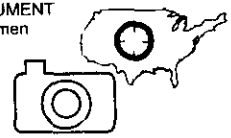
OVERVIEW OF EXPERIMENTAL METHODS

I. COLLECT, DOCUMENT, AND IDENTIFY SPECIMENS

COLLECT specimen

DOCUMENT specimen



IDENTIFY specimen

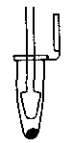
COLLECT tissue sample

II. ISOLATE DNA FROM PLANT OR ANIMAL TISSUE

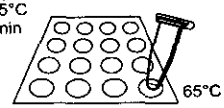
ADD specimen tissue sample

ADD nuclei lysis solution

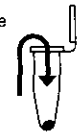
GRIND sample in solution



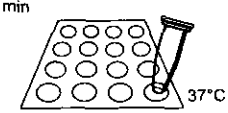
INCUBATE sample at 65°C 15 min



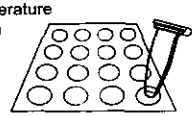
ADD RNAse



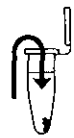
INCUBATE 37°C 15 min



INCUBATE at room temperature 5 min

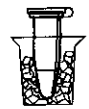


ADD protein precipitation solution

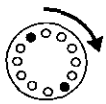


VORTEX

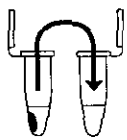
CHILL on ice 5 min



CENTRIFUGE 4 min

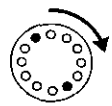


TRANSFER to fresh tube with isopropanol

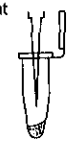


MIX

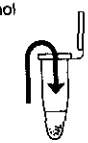
CENTRIFUGE 1 min



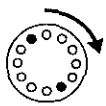
REMOVE supernatant



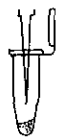
ADD ethanol



CENTRIFUGE 1 min

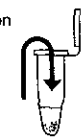


REMOVE ethanol

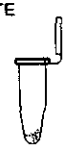


DRY pellet 10 min

ADD rehydration solution



REHYDRATE at 65°C 60 min or 4°C overnight



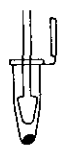
STORE at -20 °C

IIa. ISOLATE DNA FROM PLANT TISSUE (ALTERNATE

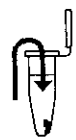
ADD plant tissue

ADD Edward's buffer

GRIND

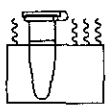


ADD Edward's buffer

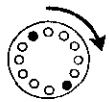


VORTEX

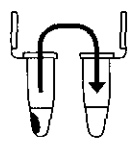
BOIL on heat block 5 min



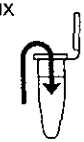
CENTRIFUGE 2 min



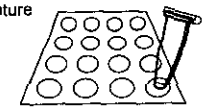
TRANSFER supernatant



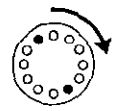
ADD and MIX isopropanol



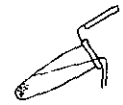
INCUBATE
at room
temperature
5 min



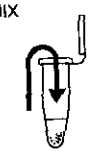
CENTRIFUGE
5 min



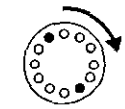
POUR OFF
supernatant



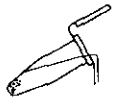
ADD and MIX
ethanol



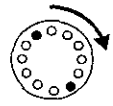
CENTRIFUGE
1 min



POUR OFF
supernatant



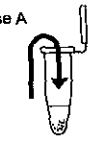
CENTRIFUGE
30 sec



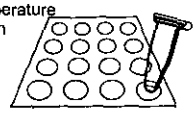
REMOVE
supernatant

DRY
pellet
10 min

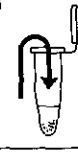
ADD
TE/RNAse A



INCUBATE
at *room*
temperature
5 min

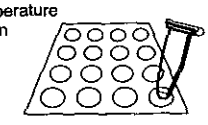


ADD and MIX
water and
sodium
acetate

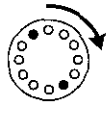


ADD and MIX
isopropanol

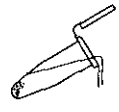
INCUBATE
at room
temperature
3 min



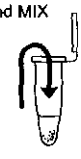
CENTRIFUGE
(5 min)



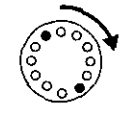
POUR OFF
supernatant



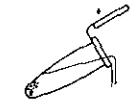
ADD and MIX
ethanol



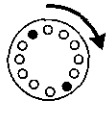
CENTRIFUGE
1 min



POUR OFF
supernatant



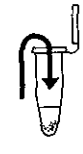
CENTRIFUGE
30 sec



REMOVE
supernatant

DRY
pellet
10 min

ADD
water



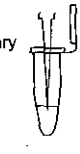
STORE
at -20 °C

III. AMPLIFY DNA BY PCR

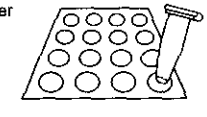
ADD
primer
mix

ADD
DNA

ADD
mineral oil
(if necessary



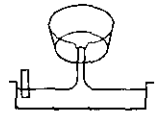
AMPLIFY
in thermal
cycler



STORE
at -20 °C

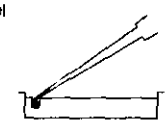
IV. ANALYZE PCR PRODUCTS BY GEL ELECTROPHORESIS

POUR
gel



SET
20 min

LOAD
gel



ELECTROPHORESE
130 volts
30 min

SEQUENCE PCR PRODUCT AND ANALYZE RESULTS

SEND
sample
for
sequencing

ANALYZE
results
using
bioinformatics