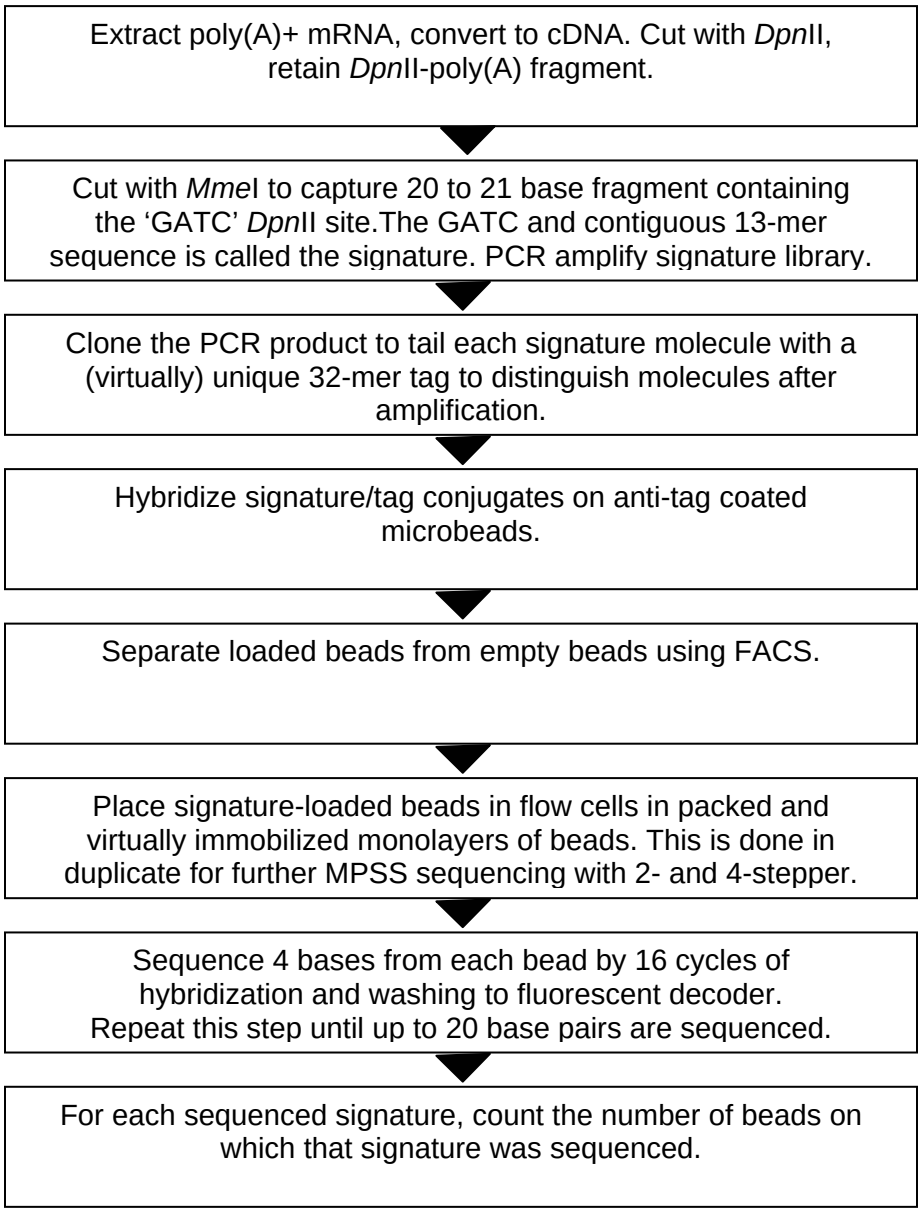
Extract poly(A)+ mRNA, convert to cDNA. Cut with *Dpn*II, retain *Dpn*II-poly(A) fragment.
Cut with *Mme*I to capture 20 to 21 base fragment containing the 'GATC' *Dpn*II site.The GATC and contiguous 13-mer sequence is called the signature. PCR amplify signature library.
Clone the PCR product to tail each signature molecule with a (virtually) unique 32-mer tag to distinguish molecules after amplification.
Hybridize signature/tag conjugates on anti-tag coated microbeads.
Separate loaded beads from empty beads using FACS.
Place signature-loaded beads in flow cells in packed and virtually immobilized monolayers of beads. This is done in duplicate for further MPSS sequencing with 2- and 4-stepper.
Sequence 4 bases from each bead by 16 cycles of hybridization and washing to fluorescent decoder. Repeat this step until up to 20 base pairs are sequenced.
For each sequenced signature, count the number of beads on which that signature was sequenced.