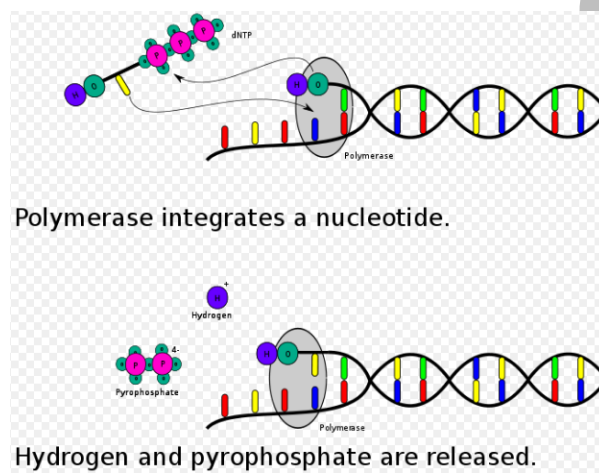


# Ion Torrent

## Sudip Samadder, PhD

Ion semiconductor sequencing is a method of DNA sequencing based on the detection of hydrogen ions that are released during the polymerization of DNA. This is a method of "*sequencing by synthesis*", during which a complementary strand is built based on the sequence of a template strand.

**Sequencing chemistry:** The incorporation of a deoxyribonucleoside triphosphate (dNTP) into a growing DNA strand involves the formation of a covalent bond and the release of pyrophosphate and a positively charged hydrogen ion.

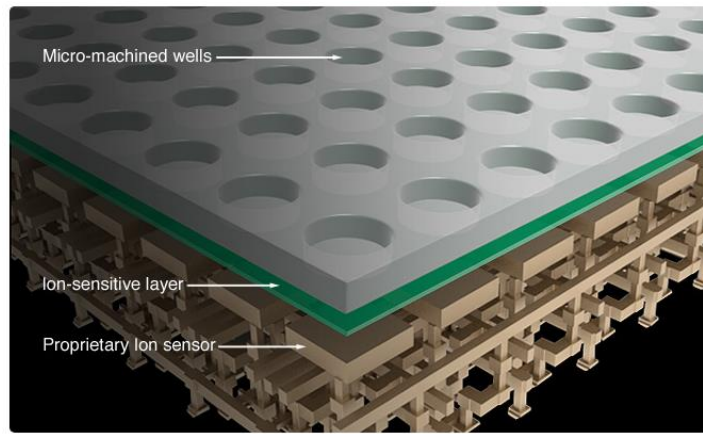


A dNTP will only be incorporated if it is complementary to the leading unpaired template nucleotide. Ion semiconductor sequencing exploits these facts by determining if a hydrogen ion is released upon providing a single species of dNTP to the reaction.

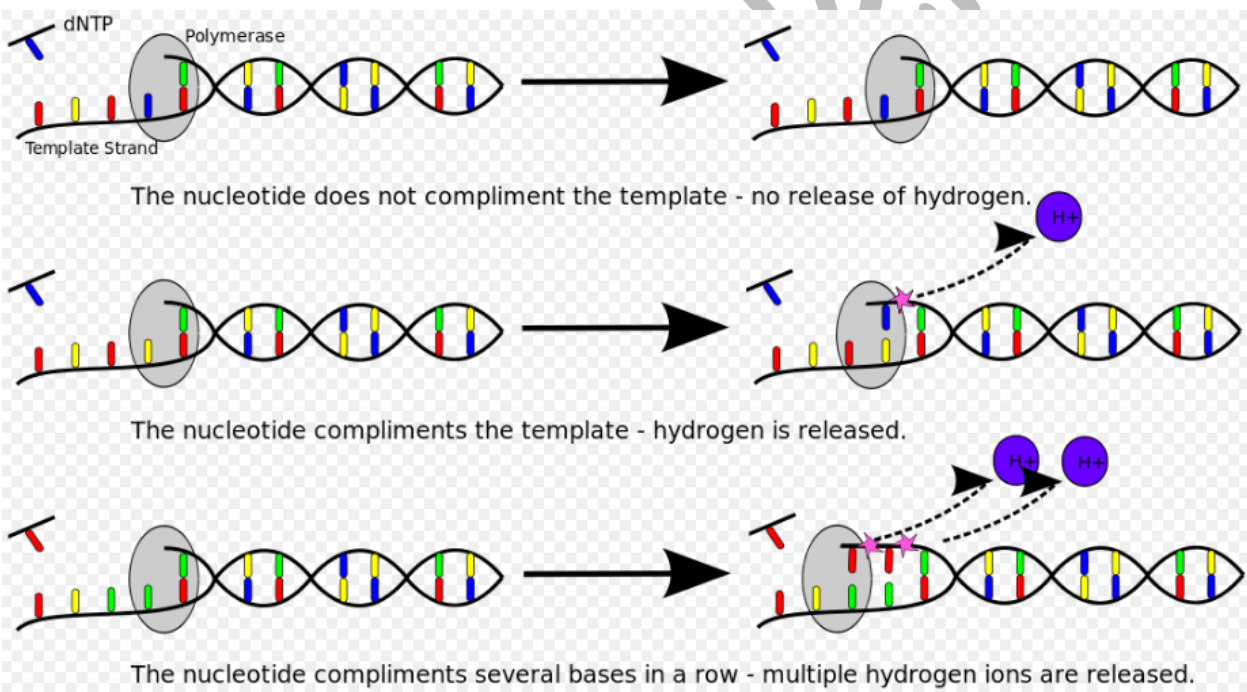
### Procedure

1) **Emulsion PCR:** Emulsion PCR allows for enriched beads to be placed in microwells (see micro-machined well). Just underneath these microwells are pH sensors that are able to detect the most miniscule changes in pH.

2) **dNTP's flooded:** Microwells on a semiconductor chip that each contain many copies of one single-stranded template DNA molecule to be sequenced and DNA polymerase are sequentially flooded with unmodified A, C, G or T dNTP. If an introduced dNTP is complementary to the next unpaired nucleotide on the template



strand it is incorporated into the growing complementary strand by the DNA polymerase. If the introduced dNTP is not complementary there is no incorporation and no biochemical reaction. The unattached dNTP molecules are washed out before the next cycle when a different dNTP species is introduced.



**3) Signal detection:** The signal is picked up by the **ISFET** sensor and translated into a base call.

**4) Wash and repeat:** Unattached dNTP molecules are washed out, and the cycle repeats with a new dNTP.

**ISFET:** ion-sensitive field-effect transistor, that is a field-effect transistor used for measuring ion concentrations in solution; when the ion concentration (such as  $H^+$ ) changes, the current through the transistor will change accordingly. Here, the solution is used as the gate electrode. A voltage between substrate and oxide surfaces arises due to an ion sheath.