<u>Ethanol precipitation of DNA with salts - Theory.</u>

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The purpose of adding salts is to neutralize the charge on the sugar-phosphate backbone of the DNA.

Ethanol's task is a little more complex than "removing" the water. For a precipitation, you're interested in forming ion pairs between the polyanion (DNA) and the cation (Na+, Mg++, etc).

In dilute aqueous solution, DNA and counterions like Na+ and Mg++ are more or less in the free ion form rather than the ion pair form (that is, each ion is surrounded by one or more layers of water molecules).

Water is a high dielectric insulator, which means that the electrostatic force between two ions of opposite charge is very low in water:

Adding organic solvent "decreases" the dielectric constant of the solution (in this case water no longer insulates individual ions; anion and cation form an ion pair and promptly pop out of solution.

NaCl increase the stability of DNA duplexes, although you might expect salts to interfere with hydrogen bonds, rather than strengthen them.

The Na+ neutralizes the charge on DNA. Each strand of DNA has an enormous charge density (charge per unit volume), so the two strands tend to push each other apart.

Cations added to the solution form a "cloud" of positive charges around the DNA. This cloud of counterions lowers the effective charge density and relieves the repulsion between the strands.
As for the effect of salt on hydrogen bonds; the hydrogen bonds formed between bases in duplex DNA contribute little to the stability of the duplex.

For an interaction to stabilize the duplex, the interaction between bases must be stronger than the interaction of the bases with water (if bases are not paired with one another in a duplex, then they are surrounded by water). Hydrogen bonding between the bases is of the same energy (sometimes even less) than the hydrogen bonds the same bases would form with water if the DNA were single-stranded.

So, what drives DNA strands together? Entropy and enthalpy.
Entropy in the form of "hydrophobic" interactions between the bases (those big aromatic rings are quite hydrophobic).
Enthalpy in the form of favourable, stabilizing interactions between the electrons of the aromatic rings of bases as they stack on top of one another.

So, what do hydrogen bonds do, if they don't stabilize the duplex?
The H-bonds do contribute *something*: GC base pairs with three H-bonds are harder to melt than AT pairs with two.
Thus they enforce the *specificity* of base pairing. Correct base pairing is nice, but doesn't add much

Isopropanol or alcohols decrease the dielectric constant of the solution, as seen above water has a high dielectric constant so in an aqueous solution, DNA and counter ions like Na+ and Mg++ are in the free ion form rather than the ion pair form i.e. they are surrounded by one or more layers of water molecules.

Adding organic solvent decreases the dielectric constant of the solution so anion and cation form an ion pair and result in precipitation of DNA.

Adding NaAce at pH 5.5 is to help the DNA into ionized form which is more soluble in water.

Alcohol will dehydrate the DNA to bring it into the insoluble form. Less DNA will be dissolved in water fully, and more water molecules are left over, so to disturb this water needs more alcohol.
If you add NaAce, this will also involve water:NaOAc interactions and intrupts the DNA:water intraction. This puts the DNA in a more ionised form, so there is a requirement for only 1.5 to 2 fold alcohol to precipitate the DNA.
In high conc. of DNA the water DNA intraction is high and “left over” water molecule availability is very low so there is no requirement for NaAce or any other salt.

Some time you will need more alcohol to precipitate some nucleotides; for example small mol. RNA needs 2.5 to 3 fold at pH4.5.

DNA is soluble in a hydrophilic solvent because it interacts with the solvent molecules.
Since ethanol has more affinity towards water molecules than DNA/RNA, it breaks the interaction of these two molecules and it itself associates with the water molecule resulting in the precipitation of DNA/RNA (it is equivalent to salting out).
Capacity to precipitate increases with the " C" no. of the alcohol i.e., methanol (CH3OH) < ethanol (CH3CH2OH) < propanol (CH3CH2CH2OH) . But evaporation decreases with C no.
Since ethanol can precipitate DNA fairly and evaporates quickly (compared to propanol), it is preferred for this reason, but propanol requires smaller volumes than ethanol so is often used for convenience.