10. Spin down precipitate at 1000*g* for 15 min.
11. Drain tubes well and resuspend the pellet in 1*M* NaCl (1 mL/g starting material) (*see* **Note 5**).
12. Add 2X vol of ethanol to precipitate nucleic acids. Store at –20°C for 30 min. Spin down nucleic acids and rinse the pellet twice with 70% ethanol (*see* **Note 6**).
13. Finally spin down nucleic acids for 5 min at 1000*g*, remove all 70% ethanol, and vacuum-dry pellets for 5 min.
14. Resuspend the pellet in TE (100 µL/g of starting material) (*see* **Note 7**).
15. If RNA-free DNA is required, **steps 16–20** can be followed (*see* **Note 8**).
16. Add 10 µL of RNase stock to the solution and incubate at 37°C for 30 min.
17. Add an equal volume of phenol:chloroform:isoamyl alcohol and emulsify by inversion. Centrifuge at 15,000*g* in a microcentrifuge for 2 min.
18. Remove the aqueous phase to a fresh Eppendorf tube and add 0.05 vol 5*M* NaCl and 2 vol of ethanol. Store at –20°C for 30 min.
19. Spin down nucleic acids and rinse the pellet with 70% ethanol.
20. Remove all 70% ethanol and vacuum-dry pellet for 5 min, before resuspending in TE.
21. Analyze the DNA by fractionating 1 µL on a 1% agarose gel.

3.2. Southern Blotting

3.2.1. Restriction Enzyme Digestion of Genomic DNA and Agarose Gel Electrophoresis

It is important to obtain as much information as possible on the structure and organization of transferred genes within the plant genome. The number of copies of T-DNA, whether they are tandemly linked or dispersed, and the position within the genome can have a great effect on the expression levels and inheritance patterns of introduced genes.

By selecting appropriate restriction enzymes to cut the plant genomic DNA, it is possible to gain information about the organization of the T-DNA within a particular transformant. The number of T-DNA copies can be estimated by two commonly used methods. The first method involves digesting genomic DNA with restriction enzymes that cut twice within the target T-DNA, to yield an internal fragment. The copy number is estimated by calibrating the intensity (following autoradiography) of the internal fragment band against samples of known copy numbers of the foreign DNA (usually plasmid DNA) run on the gel. Genomic DNA from untransformed plants must be added to the plasmid copy number control samples, to provide a similar background to the transgenic sample for hybridization. In the second method, the genomic DNA is digested with a restriction enzyme that cuts once in the target DNA. Here, border fragments are generated, where the second site of cleavage is in the genomic DNA. Since the site of the second cleavage is expected to be different for each independent insertion event, the number of bands observed on the