

# Supplemental Data

## Instructions for viewing ICP screen data of fast neutron (FN)mutagenized *Arabidopsis*

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### 1): How are the data formatted?

All data for fast neutron mutagenized seeds are saved as Excel files, allowing user to either view the data online or download easily:

#### **M2: Analyzed M2 ICP data**

M2 data are split into 11 segments labeled by their *Arabidopsis* line numbers. For example: M2: FN001-080 contains files with line number from 1 to 80. M2 data can be viewed in 2 formats: the files are both organized such that for a given element (e.g.Ba2+) the data for all lines can be viewed or for a given line (e.g.FN565) the values for all elements can be viewed.

#### **M3: Analyzed M3 ICP data**

M3 data files are labeled by the date the data was collected. Each file was formatted by *Arabidopsis* line numbers.

### 2): How do we analyze data?

A): M2 data: For data from initial pilot experiments with lines FN001-FN366, we normalized the ICP-derived concentrations (PPM) to the concentration of Mg, and the results are shown in column "ppm/Mg".

For the remaining FN367-FN1191 data, we normalized to dry weight (DW), and the results are shown in column "ppm/DW". The Ave and SD values of each element (Ave: Average value; SD: Standard deviation value) are calculated from the whole population data of that element in one experiment.

B): M3 data: All data are normalized to dry weight, and shown in column "ppm/DW". The Ave (Average) and SD (standard deviation) values are calculated from only wild type data. And then the Ave and SD were applied to FN analyses.

### 3): How were putative mutants selected?

A): In screens of M2 fast neutron lines, Z values larger than 3 (Z>3, i.e. 3 standard deviations larger or smaller than the average value) were used as a threshold to define a putative mutant. If the data

of a specific M2 plant (ppm/DW) was larger than "Ave+3SD" (i.e. Z>3), then it is shown as a "Putant", otherwise it is labeled as "-" (Fig. 1).

In addition, we list the "Ave+2SD" and "Ave-2SD" data, so the reader can evaluate borderline cases.

"Putant" or "-"

Fig. 1 M2 data

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Solution Label	Element	Corr Con	Units	DW	ppm/DW	Ave	SD	Z	Ave+2SD	Ave-2SD	Ave+3SD	M? >+2SD	M? >-2SD	M? >+3SD
col-0-1	Ba 233.527	0.12839122	ppm	29.5	0.00435	0.00524	0.00327	-0.2726	0.01179	-0.0013	0.015063	-	-	-
col-0-2	Ba 233.527	0.19896919	ppm	32.8	0.00607	0.00524	0.00327	0.25104	0.01179	-0.0013	0.015063	-	-	-
JIS.fn367	Ba 233.527	0.18174452	ppm	32.7	0.00556	0.00524	0.00327	0.09576	0.01179	-0.0013	0.015063	-	-	-
JIS.fn368	Ba 233.527	0.1610786	ppm	28.9	0.00557	0.00524	0.00327	0.10056	0.01179	-0.0013	0.015063	-	-	-
JIS.fn369	Ba 233.527	0.04205671	ppm	7.2	0.00584	0.00524	0.00327	0.18231	0.01179	-0.0013	0.015063	-	-	-
JIS.fn370	Ba 233.527	0.04859879	ppm	8.8	0.00552	0.00524	0.00327	0.08496	0.01179	-0.0013	0.015063	-	-	-
JIS.fn371	Ba 233.527	0.06587971	ppm	9.7	0.00679	0.00524	0.00327	0.47274	0.01179	-0.0013	0.015063	-	-	-
JIS.fn372	Ba 233.527	0.22947109	ppm	43.8	0.00524	0.00524	0.00327	-0.0017	0.01179	-0.0013	0.015063	-	-	-
JIS.fn373	Ba 233.527	0.1921826	ppm	41.4	0.00464	0.00524	0.00327	-0.1841	0.01179	-0.0013	0.015063	-	-	-
JIS.fn374	Ba 233.527	0.0712181	ppm	14	0.00509	0.00524	0.00327	-0.0481	0.01179	-0.0013	0.015063	-	-	-
JIS.fn375	Ba 233.527	0.21456969	ppm	41.7	0.00515	0.00524	0.00327	-0.0302	0.01179	-0.0013	0.015063	-	-	-
JIS.fn376	Ba 233.527	0.0922318	ppm	19.3	0.00478	0.00524	0.00327	-0.1423	0.01179	-0.0013	0.015063	-	-	-
JIS.fn377	Ba 233.527	0.12359632	ppm	24.1	0.00513	0.00524	0.00327	-0.0355	0.01179	-0.0013	0.015063	-	-	-
JIS.fn378	Ba 233.527	0.0839856	ppm	17.1	0.0049	0.00524	0.00327	-0.1041	0.01179	-0.0013	0.015063	-	-	-
JIS.fn379	Ba 233.527	0.12855527	ppm	19.5	0.00659	0.00524	0.00327	0.41189	0.01179	-0.0013	0.015063	-	-	-
JIS.fn380	Ba 233.527	0.0662686	ppm	9.8	0.00676	0.00524	0.00327	0.46368	0.01179	-0.0013	0.015063	-	-	-
JIS.fn381	Ba 233.527	0.09070747	ppm	17.1	0.0053	0.00524	0.00327	0.01834	0.01179	-0.0013	0.015063	-	-	-
JIS.fn382	Ba 233.527	0.19359139	ppm	43.5	0.00445	0.00524	0.00327	-0.2426	0.01179	-0.0013	0.015063	-	-	-
JIS.fn383	Ba 233.527	0.13358292	ppm	28.9	0.00462	0.00524	0.00327	-0.1901	0.01179	-0.0013	0.015063	-	-	-
JIS.fn384	Ba 233.527	0.12129249	ppm	26.1	0.00465	0.00524	0.00327	-0.1825	0.01179	-0.0013	0.015063	-	-	-
JIS.fn385	Ba 233.527	1.12531281	ppm	30.4	0.03702	0.00524	0.00327	9.70777	0.01179	-0.0013	0.015063	Putant	-	Putant
JIS.fn386	Ba 233.527	0.09990774	ppm	21.9	0.00456	0.00524	0.00327	-0.2085	0.01179	-0.0013	0.015063	-	-	-
JIS.fn387	Ba 233.527	0.11766249	ppm	23.1	0.00509	0.00524	0.00327	-0.0461	0.01179	-0.0013	0.015063	-	-	-
JIS.fn388	Ba 233.527	0.13077827	ppm	23.6	0.00554	0.00524	0.00327	0.09073	0.01179	-0.0013	0.015063	-	-	-
JIS.fn389	Ba 233.527	0.07706961	ppm	17.1	0.00451	0.00524	0.00327	-0.2253	0.01179	-0.0013	0.015063	-	-	-
JIS.fn390	Ba 233.527	0.12387797	ppm	25.8	0.0048	0.00524	0.00327	-0.1354	0.01179	-0.0013	0.015063	-	-	-
JIS.fn391	Ba 233.527	0.10551994	ppm	28.4	0.00372	0.00524	0.00327	-0.4672	0.01179	-0.0013	0.015063	-	-	-
JIS.fn392	Ba 233.527	0.10899284	ppm	25.4	0.00429	0.00524	0.00327	-0.2913	0.01179	-0.0013	0.015063	-	-	-
JIS.fn393	Ba 233.527	0.09204909	ppm	16.8	0.00548	0.00524	0.00327	0.07168	0.01179	-0.0013	0.015063	-	-	-
JIS.fn394	Ba 233.527	0.17429325	ppm	36.75	0.00474	0.00524	0.00327	-0.1533	0.01179	-0.0013	0.015063	-	-	-
JIS.fn395	Ba 233.527	0.13264874	ppm	22.7	0.00584	0.00524	0.00327	0.18303	0.01179	-0.0013	0.015063	-	-	-
JIS.fn396	Ba 233.527	0.1774611	ppm	37	0.0048	0.00524	0.00327	-0.137	0.01179	-0.0013	0.015063	-	-	-
JIS.fn397	Ba 233.527	0.08012018	ppm	14.9	0.00538	0.00524	0.00327	0.04054	0.01179	-0.0013	0.015063	-	-	-

Note to Fig.1:

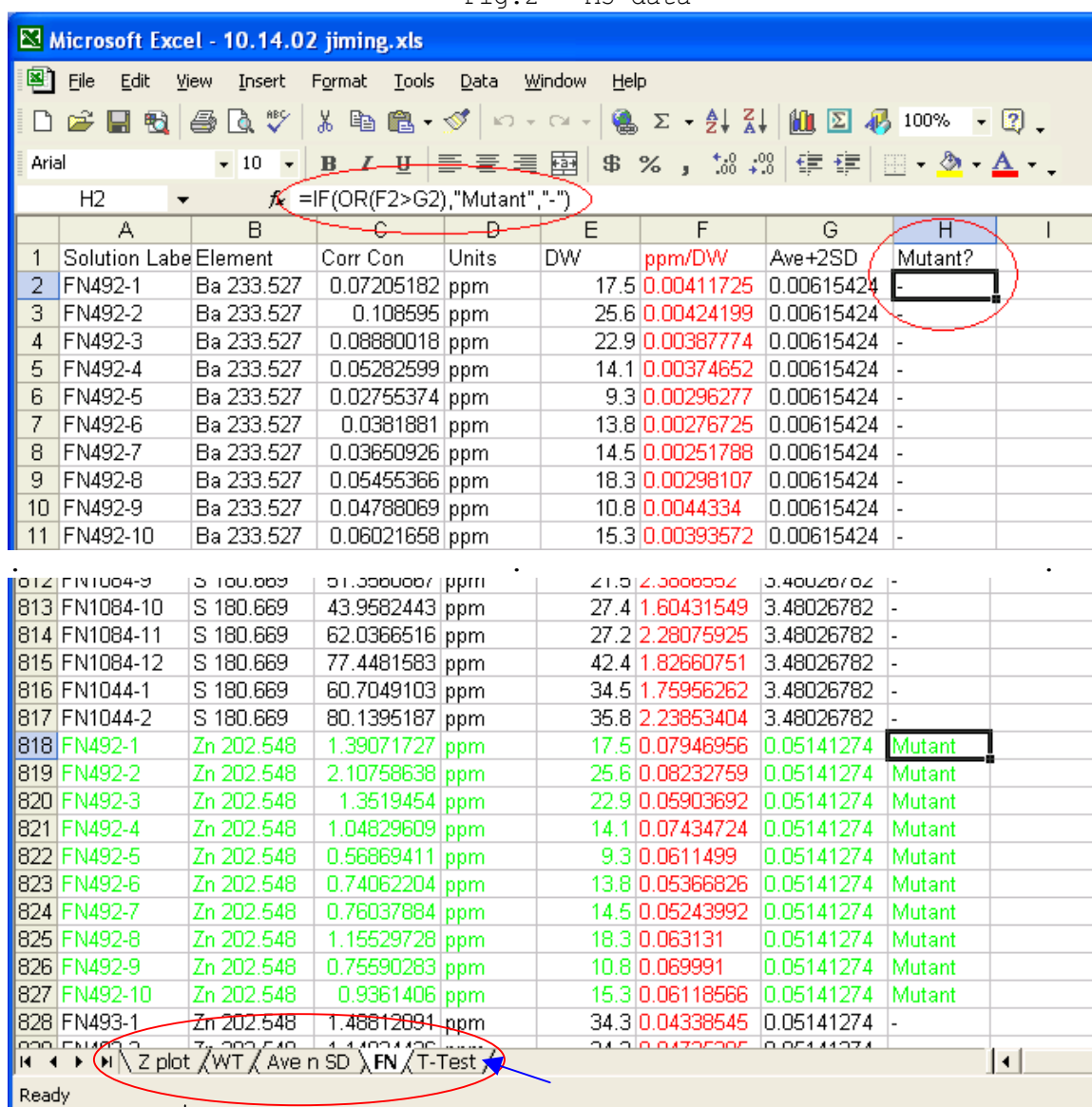
"Column" # may change in different experiments, but the "Title" never

Column	Title
A	<u>Solution Label</u> : Sample name (of the FN lines)
B	<u>Element</u> : Elements analyzed
C	<u>Corr Con</u> : ICP derived concentrations of analyzed elements
D	<u>Units</u> : The unit of concentration in column C (PPM)
E	<u>DW</u> : Dry weight of each sample
F	<u>ppm/DW</u> : ICP data normalized to DW(or ppm/Mg, if to Mg).
G	<u>Ave</u> : Average value
H	<u>SD</u> : Standard deviation value
I	<u>Z</u> : = (Normalized data-Ave)/SD
J/K/L	<u>Ave+2SD/Ave-2SD/Ave+3SD</u> : Average value plus/minus 2SD, or plus 3SD

M/N/O  $M? >2SD/M? <-2SD/M? >3SD$ : If the normalized data of a specific sample was larger than Ave+2SD/Ave+3SD or smaller than Ave-2SD, then it is a putative mutant.

B): In M3,  $Z > 2$  was used as a threshold to define a mutant, in line with the analyses of D.Salt and colleagues. For each putative mutant, 7-12 M3 plants of each line were re-screened.

Fig.2 M3 data



If most of the M3 plants passed (as shown in Fig.2 in green color), then the data were used to perform T-Tests (indicated by blue arrow in Fig.2).

**Note to Fig.2**

Z plot: Worksheet in which Z values of WT were plotted

WT: Worksheet in which data of WT were analyzed to calculate Ave & SD.

Ave n SD: Worksheet in which Ave and SD of WT population were listed

FN: Worksheet in which data of FN was analyzed to confirm a putative mutant.

T-Test: Worksheet in which mutants passed the  $Z > 2$  criteria were chosen for the two tailed, heteroscedastic T-test.

In some cases, a putative mutant didn't show a statistically clear phenotype in the M3 screen for a specific element (e.g Cd accumulation in M2), but showed other apparent phenotypes ( $P < 0.05$ ) (e.g Zn accumulation in all M3s). In such cases, we went back to the M2 data of that line and checked if there was such a phenotype ( $> 2SD$  or  $> Ave$ ). If it showed a positive result, we also listed it as a mutant.