Assay of in vitro transcription / DNA synthesis activities of organelle-nuclei (nucleoids) isolated from tobacco

1. To start the in vitro transcription reaction, mix the suspension of isolated organelle-nuclei (normally 6 μl) with 1.5 volumes (9 μl) of concentrated assay mixture that is pre-warmed to 26°C. The final reaction mixture contains 40 mM Tris-HCl (pH 7.6), 9 mM $MgCl_2$, 24 μM $(NH_4)_2SO_4$, 0.01% Nonidet P-40, 180 μM ATP, 180 μM GTP, 180 μM CTP, 5 μM [5,6-^{3}H]UTP (0.2 TBq $mmol^{-1}$), and organelle-nuclei equivalent to 10 ng DNA ml^{-1}. In addition, 0.2 M sucrose, 0.2 mM EDTA, 2.8 mM 2-mercaptoethanol, 0.5 mM spermidine, and 0.16 mM PMSF are introduced from the isolation buffer suspending the organelle-nuclei. If DNA synthesis activities are to be examined, replace the ribonucleoside triphosphates with 180 μM dATP, 180 μM dGTP, 180 μM dTTP, and 5 μM [5, -^{3}H]dCTP (0.2 TBq $mmol^{-1}$).
2. Incubate the reaction mixture for the desired duration at 26°C.
3. Spot the reaction mixture onto DE-81 ion exchange filter discs and dry them thoroughly.
4. Wash the DE-81 discs four times in 5% (w/v) Na_2HPO_4, twice in water and twice in ethanol (2.5 ml per disc), and dry them thoroughly.
5. Measure the radioactivity bound to the DE-81 discs by liquid scintillation counting.