

MinimalMarker on web Manual

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1. Overview of MinimalMarker on web

- This site provides MinimalMarker analysis on the web. MinimalMarker is a computer program that detects marker set(s) with the smallest number of markers to identify all cultivars from polymorphism data obtained by applying co-dominant markers to many cultivars.
- Target organisms are diploids only. The algorithm is based on perl script.

2. Data available for analysis on MinimalMarker on web

1. Target organisms are diploids only.
2. Genotypes of codominant and dominant markers can be analyzed.
3. The genotype should be written in ASCII characters.
4. Examples of genotype notations are as follows. String type 'AB', numeric type "120/200" like SSR, and dominant marker '+'.
5. MinimalMarker on web requires input files in CSV or xls/xlsx.
6. The first term of the data should be 'Variety' in the first line, and the name of the cultivars must be written in the first line. After the second line, the names of markers must be written in the first column and following marker genotypes. Refer to the sample input format in this manual for more information.
7. Please see the sample data linked from the top page as an example input file.

3. Notes on input data preparation

A) String order, B) Case insensitive, C) Dominant marker

- A) String order: MinimalMarker on web judges genotypes 'AB' and 'BA' as different genotypes. However, it does not distinguish between 'AB' and 'BA' if the option "-g" described below is turned ON. Generally, we do not distinguish between 'AB' and 'BA', so the default is -g ON.
- B) Case sensitivity: MinimalMarker on the web is not case sensitive. However, to avoid unexpected errors, it is preferable to use one or the other.
- C) Dominant marker : The genotypes of dominant markers can be analyzed. However, a caution must be taken. In this case, MinimalMarker on web cannot detect whether all genotypes are matched among cultivars. Therefore, this verification must be done by the user.

4. Example of input data format co-dominant marker: string type

CSV

```
Variety,Akatsuki,Yuuzora,Saotome,Yoshihim
e,Masahime,Akizora,Natsutome,Gyousei,K
ouyou Hakutou,Ookubo
M1a,AB,BA,BB,AB,AA,AA,AB,BA,AA,AB
M4c,BE,EB,BD,CE,EC,AE,CE,BE,CE,BC
M6a,AC,CA,BC,AC,CA,AC,CA,AC,CD,CD
M12a,AB,BB,AB,AB,AB,AB,AB,AB,AB,AB
M15a,CC,CC,BC,CC,CC,AC,CC,CC,CC,CC
MA006b,AA,AA,AB,AA,AA,AA,AA,AA,AA,AA
MA007a,AC,CA,AA,CC,CD,CC,CA,AC,BB,AC
MA013a,AC,AC,BD,AC,AC,AB,AC,AC,AC,AC
MA014a,DD,DD,AD,CD,DD,DC,CD,DD,BD,CD
MA015a,AA,AD,BC,AA,AD,AC,AA,AA,AD,AC
```

xls/xlsx

Be sure to fill in 'Variety'. Recognize the starting position of the data

Cultivar name

Variety	Hara	Yoshiwara	Kanbara	Yui	Okitsu	Ejiri	Fuchu	Mariko	Okabe	Fujieda
M1	AB	AB	BB	AB	AA	AA	AB	AB	AA	AB
M2	BE	BE	BD	CE	CE	AE	CE	BE	CE	BC
M3	AC	AC	BC	AC	AC	AC	AC	AC	CD	CD
M4	AB	BB	AB	AB	AB	AB	AB	AB	AB	AB
M5	CC	CC	BC	CC	CC	AC	CC	CC	CC	CC
M6	AA	AA	AB	AA	AA	AA	AA	AA	AA	AA
M7	AC	AC	AA	CC	CD	CC	AC	AC	BB	AC
M8	AC	AC	BD	AC	AC	AB	AC	AC	AC	AC
M9	DD	DD	AD	CD	DD	CD	CD	DD	BD	CD
MX	AA	AD	BC	AA	AD	AC	AA	AA	AD	AC

Marker name

Genotype

Please order the data alphabetically and use either upper- or lower-case letters to avoid unexpected errors. **Also, please remove spaces in the data.**

5. Example of input data format co-dominant marker: numerical type

CSV

```
Variety,Akatsuki,Yuuzora,Saotome,Yoshihime,Masahime,Akizora,Natsutome
,Gyosei,Kouyou Hakutou,Ookubo

M1a,80/84,80/84,84/84,80/84,80/80,80/80,80/84,80/84,80/80,80/84

M4c,78/94,78/94,78/88,80/94,80/94,74/94,80/94,78/94,80/94,78/80

M6a,193/197,193/197,195/197,193/197,193/197,193/197,193/197,193/197,193/197
,197/201,197/201

M12a,177/195,195/195,177/195,177/195,177/195,177/195,177/195,177/195,177/19
5,177/195,177/195

M15a,136/136,136/136,132/136,136/136,136/136,116/136,136/136,136/13
6,136/136,136/136

MA006b,295/295,295/295,295/301,295/295,295/295,295/295,295/295,295/
295,295/295,295/295

MA007a,111/133,111/133,111/111,133/133,133/135,133/133,111/133,111/
133,121/121,111/133

MA013a,197/213,197/213,211/227,197/213,197/213,197/211,197/213,197/
213,197/213,197/213

MA014a,167/167,167/167,150/167,163/167,167/167,163/167,163/167,167/
167,160/167,163/167

MA015a,178/178,178/263,180/185,178/178,178/263,178/185,178/178,178/
178,178/263,178/185
```

xls/xlsx

Be sure to fill in 'Variety'. Recognize the starting position of the data

Cultivar name

Variety	Hara	Yoshiwara	Kanbara	Yui	Okitsu	Ejiri	Fuchu	Mariko	Okabe	Fujieda
M1	80/84	80/84	84/84	80/84	80/80	80/80	80/84	80/84	80/80	80/84
M2	78/94	78/94	78/88	80/94	80/94	74/94	80/94	78/94	80/94	78/80
M3	193/197	193/197	195/197	193/197	193/197	193/197	193/197	193/197	197/201	197/201
M4	177/195	195/195	177/195	177/195	177/195	177/195	177/195	177/195	177/195	177/195
M5	136/136	136/136	132/136	136/136	136/136	116/136	136/136	136/136	136/136	136/136
M6	295/295	295/295	295/301	295/295	295/295	295/295	295/295	295/295	295/295	295/295
M7	111/133	111/133	111/111	133/133	133/135	133/133	111/133	111/133	121/121	111/133
M8	197/213	197/213	211/227	197/213	197/213	197/211	197/213	197/213	197/213	197/213
M9	167/167	167/167	150/167	163/167	167/167	163/167	163/167	167/167	160/167	163/167
MX	178/178	178/263	180/185	178/178	178/263	178/185	178/178	178/178	178/263	178/185

Marker name

Genotype

For numeric type data such as SSR, genotypes should be separated by '/'. In addition, numbers should be listed in ascending order to avoid unexpected errors. Also, please remove spaces in the data.

6. Example of input data format dominant marker

CSV

Variety,Akatsuki,Yuuzora,Saotome,Yoshihime,Masahime,Akizora,Natsutome,Gyousei,Kouyou Hakutou,Ookubo

M1a,+,-,+,,+,+,+,+,-,+,+

M4c,-,+,-,+,,+,+,+,+,-,-

M6a,+,,+,-,+,,+,+,+,+,+,+

M12a,+,-,+,,+,+,+,+,+,+,+

M15a,+,,+,-,+,,+,+,+,+,+,+

MA006b,+,,+,+,+,+,+,+,+,+,+

MA007a,+,,+,+,+,+,-,+,,+,-,+

MA013a,+,,+,-,+,,+,+,+,+,+,+

MA014a,-,-,+,,+,-,-,+,-,-,+

MA015a,+,,+,-,+,,+,+,+,-,+,,+

xls/xlsx

Be sure to fill in 'Variety'. Recognize the starting position of the data

Cultivar name

Variety	Hara	Yoshiwara	Kanbara	Yui	Okitsu	Ejiri	Fuchu	Mariko	Okabe	Fujieda
M1	+	-	-	+	+	+	+	-	+	+
M2	-	+	-	+	+	+	+	-	+	-
M3	+	+	-	+	+	+	+	+	+	+
M4	+	-	+	+	+	+	+	+	+	+
M5	+	+	-	+	+	+	+	+	+	+
M6	+	+	+	+	+	+	+	+	+	+
M7	+	+	+	+	-	+	+	+	-	+
M8	+	+	+	+	+	+	+	+	+	+
M9	-	-	-	+	-	-	+	-	-	+
MX	+	+	+	+	+	+	-	+	+	+

Marker name

Genotype

In the case of dominant markers, MinimalMarker on web cannot detect if all genotypes match between cultivars. Therefore, It must be verified by the user himself. It is not guaranteed to be the minimal marker set and to output all minimal marker sets. **Also, please remove spaces in the data.**

7. Example of input data format with missing data

CSV

Variety,Akatsuki,Yuuzora,Saotome,Yoshihime,Masahime,Akizora,Natsutome,Gyousei,Kouyou Hakutou,Ookubo

M1a,#,BA,BB,AB,AA,AA,AB,BA,AA,AB

M4c,BE,#,BD,CE,EC,AE,CE,BE,CE,BC

M6a,AC,CA,#,AC,CA,AC,CA,AC,CD,CD

M12a,AB,BB,AB,AB,AB,AB,AB,AB,AB,AB

M15a,CC,CC,BC,CC,CC,AC,CC,CC,CC,CC

MA006b,AA,AA,AB,AA,AA,AA,AA,AA,AA,AA

MA007a,AC,CA,AA,CC,CD,CC,CA,AC,BB,AC

MA013a,AC,AC,BD,AC,AC,AB,AC,AC,AC,AC

MA014a,DD,DD,AD,CD,DD,DC,CD,DD,BD,CD

MA015a,AA,AD,BC,AA,AD,AC,AA,AA,AD,AC

xls/xlsx

Variety	Hara	Yoshiwara	Kanbara	Yui	Okitsu	Ejiri	Fuchu	Mariko	Okabe	Fujieda
M1	#	AB	BB	AB	AA	AA	AB	AB	AA	AB
M2	BE	#	BD	CE	CE	AE	CE	BE	CE	BC
M3	AC	AC	#	AC	AC	AC	AC	AC	CD	CD
M4	AB	BB	AB	AB	AB	AB	AB	AB	AB	AB
M5	CC	CC	BC	CC	CC	AC	CC	CC	CC	CC
M6	AA	AA	AB	AA	AA	AA	AA	AA	AA	AA
M7	AC	AC	AA	CC	CD	CC	AC	AC	BB	AC
M8	AC	AC	BD	AC	AC	AB	AC	AC	AC	AC
M9	DD	DD	AD	CD	DD	CD	CD	DD	BD	CD
MX	AA	AD	BC	AA	AD	AC	AA	AA	AD	AC

If the genotype has a deletion value, use '#' instead of genotype and turn on option '-k'. If you use '#', MinimalMarker on web does not guarantee a minimal marker set. It also cannot detect if all genotypes match between cultivars. Therefore, users need to check by themselves if all genotypes match among cultivars. **Also, please remove spaces in the data.**

8. Operation procedures

Minimal Marker
National Agriculture and Food Research Organization

A computer program that calculates the minimal marker genotype table obtained by applying cultivar identification. Initially, the default analysis is recommended. In case of timeout, refer to the manual ([English](#), [Japanese](#)).

Data

Upload file / Use sample data
 Upload file (csv/xls/xlsx)
 Sample data

Options (Specifying multiple options may produce unexpected results, so please verify your results.)

Algorithm		
0: enumeration method [slow]		<input type="radio"/> 0: enumeration method [slow]
-m 1: branch and bound [fast] (default)		<input checked="" type="radio"/> 1: branch and bound [fast]
2: greedy algorithm	Use when a timeout occurs in -m1 (default) mode. It is not guaranteed to be the minimal marker set and to output all minimal marker sets. However, it outputs a sufficiently small number of marker sets that identify all the cultivars in a short time.	<input type="radio"/> 2: greedy algorithm
Number of markers identifying all 2-cultivar combinations		
-w 1: (default)		1
2:	In the minimal marker set, for any combination of two cultivars in a given genotype table, the marker set is organized so that there is at least one marker that identifies the two cultivars. This "at least one marker" can be changed to "at least two markers" by setting -w2 . 2 or more can also be specified.	
n: (max: 5)		
-g Ignore order of characters (default: '-g')	When '-g' is turned on, genotypes 'AB' and 'BA' are considered to be the same genotype. Not case sensitive, but case-sensitive is preferable to avoid unexpected errors.	<input type="checkbox"/> Consider the order of genotype strings
-b 1: Bitwise operation [fast] (default)	0: arithmetic operation [slow] 1: bit operation [fast]	<input type="checkbox"/> 0: Arithmetic operation [slow]
-k Replace a missing value (default: none)	'#' is used when genotypes has a missing value. It is not guaranteed to be the minimal marker set and to output all minimal marker sets.	<input checked="" type="checkbox"/> Replace a missing value with '#'
-v Identify specified cultivar(s) from other cultivars	Specify the cultivar(s) to be distinguished from other cultivars. Specify the numerical value of the order of the cultivars (columns) in the genotype table. Multiple entries can be specified.	<input type="text"/> (Example: 3 or 3,4)
-s Number of markers start searching (default: 1, max: 30)		1
-n Specify the marker(s) the user wants to use	Specify the numerical value of the order of the markers (lines) in the genotype table. Multiple entries can be specified.	<input type="text"/> (Example: 3 or 3,4)
-l Specifies the number of the minimum	are detected. However, if number of output minimal	<input type="text"/> (Example: 10)
-p Print data table (default: Search)		<input type="text"/>

Print only Print and Search Search

1. Select "upload file" by radio button.

2. Select upload file.

Initially, the default analysis is recommended

3. Select options. The results can be obtained with the defaults. See the manual for a description of the options.

4. Click on "Search" to begin analysis.

Print discrimination possibility table. Refer Table2 in Fujii et al. 2013 Journal of Bioinformatics and Computational Biology 11(2):1250022.

Both print and analysis

9. Options in MinimalMarker on web

Options	Argument	Description
-m	Algorithm	
	0: enumeration method [slow]	
	1: branch and bound [fast] (default)	
	2: greedy algorithm	Use when a timeout occurs in -m1 (default) mode. It is not guaranteed to be the minimal marker set and to output all minimal marker sets. However, it outputs a sufficiently small number of marker sets that identify all the cultivars in a short time.
-w	Number of markers identifying all 2-cultivar combinations	
	1: (default)	
	2: n:	In the minimal marker set, for any combination of two cultivars in a given genotype table, the marker set is organized so that at least one marker identifies the two cultivars. This "at least one marker" can be changed to "at least two markers" by setting -w2 . 2 or more can also be specified.
-g	Ignore order of characters (default: '-g' is ON) Genotypes 'AB' and 'BA' are considered to be the same genotype	When '-g' is turned on, genotypes 'AB' and 'BA' are considered to be the same genotype. Not case-sensitive, but case-sensitive is preferable to avoid unexpected errors.
-b	Operation method	
	0: arithmetic operation [slow] 1: Bitwise operation [fast] (default)	
-k	Replace a missing value (default: none)	#' is used when genotypes have missing values. It is not guaranteed to be the minimal marker set and to output all minimal marker sets.
-v	Identify specified cultivar(s) from other cultivar	
	Specify the cultivar(s) to be distinguished from other cultivars.	
	Specify the numerical value of the order of the cultivars (columns) in the genotype table. Multiple entries can be specified.	Outputs minimal marker set(s) to identify specified cultivars and other cultivars
-s	Number of markers start searching (default: 1)	
-n	Specify the marker(s) the user wants to use	
	Specify the numerical value of the order of the markers (lines) in the genotype table. Multiple entries can be specified.	Outputs a minimal marker set(s) that includes the specified marker(s)
-l	Specifies the number of outputs of the minimal marker set	By default, MinimalMarker does not stop analyzing until all minimal marker sets are detected. However, if the computation time becomes too long, it times out. To avoid this, specify the number of output minimal marker sets.
-p	Print data table / Search (default: Search)	See Table 2 in Fujii et al. 2013 Journal of Bioinformatics and Computational Biology 11(2):1250022 for "print data".

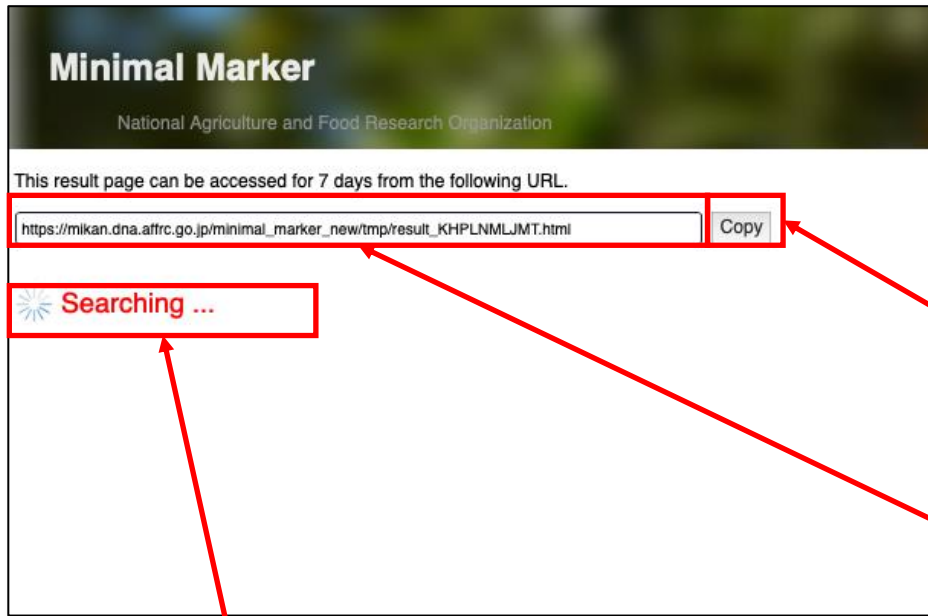
10. How to specify options -v, -n

Use the "-v" option to identify specific cultivar(s) from other cultivars. To specify cultivar(s), specify the column number where the cultivar is located, counting excluding the first column. For example, to specify "Yoshiwara", use "2". More than one is possible, and "Yoshiwara" and "Fujieda" would be "2,10".

Use the "-n" option to identify a specific marker the user wants. To specify marker(s), specify the line number on which the marker is listed, except for the first line. For example, to specify "M2", use "2". Multiple designations are also possible. For example, "M2" and "MX" are "2,10".

		1	2	3	4	5	6	7	8	9	10
	Variety	Hara	Yoshiwara	Kanbara	Yui	Okitsu	Ejiri	Fuchu	Mariko	Okabe	Fujieda
1	M1	AB	AB	BB	AB	AA	AA	AB	AB	AA	AB
2	M2	BE	BE	BD	CE	CE	AE	CE	BE	CE	BC
3	M3	AC	AC	BC	AC	AC	AC	AC	AC	CD	CD
4	M4	AB	BB	AB	AB	AB	AB	AB	AB	AB	AB
5	M5	CC	CC	BC	CC	CC	AC	CC	CC	CC	CC
6	M6	AA	AA	AB	AA	AA	AA	AA	AA	AA	AA
7	M7	AC	AC	AA	CC	CD	CC	AC	AC	BB	AC
8	M8	AC	AC	BD	AC	AC	AB	AC	AC	AC	AC
9	M9	DD	DD	AD	CD	DD	CD	CD	DD	BD	CD
10	MX	AA	AD	BC	AA	AD	AC	AA	AA	AD	AC

11. Display of analysis in progress and timeout



- Select the "Search" button shown on the previous page, and the screen shown on the left will appear in a new window.
- When the calculation is completed, this screen will be overwritten with the output of the results.
- Depending on the contents of the data, the calculation may take a long time. In this case, select the "COPY" button on the left screen to copy and save the URL. You can view the results from the URL within 7 days.
- The results are retained on the server side and can be viewed even if the power of the terminal is turned off.

"Searching" is displayed during calculation and the progress indicator rotates.

To be precise, at 03:10 every day, result files older than 7 days are deleted.

❑ Timeout

A timeout occurs at midnight 24 hours after the start of the calculation, and the calculation is terminated.

See the next page for how to avoid the timeout.

12. How to avoid timeouts

- MinimalMarker is configured by default to detect all minimal marker sets. Therefore, if the number of markers or varieties is large, the number of combinations to be considered will increase, the calculation will not be completed, and the program will time out.
- In this case, it is recommended to use the greedy method with the option "-m2".
- Since the greedy method does not guarantee that the least marker set is the one and that all least marker sets are output, if you want to detect the least marker set correctly, download the MinimalMarker Perl script from the link below and run it on a high-performance terminal. If you want to find the correct minimal marker set, download the MinimalMarker Perl script from the following link and run it on a high-performance terminal.

[Translated with DeepLhttps://www.naro.go.jp/english/laboratory/nifts/minimal_marker/index.html](https://www.naro.go.jp/english/laboratory/nifts/minimal_marker/index.html)

13. Elapsed time with option -m2 (greedy algorithm)

□ Condition

- ◆ SNP marker
- ◆ Number of markers : 277
- ◆ Number of cultivars : 101

□ Option and Elapsed time

- ◆ Default : Timeout
- ◆ Option -m2 : 94 sec.
(greedy algorithm)

■ Result

- Number of Marker sets :83
- Number of markers
comprising a marker set:7

- This is only a guide

14. Example of output data for sample data option: default

```
*Time stamp
Started on Mon Mar 6 15:18:49 2023
*Input data
Inputfile name: sample_data.xls
Number of markers: 10
Number of cultivars: 10
```

```
*Option
-m Algorithm: 1
-w Number of markers identifying all 2-cultivar combinations: 1
-g Ignore order of characters: 0n
-b bitwise operation: 1
-k replace missing value:
-v Identify specified cultivar(s) from other cultivars:
-s Number of markers start searching: 1
-n Specify the marker(s) the user wants to use:
-l maximum number of marker sets:
-p Print/ Print & Search/ Search: Search
```

Indicates option setting status

```
*Result
!!!!Caution!!!!
No marker can discriminate between the cultivars listed below!
Hara x Mariko
Under the above conditions, the computation of the MINIMAL MARKER SET(s)
continues!
But recommend adding the marker(s) to resolve the above condition!
```

The combination of two unidentifiable cultivars is displayed when it exists. Note that in the case of dominant markers, even if there are combinations that cannot be identified, this part is not output. Note that this may not be indicated depending on the option settings. For this reason, we recommend that you first analyze with the default settings.

```
Number of minimal marker set(s) found: 3
The list of minimal marker sets found by MinimalMarker
```

```
M2,M4,M7
M2,M7,MX
M7,M9,MX
```

Each row is one minimum marker set. Here there are three.

```
*Time stamp
Completed at Mon Mar 6 15:18:49 2023
Elapsed time: 0 sec.
```

15. Example of output data for sample data option -v4

```
*Time stamp
Started on Mon Mar 6 15:22:54 2023
*Input data
Inputfile name: sample_data.xls
Number of markers: 10
Number of cultivars: 10
*Option
-m Algorithm: 1
-w Number of markers identifying all 2-cultivar combinations: 1
-g Ignore order of characters: On
-b bitwise operation: 1
-k replace missing value:
-v Identify specified cultivar(s) from other cultivars: Yui
-s Number of markers start searching: 1
-n Specify the marker(s) the user wants to use:
-l maximum number of marker sets:
-p Print/ Print & Search/ Search: Search
```

```
*Result
Number of minimal marker set(s) found: 5
The list of minimal marker sets found by MinimalMarker
```

```
M1,M7
M2,M7
M5,M7
M7,M8
M7,MX
```

```
*Time stamp
Completed at Mon Mar 6 15:22:54 2023
Elapsed time: 0 sec.
```

The "Yui" specified cultivar in option -v4 is displayed

With option -v, even if there is a combination of two cultivars with identical genotypes all, it will not be displayed. we recommend that you first analyze with the default settings.

Each row is one minimal marker set. Here five are three.

16. Example of output data for sample data option -n4

```
*Time stamp
Started on Mon Mar 6 15:28:45 2023
*Input data
Inputfile name: sample_data.xls
Number of markers: 10
Number of cultivars: 10
*Option
-m Algorithm: 1
-w Number of markers identifying all 2-cultivar combinations: 1
-g Ignore order of characters: On
-b bitwise operation: 1
-k replace missing value:
-v Identify specified cultivar(s) from other cultivars:
-s Number of markers start searching: 1
-n Specify the marker(s) the user wants to use: M4
-l maximum number of marker sets:
-p Print/ Print & Search/ Search: Search
*Result
```

```
!!!!Caution!!!!
No marker can discriminate between the cultivars listed below!
Hara x Mariko
Under the above conditions, the computation of the MINIMAL MARKER
SET(s) continues!
But recommend adding the marker(s) to resolve the above condition
```

```
Number of minimal marker set(s) found: 1
The list of minimal marker sets found by MinimalMarker
```

```
M2,M4,M7
```

```
*Time stamp
Completed at Mon Mar 6 15:28:45 2023
Elapsed time: 0 sec.
```

The "M4" marker specified in option -n4 is displayed

The combination of two unidentifiable cultivars is displayed when it exists. Note that in the case of dominant markers, even if there are combinations that cannot be identified, this part is not output.

Each row is one minimum marker set. Here there are one.

17. Citation

When publishing an academic paper using the results obtained using the MinimalMarker on web, please describe the use of MinimalMarker in the paper and cite the following literature.

- MinimalMarker

FUJII Hiroshi, OGATA Tatsushi, SHIMADA Takehiko, ENDO Tomoko, IKETANI Hiroyuki, SHIMIZU Tokuro, YAMAMOTO Toshiya and OMURA Mitsuo (2013) Minimal marker: an algorithm and computer program for the identification of minimal sets of discriminating DNA markers for efficient variety identification. *Journal of Bioinformatics and Computational Biology* 11(2):1250022.

doi: 10.1142/S0219720012500229

- MiGD

KAWAHARA Yoshihiro, ENDO Tomoko, OMURA Mitsuo, TERAMOTO Yumiko, ITHO Tsuyoshi, FUJII Hiroshi, SHIMADA Takehiko (2020) Mikan Genome Database (MiGD): integrated database of the genome annotation, genomic diversity, and CAPS marker information for mandarin molecular breeding. *Breeding Science*.

doi: 10.1270/jsbbs.19097

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23. History of MinimalMarker on web Manual

- English Version 1.0 March 8, 2023
- English Version 1.1 July 12, 2023