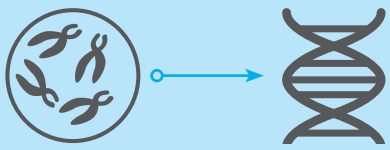
NEXT GENERATION SEQUENCING

ANZPAA
Australia New Zealand Policing Advisory Agency

NIFS
National Institute of Forensic Science Australia

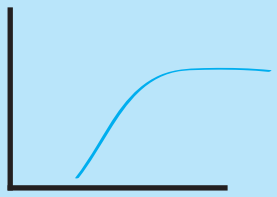
1 DNA EXTRACTION

Release DNA from inside cells.



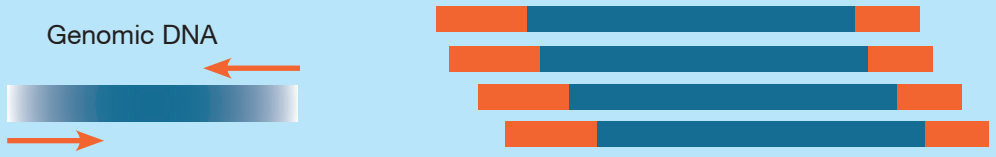
2 DNA QUANTITATION

Measure the amount of DNA obtained.



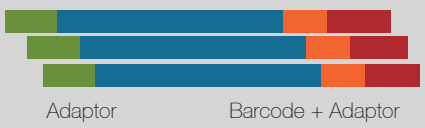
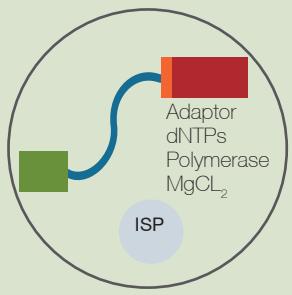
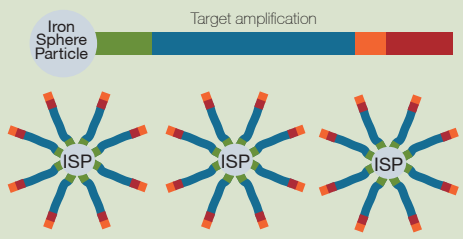
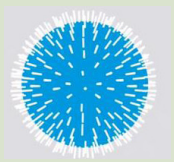
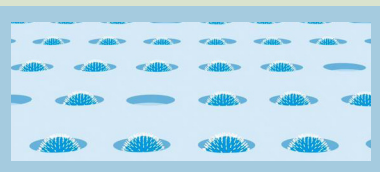
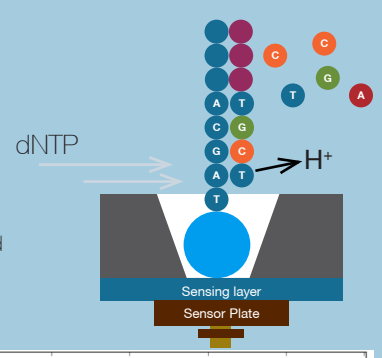
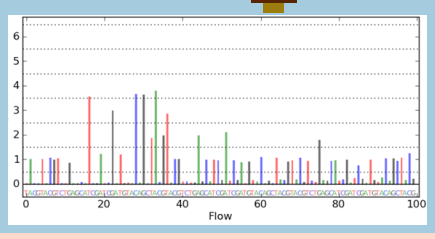
3 DNA AMPLIFICATION

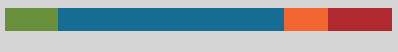
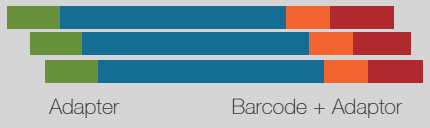
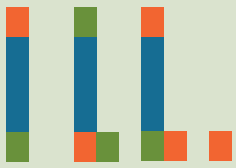
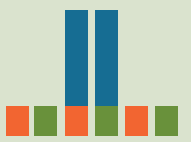
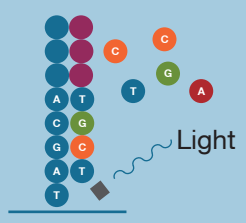
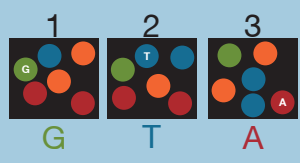
Replicate DNA using forensically relevant primers to target specific sites.



ION TORRENT (Life Technologies)

MISEQ (Illumina)

PROCESS	STEP	EXPLANATION	DIAGRAM
4 LIBRARY PREPARATION	Create blunt ends		
	Add adapters and barcode		 Barcode plus adapter Adaptor
	Clean-up	Removes excess chemicals	
	Library		 Adaptor Barcode + Adaptor
5 CLONAL AMPLIFICATION	Clonal Amplification by Emulsion PCR	DNA fragments bind to Ion Sphere Particles (ISP)	 Adaptor dNTPs Polymerase $MgCL_2$ ISP
		DNA is replicated and new fragments bind to ISPs.	 Iron Sphere Particle Target amplification ISP ISP ISP
	Clean-up	Removes oil and excess chemicals from the PCR reaction.	
6 SEQUENCING REACTION		Load Spheres onto semi-condensed chip into individual wells.	
		Wells are flooded and drained successively with dNTPs.	 dNTP H^+ Sensing layer Sensor Plate
		Addition of a nucleotide causes a change in pH. The higher the voltage charge the more nucleotides that are added in the sequence.	
		Ionogram is produced.	 Flow
7 DATA ANALYSIS		Quality sequencing checking is performed. Sequence data is aligned and corrected. Target sequence genotype is obtained.	

STEP	EXPLANATION	DIAGRAM
Repair ends and add A overhang		
Add adapters and barcode		 Barcode plus adapter Adaptor
Clean up	Removes excess chemicals	
Library		 Adapter Barcode + Adaptor
Clonal Amplification by Bridge Amplification	Hybridise fragments to glass flow cell	
	Single-stranded DNA flips to bind to molecule to form a bridge, fragment extended by polymerase	
	Double-stranded bridge denatured leaving two single stranded fragments	
Cluster generation	Process repeated to form clusters of fragments	
Clean up	Reverse strands cleaved and washed away (leaving forward strands in clusters on flow cell)	
Sequencing Reaction	Sequencing primer added	
	Perform sequencing on forward strands. As dNTPs are added, fluorescence is emitted. Light emission is measured on a detector.	 Light
	Regenerate DNA fragments for reverse strand on flow cell.	
	Sequencing is repeated on reverse strands. Light signals are converted to sequence.	 1 2 3 G T A

Produced by: ANZPAA NIFS 2014. With thanks to Life Technologies and Illumina for their generous assistance and use of images.