Pulsed-Field Gel Electrophoresis of Mammalian Genomic for DNA Damage Determination

Reagents:
Standard Tissue Culture conditions, equipment, and protocols.
BioRad Low Melt Agarose
BioRad MegaBase Agarose
BioRad PFGE CHEF-DRII System
BioRad Plug Molds

Digestion Buffer:
- 1% Sodium Laurel Sarcosine
- 0.2% Sodium Deoxycholate
- 1 mg/mL Proteinase K
- 100 mM EDTA, pH 8.0

1x Wash Buffer:
- 20 mM Tris-HCl, pH 8.0
- 50 mM EDTA, pH 8.0

Protocol:

(1) Harvest Cells via Trypsinization, first rinse cells briefly in 1xPBS, trypsinize and quench with media. Pellet cells, resuspend in 1x PBS.
(2) Count cells with a hemacytometer.
(3) Pellet cells again, flash freeze pellet in Dry Ice/EtOH, transfer to -80°C freezer.
(4) Resuspend pellet in 2% Low Melt Agarose in 0.5xTBE buffer, resuspend each sample so as to equalize cell concentration in the LMT solution, mix well.
(5) While still liquid transfer Cell/LMT solution to Mold Plugs, taking care not to introduce bubbles to plugs.
(6) Solidify plugs by placing on ice.
(7) Lyse cells in plugs, by incubating in Digestion buffer at 55°C for more than 24hrs.
(8) Wash plugs in Wash Buffer, 4x.
(9) Store plugs in 0.1x Wash Buffer.
(10) Prepare 0.7% gel in BioRad MegaBase agarose in 0.5x TBE. Insert plugs, and seal with LMT, so they are stable inside wells. NO BUBBLES.
(11) Add a sufficient volume of 0.5x TBE to the CHEF-DRII apparatus and set to 14°C. Pump strength of 70%.
(12) Transfer gel to CHEF-DRII apparatus.
(13) Run first stage of Electrophoresis: 30 hours, pulse gradient from 30s to 120s, and a field strength of $1.9 V/cm^3$.
(14) Run second stage of Electrophoresis: 51 hours, pulse gradient from 120s to 42 minutes, and a field strength of $1.9 V/cm^3$.
(15) Remove gel from apparatus.
(16) Stain gel in EtBr (0.5 μg/mL) in dH_2O for 30 minutes.
(17) Destain gel in dH_2O for 3 hours.
(18) Image gel