METAGENOMIC AND NGS

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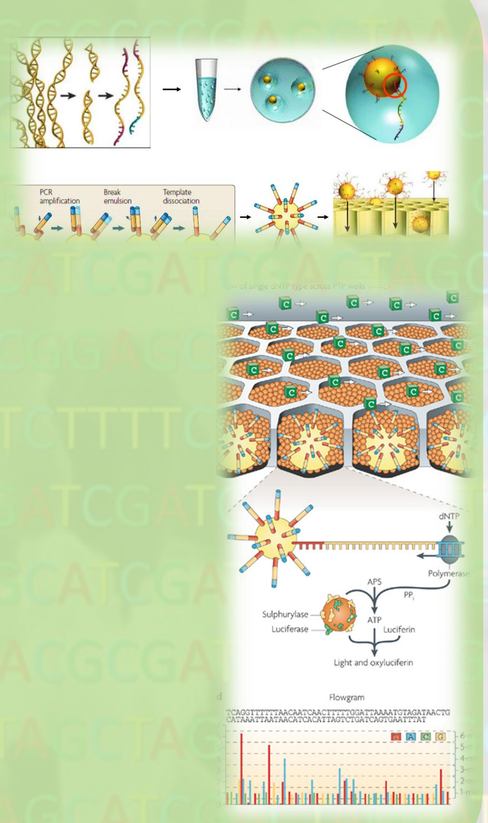
NEXT GENERATION SEQUENCING

454 - ROCHE

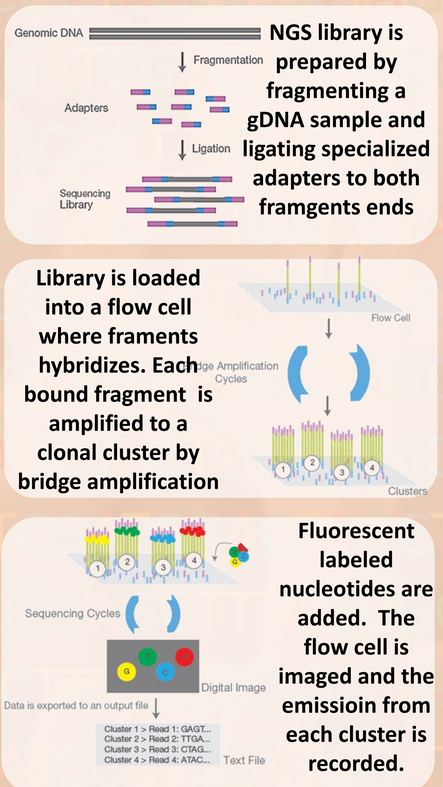
PYROSEQUENCING



ILLUMINA

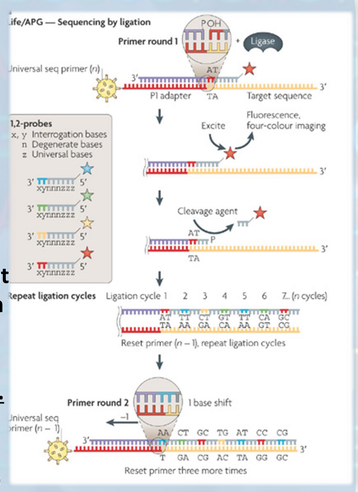
SEQ BY SYNTHESIS

LIBRARY CLUSTER SEQ.



SOLID

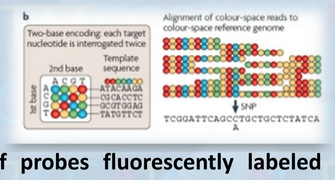
SEQ BY LIGATION



Library is prepared. An universal adaptor P1 is used so that the starting sequence of every fragment is both known and identical.

<u>Emulsion PCR.</u>

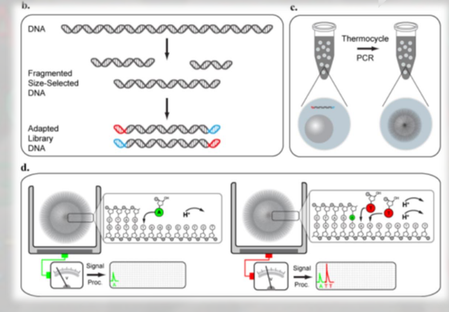
Primers hybridizing to the adapter sequence mold library is added



Four types of probes fluorescently labeled two bases compete for binding to the first used for sequencing . Specificity is achieved by interrogating each first and second base in the ligation reaction . several cycles are performed . The product is removed and the mold is reused with a first complementary to the n-1 position to a second round of cycles

ION TORRENT

Prepare Genomic Library | Prepare Template on Bead | Sequence on Ion Chip | Signal processing and Base Calling



First, DNA is isolated and cut into smaller fragments , which bind small balls called bead. Each , together with millions of DNA fragments enter the microwells of a microchip. Then the micro- chip with a solution of one of nucleotides bathes. If the nucleotide is incorporated into the DNA chain ,a proton is released. This process is repeated every 15 seconds with a new solution of each nucleotide.

METAGENOMIC

TIMELINE

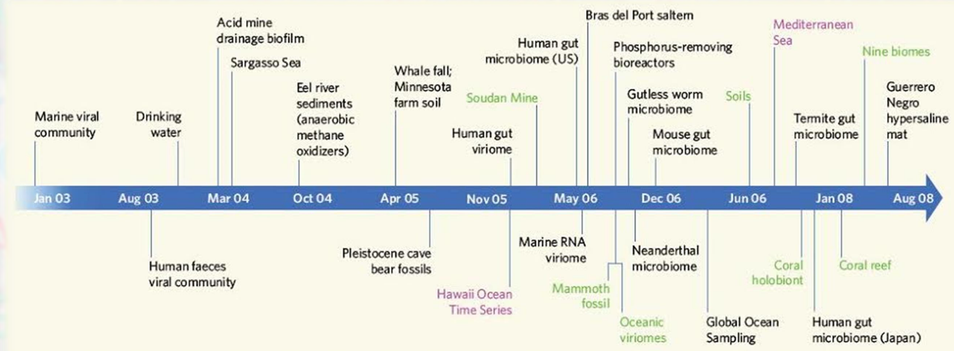


Figure 1 | Timeline of sequence-based metagenomic projects showing the variety of environments sampled since 2002. The oceanic viriomes (all viruses in a habitat) (August 2006) were from the Sargasso Sea, Gulf of Mexico, coastal British Columbia and the Arctic Ocean. The nine biomes (March 2008) were stromatolites, fish gut, fish ponds, mosquito viriome, human-lung viriome, chicken gut, bovine gut and marine viriome. The different technologies used are dye-terminator shotgun sequencing (black), fosmid library sequencing (pink) and pyrosequencing (green). (Graphic based on data sets represented at www.genomesonline.org.)

STRATEGY

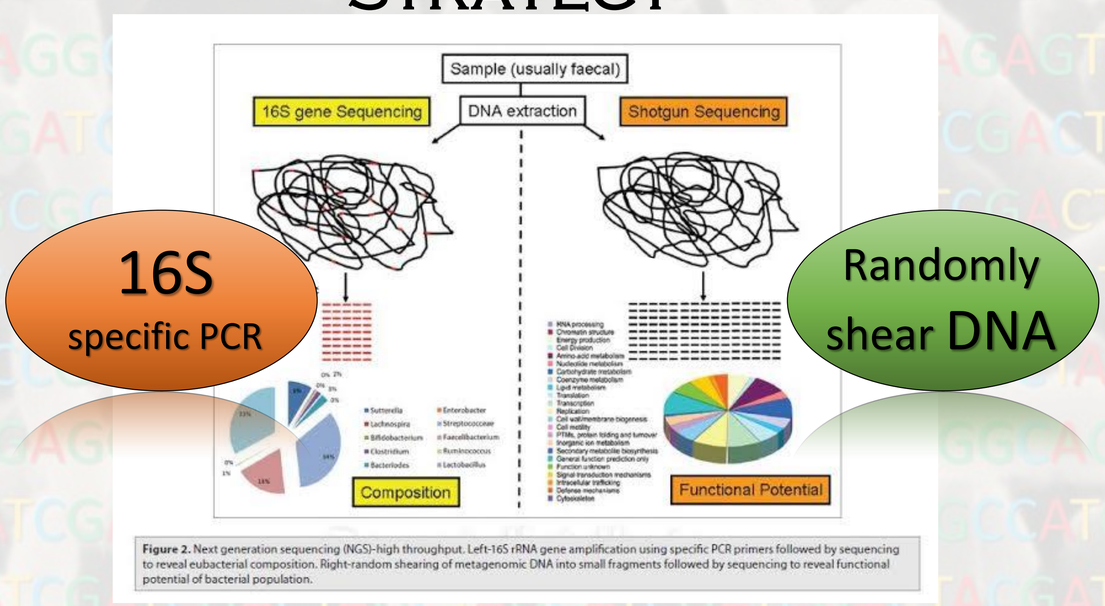


Figure 2. Next generation sequencing (NGS)-high throughput. Left-16S rRNA gene amplification using specific PCR primers followed by sequencing to reveal eubacterial composition. Right-random shearing of metagenomic DNA into small fragments followed by sequencing to reveal functional potential of bacterial population.

APPLICATION

1. Meta genomics allows to **identify most microorganisms cannot be grown in laboratory**.

2. Meta genomics provides a view not only of the community structure (species richness and distribution) but also of **the functional (metabolic) potential of a community**.

3. Meta genomics can analyze any environment as long as nucleic acids can be extracted from sample material. However, most interest has centred on the marine environment (the largest meta genomic study to date is *the Global Ocean Sampling Expedition*) and in medicine (of particular note is an international initiative, *the Human Microbiome Project*, which aims to map human-associated microbial communities)

4. Metagenomics **can be used for viruses, eukaryotes and prokaryotes.**

5. Meta genomics has a **high potential for serendipitous discovery**. For instance, discoveries such as proteorhodopsin proteins or archaeal ammonia oxidizers.

6. Meta genomics **can reconstruct whole genomes from an environmental sample** by means random sequencing.

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