APPENDIX

5E Seed Bank Testing Protocol

Seed Bank Composition of Wetlands – Seed Emergence Methodology

Developed by Jeff Lee of Barr Engineering

Field Collection

One composite sediment sample is collected for approximately every 70 acres of wetland area. Five sediment core samples are collected from random representative locations within each ~70 acre parcel of the wetland. The five sediment samples are collected using a 5 cm-diameter (~2") core to a depth of 7 cm (~3").
Sediment from the five cores collected for each area are combined, mixed, bagged as one sample, and stored in a cooler (at ~ 4 °C) for later processing. Samples can be stored wet in plastic bags at 4°C for up to 5 days after collection.

Seedling Emergence

Each composite sample is thoroughly mixed by hand and any large rhizomes removed. The soil from each composite sample is then divided into two equal parts. Each subsample is spread over 2 cm of sterilized potting soil in plastic flats filled with 500 cm3 of sterilized sand.

Samples are placed in a greenhouse with daytime temperatures of ~20° C, night temperature of ~12 ° C and a ~14-hour photoperiod. Alternatively, if this diurnal temperature variation is not possible under greenhouse conditions, flats should be placed outdoors in a covered sunny area that protects the flats from rain but allows full filtered sun.

One control flat per 10 sample flats, containing only sterile loam and sand, are to be placed at random with the samples to test for contamination of samples from greenhouse or nearby seed sources.

One subsample per site the soil is exposed and kept moist by watering once or twice daily to maintain saturated conditions during the assay period of four months. In the other subsample, water levels are kept 2-3 cm (3/4" to 1-1/4") above the soil surface for the duration of the four-month period.

Flats are to be checked frequently, ideally at three to four-day intervals, for newly emerged seedlings to assure that no plants emerge and die between census intervals. Seedlings of vascular plants which can be identified are counted and immediately removed once they reach an identifiable stage. Most viable seeds should germinate within the four-month assay period as past studies have shown that 90% of temperate wetland seedlings emerged within the first three months. Some of the hard to identify seedlings will need to be replanted into 4" pots for further growing to an identifiable stage. Some of the hard-to-identify species may need to be stressed to induce flowering in order to allow for complete identification. At the end of the four month germination period, all remaining unknown seedlings in the flats are transplanted into pots to encourage flowering and grown to an identifiable stage. Species and number of seedlings should be recorded for each flat.

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