Volume 16 Number 9 1988 Nucleic Acids Research

Improved hybridization conditions for DNA 'fingerprints' probed with M13

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Submitted March 23, 1988

Wildtype M13 phage DNA has been shown to detect highly variable minisatellite sequences (DNA fingerprints) in humans and several other mammals (1). We report here new hybridization conditions that significantly improve the resolution and consistency of DNA fingerprints obtained with the M13 probe. The conditions suggested by Vassart et al. (1), using the skim milk based "blotto" hybidization cocktail, were found to give inconsistant hybridization and often high levels of background hybridization to the membrane (nitrocellulose or nylon). This may reflect variation in the components of different lots and/or brands of dried skim milk used. These problems are eliminated by the use of prehybridization and hybridization conditions based on SDS, BSA and sodium phosphate (2). These conditions function in a similar manner to "blotto" in that no vertebrate carrier DNA (such as that from salmon sperm) is present during hybridization. This new protocol works equally well with nylon or nitrocellulose membranes and has substantially improved the DNA fingerprints obtained with M13 DNA as a minisatellite probe. The use of this protocol with Jeffreys' 33.15 minisatellite probe has also yielded improved DNA fingerprints in our laboratory. We recommend the following Southern blot protocol: **Southern transfer**: After electrophoresis, soak gel in 1.5 M NaCl, 1.5 M NaOH twice for 15 min. each, followed by two 15 min. washes in 1 M ammonium acetate, 0.04 M NaOH. Before placing the membrane (nitrocellulose (Schleicher and Schuell) or nylon (Zetabind, AMF Cuno)) on the gel, it is wetted in water for 5-10 min. followed by 1 M ammonium acetate for 5 min. Tranfer is carried out overnight in 1 M ammonium acetate/0.04 M NaOH and the filter is dried and baked at 80°C for 2 hr. **Prehybridization**: Wet the filter in 5xSSC briefly, then place in a heatseal bag containing 7% SDS, 1mM EDTA (pH 8.0), 0.263 M Na_2HPO_4 and 1% bovine serum albumin (fraction V) [0.5 M Na_2HPO_4 (ph 7.2) stock is composed of 134 g of $Na_2HPO_4 \cdot 7H_2O$ and approximately 4 ml of 85% H_3PO_4 per liter]. We use 10 ml for a 400 cm^2 filter. Prehybridization is carried out at 60°C overnight. **Hybridization** is carried out in the same solution at 60°C for 24-48 hr with the addition of ^{32}P-labeled probe (we used random-primed probes). **Washes**: For M13 probe: 1) Twice each for 15 min. in 2xSSC, 0.1% SDS at room temperature, followed by 2) one 15 min. wash in the same solution at 60°C. 3) The filter is rinsed briefly at room temperature in 1xSSC before being wrapped for exposure to film. For Jeffreys' 33.15 probe: As above for wash 1, but the temperature of washes 2 and 3 are both raised to 65°C and wash 3 lasts for 30 min. Exposure times are typically 1 day with 2 intensifying screens and 6-7 days with no screens. Under the latter wash conditions, we detect very little cross-hybridization of minisatellites in indigo buntings (Passerina cyanea) by Jeffreys' 33.15 probe (a repeated core sequence cloned in M13) and wildtype M13. Use of probes specific to the core sequence of the 33.15 probe should allow detection of minisatellite sequences distinct from those detected by random-primed M13 DNA.

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