Instructions for Silique Screening Forms

A. General Guidelines:

-Avoid siliques with < 40 seeds and many unfertilized ovules.
-Pick mature green siliques to get a terminal embryo phenotype.
-Mutant seeds can be deflated but not desiccated if embryo is to be removed.
-Avoid bias when selecting alternate mutant seeds for dissection.
-Do not count spontaneously aborted seeds if they are outside the range of typical mutant seeds.
-Do not count unfertilized ovules; make a note if they are unusually abundant.

B. Line Above Silique Grid:

Plant: Subfamily number (1A,1B,1C,1D,…)

Age: Stage of wild-type seeds in silique:

- M: Mature green
- C: Curled cotyledon
- E: Early curled cotyledon
- L: Linear – torpedo

Seeds: Total number of seeds in the silique.

Mutant: Total number of mutant seeds in the silique.

Top Half: -Number of mutant seeds in the top half of the silique.
-The top refers to the tip of the silique not directly attached to the stem.
-Draw a vertical line in red to mark halfway point for each valve.
-Draw 2 lines if the valve contains an odd number of seeds.
-If a mutant seed is found between 2 lines, include it in the top half in one valve, then in the bottom half in the next valve, alternating through all the siliques.

Initials: Person doing the screening.

Date: Date of screening (MM, DD, YY).

C. Silique Grid and Seed Dissection:

-Each square marks the location of a seed. Dots represent mutant seeds.
-Note the tip of each row of seeds (in the valves) with a bracket ( ]) to the right of the last seed.
-Randomly select four mutant seeds for measurement and dissection.

Seed Color: 1 – white, 2 – pale yellow-green, 3 – pale green, 4 – green (like the wild-type seed), 5 – fusca (appears purple/brown)
Seed Length: Length of seed in microns (measure with stage micrometer). Round the seed size to the nearest 50 μm.

-Remove embryo from mutant seed and make quick measurements.
-If embryo is seen but destroyed upon dissection, move to next seed.
-If embryo is too small and cannot be found, use “X” for Embryo Class.

Embryo Color: 1 – white, 2 – pale yellow-green, 3 – pale green, 4 – green (like the wild-type embryo) 5 – fusca (appears purple/brown)
Include 2 numbers if portions of embryo differ in color (specify “P” in “Note Class”).

Embryo Length: Length of embryo in microns (measure with stage micrometer).
Round the embryo size to the nearest 25 μm.
Measure the greatest distance without unfolding cotyledons (exclude the suspensor).

Embryo Class: Assign embryo to a morphological class based on standard diagram of embryo phenotypes. Ask about adding a new class if the morphology is not represented. Make drawings in "Other Notes".

Note Class:
- A: Apical meristem (SAM) is enlarged (distinct from multiple cotyledons).
- B: Seed coat brown or turning brown.
- C: Clear / watery seeds like titan class.
- D: Deflated seed (starting to desiccate).
- F: Flattened seed (completely desiccated).
- G: Embryo turns greener when dried.
- M: Multiple cotyledons evident.
- P: Pigmentation uneven in embryo.
- X: Embryo partially damaged on dissection.

Other Notes: Special features not represented in previous sections. Drawings should be made when the embryo phenotype does not match any class in the diagram.

D. Summary of Screening Data:

Total Seeds: Sum of numbers in "Seeds" section above silique grids.
Mutant Seeds: Sum of numbers in "Mutant" section above silique grids.
Mutant Top Half: Sum of numbers in "Top Half" section above silique grids.

Percent Mutant: Percent (Mutant/Total) rounded to nearest 0.1 decimal. Expect 25% mutant seeds.
Chi-Square: =[(#mutants observed - #mutants expected)squared / #mutants expected]+
[(#wild-types observed - #wild-types expected)squared / #wild-types expected]

Percent Top Half: Percent (Top/Mutant) rounded to nearest 0.1 decimal. Expect 50% mutant seeds in top half.
Chi-Square: =[(#top half observed - #top half expected)squared / #top half expected]+
[(#bottom half observed - #bottom half expected)squared / #bottom half expected]

Avg.Seed Length: Transfer numbers from screening sheet to calculation sheet.
Calculate average. The "S.E" entry can be ignored. Save numbers for the histogram.
Avg.Embryo Length: Same procedure as for average seed length.

Embryo Classes: Transfer information from screening sheet to calculation sheets.
Transfer final numbers from the calculation sheet to the summary sheet.
S. and E. Colors: Same procedure as for the embryo class.
Average Seed: Calculate average seed color.
Average Embryo: Calculate average embryo color.

Phenotype Summary: Example: "Standard globular with an occasional abnormal suspensor"
Check with Dr. Meinke if uncertain about summary statements.
Further information will be presented on Nomarski summary page.
Special Features: Anything particularly interesting or noteworthy.

Pollen Examined: Yes (Y) or No (N). Defect: Yes (Y) or No (N).
Percent Defective: If the pollen appears abnormal, view approximately 100 grains and estimate the percent that appear to be abnormal.
Nature of Defect: Note whether grains are deflated, distorted, or normal shape but reduced in size.

Embryo Phenotypes

Globular (G): No Distinct Cotyledons

A Globular Shape
- A1 Standard shape
- A2 Tear shape

B Globular with Suspensor
- B1 Thin, straight suspensor
- B2 Long, curled suspensor

C C1 Globular with Giant Suspensor

D D1 Globular with Irregular Surface

Transition (T): Elongation / Cotyledon Initiation

E Early Transition to Heart Shape
- E1 Triangular with no primordia
- E2 Short cotyledon primordia
- E3 Multiple bumps / primordia
- E4 Wide suspensor

F Elongate Shape
- F1 Small elongate / blimp (150-200 um)
- F2 Large elongate / blimp (>200-300 um)

G Small Cotyledons and Hypocotyl
(≤ 175 um in length)
- G1 small heart
- G2 small heart with early development of hypocotyls
- G3 small thin coytyledons with early development of hypocotyls
- G4 small thin open cotyledons – no hypocotyl

Cotyledon (C): Increased Size and Differentiation

H Cotyledons and Hypocotyl
(>175 um in length)
- H1 Heart shape
- H2 Thin cotyledons and hypocotyl
- H3 Wide hypocotyl
- H4 Bent hypocotyl
- H5 Irregular surface

I Reduced Cotyledons
- I1 Thin hypocotyl - cotyledons are very small
- I2 Broad hypocotyl – cotyledons are very small
- I3 Standard hypocotyl – cotyledons are very small
- I4 No cotyledons distinguishable (>300 um)

J Reduced Hypocotyl
- J1 Open thin cotyledons – no hypocotyl
- J2 Closed cotyledons – no hypocotyl
- J3 Mature cotyledons – short hypocotyl
- J4 Single cotyledon – short bent hypocotyl

K Large Heart with Multiple Apical Primordia

L Early Curled Cotyledons
- L1 Short cotyledons
- L2 Irregular short cotyledons
- L3 Bent irregular short cotyledons
- L4 Standard curled or mature cotyledons, bent or twisted hypocotyl

M Prominent, Fused or Folded Cotyledons
- M1 Cup shaped cotyledons
- M2 Sperical – short hypocotyl
- M3 Partially fused – irregular surface

N Advanced Cotyledons; Signs of Vivipary
- N1 Linear stage - clear tipped hypocotyl
- N2 Curled stage - clear tipped hypocotyl

O Normal Mature Cotyledon Shape

T Twin Embryos
- T1 Two embryos formed – cotyledon stage of development in at least one of the embryos