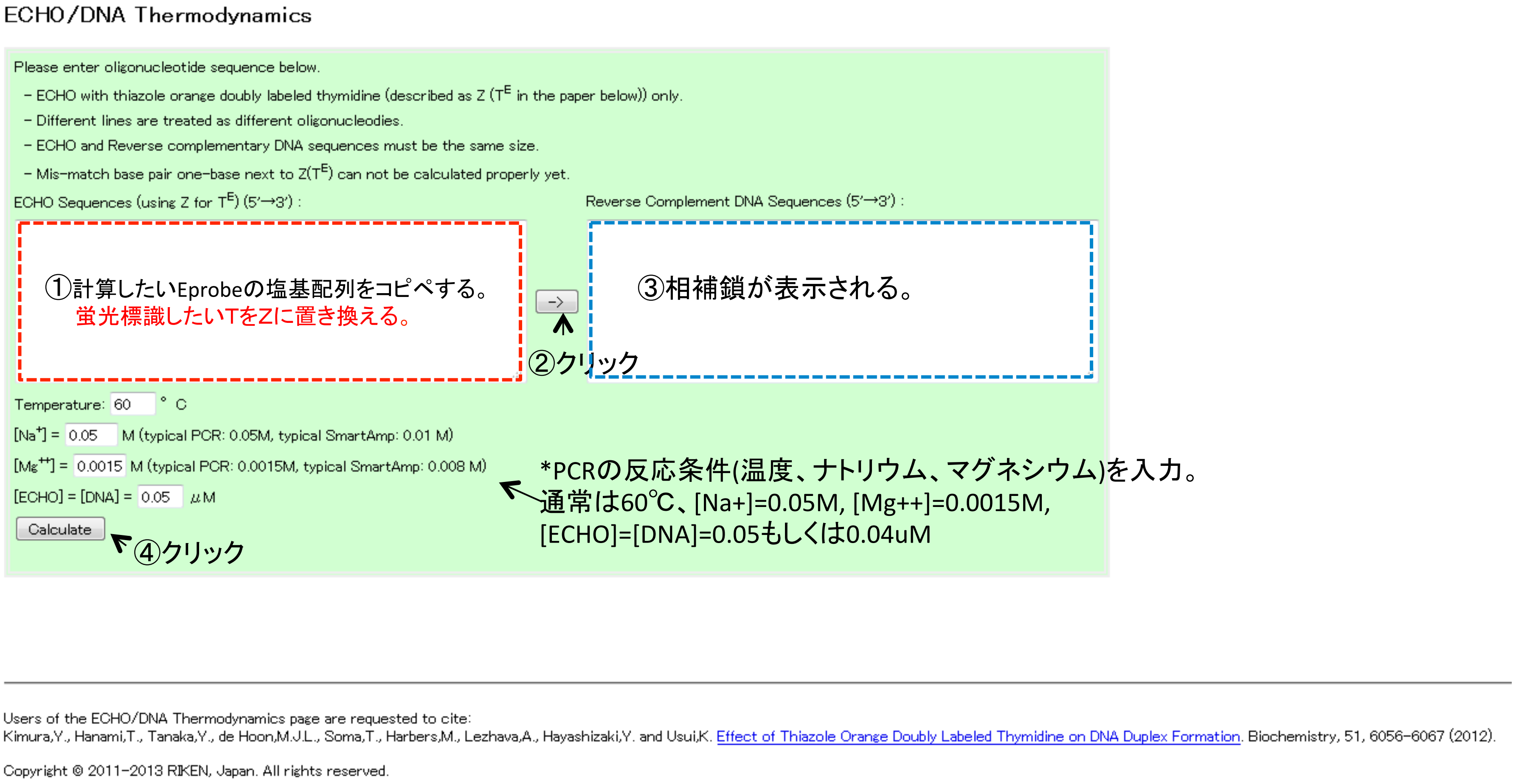
ECHO DNA Thermodynamics (https://www.dnaform.com/devel/echo/thermodynamics)にアクセスする。



ECHO/DNA Thermodynamics

Please enter oligonucleotide sequence below.

- ECHO with thiazole orange doubly labeled thymidine (described as Z (T^E in the paper below)) only.
- Different lines are treated as different oligonucleodies.
- ECHO and Reverse complementary DNA sequences must be the same size.
- Mis-match base pair one-base next to Z(T^E) can not be calculated properly yet.

ECHO Sequences (using Z for T^E) (5'→3') :

①計算したいEprobeの塩基配列をコピペする。
蛍光標識したいTをZに置き換える。

Reverse Complement DNA Sequences (5'→3') :

③相補鎖が表示される。

Temperature: 60 ° C

[Na$^+$] = 0.05 M (typical PCR: 0.05M, typical SmartAmp: 0.01 M)

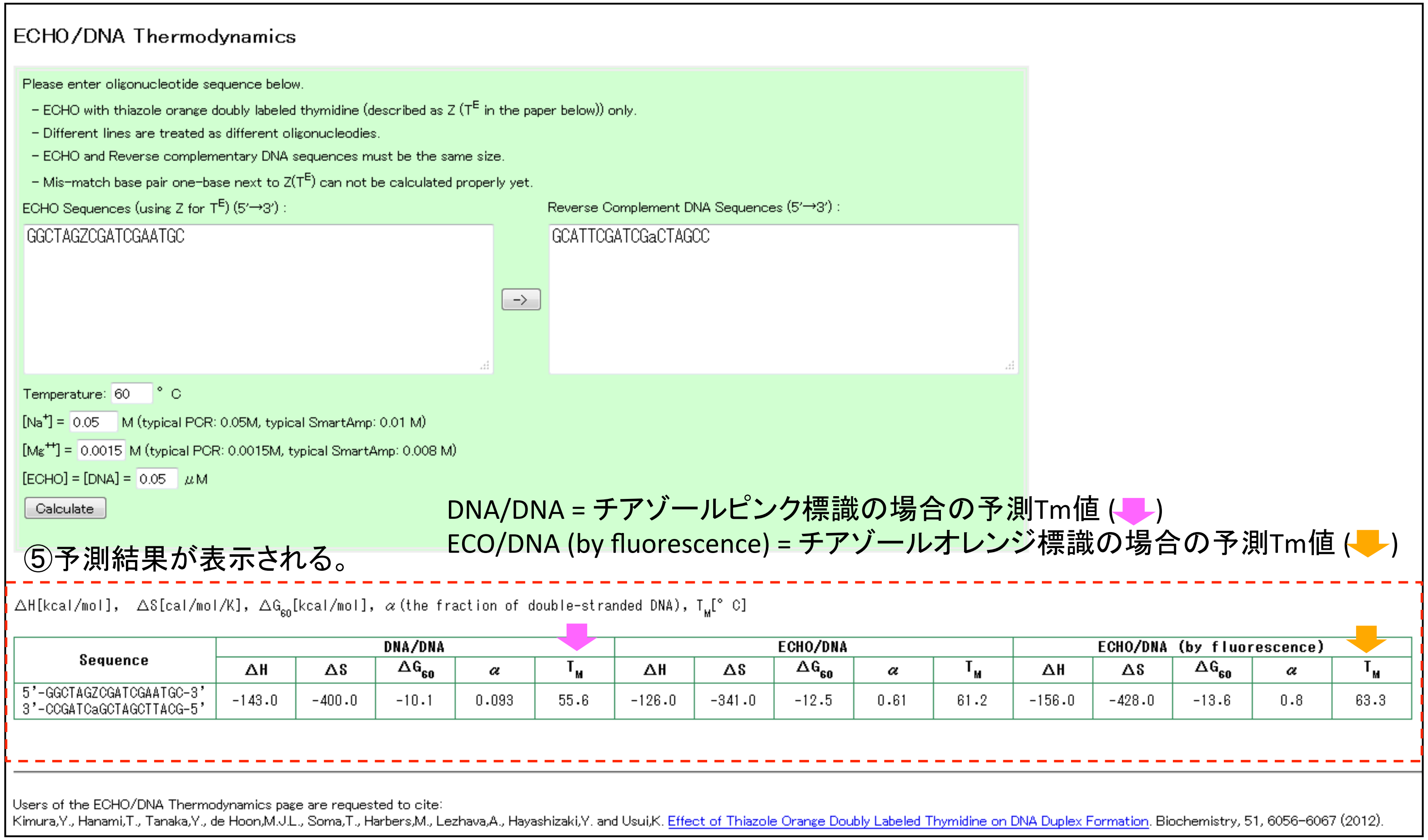
[Mg^{++}] = 0.0015 M (typical PCR: 0.0015M, typical SmartAmp: 0.008 M)

[ECHO] = [DNA] = 0.05 µM

*PCRの反応条件(温度、ナトリウム、マグネシウム)を入力。
通常は60℃、[Na+]=0.05M, [Mg++]=0.0015M,
[ECHO]=[DNA]=0.05もしくは0.04uM

Users of the ECHO/DNA Thermodynamics page are requested to cite:
Kimura,Y., Hanami,T., Tanaka,Y., de Hoon,M.J.L., Soma,T., Harbers,M., Lezhava,A., Hayashizaki,Y. and Usui,K. Effect of Thiazole Orange Doubly Labeled Thymidine on DNA Duplex Formation. Biochemistry, 51, 6056-6067 (2012).

Tm計算例（5’-GGCTAGZCGATCGAATGC-3’のTm予測）



ECHO/DNA Thermodynamics

Please enter oligonucleotide sequence below.

- ECHO with thiazole orange doubly labeled thymidine (described as Z (T^E in the paper below)) only.
- Different lines are treated as different oligonucleodies.
- ECHO and Reverse complementary DNA sequences must be the same size.
- Mis-match base pair one-base next to Z(T^E) can not be calculated properly yet.

ECHO Sequences (using Z for T^E) (5’→3’) :

```
GGCTAGZCGATCGAATGC
```

Reverse Complement DNA Sequences (5’→3’) :

```
GCATTCGATCGaCTAGCC
```

Temperature: 60 ° C

$[Na^+]$ = 0.05 M (typical PCR: 0.05M, typical SmartAmp: 0.01 M)

$[Mg^{++}]$ = 0.0015 M (typical PCR: 0.0015M, typical SmartAmp: 0.008 M)

[ECHO] = [DNA] = 0.05 μM

DNA/DNA = チアゾールピンク標識の場合の予測Tm値 (⬇)

ECO/DNA (by fluorescence) = チアゾールオレンジ標識の場合の予測Tm値 (⬇)

⑤予測結果が表示される。

ΔH[kcal/mol], ΔS[cal/mol/K], ΔG_{60}[kcal/mol], α (the fraction of double-stranded DNA), T_M[° C]

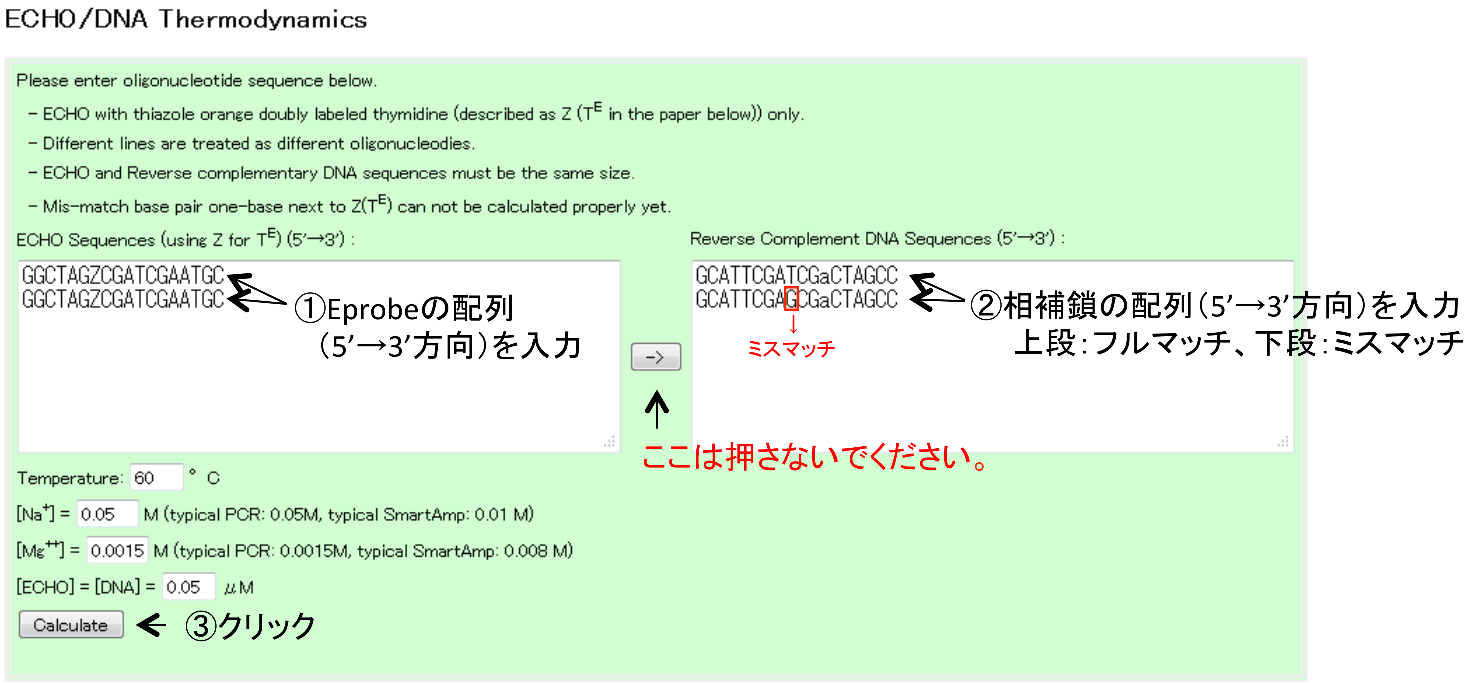
Sequence	DNA/DNA					ECHO/DNA					ECHO/DNA (by fluorescence)				
	ΔH	ΔS	ΔG_{60}	α	T_M ⬇	ΔH	ΔS	ΔG_{60}	α	T_M	ΔH	ΔS	ΔG_{60}	α	T_M ⬇
5’-GGCTAGZCGATCGAATGC-3’ 3’-CCGATCaGCTAGCTTACG-5’	-143.0	-400.0	-10.1	0.093	55.6	-126.0	-341.0	-12.5	0.61	61.2	-156.0	-428.0	-13.6	0.8	63.3

Users of the ECHO/DNA Thermodynamics page are requested to cite:
Kimura,Y., Hanami,T., Tanaka,Y., de Hoon,M.J.L., Soma,T., Harbers,M., Lezhava,A., Hayashizaki,Y. and Usui,K. Effect of Thiazole Orange Doubly Labeled Thymidine on DNA Duplex Formation. Biochemistry, 51, 6056-6067 (2012).

フルマッチとミスマッチのTm値予測例

フルマッチ(上側がEprobe配列、下側が相補鎖)
5’ GGCTAGZCGATCGAATGC 3’
3’ CCGATCaGCTAGCTTACG 5’

ミスマッチ(上側がEprobe配列、下側がミスマッチを含む相補鎖)
5’ GGCTAGZCGATCGAATGC 3’
3’ CCGATCaGCGAGCTTACG 5’



ECHO/DNA Thermodynamics

Please enter oligonucleotide sequence below.

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- Different lines are treated as different oligonucleodies.
- ECHO and Reverse complementary DNA sequences must be the same size.
- Mis-match base pair one-base next to Z(T^E) can not be calculated properly yet.

ECHO Sequences (using Z for T^E) (5’→3’) :

```
GGCTAGZCGATCGAATGC
GGCTAGZCGATCGAATGC
```

①Eprobeの配列
（5’→3’方向）を入力

Reverse Complement DNA Sequences (5’→3’) :

```
GCATTCGATCGaCTAGCC
GCATTCGAGCGaCTAGCC
```

②相補鎖の配列（5’→3’方向）を入力
上段：フルマッチ、下段：ミスマッチ

ミスマッチ

ここは押さないでください。

Temperature: 60 ° C

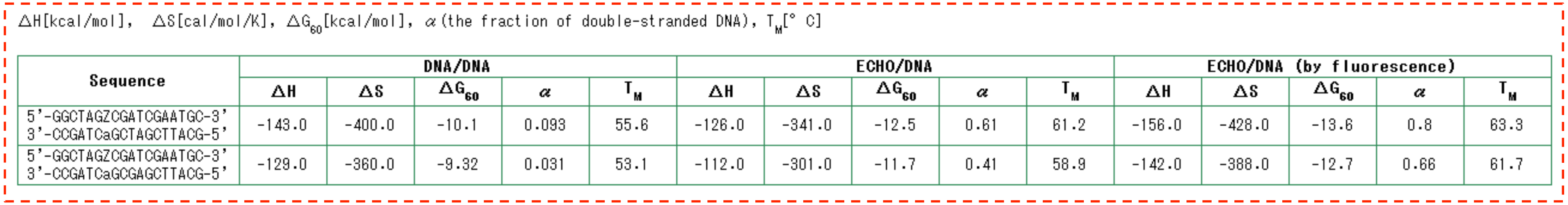
[Na⁺] = 0.05 M (typical PCR: 0.05M, typical SmartAmp: 0.01 M)

[Mg⁺⁺] = 0.0015 M (typical PCR: 0.0015M, typical SmartAmp: 0.008 M)

[ECHO] = [DNA] = 0.05 μM

③クリック

④予測結果が表示される（上段：フルマッチ、下段：ミスマッチ）。



ΔH[kcal/mol], ΔS[cal/mol/K], ΔG_{60}[kcal/mol], α (the fraction of double-stranded DNA), T_M[° C]

Sequence	DNA/DNA					ECHO/DNA					ECHO/DNA (by fluorescence)				
	ΔH	ΔS	ΔG_{60}	α	T_M	ΔH	ΔS	ΔG_{60}	α	T_M	ΔH	ΔS	ΔG_{60}	α	T_M
5’-GGCTAGZCGATCGAATGC-3’ 3’-CCGATCaGCTAGCTTACG-5’	-143.0	-400.0	-10.1	0.093	55.6	-126.0	-341.0	-12.5	0.61	61.2	-156.0	-428.0	-13.6	0.8	63.3
5’-GGCTAGZCGATCGAATGC-3’ 3’-CCGATCaGCGAGCTTACG-5’	-129.0	-360.0	-9.32	0.031	53.1	-112.0	-301.0	-11.7	0.41	58.9	-142.0	-388.0	-12.7	0.66	61.7

Users of the ECHO/DNA Thermodynamics page are requested to cite:
Kimura,Y., Hanami,T., Tanaka,Y., de Hoon,M.J.L., Soma,T., Harbers,M., Lezhava,A., Hayashizaki,Y. and Usui,K. Effect of Thiazole Orange Doubly Labeled Thymidine on DNA Duplex Formation. Biochemistry, 51, 6056-6067 (2012).