#2. You perform a Southern blot in which your probe should hybridize to a single DNA band.

**Blot I:** Name **THREE** possible problems that could cause this (blank blot, no bands).

1. Failure of DNA to transfer to membrane
2. Forgot to bake membrane & DNA washed off
3. Didn’t digest enough DNA to detect
4. Wrong probe (sequence doesn’t match your target)
5. Bad probe (not properly labeled, or label has decayed)
6. Probe concentration too low
7. Conditions too stringent (temp too high, salt too low)
8. Hybridization too brief
9. Didn’t denature the DNA or the probe
10. MORE!!!

**ANOTHER ATTEMPT:**

**Blot II:** Name **ONE** problem that could cause this kind of experimental failure.

1. Inadequate prehybridization (regions of membrane were left unblocked)
2. You touched the membrane with ungloved hands.
   
   **NOTE:** This would be because probe stuck to oils or other debris from your hands. It would NOT be because DNA from cells on your fingers hybridized to the probe. {Why? Even if the DNA in your cells was complementary to the probe, it is not available for hybridization as it is inside cells, and not denatured.}

3. Inadequate washing

**ANOTHER ATTEMPT:**

**Blot III:** What could you do to correct this experimental failure?

1. Increase stringency of hybe & washes (increase temp, decrease [salt])
2. Use a different probe sequence
3. Do more washes

Increasing probe length alone will NOT correct this problem. You would also need to increase stringency (a longer probe under the same conditions will still bind to these 3 DNAs).