Nucleoprotein gel assay for DNA-Rad51 complexes

The assay is based on the difference in electrophoretic mobility of glutaraldehyde-fixed DNA-Rad51 complexes formed at different DNA/protein ratios. The molecular weight of a saturated nucleoprotein filament with a stoichiometry of 3 bp (ntd) per Rad51 is increased approximately 20 (40) times compared to protein-free ds (ss) DNA resulting in a significantly reduced mobility; decreasing the protein/DNA ratio results in a proportional increase of complex mobility.

Materials and stock solutions:

ΦX174 ssDNA (virion), ΦX174 dsDNA (RF I), pUC19 dsDNA from NEB (1 mg/ml)
Rad51 protein
Reaction buffer (1 x): prepare 10 x concentrated stock for use

25 mM triethanolamine acetate (TEA) pH 7.5
13 mM magnesium acetate
1.8 mM DTT
supplemented with: 5 mM ATP (from 0.1 M stock)
100 µg/ml BSA (from NEB 10 mg/ml stock)
optional: ATP regeneration system consisting of 20 mM phosphocreatine and 0.1 µg/µl creatine kinase

20 x concentrated glutaraldehyde (GA), freshly made (5% v/v buffered wth 0.1 M TEA pH 7.5)

Reaction conditions:

Volume: 10-20 µl, 10 µl loaded on gel
30 µM (ntd/bp) ss/dsDNA
bp (ntd)/Rad51: 3/1, 4/1, 6/1, 12/1, 24/1
Incubation:

1. with Rad51, 15 minutes at 30°C
2. add GA, 15 minutes at 30°C

Gel:

1% agarose in 1 x TAE buffer (70 ml agarose per wide tray, 12 or 20 well combs, 2 per tray)
Load 10 µl of material (about 200 ng dsDNA or 100 ng ssDNA) + 1/10 volume of 10 x loading buffer
Run 2 hours at 80 V (4 V/cm)
Stain first with SYBR Gold, make photo, then stain with EtBr.