Protein-Profiling of Genomic Instability in Endometrial Cancer

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Two-dimensional gel-electrophoresis

Before application, 75µg of each sample was diluted to 300µL of 7M urea, 2M thiourea, 1% 3-(3-chloramidopropyl)dimethylammonio-1-propanesulfonate (CHAPS), 0,5% immobilized pH gradient buffer (IPG), 18mM dithiothreitol (DTT) and a trace of bromphenol blue. This mixture was applied to a Ready Strip (17cm, pH 4–7 linear; Bio-Rad) and rehydrated at 50V at 20C for 16h. Proteins were isoelectrically focused stepwise from 500V to 8000V for a total of 52,900Vh per gel. Ready Strips were stored at -80°C until second dimension processing.

After isoelectric focusing, the strips were equilibrated for 2 x 15min with 50mM Tris-HCl, pH 8.8, in 6M urea, 30% glycerol and 2% SDS. DTT (1%) was included in the first and iodacetamide (2.5%) in the second equilibration step to reduce SS-bridges and alkylate thiols. We used 10-13% linear acrylamide gradient gels (1.5 x 200 x 230mm) with 1% SDS for second-dimension gel electrophoresis. The IPG strip was placed on the surface of the second-dimension gel and sealed with 0.5% agarose in SDS-electrophoresis buffer (25mM Tris base, 192mM glycine, 0,1% SDS). The gels were run overnight at 100V, 12C, and then fixed for 2x30 min in 30% ethanol,10% acetic acid, and sensitized for 30min in 20% ethanol, 0.5M potassium acetate, and 8.3M potassium tetrathionate. After washing with distilled water, the gels were stained in 0.2% silver nitrate for 120min and washed twice in water for 1min each. Development was performed in a solution of 0.22M potassium carbonate, 0.05mM sodium thiosulfate and 0.009% formaldehyde. After 10min, a termination solution of 5% acetic acid was used to stop development, after which the stained gels were washed three times in water for 5min each. They were then scanned by a flatbed

densitometer (GS 710, Bio-Rad) and analyzed with PDQuest 2-DE analysis software (BioRad, CA, USA, version 8.0.1). Image analysis included spot detection, spot editing, background subtraction, spot matching, and normalization.