

* Inoculate Lib2, Plate 3, 4, 5, 6

* Inoculate Lib2, Plate 2 IPTG (+)ve clones for
MIC ← Plasmid isolation.

21/4/15

① MIC on Amp :- ② inoculate rest of Lib2. Plate 2.

For # 1 5 96 well plates

20ml per Plate (150 μ l each well.)

100ml

200 ml. LB + 200 μ l Kan. + IPTG Final 1mM

→ Take 20ml of above. 100v = 1 x 200 ml
= 2 ml.

↓

add 40 μ l of 166 μ g/ μ l Amp

$$40 \mu\text{l} \times 166 = 6640 \mu\text{g in } 20\text{ml}$$
$$= 332 \mu\text{g/ml Amp.}$$

MIC on Amp

Plate 1; WT, A1, A3, A5, A8, A9, A11, A12

2: WT, B2, B3, B4, B5, B6, B7, B8

3: WT, B11, B12, C1, C2, C3, C4, C7

4: WT, C10, C11, C12, D1, D3, D4, D5

5: WT, D7, D10, E1, E5, E6, E6A Repeat

Lib2

Plate 2

* Reinoculate Lib2, Plate 2 IPTG (+)ve clones for MIC values. for fresh culture.
- MIC on Cephalothin.

Lib2, Plate 2: MIC on Cephalothin.

- #1 WT, A1, A3, A5, A8, A9, A11, A12
- #2 WT, B2, B3, B4, B5, B6, B7, B8.
- #3 WT, B11, B12, C1, C2, C3, C4, C7,
- #4 WT, C10, C11, C12, D1, D3, D4, D5.
- #5 WT, D7, D10, E1, E5, E6, E5, E6

LB Media: 5 Plates x 96 well x 150 μ l each well
= 72 ml.

Make it 120 ml.

① 120 ml LB + 120 μ l Kanamycin
+ 1 mM IPTG final
(1.2 ml IPTG of 1000 mM) $1000 \text{ mM} \times V = 1 \times 120$
 $V = \frac{120}{1000} = 1.2$
ml

② Take 20 ml from #1
Add:- 240 μ l of 200 μ g/ml Cephalothin.

$$= 240 \times 200 = 48000 \text{ } \mu\text{g} / 20 \text{ ml}$$
$$= 2400 \text{ } \mu\text{g} / \text{ml}$$

Serial dilute. ~~star~~

Lib2, Plate 2; MIC on Ceftezidime.

2/11/15

WT, A1, A3, A5, A8, A9, A11, A12

WT, B2, B3, B4, B5, B6, B7, B8

WT, ~~B1~~ B11, B12, C1, C2, C3, C4, C7

WT, C10, C11, C12, D1, D3, D4, D5

WT, D7, D10, E1, E5, E6, E5, E6.

120ml LB media + 120 μ l Kan.

+ 1.2 ml IPTG \Rightarrow 1mM Final
(of 100mM)

Take 20 ml of #1

add 100 μ l of 5 μ g/ μ l Ceftezidime

$$= 500 \mu\text{g} / 20 \text{ml}$$

$$= 25 \mu\text{g} / \text{ml} \text{ Final } \checkmark$$

then serial dilute it.

Lib2, Plate 2:-

2/16/15

E7, E8, E11, E12, F3, F4, F7
F11, G1, G2, G5, G8, G9, G11
H1, H3, H4, H10, H11, WT,

2/17/15

Plate 1; WT, E7, E8, E11, E12, F3, F4, F7
2: WT, F11, G1, G2, G5, G8, G9, G11
3: WT, H3, H4, H10, H11, C4, ~~WT~~, ~~WT~~
WT, H1, H3, H4, H10, H11, C4, WT
~~4~~ 50ml / set.

①* 200 ml LB + 200 μ l Kan.

② add $100 \text{ mM} \times V = 1 \times 200 \text{ ml}$
 $V = 2 \text{ ml IPTG (of } 100 \text{ mM)}$
(1 mM final)

③ 10 ml of #2

(3a) add 20 μ l Amp. (= 332 μ g/ml)

(3b) add 120 μ l cephalothin (= 2400 μ g/ml)

(3c) add 50 μ l ceftazidime (= 25 μ g/ml)

Exp done 2/4/15
Result 2/5/15

Amp

Lib2, Plate 2
#1

Antibiotic:		Date: 2/5/15											
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	+	+	+	+	+	+	+	+	+	+	+
A1	B	-	-	+	+	+	+	+	+	+	+	+	+
A3	C	-	-	-	-	-	+	+	+	+	+	+	+
A5	D	-	-	-	-	-	+	+	+	+	+	+	+
A8	E	-	-	-	-	-	+	+	+	+	+	+	+
A9	F	-	-	-	-	-	+	+	+	+	+	+	+
A11	G	-	-	-	-	-	+	+	+	+	+	+	+
A12	H	-	-	-	+	+	+	+	+	+	+	+	+

Amp $\mu\text{g/ml}$
332 166 83 41.5 20.7 10.3 5.1 2.5 1.2 0.6 0.3 0.16

Antibiotic:		Date:											
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	+	+	+	+	+	+	+	+	+	+	+
B2	B	-	-	-	+	+	+	+	+	+	+	+	+
B3	C	-	-	-	-	-	+	+	+	+	+	+	+
B4	D	-	-	-	-	-	+	+	+	+	+	+	+
B5	E	-	-	-	-	-	+	+	+	+	+	+	+
B6	F	-	-	-	-	-	+	+	+	+	+	+	+
B7	G	-	-	-	-	-	+	+	+	+	+	+	+
B8	H	-	-	-	-	-	+	+	+	+	+	+	+

Amp $\mu\text{g/ml}$
332 166 83 41.5 20.7 10.3 5.1 2.5 1.29 0.64 0.32 0.16

#2

Antibiotic:		Date:											
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	+	+	+	+	+	+	+	+	+	+	+
B11	B	-	+	+	+	+	+	+	+	+	+	+	+
B12	C	-	-	-	-	-	+	+	+	+	+	+	+
C1	D	-	-	-	+	+	+	+	+	+	+	+	+
C2	E	-	-	-	-	-	+	+	+	+	+	+	+
C3	F	-	-	-	-	-	+	+	+	+	+	+	+
C4	G	+/	+	+	+	+	+	+	+	+	+	+	+
C7	H	-	-	-	-	-	+	+	+	+	+	+	+

*

#3

Lib2, Plate 2
MIC - Amp.

Exp date 2/4/15
 Result 2/5/15

Amp

Antibiotic:		Date: 8/5/15											
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	+	+	+	+	+	+	+	+	+	+	+	+
C10	B	-	-	-	-	+	+	+	+	+	+	+	+
C11	C	-	-	-	-	+	+	+	+	+	+	+	+
C12	D	-	-	-	-	+	+	+	+	+	+	+	+
D1	E	-	-	-	-	+	+	+	+	+	+	+	+
D3	F	-	-	-	-	+	+	+	+	+	+	+	+
D4	G	-	-	-	-	+	+	+	+	+	+	+	+
D5	H	-	-	-	-	+	+	+	+	+	+	+	+

Lib2, Plate 2

#4

Amp
 µg/ml
 332 166 83 41.5 20.7 10.3 5.1 2.5 1.2 0.6 0.3 .16

Antibiotic:		Date:											
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	+	+	+	+	+	+	+	+	+	+	+
D7	B	-	-	-	-	+	+	+	+	+	+	+	+
D10	C	-	-	-	-	+	+	+	+	+	+	+	+
E1	D	-	-	-	-	+	+	+	+	+	+	+	+
E5	E	-	-	-	+	+	+	+	+	+	+	+	+
E6	F	-	-	-	-	+	+	+	+	+	+	+	+
E5	G	-	-	-	+	+	+	+	+	+	+	+	+
E6	H	-	-	-	-	+	+	+	+	+	+	+	+

#5

Antibiotic:		Date:											
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
	A												
	B												
	C												
	D												
	E												
	F												
	G												
	H												

#6

Lib2, Plate 2
 mFC - Amp

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Cephalothin

~~2/11/15~~ 2/11/15

Lib2, Plate 2

A1
A3
A5
A8
A9
A11
A12

Antibiotic:		Date:											
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	+	+	+	+	+	+	+	+	+	+	+
A1	B	-	-	-	-	-	-	+	+	+	+	+	+
A3	C	-	-	-	-	-	-	-	-	-	-	-	+
A5	D	-	-	-	-	-	-	-	-	-	-	-	+
A8	E	-	-	-	-	-	-	-	-	-	-	-	+
A9	F	-	-	-	-	-	-	-	-	-	-	+	+
A11	G	-	-	-	-	-	-	-	-	-	-	+	+
A12	H	-	-	-	-	-	-	+	+	+	+	+	+

#1

μg/ml
2400 1200 600 300 150 75 37.5 18.7 9.3 4.6 2.3 1.1

Antibiotic:		Date:											
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	+	+	+	+	+	+	+	+	+	+	+
B2	B	-	-	-	-	-	-	+	+	+	+	+	+
B3	C	-	-	-	-	-	-	-	-	+	+	+	+
B4	D	-	-	-	-	-	-	-	-	-	+	+	+
B5	E	-	-	-	-	-	-	-	-	-	-	-	+
B6	F	-	-	-	-	-	-	-	-	-	+	+	+
B7	G	-	-	-	-	-	-	-	-	+	+	+	+
B8	H	-	-	-	-	-	-	-	-	-	-	-	+

#2

2400 1200 600 300 150 75 37.5 18.7 9.3 4.6 2.3 1.1

Antibiotic:		Date:											
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	+	+	+	+	+	+	+	+	+	+	+
B11	B	-	-	-	-	+	+	+	+	+	+	+	+
B12	C	-	-	-	-	-	-	-	-	-	-	+	+
C1	D	-	-	-	-	-	-	-	+	+	+	+	+
C2	E	-	-	-	-	-	-	-	-	-	-	+	+
C3	F	-	-	-	-	-	-	-	-	-	-	+	+
C4	G	-	-	-	+	+	+	+	+	+	+	+	+
C7	H	-	-	-	-	-	-	-	-	-	-	-	+

#3

*

Cephalothin

2/11/15

Lib2, Plate 2

Antibiotic:		Date:											
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	+	+	+	+	+	+	+	+	+	+	+
C10	B	-	-	-	-	-	-	-	+	+	+	+	+
C11	C	↓	↓	↓	↓	↓	↓	↓	-	-	-	+	+
C12	D	↓	↓	↓	↓	↓	↓	↓	↓	↓	-	+	+
D1	E	↓	↓	↓	↓	↓	↓	↓	↓	↓	-	+	+
D3	F	↓	↓	↓	↓	↓	↓	↓	↓	↓	+	+	+
D4	G	↓	↓	↓	↓	↓	↓	↓	↓	↓	-	-	+
D5	H	↓	↓	↓	↓	↓	↓	↓	↓	↓	-	-	+

#4

2400 120 600 300 150 75 37.5 18.7 9.3 4.6 2.3 1.1

Antibiotic:		Date:											
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	+	+	+	+	+	+	+	+	+	+	+
D7	B	-	-	-	-	-	-	-	-	-	+	+	+
D10	C	↓	↓	↓	↓	↓	↓	↓	-	-	-	-	+
E1	D	↓	↓	↓	↓	↓	↓	↓	-	-	-	+	+
E5	E	↓	↓	↓	↓	↓	↓	↓	+	+	+	+	+
E6	F	↓	↓	↓	↓	↓	↓	↓	-	-	-	-	+
E5	G	↓	↓	↓	↓	↓	↓	↓	+	+	+	+	+
E6	H	↓	↓	↓	↓	↓	↓	↓	-	-	-	-	+

#5

Optical center

Antibiotic:		Date:											
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
	A												
	B												
	C												
	D												
	E												
	F												
	G												
	H												

Ceftazidime

Exp 2/11/15

Result 2/12/15

Antibiotic:		Date:											
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	-	-	+	+	+	+	+	+	+	+	+
A1	B	-	-	-	-	-	-	+	+	+	+	+	+
A3	C	↓	↓	↓	↓	↓	↓	↓	↓	↓	-	+	+
A5	D	↓	↓	↓	↓	↓	↓	↓	↓	↓	-	+	+
A8	E	↓	↓	↓	↓	↓	↓	↓	↓	↓	-	+	+
A9	F	↓	↓	↓	↓	↓	↓	↓	↓	↓	-	+	+
A11	G	↓	↓	↓	↓	↓	↓	↓	↓	↓	-	+	+
A12	H	↓	↓	↓	↓	↓	↓	↓	↓	↓	+	+	+

Lib2, Plat2

#1

95 12.5 6.25 3.1 1.5 .78 .39 .19 .09 .04 .02 .012

Antibiotic:		Date:											
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	-	-	+	+	+	+	+	+	+	+	+
B2	B	↓	↓	↓	-	-	-	-	-	-	-	+	+
B3	C	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	+	+
B4	D	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	+	+
B5	E	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	+	+
B6	F	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	+	+
B7	G	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	+	+
B8	H	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	+	+

#2

95 12.5 6.25 3.1 1.5 .78 .39 .19 .09 > .04 .02 .012

Antibiotic:		Date:											
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	-	-	+	+	+	+	+	+	+	+	+
B11	B	↓	↓	↓	-	-	-	-	-	-	+	+	+
B12	C	↓	↓	↓	↓	↓	↓	↓	↓	↓	-	+	+
C1	D	↓	↓	↓	↓	↓	↓	↓	↓	↓	-	+	+
C2	E	↓	↓	↓	↓	↓	↓	↓	↓	↓	-	+	+
C3	F	↓	↓	↓	↓	↓	↓	↓	↓	↓	-	+	+
C4	G	+	-	+	+	+	+	+	+	+	+	+	+
C7	H	-	-	-	-	-	-	-	-	-	-	-	+

#3

→ Report

#

Ceftazidime

2/12/15

		Antibiotic:		Date:									
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	-	±	+	+	+	+	+	+	+	+	+
C10	B	-	-	-	-	-	-	-	-	-	-	+	+
C11	C	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	+	+
C12	D	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	+	+
D1	E	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	+	+
D3	F	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	+	+
D4	G	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	+	+
D5	H	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	-	+

25 12.5 6.25 3.12 1.56 .78 .39 .19 .097 .048 .02 .01

		Antibiotic:		Date:									
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	-	±	+	+	+	+	+	+	+	+	+
D7	B	↓	↓	-	-	-	-	-	-	-	+	+	+
D10	C	↓	↓	↓	↓	↓	↓	↓	↓	↓	-	+	+
E1	D	↓	↓	↓	↓	↓	↓	↓	↓	↓	-	+	+
E5	E	↓	↓	↓	↓	↓	↓	↓	↓	↓	+	+	+
E6	F	↓	↓	↓	↓	↓	↓	↓	↓	↓	-	+	+
E5	G	↓	↓	↓	↓	↓	↓	↓	↓	↓	+	+	+
E6	H	↓	↓	↓	↓	↓	↓	↓	↓	↓	-	-	+

		Antibiotic:		Date:									
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
	A												
	B												
	C												
	D												
	E												
	F												
	G												
	H												

Paq

Amp.

Lib2, Plate 2

Exp 2/17/15
Result 2/18/15

Antibiotic:		Date:											
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	+	+	+	+	+	+	+	+	+	+	+
E7	B	-	-	-	-	-	-	+	+	+	+	+	+
E8	C	-	-	-	-	-	-	+	+	+	+	+	+
* E11	D	+	+	+	+	+	+	+	+	+	+	+	+
E12	E	-	-	-	-	-	+	+	+	+	+	+	+
F3	F	-	-	-	-	-	-	+	+	+	+	+	+
F4	G						+	+	+	+	+	+	+
F7	H	+	-	+	+	+	+	+	+	+	+	+	+

$\mu\text{g/ml}$
 332 166 83 41.5 20.7 10.3 5.1 2.5 1.2 .6 .3 .16

* # 1

Antibiotic:		Date:											
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	* -	+	+	+	+	+	+	+	+	+	+	+
F11	B	-	-	-	-	-	-	+	+	+	+	+	+
G1	C	↓	↓	↓	↓	↓	-	+	+	+	+	+	+
G2	D	↓	↓	↓	↓	↓	+	+	+	+	+	+	+
G5	E	↓	↓	↓	↓	↓	-	+	+	+	+	+	+
G8	F	↓	↓	↓	↓	↓	-	+	+	+	+	+	+
G9	G	↓	↓	↓	↓	↓	-	+	+	+	+	+	+
G11	H	↓	↓	↓	↓	↓	-	+	+	+	+	+	+

$\mu\text{g/ml}$
 332 166 83 41.5 20.7 10.3 5.1 2.5 1.2 .6 .3 .16

2

Antibiotic:		Date:											
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	+	+	+	+	+	+	+	+	+	+	+
H1	B	-	-	-	-	-	-	+	+	+	+	+	+
H3	C	↓	↓	↓	↓	↓	+	+	+	+	+	+	+
H4	D	↓	↓	↓	↓	↓	-	+	+	+	+	+	+
H10	E	↓	↓	↓	↓	↓	-	+	+	+	+	+	+
H11	F	↓	↓	↓	↓	↓	-	+	+	+	+	+	+
C4	G	+/-	+	+	+	+	+	+	+	+	+	+	+
WT	H	-	+	+	+	+	+	+	+	+	+	+	+

3

E11, C4

Cephalothin

Lib2, Plat2

		Antibiotic:						Date:					
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	+	+	+	+	+	+	+	+	+	+	+
E7	B	-	-	-	-	-	-	-	-	-	-	+	+
E8	C	-	-	-	-	-	-	-	-	-	-	+	+
E11	D	-	+	+	+	+	+	+	+	+	+	+	+
E12	E	-	-	-	-	-	-	-	-	-	+	+	+
F3	F	-	-	-	-	-	-	-	-	-	+	+	+
F4	G	-	-	-	-	-	-	-	-	-	-	+	+
F7	H	-	-	-	-	-	-	-	+	+	+	+	+

$\mu\text{g/ml}$
 2400 1200 600 300 150 75 37.5 18.7 9.3 4.6 2.3 1.1

		Antibiotic:						Date:					
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	+	+	+	+	+	+	+	+	+	+	+
F11	B	-	-	-	-	-	-	-	-	-	+	+	+
G1	C	↓	↓	↓	↓	↓	↓	↓	↓	+	+	+	+
G2	D	↓	↓	↓	↓	↓	↓	↓	↓	-	+	+	+
G5	E	↓	↓	↓	↓	↓	↓	↓	↓	↓	+	+	+
G8	F	↓	↓	↓	↓	↓	↓	↓	↓	↓	+	+	+
G9	G	↓	↓	↓	↓	↓	↓	↓	↓	↓	+	+	+
G11	H	↓	↓	↓	↓	↓	↓	↓	↓	↓	+	+	+

$\mu\text{g/ml}$
 2400 1200 600 300 150 75 37.5 18.7 9.3 4.6 2.3 1.1

		Antibiotic:						Date:					
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	+	+	+	+	+	+	+	+	+	+	+
H1	B	↓	-	-	-	-	-	-	-	-	+	+	+
H3	C	↓	↓	↓	↓	↓	↓	↓	-	-	+	+	+
H4	D	↓	↓	↓	↓	↓	↓	↓	-	-	+	+	+
H10	E	↓	↓	↓	↓	↓	↓	↓	-	+	+	+	+
H11	F	↓	↓	↓	↓	↓	↓	↓	-	-	+	+	+
C4	G	-	-	-	+	+	+	+	+	+	+	+	+
WT	H	⊖	+	+	+	+	+	+	+	+	+	+	+

Ceftazidime.

Lib2, Plate 2

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		Antibiotic:						Date:					
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	-	-	+	+	+	+	+	+	+	+	+
E7	B	-	-	-	-	-	-	-	-	-	-	+	+
E8	C	-	-	-	-	-	-	-	-	-	-	+	+
E11	D	-	-	-	+	+	+	+	+	+	+	+	+
E12	E	-	-	-	-	-	-	-	-	-	-	+	+
F3	F	-	-	-	-	-	-	-	-	-	-	+	+
F4	G	-	-	-	-	-	-	-	-	-	-	+	+
F7	H	-	-	-	-	-	+	+	+	+	+	+	+

µg/ml
 25 12.5 6.25 3.1 1.5 .78 .39 .19 .09 .04 .02 .01

		Antibiotic:						Date:					
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	-	-	+	+	+	+	+	+	+	+	+
F11	B	-	-	-	-	-	-	-	-	-	-	+	+
G1	C	-	-	-	-	-	-	-	-	-	-	+	+
G2	D	-	-	-	-	-	-	-	-	-	-	+	+
G5	E	-	-	-	-	-	-	-	-	-	-	+	+
G8	F	-	-	-	-	-	-	-	-	-	-	+	+
G9	G	-	-	-	-	-	-	-	-	-	-	+	+
G11	H	-	-	-	-	-	-	-	-	-	-	+	+

µg/ml
 25 12.5 6.25 3.1 1.5 .78 .39 .19 .09 .04 .02 .01

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		Antibiotic:						Date:					
Clone	Lane	1	2	3	4	5	6	7	8	9	10	11	12
WT	A	-	-	-	+	+	+	+	+	+	+	+	+
H1	B	-	-	-	-	-	-	-	-	-	-	+	+
H3	C	-	-	-	-	-	-	-	-	-	-	+	+
H4	D	-	-	-	-	-	-	-	-	-	-	+	+
H10	E	-	-	-	-	-	-	-	-	-	-	+	+
H11	F	-	-	-	-	-	-	-	-	-	-	+	+
C4	G	+	+	+	+	+	+	+	+	+	+	+	+
WT	H	-	-	-	+	+	+	+	+	+	+	+	+