VIBRATOME SECTIONING

Need:

Agarose -~3% is good for most roots – use low-strength gel agarose
1-2% for very soft roots
>3% for harder roots and other tissues
Sigma Type V agarose, gels at 35-37°C, melts at >90°C, is OK

For harder tissues, Sigma Type IIB agarose – high strength gel, gels at ~42°C, at ~5% works well

Locktite or other cyanoacrylate-based superglue

Sharp razor blades - single-edged for trimming agarose blocks before glueing down
double-edged regular blades or carbon steel blades for sectioning

Procedure:

1. Melt agarose in microwave, place in 90°C waterbath.
   Place embedding moulds on support platform – above water – in waterbath chamber to warm up.
2. Fill embedding moulds half-way with molten agarose.
   Dry off tissue and lay on top of agarose - tissue must be dry or it will pop out of the agarose during sectioning.
3. Cover tissue with molten agarose, make sure no air bubbles are around tissue, and replace on support platform in waterbath for 5-10 min so agarose makes good contact with tissue.
4. Remove embedding mould from waterbath chamber, allow agarose to set firmly.
   Can place mould in fridge or on ice to speed up setting.
5. When set, pop agarose with tissue out of the mould, and trim so that tissue is exactly vertical (for cross-sections) or horizontal (for longitudinal sections).
   Block needs to be carefully trimmed so that top and bottom are parallel before glueing down.
   Line up on vibratome block first to check before glueing.
6. Glue trimmed agarose block onto centre of black vibratome block with superglue.
7. Insert half of double-edged razor blade, or injection blade, into holder.
   Set blade angle to about 10° in the first instance.
8. Fill vibratome chamber with water or buffer until it just touches the blade.
   Place vibratome block with sample into chuck and clamp firmly.
9. Wind chuck up or down so that the top of the agarose block is just below the razor blade/water surface.
   If necessary, adjust water/buffer level so it just touches the blade and covers the specimen.
10. Set amplitude and speed at about the middle to start with.
    Advance the blade, check that it does not begin to slice off a large chunk of tissue.
11. Advance the blade until it begins to section through the agarose and tissue.
    Use section thickness of 50-200 µm, depending on cell size.
12. Collect each section carefully with small paintbrush or forceps and place on a sticky slide (coated with silane, polyethyleneimine, poly-L-lysine or other adhesive material).
    Keep slides in a moist chamber until sectioning is finished.
13. Stain the sections!!