**3:1 Ethanol/Acetic Acid Fixation For Paraffin Sections**

modified from the John Harada Lab

**Name\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Date\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Day One**

Plant Info\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Fix tissue in ice-cold 3:1 (95% EtOH­­­­­­­­­­­­­­­­­­­­­­­­:Acetic Acid) fixative in the afternoon. Vacuume on ice for 15 to 60 min. Label vials with plant info and date of fixation. Fix tissues overnight at 4 degrees.

**Day Two**

Wash tissues 3 times for 5 minutes in 70% EtOH on roato and process immediately. If no time keep at 4 degrees with 70% EtOH **after** EtOH washes. Tissues need to be used for RNA isolation within a month from the date of fixation.

1 hour each:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **% EtOH with water** | **70%** | **85%** | **95%** | **\*100%** | **100%** | **100% overnight** |
| **Time of Change** |  |  |  |  |  |  |

\* if there in not time to do the seies of changes, tissues can stay overnight in the first change to 100%.

**Day Three**

Going into Histoclear at RT while shaking, 2 hours each:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Ratio of Ethanol to Histoclear** | **3:1** | **1:1** | **1:3** | **\*0:1** | **0:1** | **0:1** |
| **Time of Change** |  |  |  |  |  |  |

\* if there is no time to do the series of changes, tissues can stay overnight in the first change to 100%.

**Day Four**

|  |  |
| --- | --- |
| **Time of Changes** | **Changes (on shaker)** |
|  | 100% histoclear, 2 hours |
|  | 100% histoclear + 10-15 paraffin chips |
|  | Add 15 paraffin chips at the end of the day, overnight |

**Day Five**

|  |  |
| --- | --- |
| **Time of Changes** | **Changes (on shaker)** |
|  | Incubate at 42 degrees until paraffin is dissolved, ~1.5 hours. Quarter turn lids before putting into the incubator. |
|  | Remove paraffin: sump histoclear solution and add melted paraffin. Incubate at 60 degrees. Do not close tubes completely. |
|  | Paraffin change at the end of the day. |

**Day Six**

|  |  |
| --- | --- |
| **Time of Changes** | **Changes (on shaker)** |
|  | Paraffin change in morning. |
|  | Paraffin change in late afternoon. |
|  | Paraffin change at night. |

**Day Seven**

|  |  |
| --- | --- |
| **Time of Changes** | **Changes (on shaker)** |
|  | Paraffin change in morning. |
|  | Paraffin change in late afternoon. |
|  | Paraffin change at night, embed next day |

**Comments**