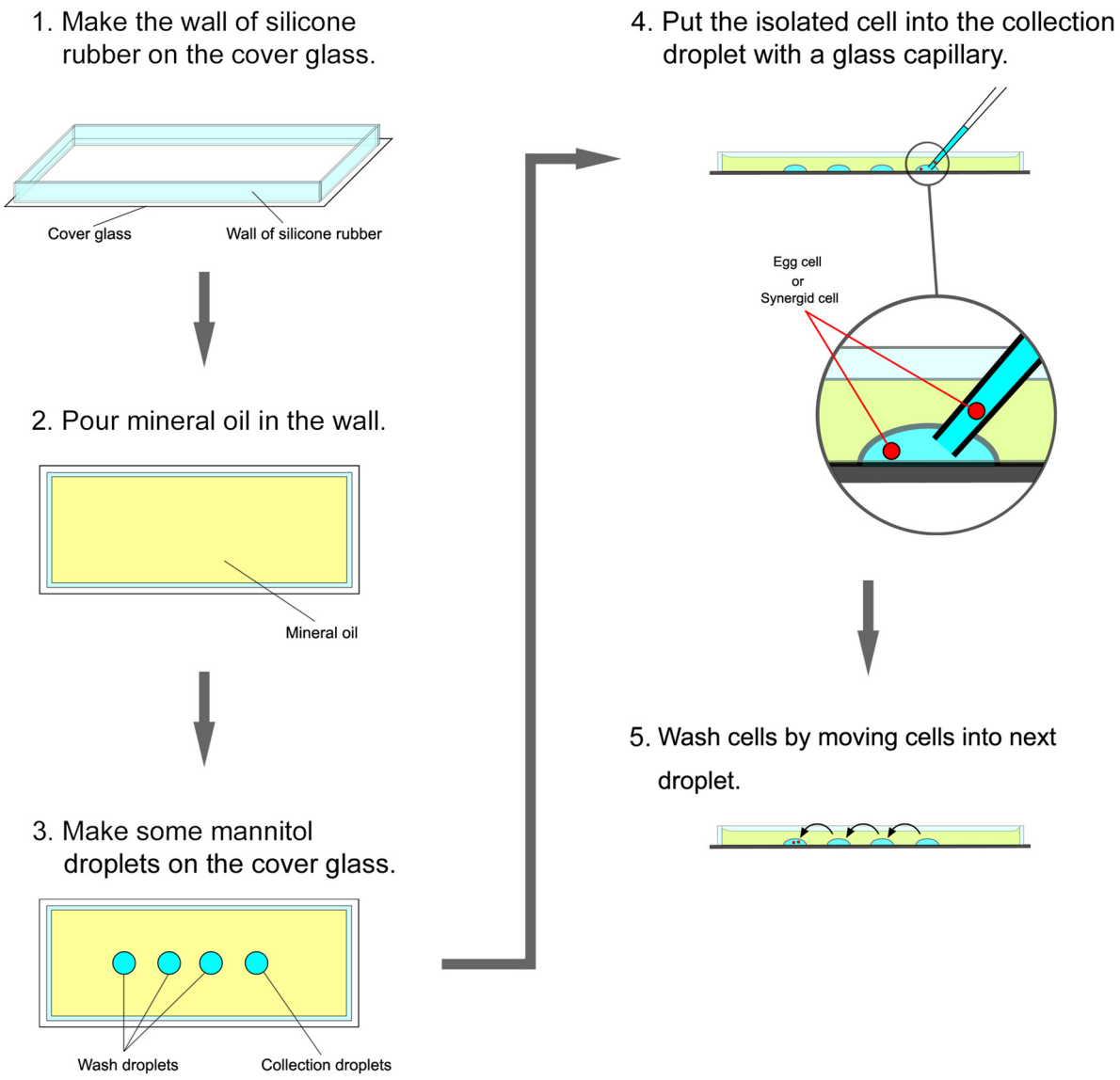
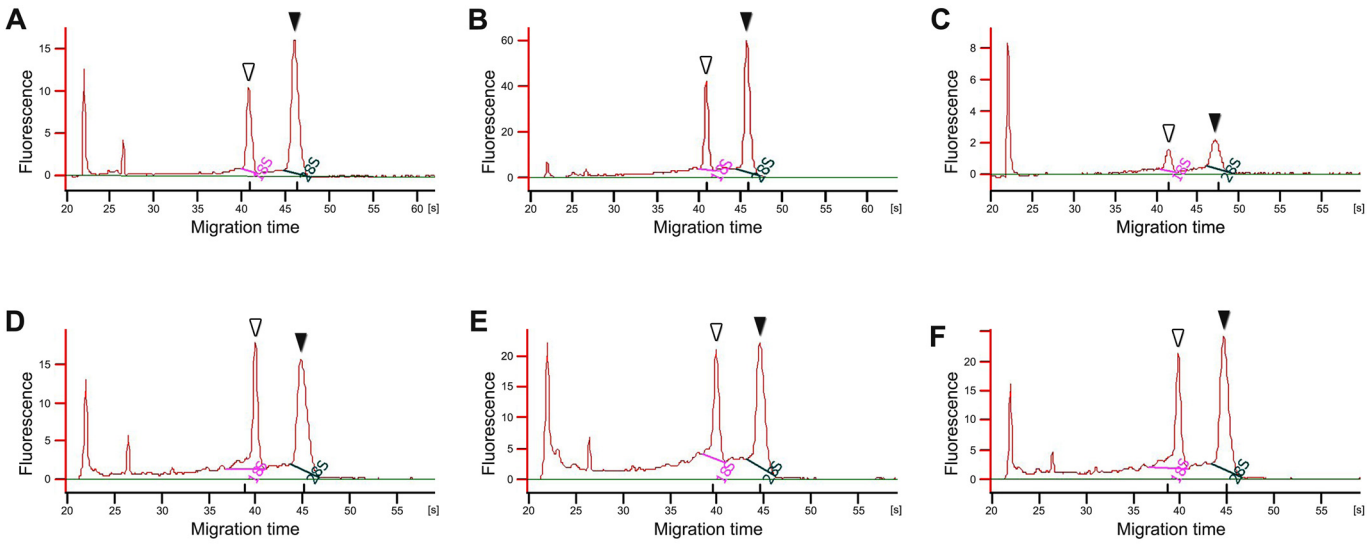


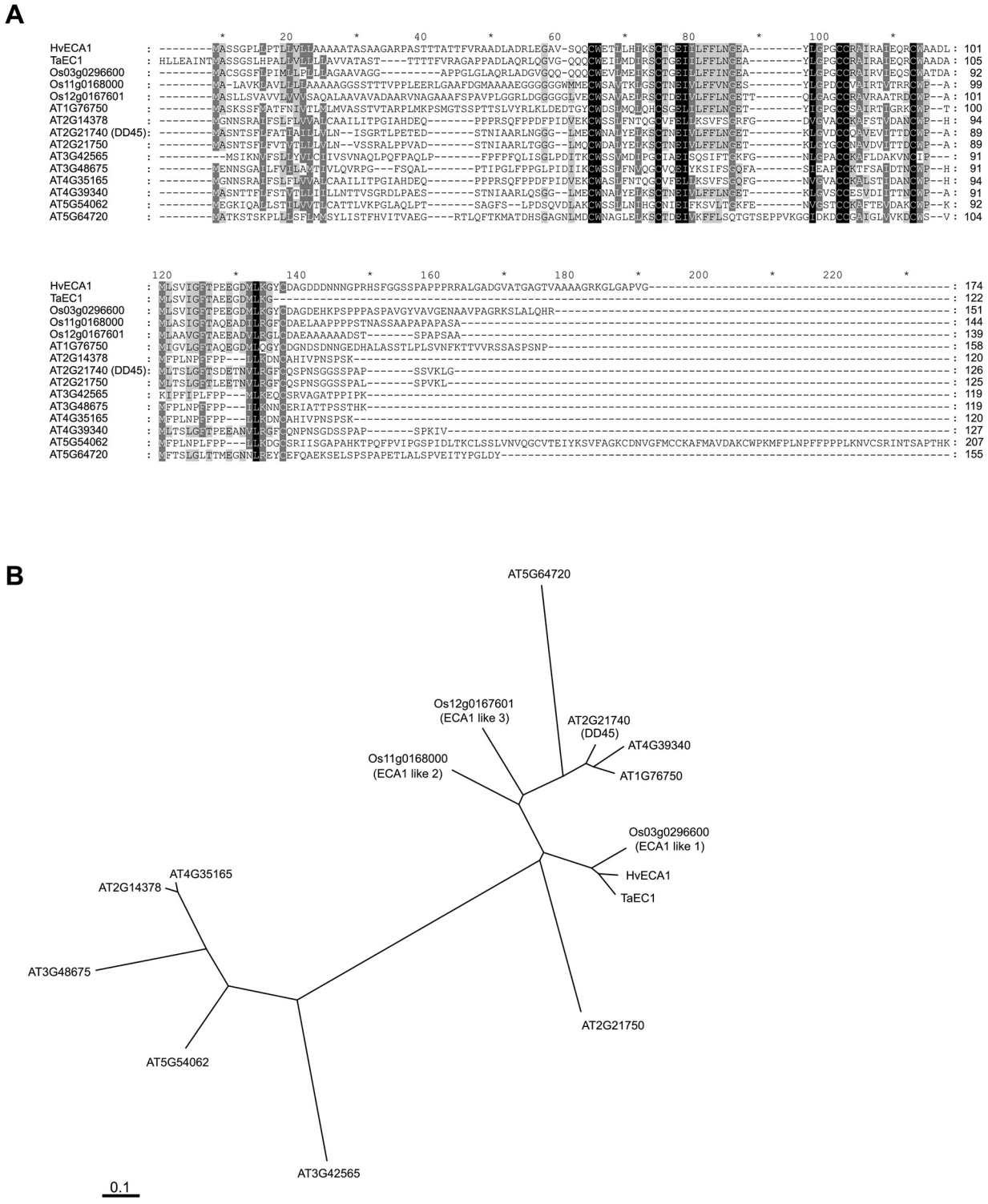
Supplemental Figure S1. How the isolated cells were collected and washed.

Schematic drawing of the chamber used for cell collection and washing. The released egg cell and synergid cell were picked up and transferred into a mannitol droplet with a microinjector. The isolated cells were washed by transferring the cells into fresh mannitol droplets three times.



Supplemental Figure S2. Assessments of integrity of RNA extracted from egg cells and synergid cells.

The quality of the total RNA extracted from egg cells and synergid cells was assessed using an RNA 6000 Pico kit on the Agilent 2100 Bioanalyzer (Agilent Technologies). RNA integrity can be judged by the ratio between the relative peak areas for 18S and 28S ribosomal RNAs and the heights of the broad peaks originating from degradation products. A, B, and C show electropherograms of egg cell RNA of replicates 1, 2, and 3, respectively. D, E, and F show electropherograms of synergid cell RNA of replicates 1, 2, and 3, respectively. Open and closed arrowheads indicate the 18S and 28S ribosomal RNA peaks, respectively.



Supplemental Figure S3. Multiple alignment and phylogenic tree analysis of ECA1 family proteins. A, Multiple alignment of the deduced amino acid sequences of ECA1 family proteins. Black, dark gray, and light gray backgrounds indicate amino acids conserved among 15, more than 11, and 8 sequences, respectively. Sequence comparisons were performed by using the Clustal W multiple alignment program (Thompson et al., 1994) and were displayed with GeneDOC (Nicholas, 1996). B, Phylogenic tree of ECA1 family proteins. Phylogenic tree analysis was performed by using the Clustal X multiple alignment program (Thompson et al., 1997) and the neighbor-joining method (Saitou and Nei, 1987). The phylogenic tree was displayed with the Tree View program (Page, 1996). The scale represents 0.1 substitutions per site. GenBank accession numbers for the proteins used in this analysis are as follows: Hordeum vulgare ECA1, AAF23356; Triticum aestivum EC1, CV973634; Oryza sativa Os03g0296600 (ECA1 like 1), BAF11745; Os11g0168000 (ECA1 like 2), BAF27687; Os12g0167601 (ECA1 like 3), ABA95905; Arabidopsis thaliana AT1G76750, NP_177801; AT2G14378, NP_001077890; AT2G21740 (DD45), ABK28194; AT2G21750, NP_179767; AT3G42565, NP_001078233; AT3G48675, NP_680118; AT4G35165, NP_680764; AT4G39340, ABK28267; AT5G54062, AAU44598; AT5G64720, NP_201277.

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