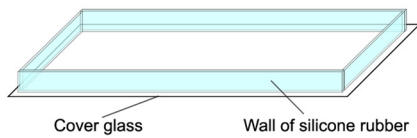
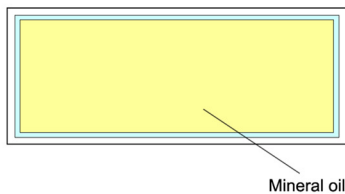


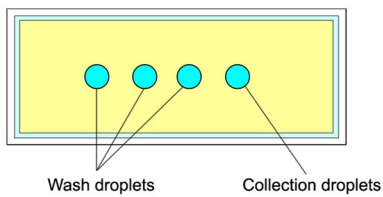
1. Make the wall of silicone rubber on the cover glass.



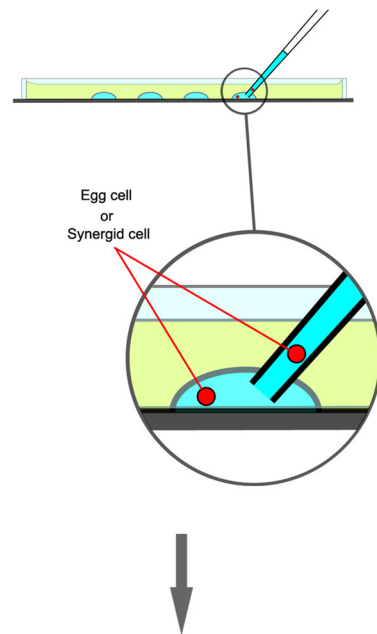
2. Pour mineral oil in the wall.



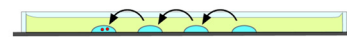
3. Make some mannitol droplets on the cover glass.



4. Put the isolated cell into the collection droplet with a glass capillary.

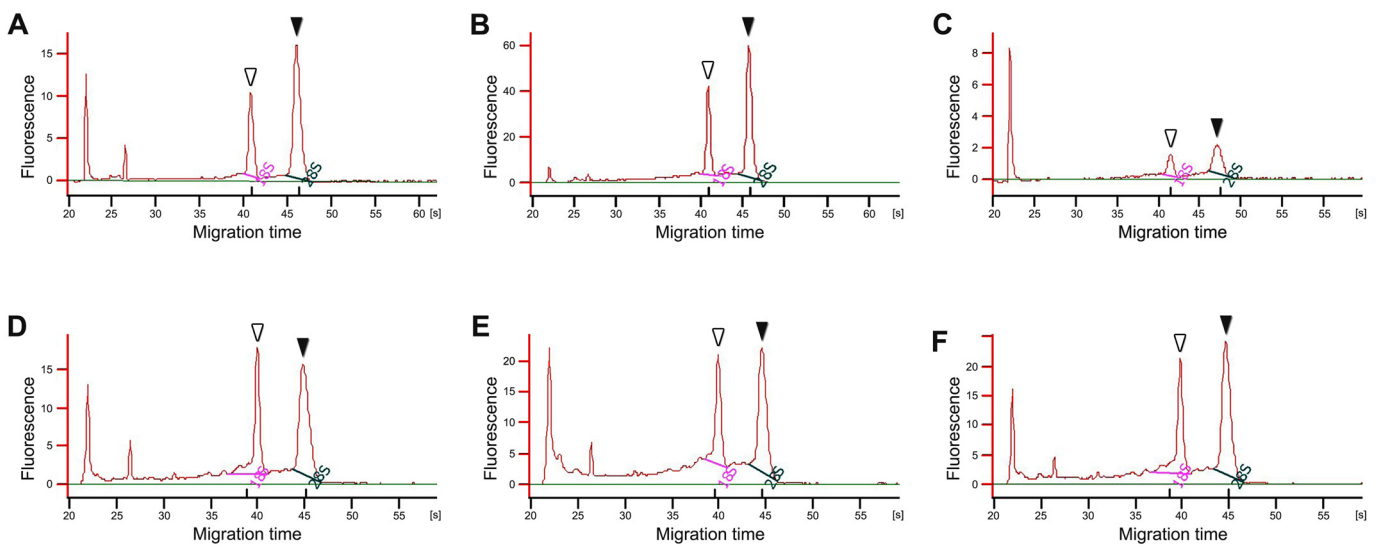


5. Wash cells by moving cells into next droplet.



**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.

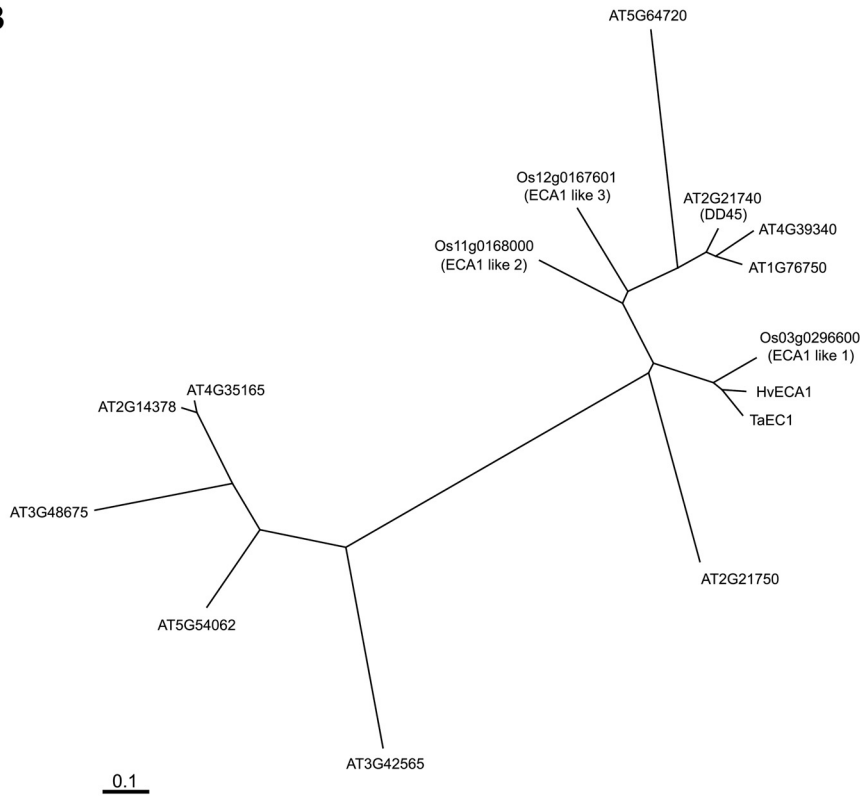
**A**

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*          *          *          *          *          *          *
      20      40      60      80      100
HvECA1 : -----*SSGSLPPTLIVLAAAAATAAAGARPASTTTATTFVRAADLADRLGSAV-SQCSTLHFKSCPCGELILFFLNSEA-----YLPCCCRIRAEQRCHAADL : 101
TaEC1  : HLLLEANLWASSGSLHPALLVLLAVVATATST---TTTTFVRAGAPPADLAQRLOQGV-QQCSTELMDKRSQPCGELILFFLNSEA-----YLPCCCRIRAEQRCHAADL : 105
Os03g0296600 : -----*ACGGSFPIMLLPLLAGAAVAGG---APPGLGQAQLADGVGQQ-QQCSTVEMDKRSQPCGELILFFLNSEA-----YLPCCCRIRAEQRCHAADL : 92
Os11g0168000 : -----*A-LAVKAVLILAAAAAGGSSTTVPLEERLGAAPDMAAAAGGGGGGMMMECSAVTKIGSCNDELVLFFVNES-----YLPCCCRIRAEQRCHAADL : 99
Os12g0167601 : -----*ASLLSVAVVILVWSAQALAAVAVADAARVNAGAAAFSPAFLGRGLDGGGGDILVECSAAVAPKRSQPCGELILFFLNSET---QLGACCRVRAATRECP---A : 101
AT1G76750 : -----*ASKSSVATFNIVTLMLVASSTVADARPLMKGSAFSPPTSLVLYRLKLDDEITGYCWSAMQIQHSCGELILFFLNSEA-----YLPCCCRIRAEQRCHAADL : 100
AT2G14378 : -----*MGNNSRAIFSLFVVAACAILITPGIAHDEQ---PPRSQFPDFPIDVEKCSSEFNTQGVFELIKSVFSRFG---DVEVACCKRSTVDANCP---H : 94
AT2G21740 (DD45) : -----*MASNTSFFATHTLILNLN--ISGRITPETED---STNIAARLNGGG--LMECNALYFKSCNDELVLFFLNSET---KLVVCCQAVVEVITTDCCP---A : 89
AT2G21750 : -----*MASNTSFFVIVLILNLN--VSSRALPPVAD---STNIAARLTGGG--LMCCWDAIYFKSCNDELVLFFLNSET---KLVVCCQAVVEVITTDCCP---A : 89
AT3G42565 : -----*MSIKNPFSLIVLIVSVNAQLPQPPAQLP---FFPFLQISGLPDIITKCSSEFMDIPGCIATISQSIPTKFG---NLEPACCKRSTVDANCP---H : 91
AT3G48675 : -----*MENNSGALFLVIAATVLOVRPG--FSQALP---PTIPGLFPPGLFDIILKCSSEFMDIPGCIATISQSIPTKFG---NLEPACCKRSTVDANCP---H : 91
AT4G35165 : -----*MGNNSRAIFSLFVVAACAILITPGIAHDEQ---PPRSQFPDFPIDVEKCSSEFNTQGVFELIKSVFSRFG---DVEVACCKRSTVDANCP---H : 94
AT4G39340 : -----*MASNTTFFSTVTLILLLNNTVSGRDLPAES---STNIAARLQSGG--LMECNALYFKSCNDELVLFFLNSET---KLVVCCQAVVEVITTDCCP---A : 91
AT5G54062 : -----*MEGRIQALSTLILVITLCATTLVKPGLAQLPT---SAGFS--LPDSQVDLAKCSSEFLMHGCONDELKSVLITKFE---NVESTCKRSTVDANCP---R : 92
AT5G64720 : -----*ATKTSKSPKLLSFLMAYLITSTFHVITVAEG---RTLQFTKMATDHSAGAGLMDLWAGLPLKSKSDELILKFFELISQGTGTSEPPVKGGLDKDCGSLGLVVKDMS--V : 104

      120      140      160      180      200      220      *
HvECA1 : MLSVIGTPEEGDMLRGYDAGDDDDNNNGPRHSFGSSPAPPVRRALGAGVATGAGTVAAGRKLGLGAPV----- : 174
TaEC1  : MLSVIGTAEEGDMLRG----- : 122
Os03g0296600 : MLSVIGTPEEGDMLRGYDAGDEHKPSPPPSPAVGVAVGENAAVPAGRKSLALQHR----- : 151
Os11g0168000 : MLSVIGTAEQADILRFGDAELAAAPPPPTNASSAAPAPAPASA----- : 144
Os12g0167601 : MLSVIGTAEQADVLRGLDAEAAAAAADST---SPAPSA----- : 139
AT1G76750 : MIVGLGTAQEGDMLRGYDGNDSDDNNGEDHALASSTLPLSVNFKTTVRRSSASPSNP----- : 158
AT2G14378 : MFLPLNPFPPP---LLRDNRAHIVNPSPSK----- : 120
AT2G21740 (DD45) : MFLSLGFTSEDTNLRGFGQSPNSGGSSPAP-----SSVKLG----- : 126
AT2G21750 : MFLSLGFTSEDTNLRGFGQSPNSGGSSPAL---SFVKL----- : 125
AT3G42565 : KLPFLPLPPP---MLRERSRVAGATPPPIPK----- : 119
AT3G48675 : MFLPLNPFPPP---LLRDNRAHIVNPSPSK----- : 119
AT4G35165 : MFLPLNPFPPP---LLRDNRAHIVNPSPSK----- : 120
AT4G39340 : MFLSLGFTPEEAVLRGFGQSPNSGGSSPAP-----SPKIV----- : 127
AT5G54062 : MFLPLNPLPPP---LLRDCSRIISGAPAKHTPQPPVIPGSPIDLTKCLSSLVNVQGVTEIYKSVFAGKCDNVGFMCCAKFMAVDAKCKWPKMPLNPFPPPLKNVCSRINTSAPTHK : 207
AT5G64720 : MFLSLGLTMEGNLREYEFQAEKSELSPAPETALSPVEITYPLGY----- : 155

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**B**

**Supplemental Figure S3. Multiple alignment and phylogenetic 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, Phylogenetic tree of ECA1 family proteins. Phylogenetic 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 phylogenetic 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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**Page RD** (1996) TreeView: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* **12**: 357-358

**Saitou N, and Nei M** (1987) The neighbor-joining method – a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**: 406-425

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**Thompson JD, Higgins DG, and Gibson TJ** (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**: 4673-4680