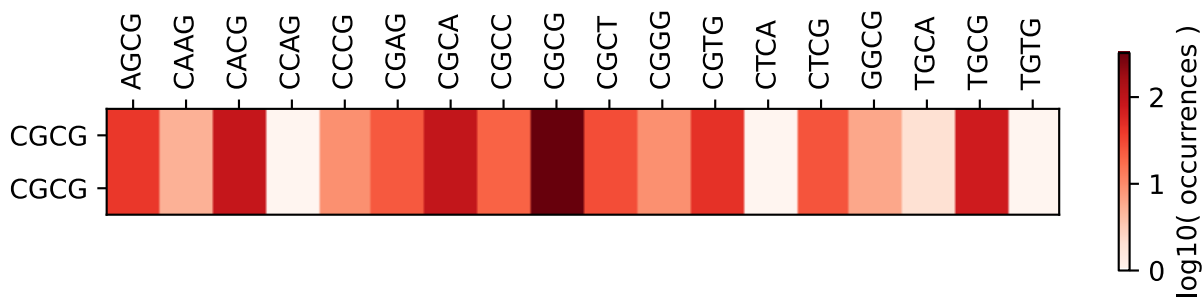
[APST][R]

R[EAVGD]

[DINSFAVRCGPLYHT]A

[APST][R]

Misannealing overhangs:







# CCCG

# CGGG

Extreme GC content: 100 %.

Has 3 identical bases in a row. However, this has not shown to be very important.

Can form the following amino acids in 6 translation frames:

P[EAVGD]

[DINSFAVRCGPLYHT]P

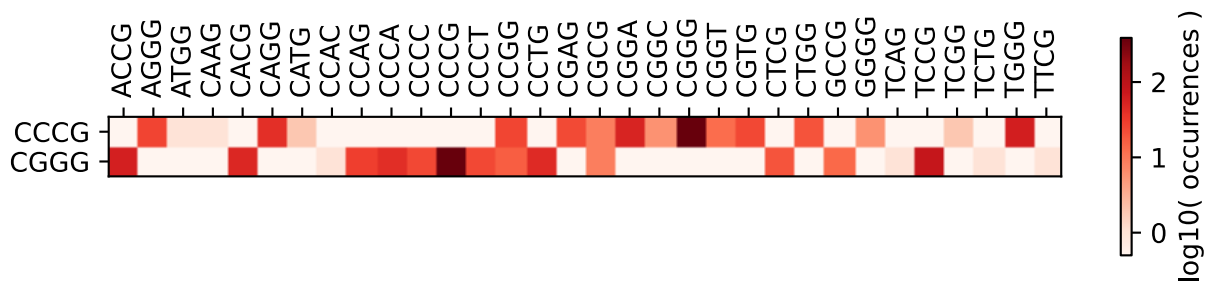
[APST][R]

R[EAVGD]

[DINSFAVRCGPLYHT]G

[APST][G]

Misannealing overhangs:





# ACCG

# CGGT

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

T[EAVGD]

[IESAV\*KQGPLRT]P

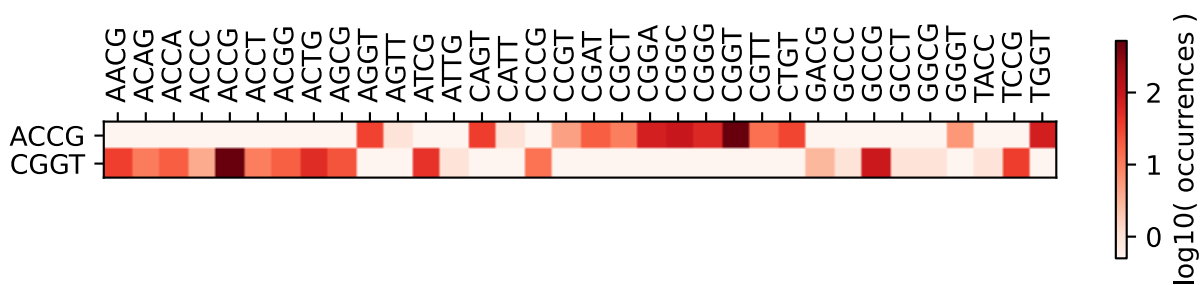
[NDYH][R]

R[SFW\*CLY]

[DINSFAVRGPLYHT]G

[APST][V]

Misannealing overhangs:





# CGTC

# GACG

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

R[PLRHQ]

[DINSFAVRCGPLYHT]V

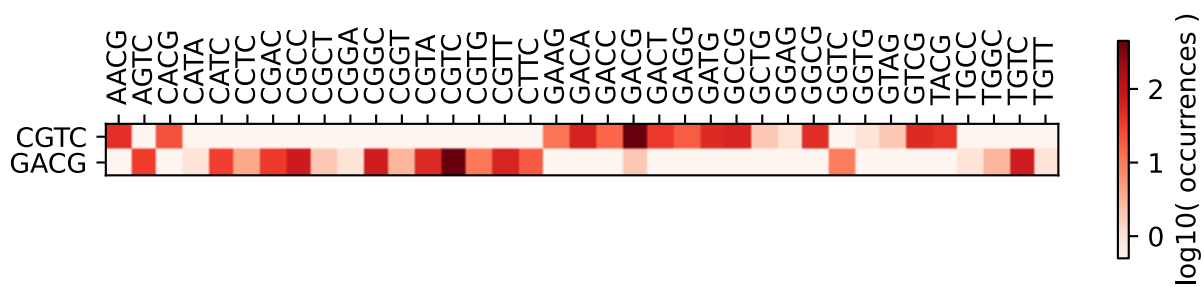
[APST][S]

D[EAVGD]

[GESWAV\*KQMPLRT]T

[G\*R][R]

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]

[DINSFAVRCGPLYHT]\*

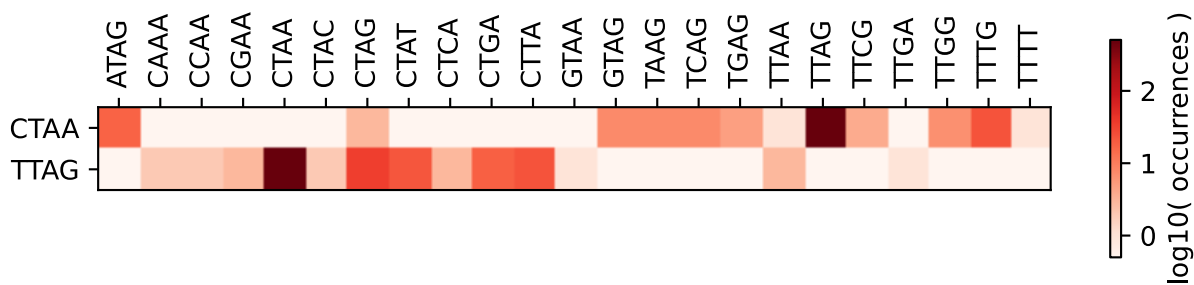
[APST][NK]

L[EAVGD]

[DINSFAVRCGPLYHT]\*

[FVIL][RS]

Misannealing overhangs:





# CTAG

# CTAG

GC content: 50 %.

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

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

Can form the following amino acids in 6 translation frames:

L[EAVGD]

[DINSFAVRCGPLYHT]\*

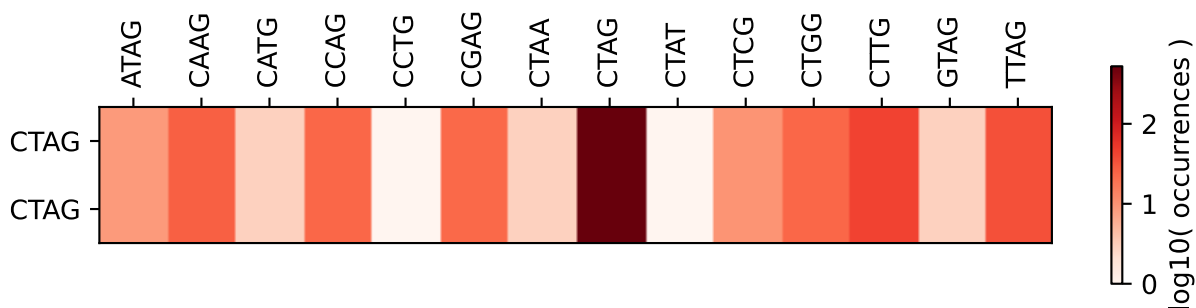
[APST][RS]

L[EAVGD]

[DINSFAVRCGPLYHT]\*

[APST][RS]

Misannealing overhangs:





# ATAG

# CTAT

GC content: 25 %.

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

Can form the following amino acids in 6 translation frames:

I[EAVGD]

[IESAV\*KQGPLRT]\*

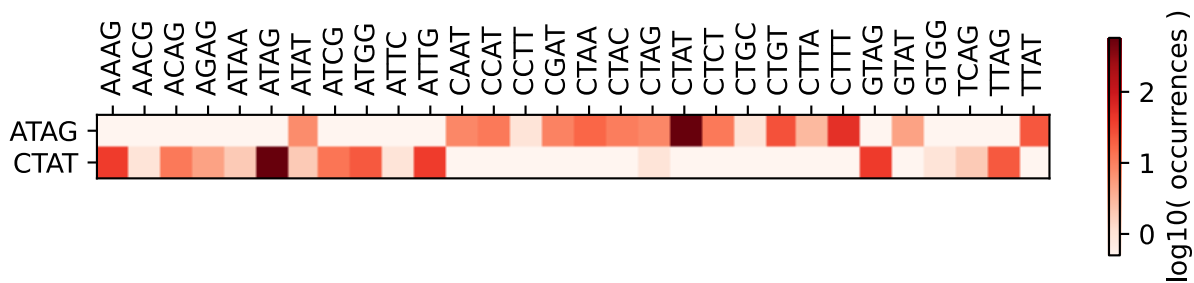
[NDYH][RS]

L[SFW\*CLY]

[DINSFAVRRCGPLYHT]Y

[APST][MI]

Misannealing overhangs:





# CTCC

# GGAG

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

L[PLRHQ]

[DINSFAVRCGPLYHT]S

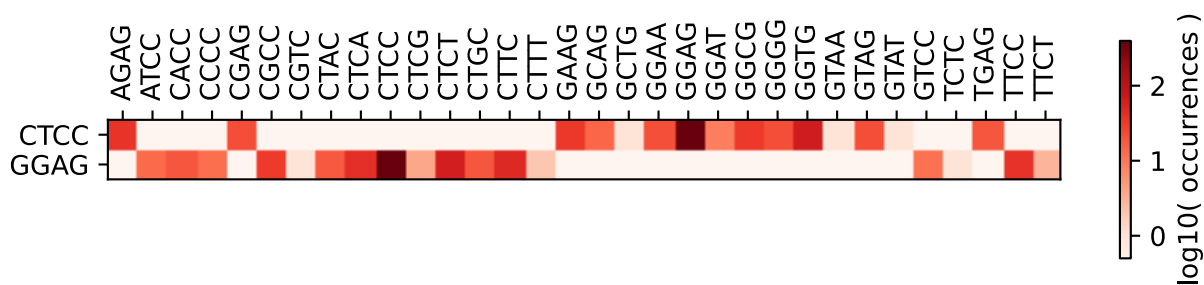
[APST][P]

G[EAVGD]

[GESWAV\*KQMPLRT]E

[WGR][RS]

Misannealing overhangs:





# AGAG

# CTCT

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

R[EAVGD]

[IESAV\*KQGPLRT]E

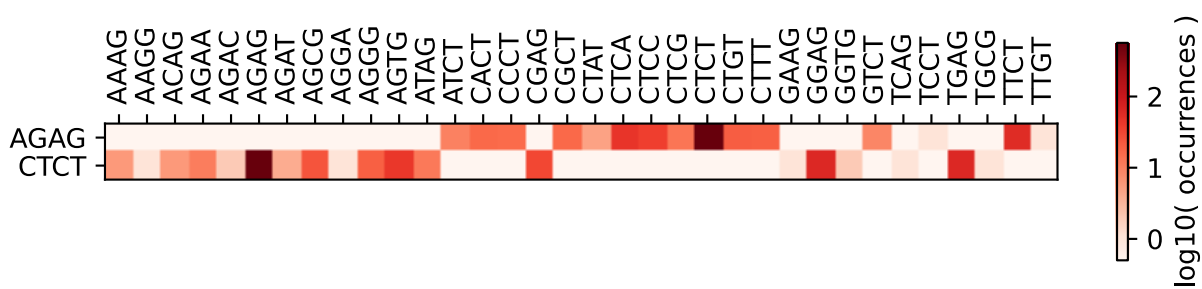
[\*EKQ][RS]

L[SFW\*CLY]

[DINSFAVRCGPLYHT]S

[APST][L]

Misannealing overhangs:







# CCAG

# CTGG

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

P[EAVGD]

[DINSFAVRCGPLYHT]Q

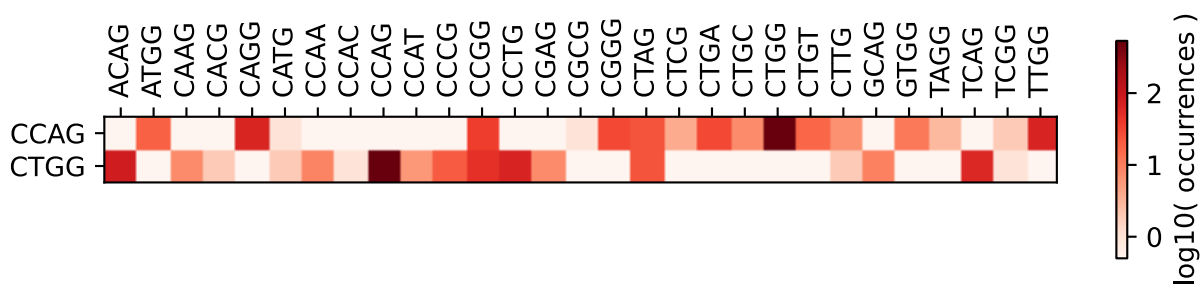
[APST][RS]

L[EAVGD]

[DINSFAVRCGPLYHT]W

[APST][G]

Misannealing overhangs:





# ACAG

# CTGT

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

T[EAVGD]

[IESAV\*KQGPLRT]Q

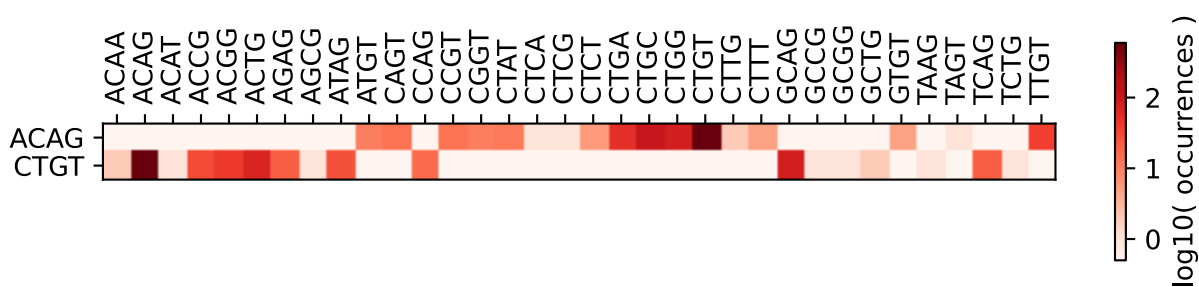
[NDYH][RS]

L[SFW\*CLY]

[DINSFAVRCGPLYHT]C

[APST][V]

Misannealing overhangs:





# CTTA

# TAAG

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]

[DINSFAVRCGPLYHT]L

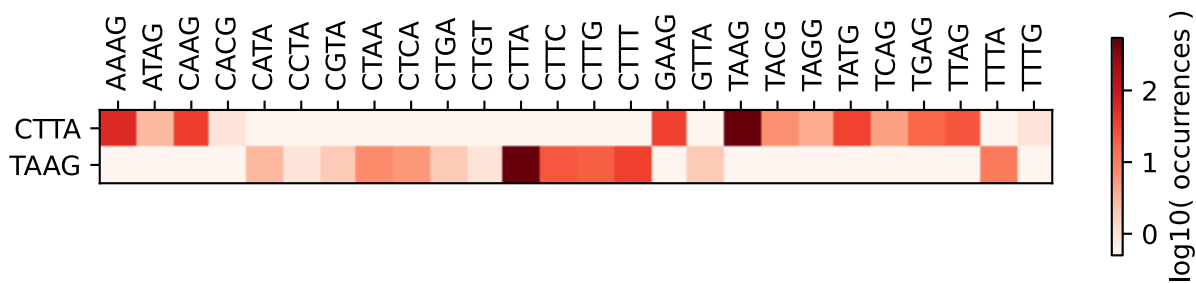
[APST][Y\*]

\*[EAVGD]

[DINSFAVRCGPLYHT]K

[VIL][RS]

Misannealing overhangs:





# CAAG

# CTTG

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

Q[EAVGD]

[DINSFAVRCGPLYHT]K

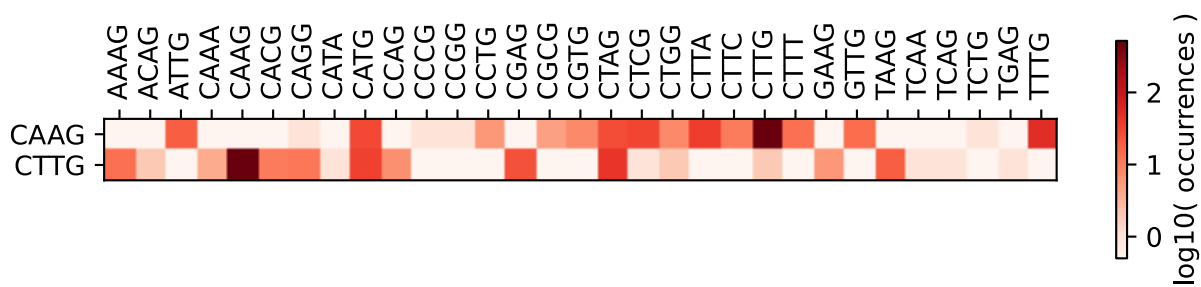
[APST][RS]

L[EAVGD]

[DINSFAVRCGPLYHT]L

[APST][W\*C]

Misannealing overhangs:





# GAAC

# GTTC

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

E[PLRHQ]

[GESWAV\*KQMPLRT]N

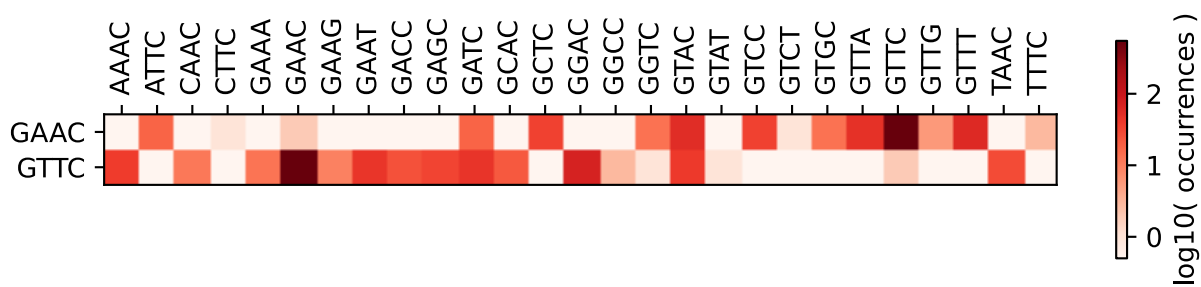
[G\*R][T]

V[PLRHQ]

[GESWAV\*KQMPLRT]F

[GRCS][S]

Misannealing overhangs:





# CTTC

# GAAG

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

L[PLRHQ]

[DINSFAVRCGPLYHT]F

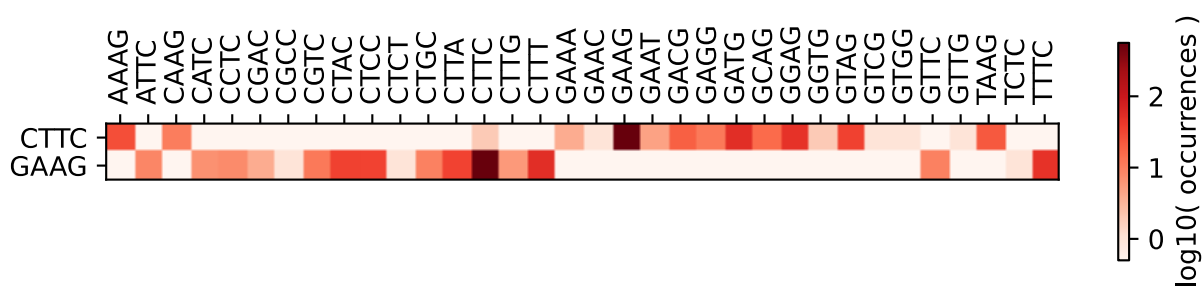
[APST][S]

E[EAVGD]

[GESWAV\*KQMPLRT]K

[G\*R][RS]

Misannealing overhangs:





# AGTC

# GACT

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

S[PLRHQ]

[IESAV\*KQGPLRT]V

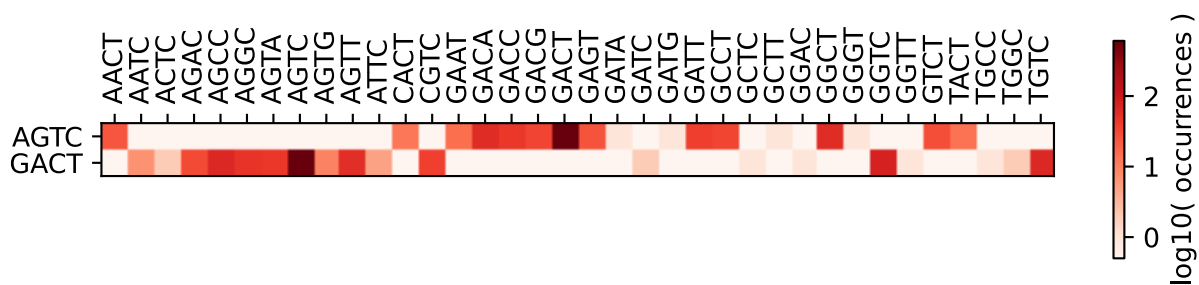
[\*EKQ][S]

D[SFW\*CLY]

[GESWAV\*KQMPLRT]T

[G\*R][L]

Misannealing overhangs:





# GAGA

# TCTC

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

E[INSKMRT]

[GESWAV\*KQMPLRT]R

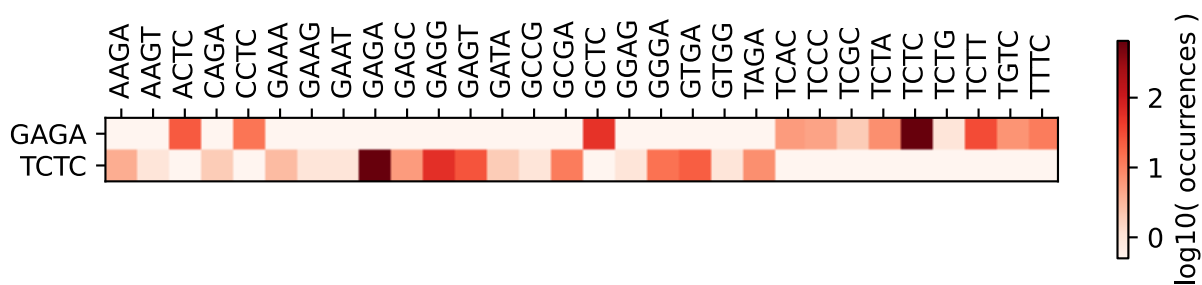
[G\*R][ED]

S[PLRHQ]

[DINSFAVRCGPLYHT]L

[FVIL][S]

Misannealing overhangs:







# GAGC

# GCTC

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

E[PLRHQ]

[GESWAV\*KQMPLRT]S

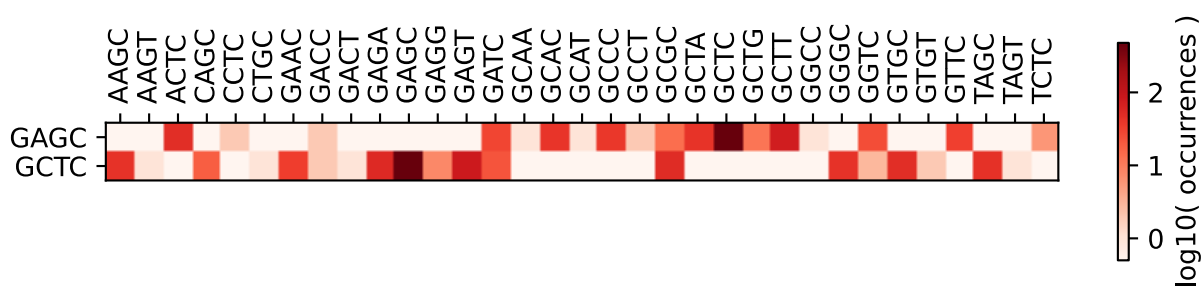
[G\*R][A]

A[PLRHQ]

[GESWAV\*KQMPLRT]L

[GRCS][S]

Misannealing overhangs:





# GATC

# GATC

GC content: 50 %.

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

Can form the following amino acids in 6 translation frames:

D[PLRHQ]

[GESWAV\*KQMPLRT]I

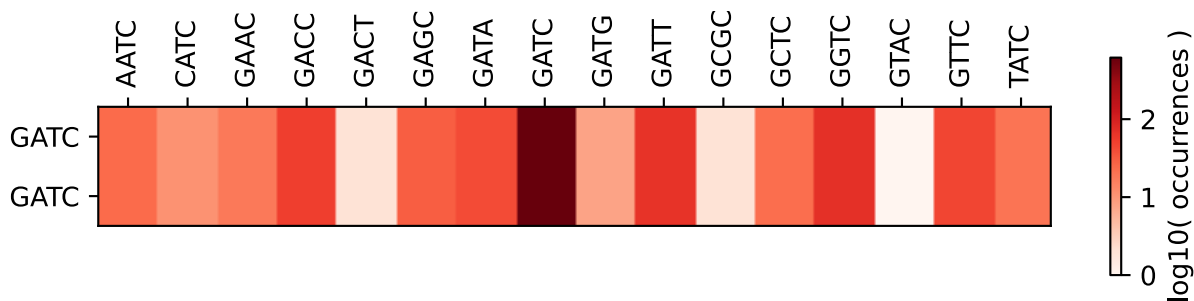
[G\*R][S]

D[PLRHQ]

[GESWAV\*KQMPLRT]I

[G\*R][S]

Misannealing overhangs:





# CATC

# GATG

GC content: **50 %**.

The overhang contains the start codon ATG.

Can form the following amino acids in 6 translation frames:

H[PLRHQ]

[DINSFAVRCGPLYHT]I

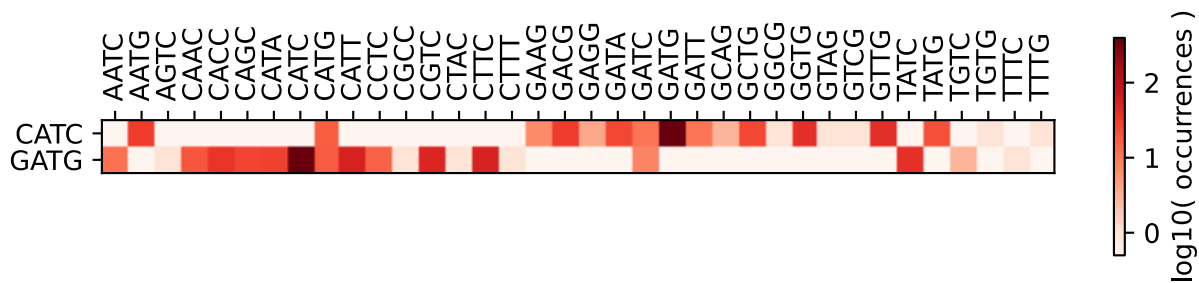
[APST][S]

D[EAVGD]

[GESWAV\*KQMPLRT]M

[G\*R][W\*C]

Misannealing overhangs:





# AATC

# GATT

GC content: **25 %**.

Can form the following amino acids in 6 translation frames:

N[PLRHQ]

[IESAV\*KQGPLRT]I

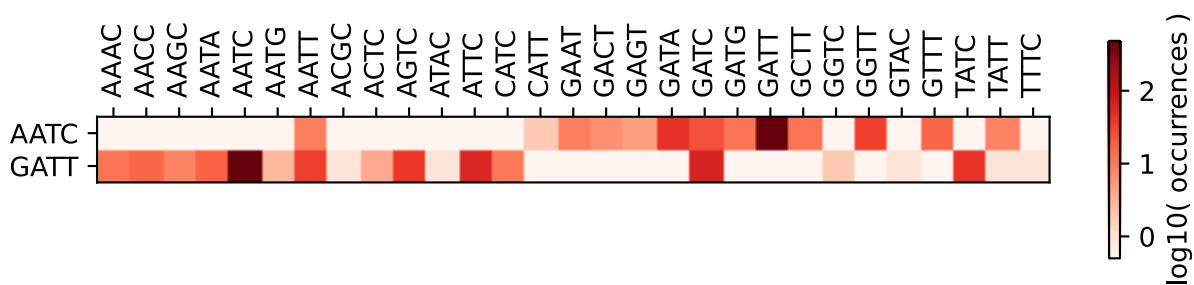
[\*EKQ][S]

D[SFW\*CLY]

[GESWAV\*KQMPLRT]I

[G\*R][FL]

Misannealing overhangs:





# CTGC

# GCAG

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

L[PLRHQ]

[DINSFAVRCGPLYHT]C

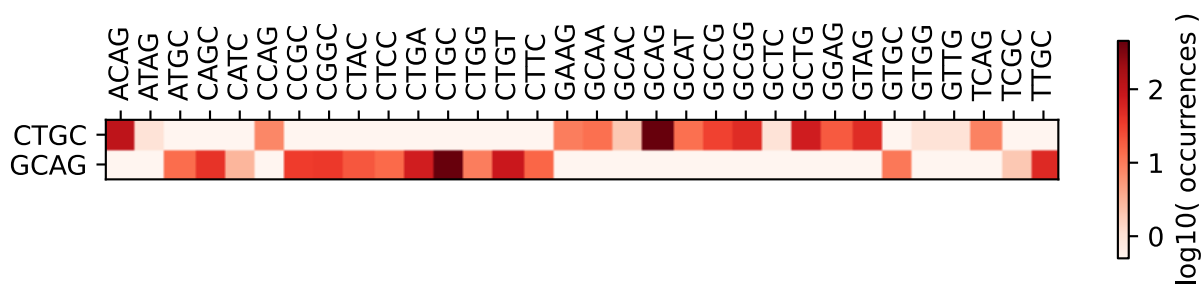
[APST][A]

A[EAVGD]

[GESWAV\*KQMPLRT]Q

[GRCS][RS]

Misannealing overhangs:





# ATGC

# GCAT

GC content: **50 %**.

The overhang contains the start codon ATG.

Can form the following amino acids in 6 translation frames:

M[PLRHQ]

[IESAV\*KQGPLRT]C

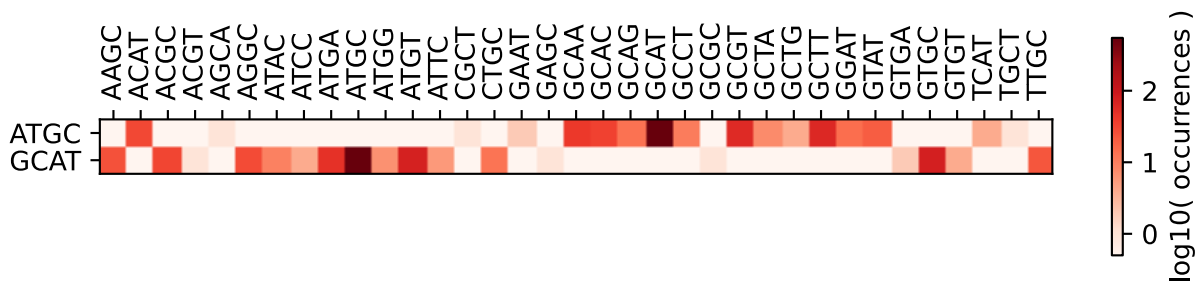
[NDYH][A]

A[SFW\*CLY]

[GESWAV\*KQMPLRT]H

[GRCS][MI]

Misannealing overhangs:





# TGGC

Can form the following amino acids in 6 translation frames:

[GESWAV\*KQMPLRT]P

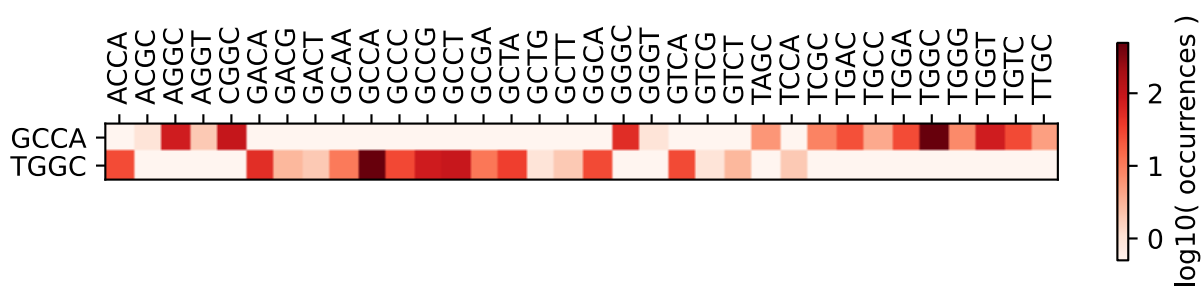
[GRCS][HQ]

W[PLRHQ]

[DINSFAVRCGPLYHT]G

 $[MVL][A]$ 

Misannealing overhangs:





# CGGC

# GCCG

Extreme GC content: 100 %.

Can form the following amino acids in 6 translation frames:

R[PLRHQ]

[DINSFAVRCGPLYHT]G

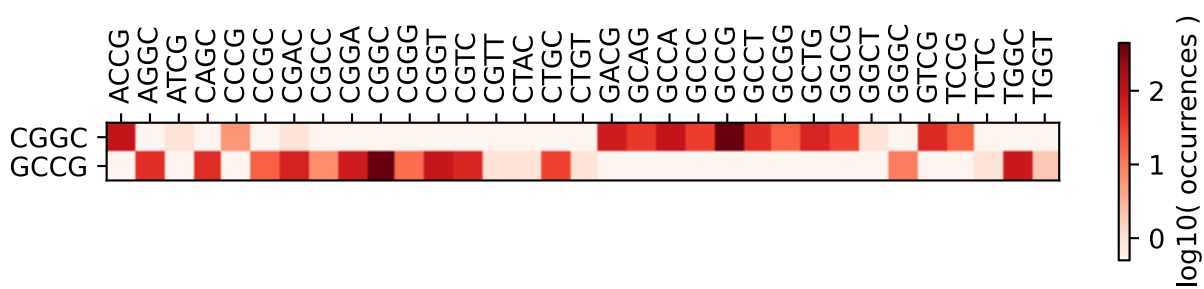
[APST][A]

A[EAVGD]

[GESWAV\*KQMPLRT]P

[GRCS][R]

Misannealing overhangs:







# GCGC

# GCGC

Extreme GC content: 100 %.

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

Can form the following amino acids in 6 translation frames:

A[PLRHQ]

[GESWAV\*KQMPLRT]R

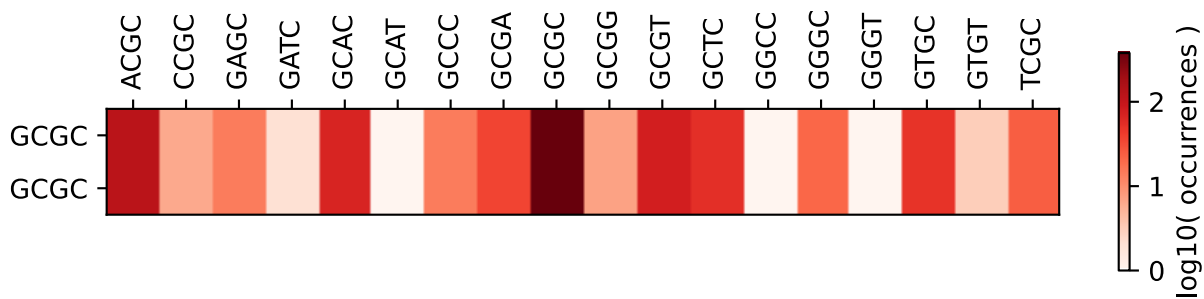
[GRCS][A]

A[PLRHQ]

[GESWAV\*KQMPLRT]R

[GRCS][A]

Misannealing overhangs:





# CCGC

# GCGG

Extreme GC content: 100 %.

Can form the following amino acids in 6 translation frames:

P[PLRHQ]

[DINSFAVRCGPLYHT]R

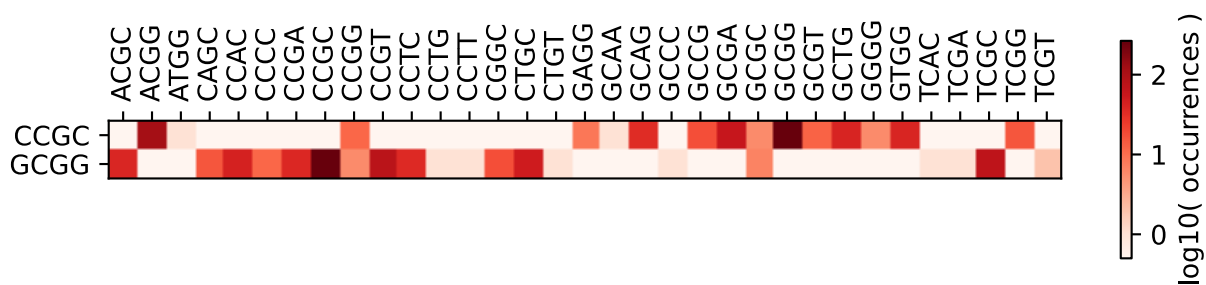
[APST][A]

A[EAVGD]

[GESWAV\*KQMPLRT]R

[GRCS][G]

Misannealing overhangs:





# GCTA

# TAGC

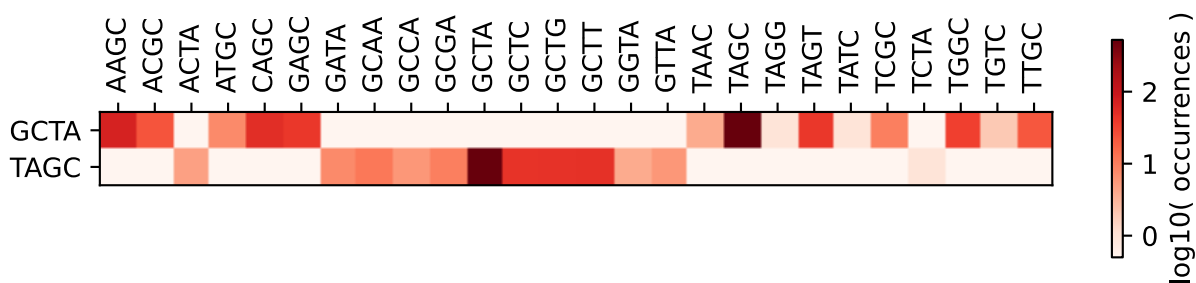
GC content: **50 %**.

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

Can form the following amino acids in 6 translation frames:

A[INSKMRT]  
[GESWAV\*KQMPLRT]L  
[GRCS][Y\*]  
\*[PLRHQ]  
[DINSFAVRRCGPLYHT]S  
[VIL][A]

Misannealing overhangs:





# CAGC

# GCTG

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

Q[PLRHQ]

[DINSFAVRCGPLYHT]S

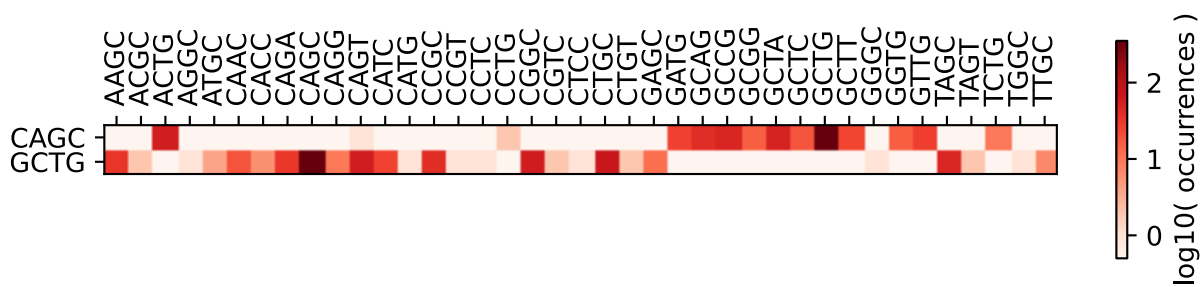
[APST][A]

A[EAVGD]

[GESWAV\*KQMPLRT]L

[GRCS][W\*C]

Misannealing overhangs:





# GGAC

# GTCC

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

G[PLRHQ]

[GESWAV\*KQMPLRT]D

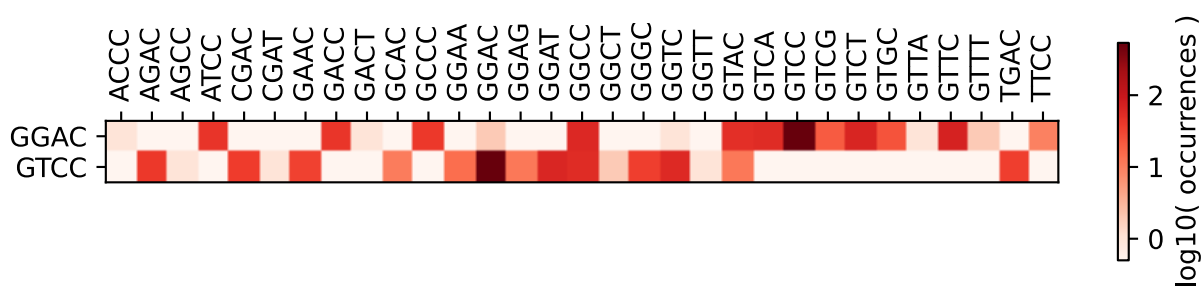
[WGR][T]

V[PLRHQ]

[GESWAV\*KQMPLRT]S

[GRCS][P]

Misannealing overhangs:





# GGCA

# TGCC

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

G[INSKMRT]

[GESWAV\*KQMPLRT]A

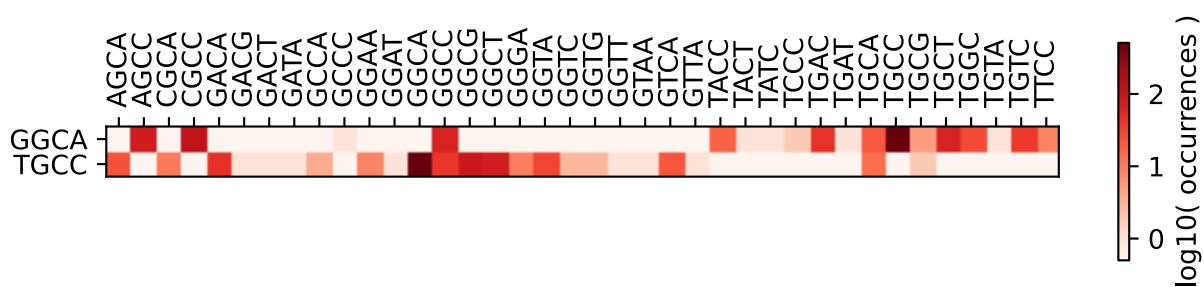
[WGR][HQ]

C[PLRHQ]

[DINSFAVRCGPLYHT]A

[MVL][P]

Misannealing overhangs:





# GGCC

# GGCC

Extreme GC content: 100 %.

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

Can form the following amino acids in 6 translation frames:

G[PLRHQ]

[GESWAV\*KQMPLRT]A

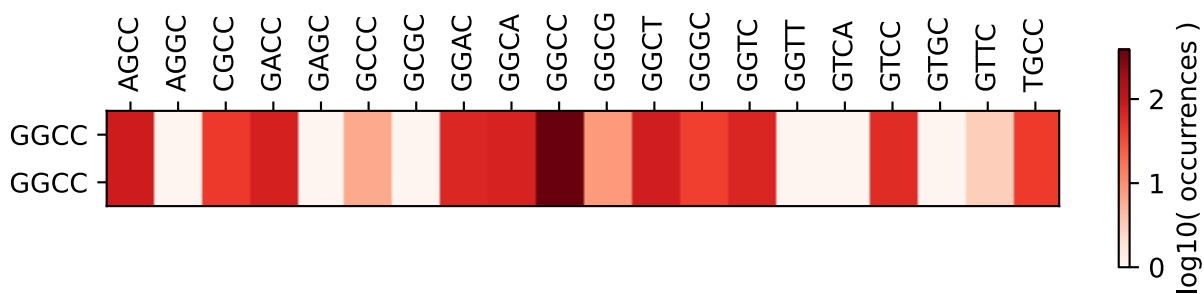
[WGR][P]

G[PLRHQ]

[GESWAV\*KQMPLRT]A

[WGR][P]

Misannealing overhangs:





GGCG

Can form the following amino acids in 6 translation frames:

[DINSFAVRCGPLYHT]A

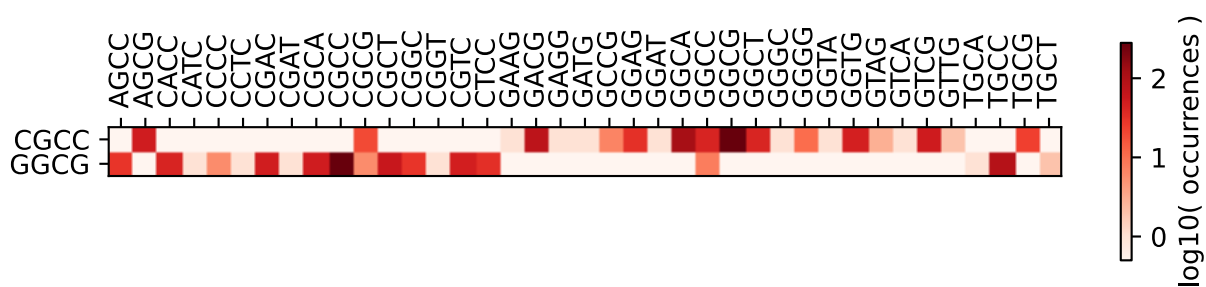
[APST][P]

G[EAVGD]

[GESWAV\*KQMPLRT]A

[WGR][R]

Misannealing overhangs:







# GGGA

# TCCC

GC content: **75 %**.

Has 3 identical bases in a row. However, this has not shown to be very important.

Can form the following amino acids in 6 translation frames:

G[INSKMRT]

[GESWAV\*KQMPLRT]G

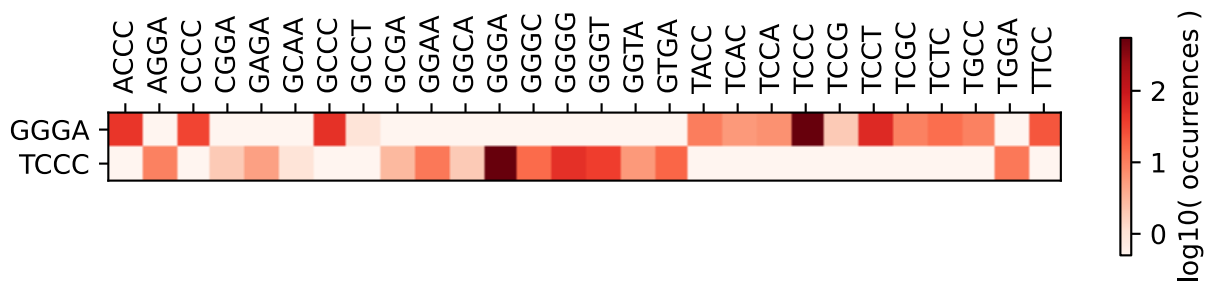
[WGR][ED]

S[PLRHQ]

[DINSFAVRRCGPLYHT]P

[FVIL][P]

Misannealing overhangs:





# GCCC

# GGGC

Extreme GC content: 100 %.

Has 3 identical bases in a row. However, this has not shown to be very important.

Can form the following amino acids in 6 translation frames:

A[PLRHQ]

[GESWAV\*KQMPLRT]P

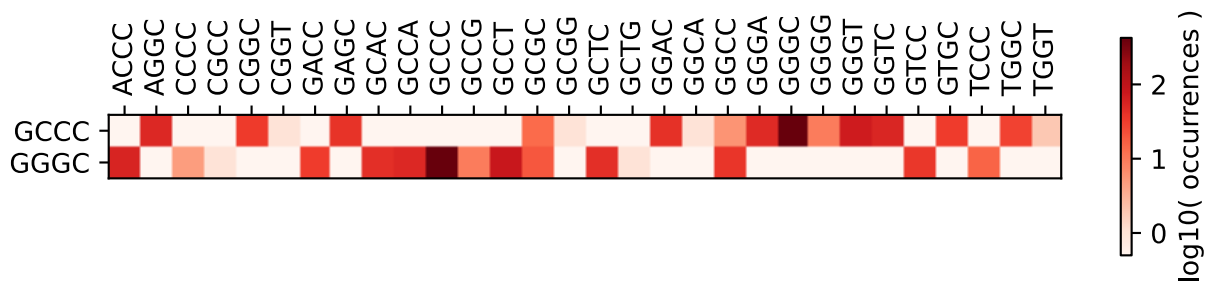
[GRCS][P]

G[PLRHQ]

[GESWAV\*KQMPLRT]G

[WGR][A]

Misannealing overhangs:





# CCCC

# GGGG

Extreme GC content: 100 %.

Has 3 identical bases in a row. However, this has not shown to be very important.

Can form the following amino acids in 6 translation frames:

P[PLRHQ]

[DINSFAVRGPLYHT]P

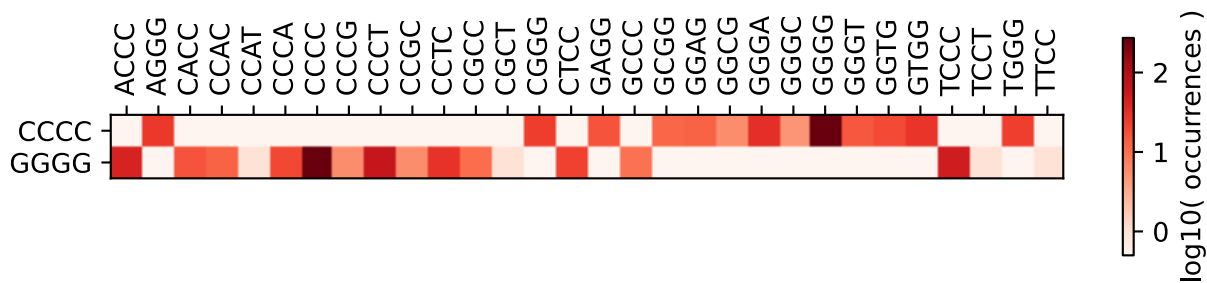
[APST][P]

G[EAVGD]

[GESWAV\*KQMPLRT]G

[WGR][G]

Misannealing overhangs:





# GGTA

# TACC

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

G[INSMRT]

[GESWAV\*KQMPLRT]V

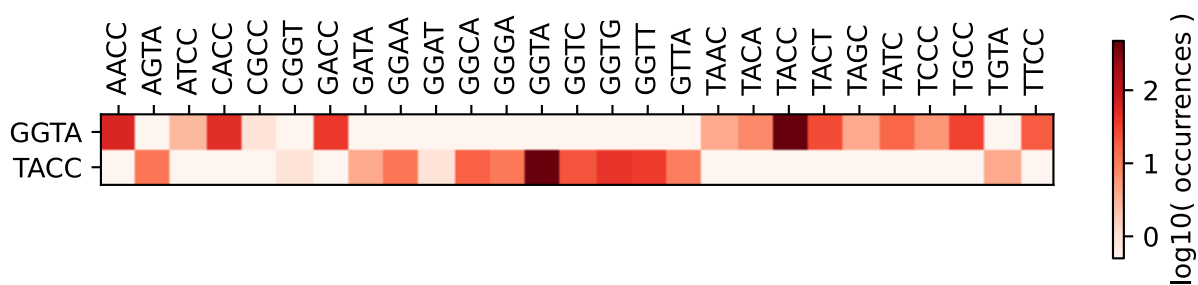
[WGR][Y\*]

Y[PLRHQ]

[DINSFAVRCGPLYHT]T

[VIL][P]

Misannealing overhangs:





# GACC

# GGTC

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

D[PLRHQ]

[GESWAV\*KQMPLRT]T

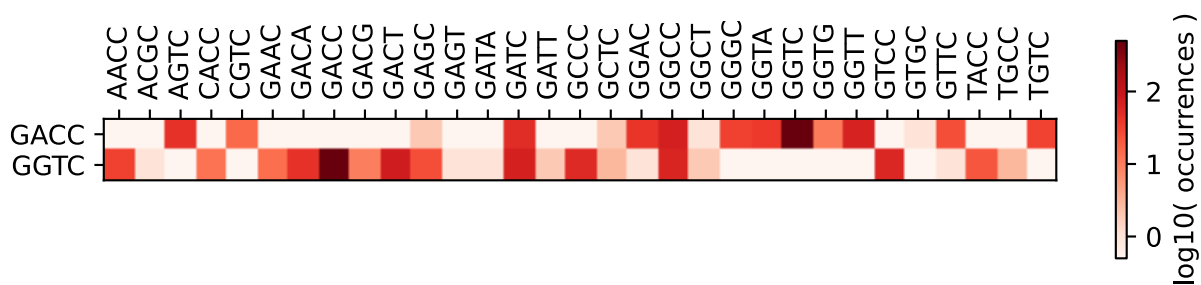
[G\*R][P]

G[PLRHQ]

[GESWAV\*KQMPLRT]V

[WGR][S]

Misannealing overhangs:





# GTAC

# GTAC

GC content: 50 %.

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

Can form the following amino acids in 6 translation frames:

V[PLRHQ]

[GESWAV\*KQMPLRT]Y

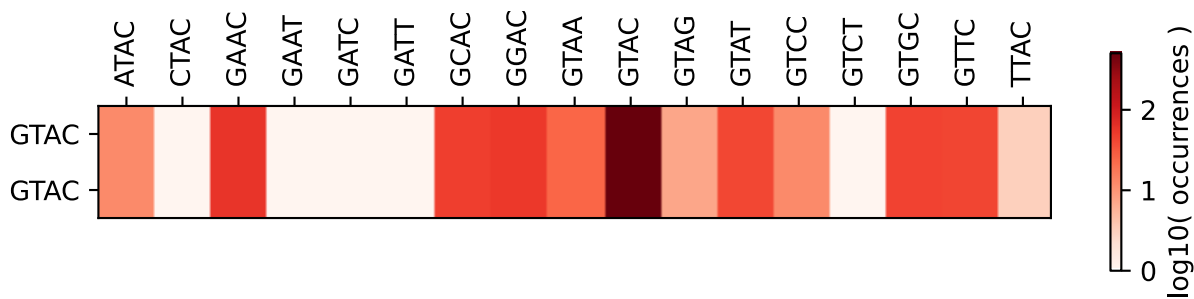
[GRCS][T]

V[PLRHQ]

[GESWAV\*KQMPLRT]Y

[GRCS][T]

Misannealing overhangs:





# CTAC

# GTAG

GC content: **50 %**.

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

Can form the following amino acids in 6 translation frames:

L[PLRHQ]

[DINSFAVRRCGPLYHT]Y

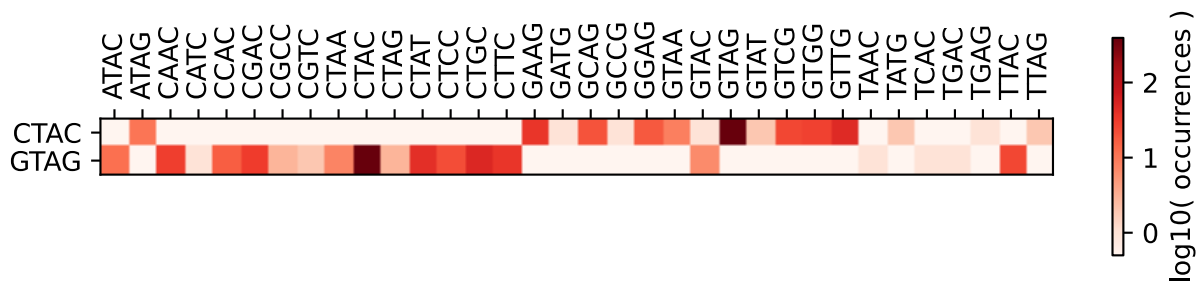
[APST][T]

V[EAVGD]

[GESWAV\*KQMPLRT]\*

[GRCS][RS]

Misannealing overhangs:





# CGAC

# GTCG

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

R[PLRHQ]

[DINSFAVRCGPLYHT]D

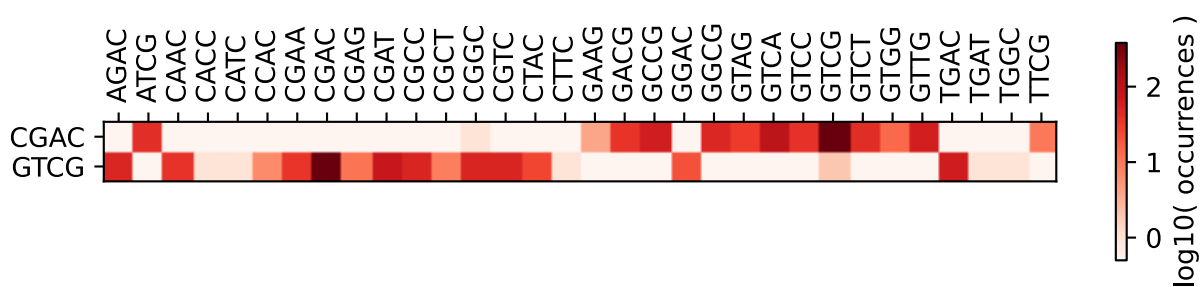
[APST][T]

V[EAVGD]

[GESWAV\*KQMPLRT]S

[GRCS][R]

Misannealing overhangs:







# AGAC

# GTCT

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

R[PLRHQ]

[IESAV\*KQGPLRT]D

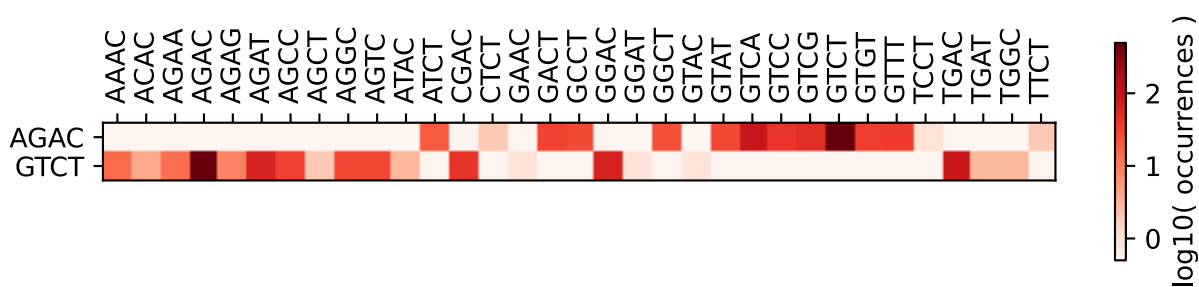
[\*EKQ][T]

V[SFW\*CLY]

[GESWAV\*KQMPLRT]S

[GRCS][L]

Misannealing overhangs:





# GTGA

# TCAC

GC content: **50 %**.

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

Can form the following amino acids in 6 translation frames:

V[INSKMRT]

[GESWAV\*KQMPLRT]\*

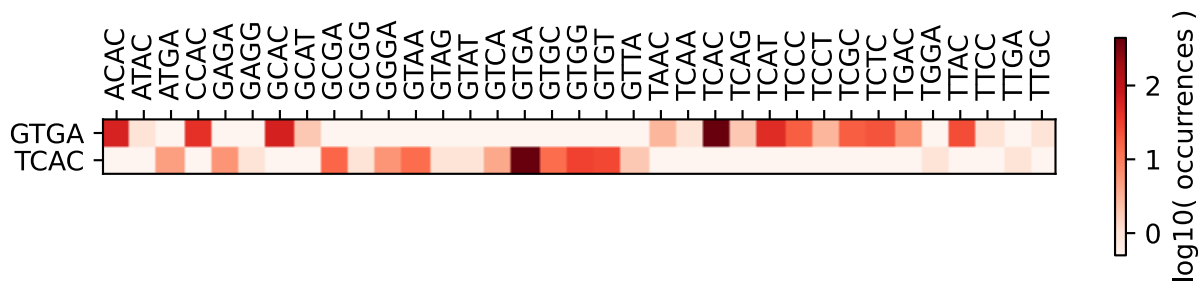
[GRCS][ED]

S[PLRHQ]

[DINSFAVRCGPLYHT]H

[FVIL][T]

Misannealing overhangs:





# GCAC

# GTGC

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

A[PLRHQ]

[GESWAV\*KQMPLRT]H

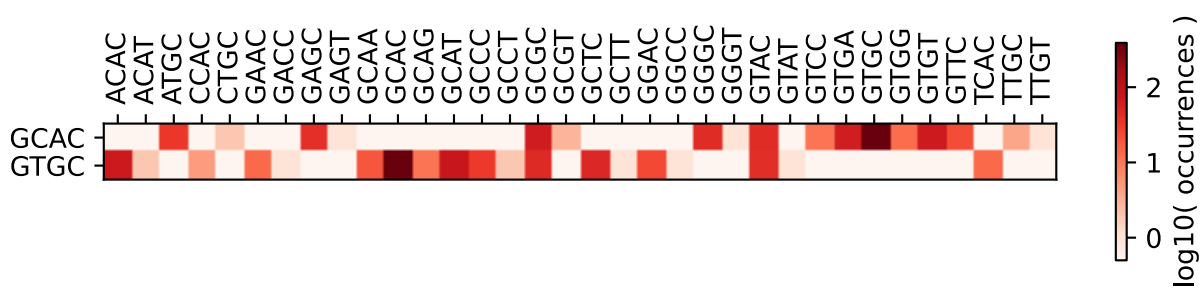
[GRCS][T]

V[PLRHQ]

[GESWAV\*KQMPLRT]C

[GRCS][A]

Misannealing overhangs:





# CCAC

# GTGG

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

P[PLRHQ]

[DINSFAVRCGPLYHT]H

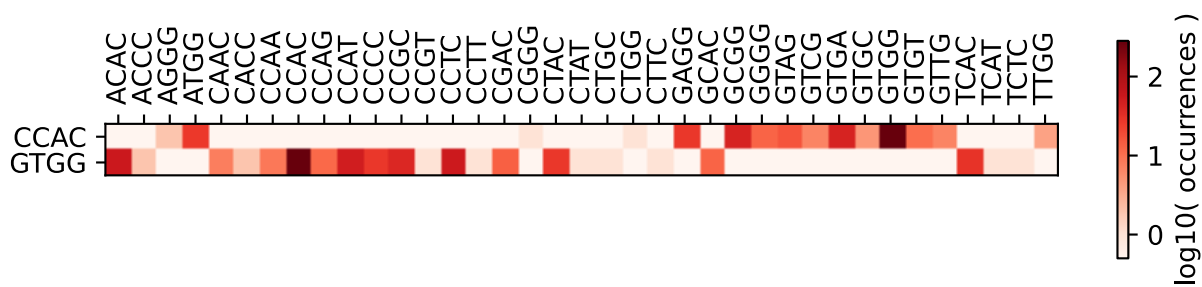
[APST][T]

V[EAVGD]

[GESWAV\*KQMPLRT]W

[GRCS][G]

Misannealing overhangs:





# ACAC

# GTGT

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

T[PLRHQ]

[IESAV\*KQGPLRT]H

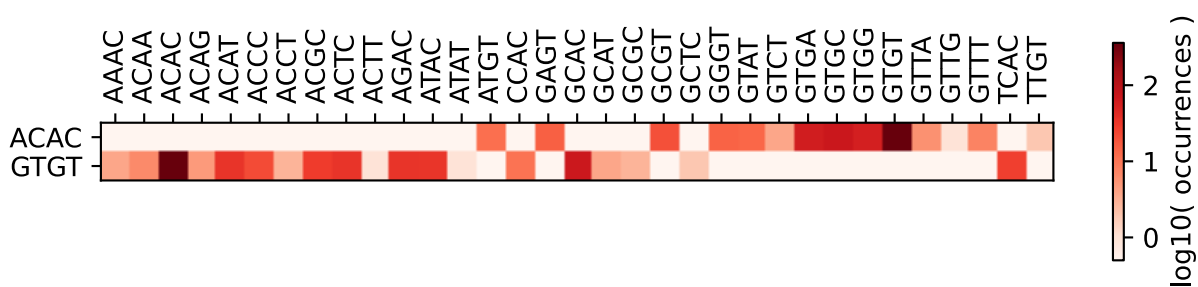
[NDYH][T]

V[SFW\*CLY]

[GESWAV\*KQMPLRT]C

[GRCS][V]

Misannealing overhangs:





# AAAC

# GTTT

GC content: 25 %.

Has 3 identical bases in a row. However, this has not shown to be very important.

Can form the following amino acids in 6 translation frames:

K[PLRHQ]

[IESAV\*KQGPLRT]N

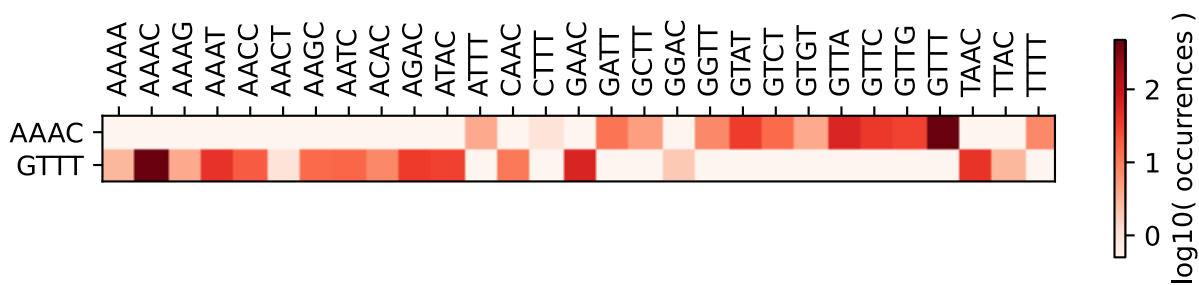
[\*EKQ][T]

V[SFW\*CLY]

[GESWAV\*KQMPLRT]F

[GRCS][FL]

Misannealing overhangs:





# GTTA

# TAAC

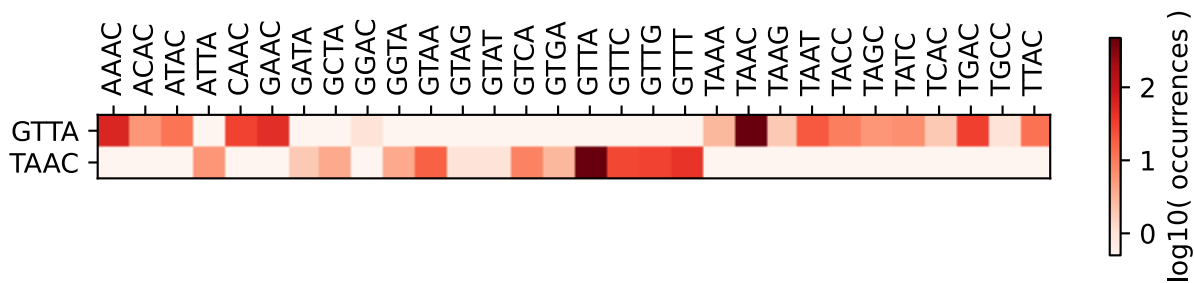
GC content: **25 %**.

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

Can form the following amino acids in 6 translation frames:

V[INSKMRT]  
[GESWAV\*KQMPLRT]L  
[GRCS][Y\*]  
\*[PLRHQ]  
[DINSFAVRGPLYHT]N  
[VIL][T]

Misannealing overhangs:





# ATTA

# TAAT

Extreme GC content: 0 %.

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

Can form the following amino acids in 6 translation frames:

I[INSKMRT]

[IESAV\*KQGPLRT]L

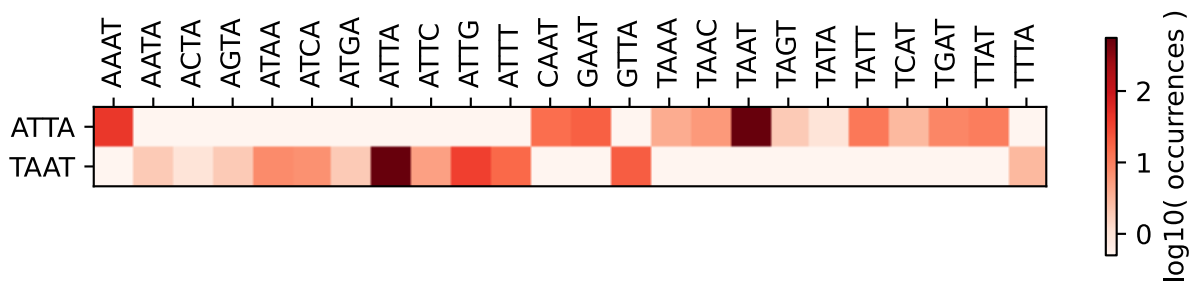
[NDYH][Y\*]

\*[SFW\*CLY]

[DINSFAVRRCGPLYHT]N

[VIL][MI]

Misannealing overhangs:







# CGTA

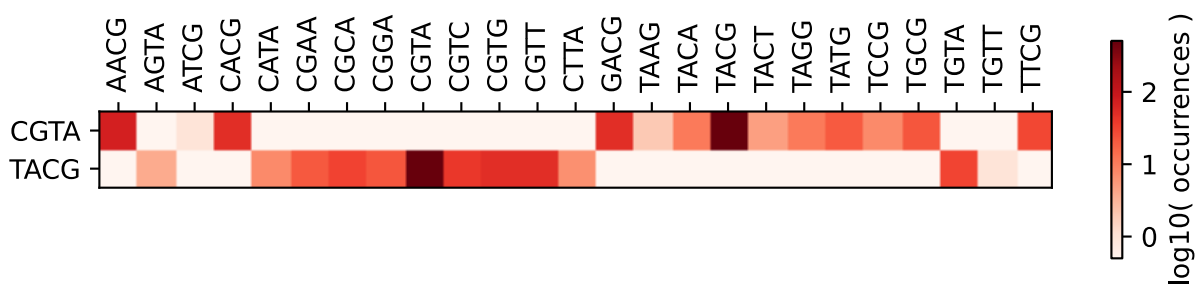
# TACG

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

R[INSMRT]  
[DINSFAVRCGPLYHT]V  
[APST][Y\*]  
Y[EAVGD]  
[DINSFAVRCGPLYHT]T  
[VIL][R]

Misannealing overhangs:





# TAGA

# TCTA

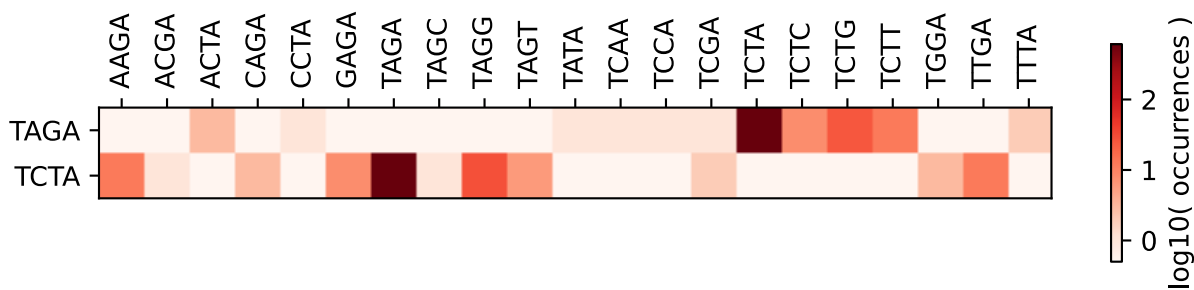
GC content: **25 %**.

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

Can form the following amino acids in 6 translation frames:

\*[INSMRT]  
[DINSFAVRCGPLYHT]R  
[VIL][ED]  
S[INSMRT]  
[DINSFAVRCGPLYHT]L  
[FVIL][Y\*]

Misannealing overhangs:





# TATA

# TATA

Extreme GC content: 0 %.

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

Can form the following amino acids in 6 translation frames:

Y[INSKMRT]

[DINSFAVRCGPLYHT]I

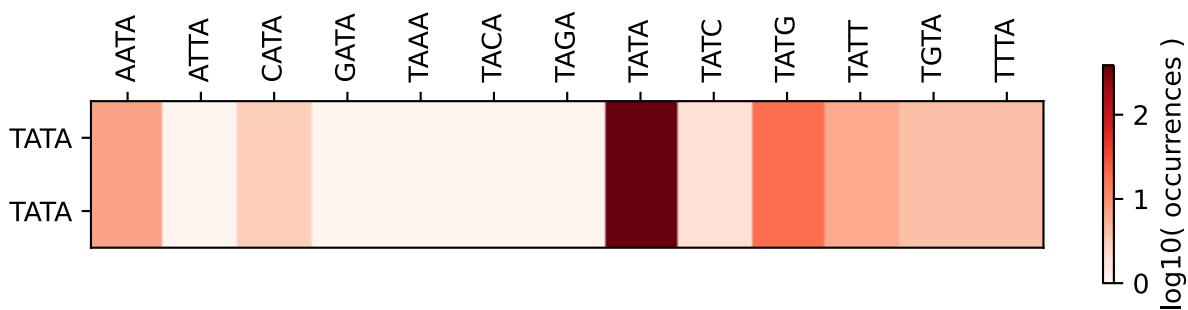
[VIL][Y\*]

Y[INSKMRT]

[DINSFAVRCGPLYHT]I

[VIL][Y\*]

Misannealing overhangs:





# GATA

# TATC

GC content: **25 %**.

Can form the following amino acids in 6 translation frames:

D[I<sup>NS</sup>KMRT]

[GESWAV\*KQMPLRT]I

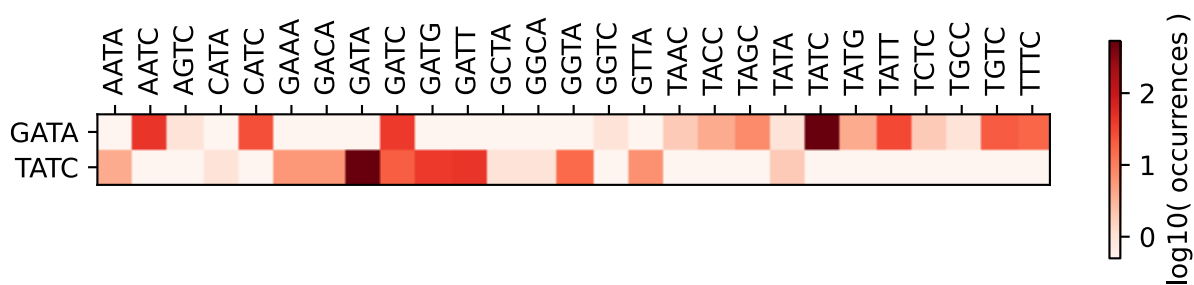
[G\*R][Y\*]

Y[PLRHQ]

[DINSFAVRCGPLYHT]I

[VIL][S]

Misannealing overhangs:





# CATA

# TATG

GC content: **25 %**.

The overhang contains the start codon ATG.

Can form the following amino acids in 6 translation frames:

H[INSKMRT]

[DINSFAVRCGPLYHT]I

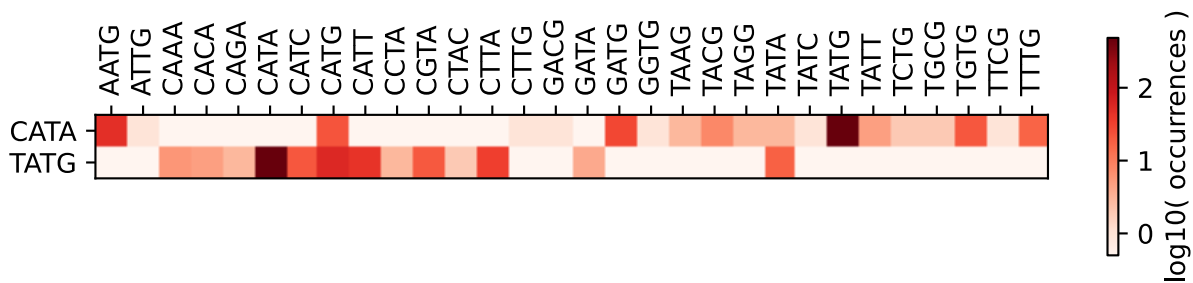
[APST][Y\*]

Y[EAVGD]

[DINSFAVRCGPLYHT]M

[VIL][W\*C]

Misannealing overhangs:





# AATA

# TATT

Extreme GC content: 0 %.

Can form the following amino acids in 6 translation frames:

N[I<sup>NS</sup>KMRT]

[I<sup>ES</sup>AV\*KQGPLRT]I

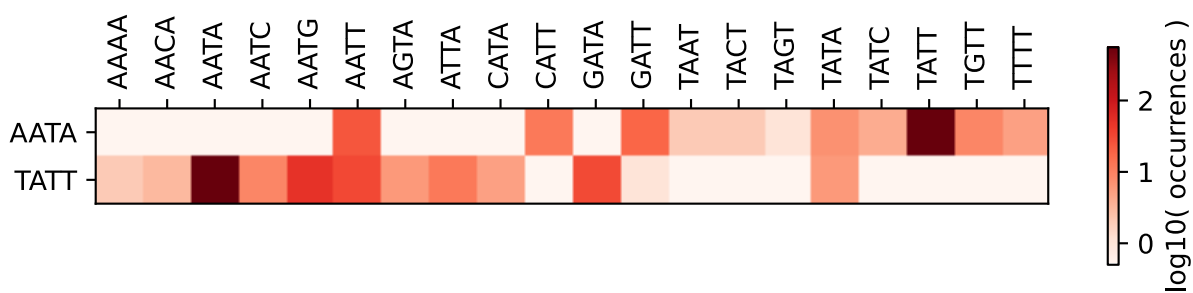
[\*EKQ][Y\*]

Y[SFW\*CLY]

[DINSFAVRCGPLYHT]I

[VIL][FL]

Misannealing overhangs:





# CTGA

# TCAG

GC content: **50 %**.

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

Can form the following amino acids in 6 translation frames:

L[INSKMRT]

[DINSFAVRCGPLYHT]\*

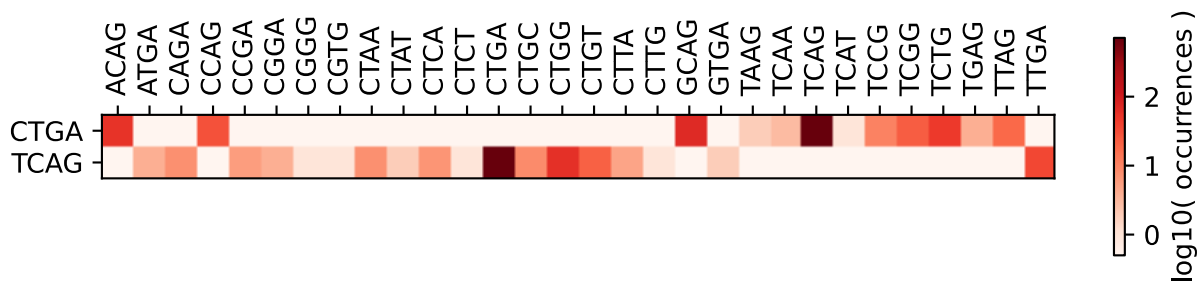
[APST][ED]

S[EAVGD]

[DINSFAVRCGPLYHT]Q

[FVIL][RS]

Misannealing overhangs:





# ATGA

# TCAT

GC content: **25 %**.

The overhang contains the start codon ATG.

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

Can form the following amino acids in 6 translation frames:

M[INSMRT]

[IESAV\*KQGPLRT]\*

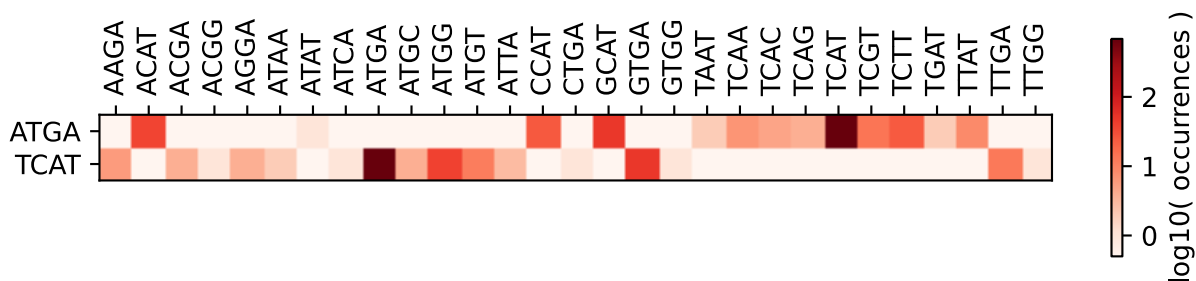
[NDYH][ED]

S[SFW\*CLY]

[DINSFAVRCGPLYHT]H

[FVIL][MI]

Misannealing overhangs:







# CGGA

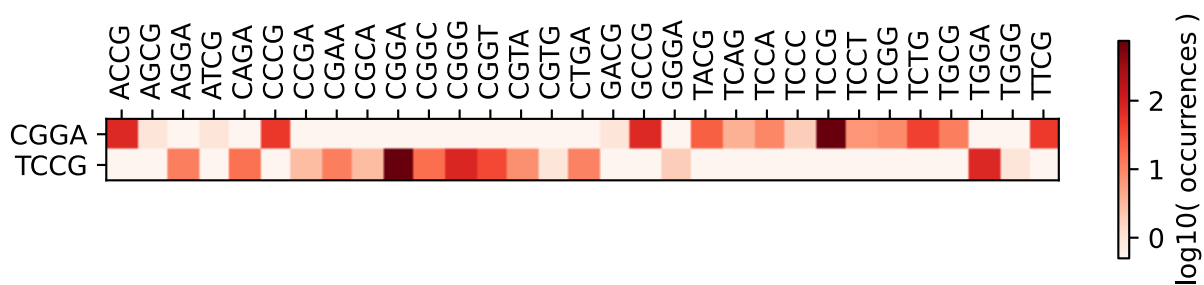
# TCCG

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

R[INSMRT]  
[DINSFAVRCGPLYHT]G  
[APST][ED]  
S[EAVGD]  
[DINSFAVRCGPLYHT]P  
[FVIL][R]

Misannealing overhangs:





# AGGA

# TCCT

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

R[INSMRT]

[IESAV\*KQGPLRT]G

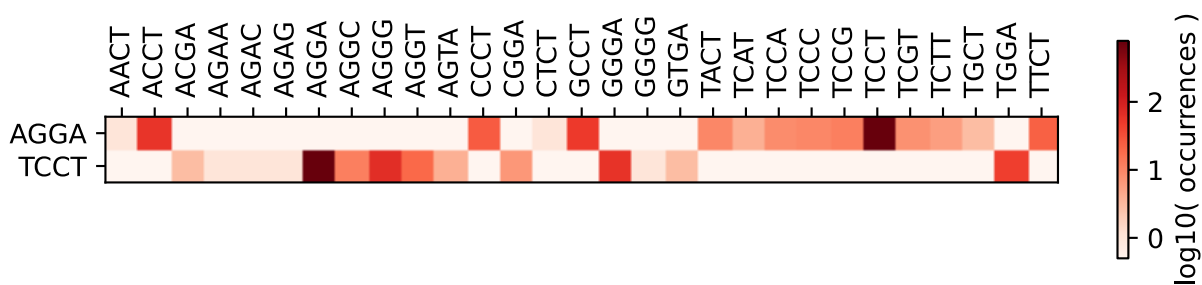
[\*EKQ][ED]

S[SFW\*CLY]

[DINSFAVRRCGPLYHT]P

[FVIL][L]

Misannealing overhangs:





# TCGA

# TCGA

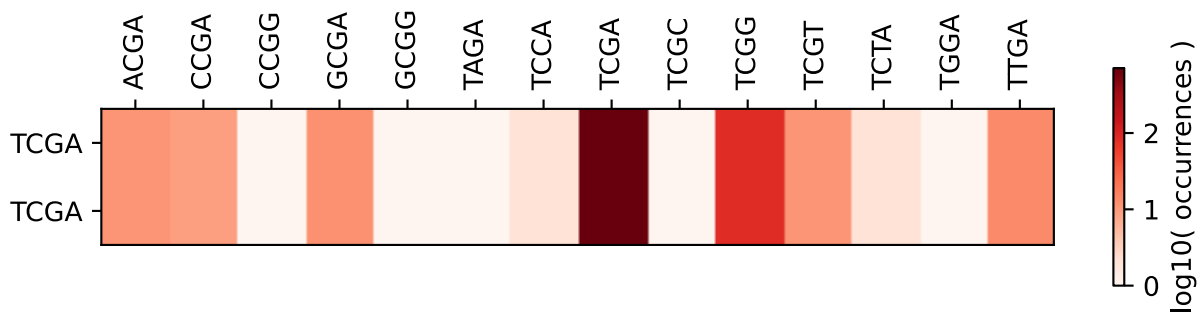
GC content: 50 %.

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

Can form the following amino acids in 6 translation frames:

S[INSKMRT]  
[DINSFAVRCGPLYHT]R  
[FVIL][ED]  
S[INSKMRT]  
[DINSFAVRCGPLYHT]R  
[FVIL][ED]

Misannealing overhangs:





# GCGA

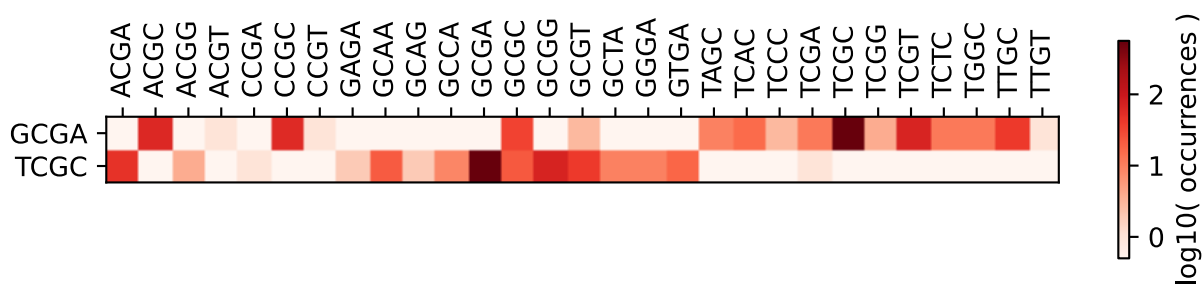
# TCGC

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

A[IINSMRT]  
[GESWAV\*KQMPLRT]R  
[GRCS][ED]  
S[PLRHQ]  
[DINSFAVRCGPLYHT]R  
[FVIL][A]

Misannealing overhangs:





# CCGA

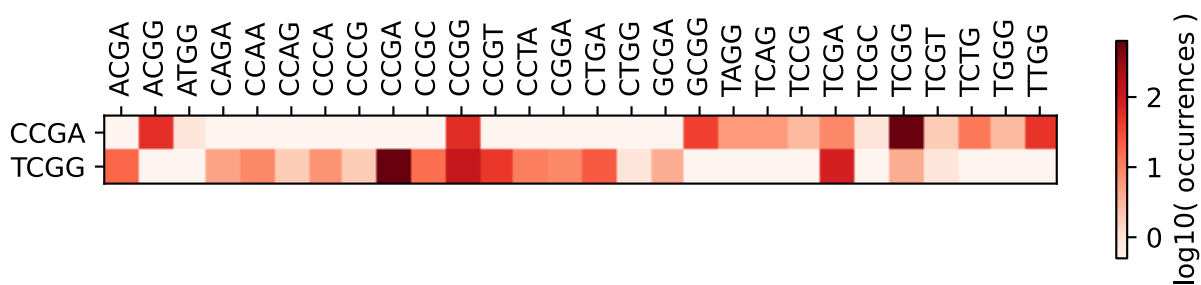
# TCGG

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

P[INSKMRT]  
[DINSFAVRCGPLYHT]R  
[APST][ED]  
S[EAVGD]  
[DINSFAVRCGPLYHT]R  
[FVIL][G]

Misannealing overhangs:





# AAGA

# TCTT

GC content: **25 %**.

Can form the following amino acids in 6 translation frames:

K[I<sup>NS</sup>KMRT]

[I<sup>ES</sup>AV\*KQGPLRT]R

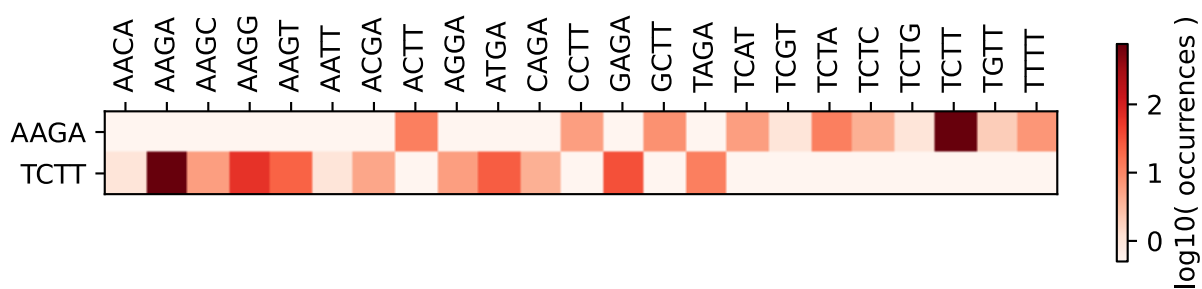
[\*EKQ][ED]

S[SFW\*CLY]

[DINSFAVRCGPLYHT]L

[FVIL][FL]

Misannealing overhangs:





# GTCA

# TGAC

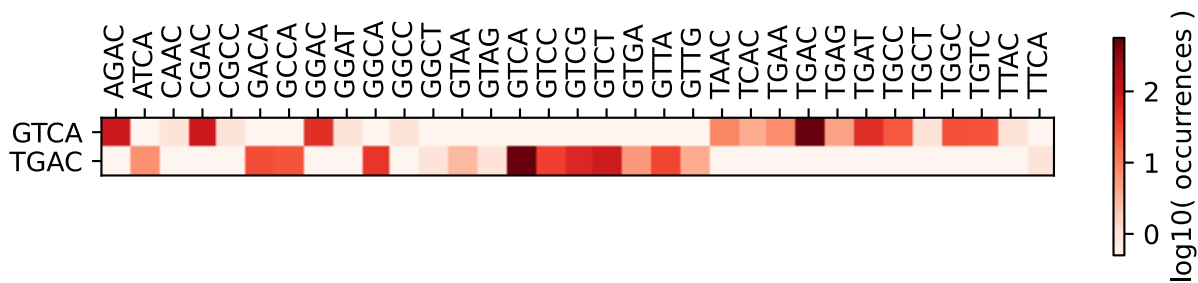
GC content: **50 %**.

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

Can form the following amino acids in 6 translation frames:

V[INSKMRT]  
[GESWAV\*KQMPLRT]S  
[GRCS][HQ]  
\*[PLRHQ]  
[DINSFAVRRCGPLYHT]D  
[MVL][T]

Misannealing overhangs:





# CTCA

# TGAG

GC content: **50 %**.

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

Can form the following amino acids in 6 translation frames:

L[INSKMRT]

[DINSFAVRCGPLYHT]S

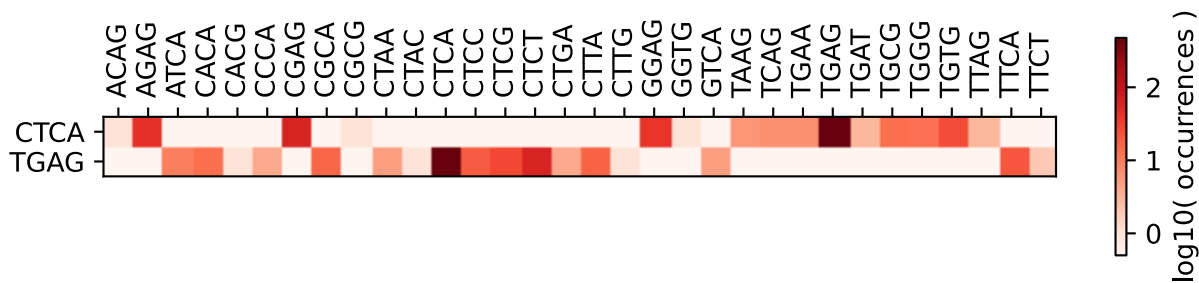
[APST][HQ]

\*[EAVGD]

[DINSFAVRCGPLYHT]E

[MVL][RS]

Misannealing overhangs:







# TGCA

# TGCA

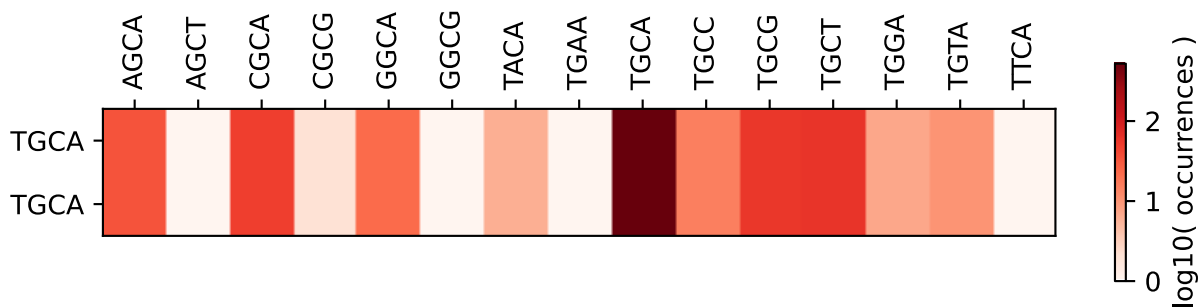
GC content: 50 %.

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

Can form the following amino acids in 6 translation frames:

C[INSKMRT]  
[DINSFAVRCGPLYHT]A  
[MVL][HQ]  
C[INSKMRT]  
[DINSFAVRCGPLYHT]A  
[MVL][HQ]

Misannealing overhangs:





# CGCA

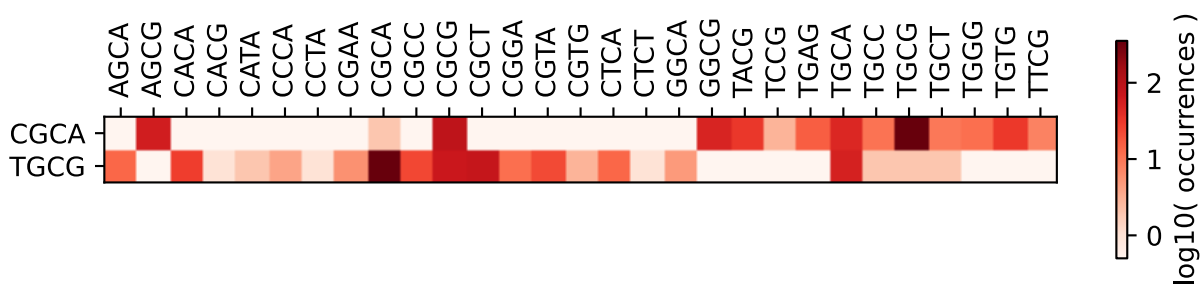
# TGCG

GC content: **75 %**.

Can form the following amino acids in 6 translation frames:

R[INSKMRT]  
[DINSFAVRCGPLYHT]A  
[APST][HQ]  
C[EAVGD]  
[DINSFAVRCGPLYHT]A  
[MVL][R]

Misannealing overhangs:





# TCCA

# TGGA

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

S[INSKMRT]

[DINSFAVRCGPLYHT]P

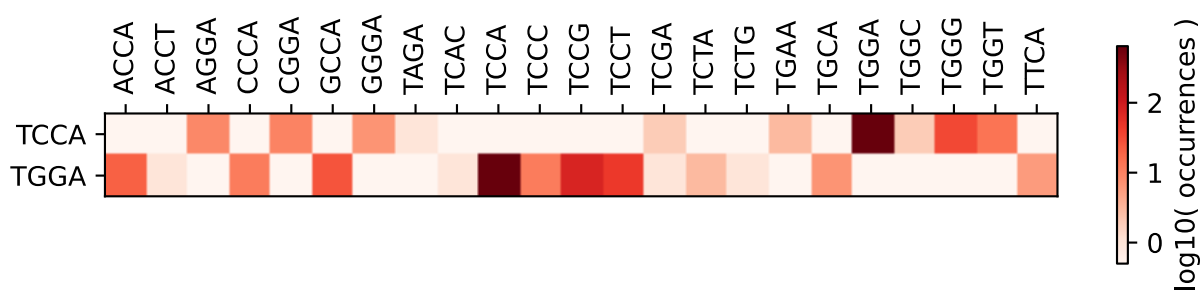
[FVIL][HQ]

W[INSKMRT]

[DINSFAVRCGPLYHT]G

[MVL][ED]

Misannealing overhangs:





# TACA

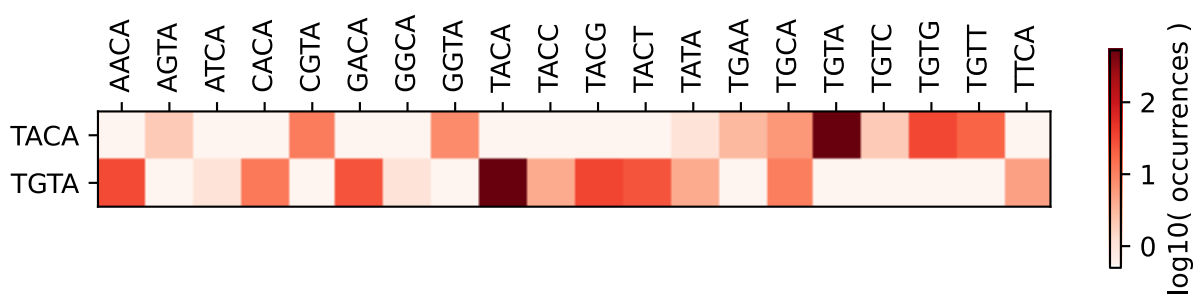
# TGTA

GC content: 25 %.

Can form the following amino acids in 6 translation frames:

Y[INSKMRT]  
[DINSFAVRCGPLYHT]T  
[VIL][HQ]  
C[INSKMRT]  
[DINSFAVRCGPLYHT]V  
[MVL][Y\*]

Misannealing overhangs:





# GACA

# TGTC

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

D[INSKMRT]

[GESWAV\*KQMPLRT]T

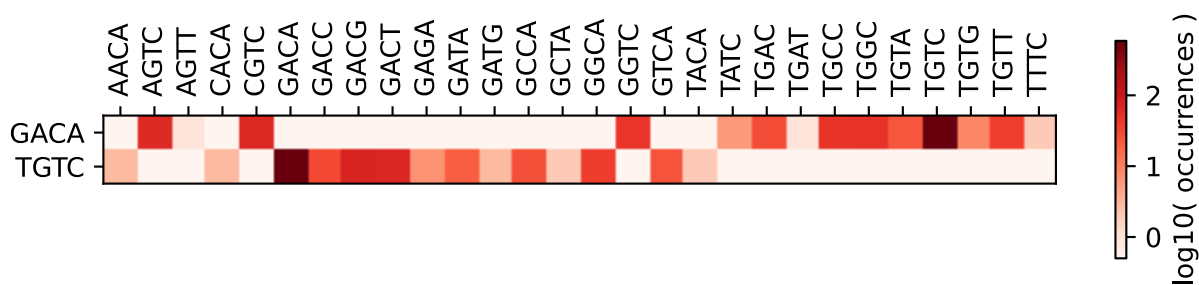
[G\*R][HQ]

C[PLRHQ]

[DINSFAVRCGPLYHT]V

[MVL][S]

Misannealing overhangs:





# AACA

# TGTT

GC content: **25 %**.

Can form the following amino acids in 6 translation frames:

N[I<sup>NS</sup>KMRT]

[I<sup>ES</sup>AV<sup>\*</sup>KQG<sup>PL</sup>RT]T

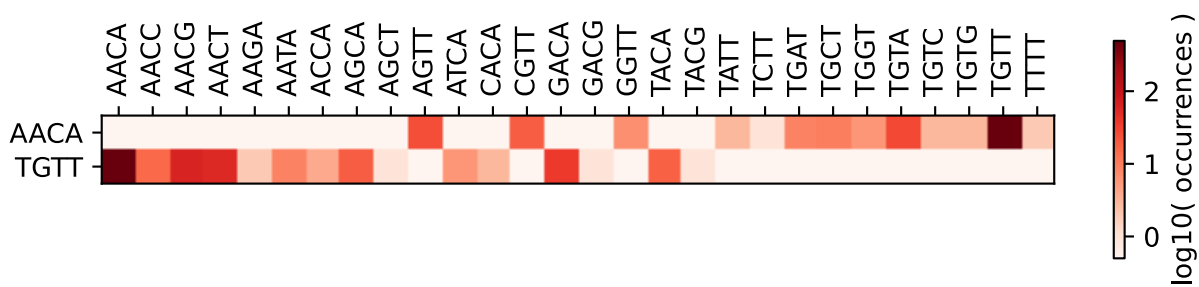
[<sup>\*</sup>EKQ][HQ]

C[SFW<sup>\*</sup>CLY]

[DINSFAVRCGPLYHT]V

[MVL][FL]

Misannealing overhangs:





# TTAA

# TTAA

Extreme GC content: 0 %.

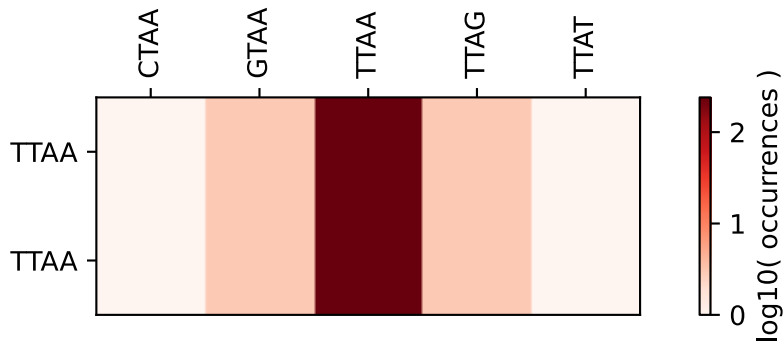
The overhang is palindromic, cannot be used for DNA assembly.

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

Can form the following amino acids in 6 translation frames:

L[INSKMRT]  
[DINSFAVRCGPLYHT]\*  
[FVIL][NK]  
L[INSKMRT]  
[DINSFAVRCGPLYHT]\*  
[FVIL][NK]

Misannealing overhangs:





# GTAA

# TTAC

GC content: 25 %.

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

Can form the following amino acids in 6 translation frames:

V[INSKMRT]

[GESWAV\*KQMPLRT]\*

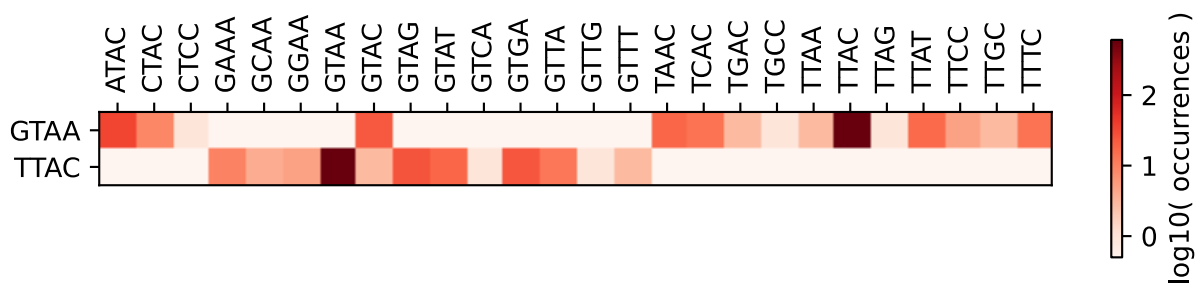
[GRCS][NK]

L[PLRHQ]

[DINSFAVRRCGPLYHT]Y

[FVIL][T]

Misannealing overhangs:







# ATAA

# TTAT

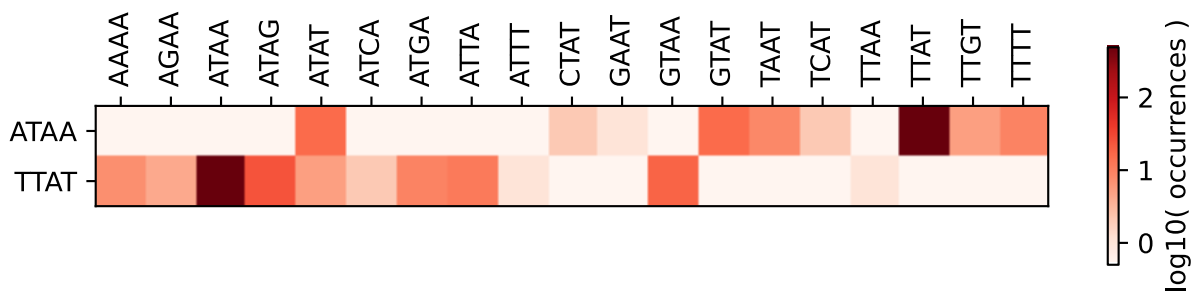
Extreme GC content: 0 %.

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

Can form the following amino acids in 6 translation frames:

I[INSKMRT]  
[IESAV\*KQGPLRT]\*  
[NDYH][NK]  
L[SFW\*CLY]  
[DINSFAVRRCGPLYHT]Y  
[FVIL][MI]

Misannealing overhangs:





# TGAA

# TTCA

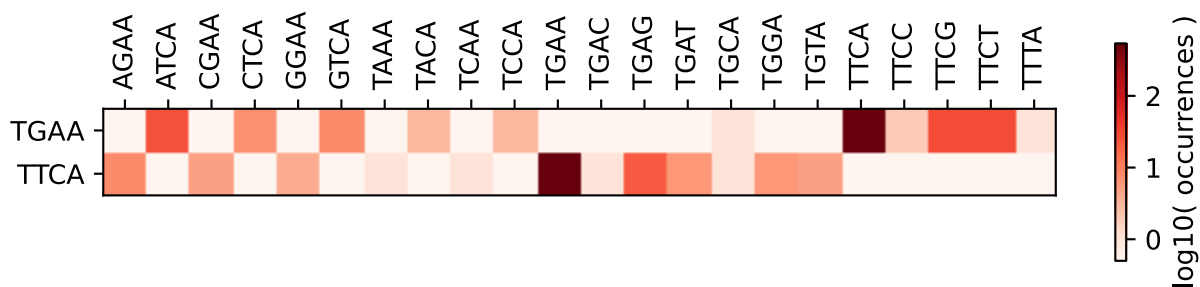
GC content: **25 %**.

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

Can form the following amino acids in 6 translation frames:

\*[INSKMRT]  
[DINSFAVRCGPLYHT]E  
[MVL][NK]  
F[INSKMRT]  
[DINSFAVRCGPLYHT]S  
[FVIL][HQ]

Misannealing overhangs:





# GGAA

# TTCC

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

G[INSKMRT]

[GESWAV\*KQMPLRT]E

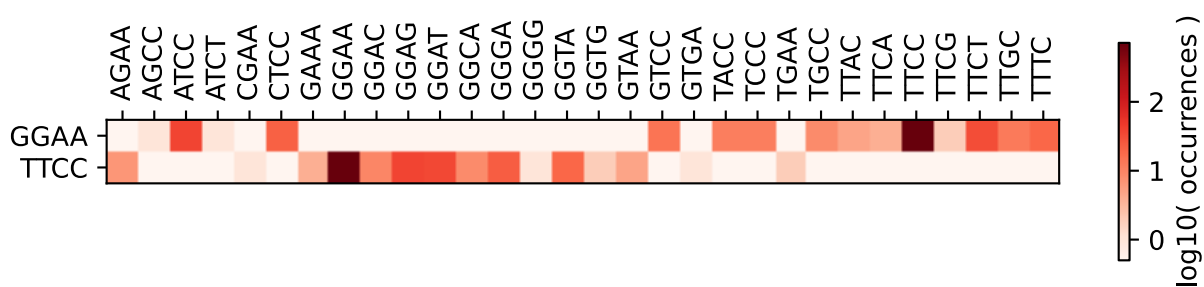
[WGR][NK]

F[PLRHQ]

[DINSFAVRCGPLYHT]S

[FVIL][P]

Misannealing overhangs:





# CGAA

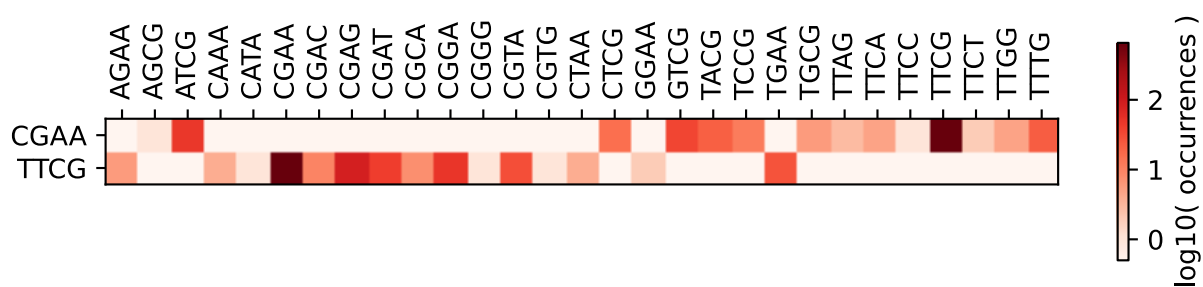
# TTCG

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

R[INSMRT]  
[DINSFAVRCGPLYHT]E  
[APST][NK]  
F[EAVGD]  
[DINSFAVRCGPLYHT]S  
[FVIL][R]

Misannealing overhangs:





# TCAA

# TTGA

GC content: 25 %.

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

Can form the following amino acids in 6 translation frames:

S[INSKMRT]

[DINSFAVRRCGPLYHT]Q

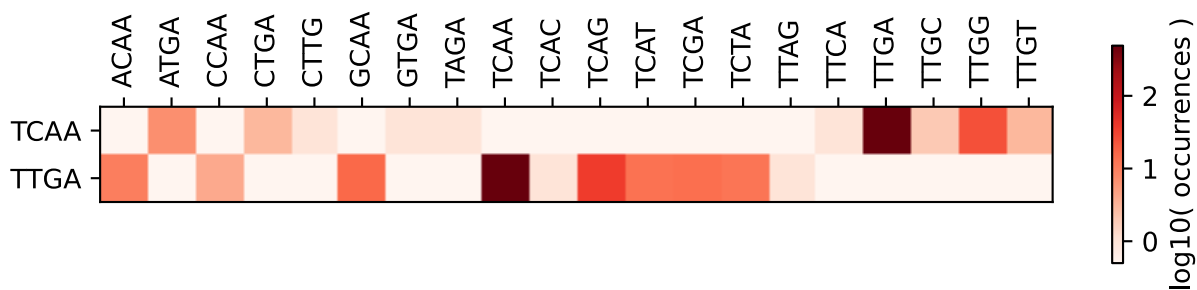
[FVIL][NK]

L[INSKMRT]

[DINSFAVRRCGPLYHT]\*

[FVIL][ED]

Misannealing overhangs:





# GCAA

# TTGC

GC content: **50 %**.

Can form the following amino acids in 6 translation frames:

A[I<sup>1</sup>NSKMRT]

[GESWAV\*KQMPLRT]Q

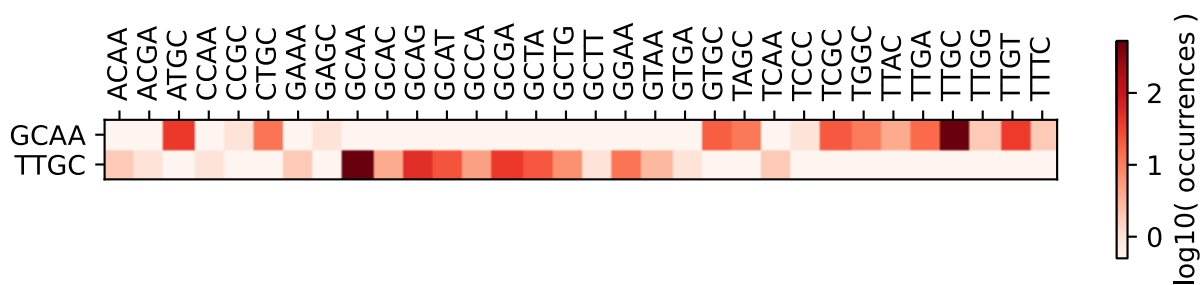
[GRCS][NK]

L[PLRHQ]

[DINSFAVRCGPLYHT]C

[FVIL][A]

Misannealing overhangs:





# TAAA

# TTTA

Extreme GC content: 0 %.

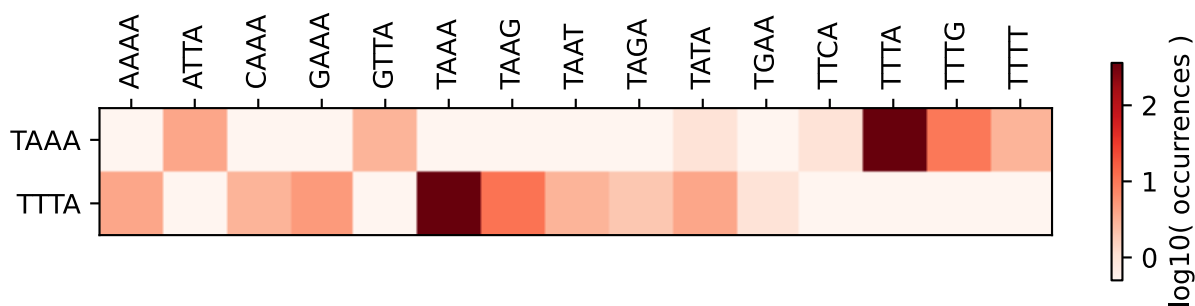
The overhang contains a stop codon (TAA, TAG or TGA).

Has 3 identical bases in a row. However, this has not shown to be very important.

Can form the following amino acids in 6 translation frames:

\*[INSKMRT]  
[DINSFAVRCGPLYHT]K  
[VIL][NK]  
F[INSKMRT]  
[DINSFAVRCGPLYHT]L  
[FVIL][Y\*]

Misannealing overhangs:





# GAAA

# TTTC

GC content: 25 %.

Has 3 identical bases in a row. However, this has not shown to be very important.

Can form the following amino acids in 6 translation frames:

E[INSKMRT]

[GESWAV\*KQMPLRT]K

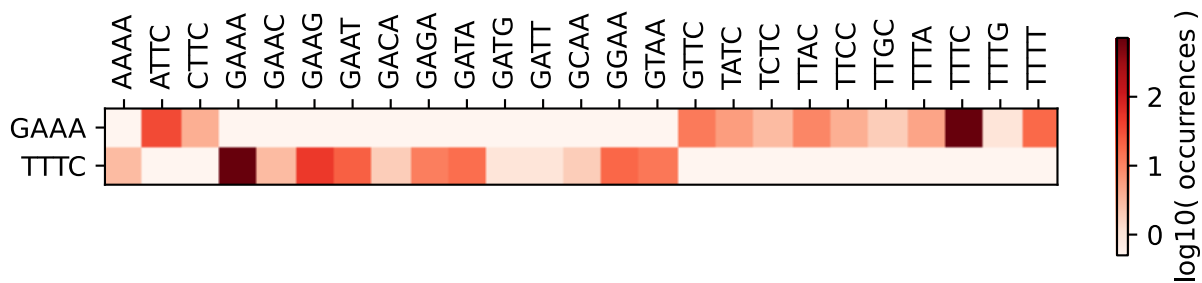
[G\*R][NK]

F[PLRHQ]

[DINSFAVRCGPLYHT]F

[FVIL][S]

Misannealing overhangs:







AAAA

TTTT

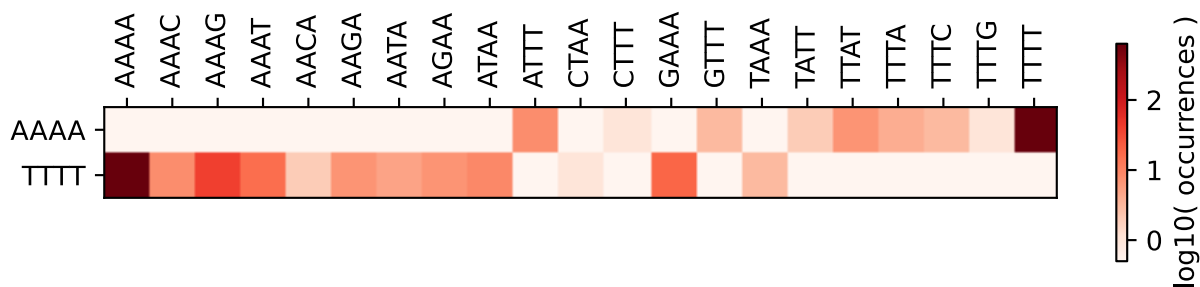
Extreme GC content: 0 %.

Has 3 identical bases in a row. However, this has not shown to be very important.

Can form the following amino acids in 6 translation frames:

K[INSKMRT]  
[IESAV\*KQGRLRT]K  
[\*EKQ][NK]  
F[SFW\*CLY]  
[DINSFAVRRCGLYHT]F  
[FVIL][FL]

Misannealing overhangs:



# Appendix

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

## Summary page(s)

The first page summarises the compendium.

## Overhang pages

Each overhang is 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.