Next generation DNA library construction (LC) for high-throughput genomics

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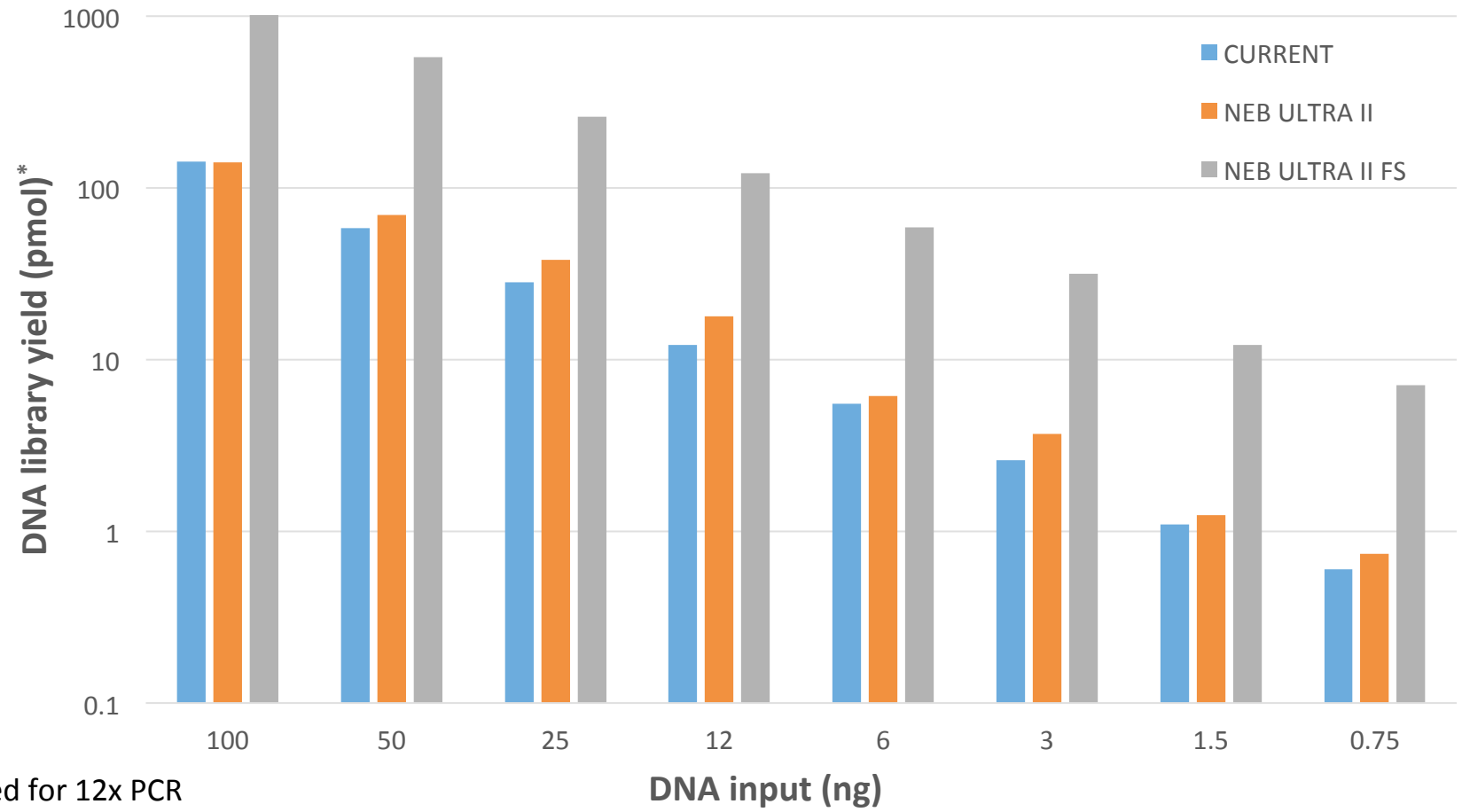
ULTRA II FS produced 10-15x more DNA library compared with other methods

DNA library yields with different LC workflows



*Normalised for 12x PCR

Human genomic DNA was subjected to DNA library construction using an existing DNA library construction workflow (CURRENT), NEB Ultra II or NEB Ultra II FS. Input for CURRENT and NEB ULTRA II was mechanically-sheared DNA. Input for NEB ULTRA II FS was intact genomic DNA. Adapter-ligated libraries were amplified by PCR (4-12 cycles), purified and quantitated using the Agilent Bioanalyzer platform. Values obtained were used to normalise DNA library yield from 12 cycles of PCR.

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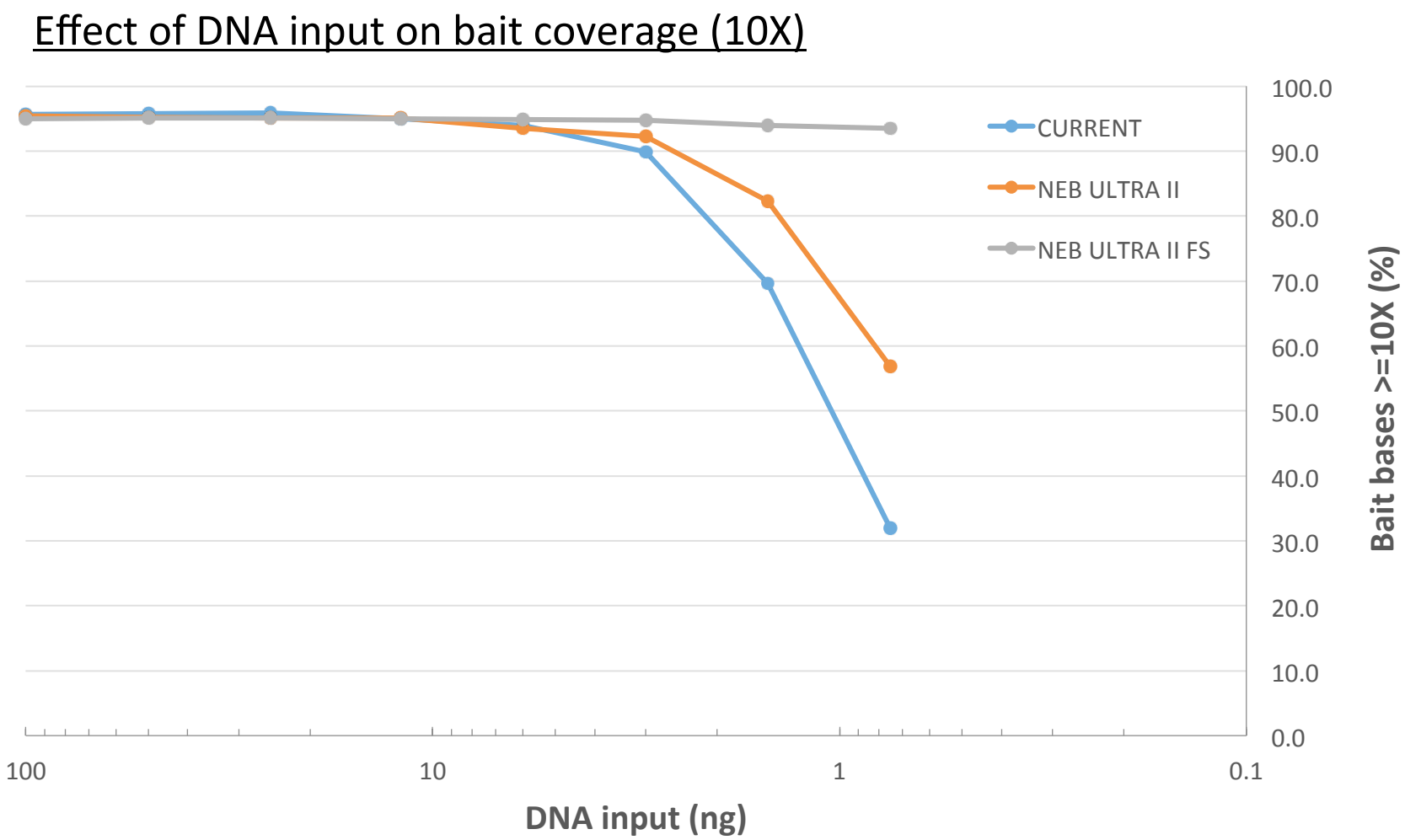
ULTRA II FS dramatically reduced DNA input required for high Q exome data



DNA libraries prepared using different DNA inputs and LC methods were subjected to exome enrichment using the SureSelect platform (Exome v5; Agilent Technologies). Captured libraries were subjected to 75 base PE sequencing using the HiSeq 2500 platform (v4 chemistry; Illumina). Data were normalised to approximately 50 million reads per sample prior to analysis.

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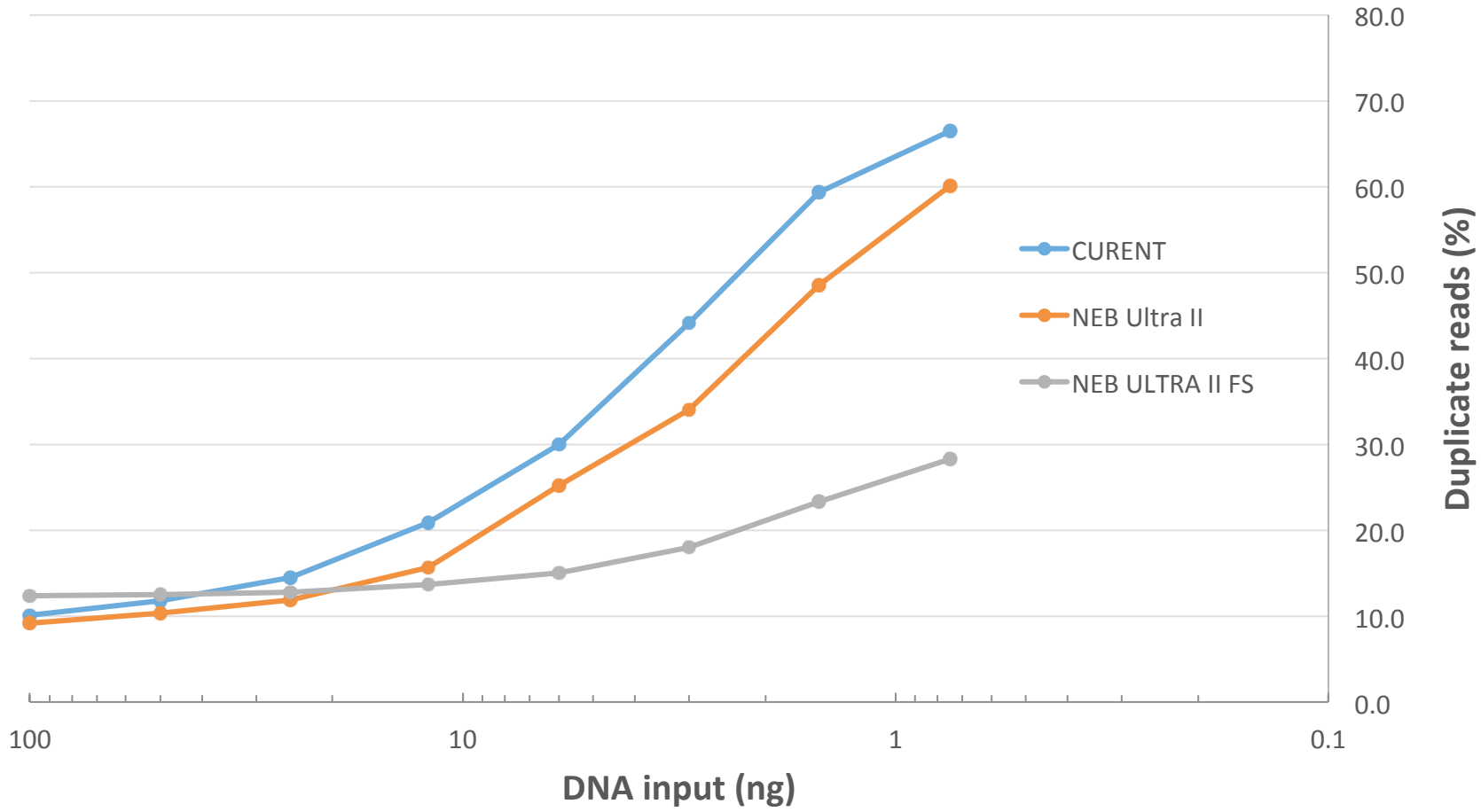
Effect of DNA input on duplicate rates



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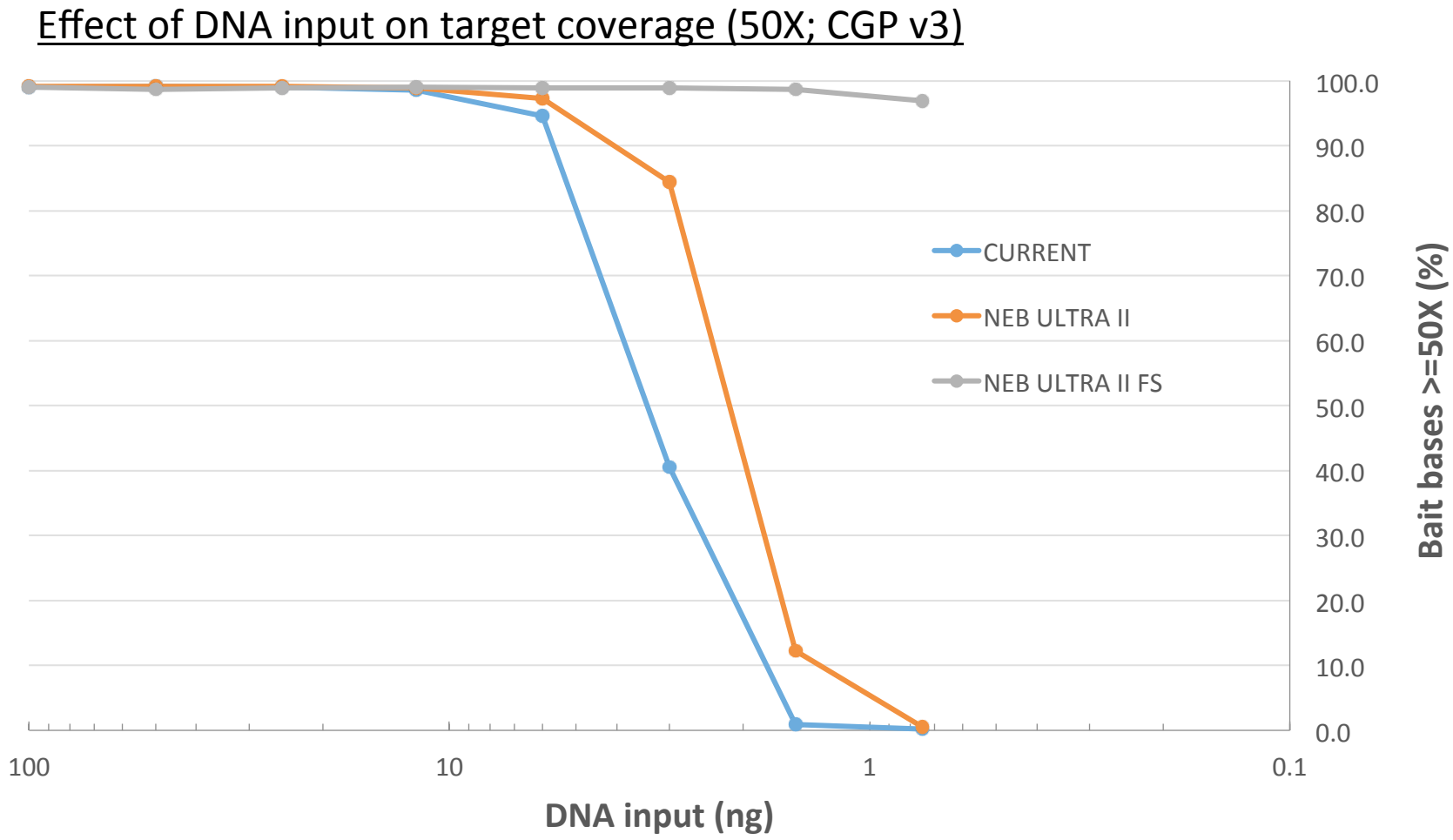
ULTRA II FS dramatically reduced DNA input required for WTSI Cancer panel



DNA libraries prepared using different DNA inputs and LC methods were subjected to custom target enrichment using the SureSelect platform (CGP v3; Agilent Technologies). Captured libraries were subjected to 75 base PE sequencing using the HiSeq 2500 platform (v4 chemistry; Illumina). Data were normalised to approximately 30 million reads per sample prior to analysis.

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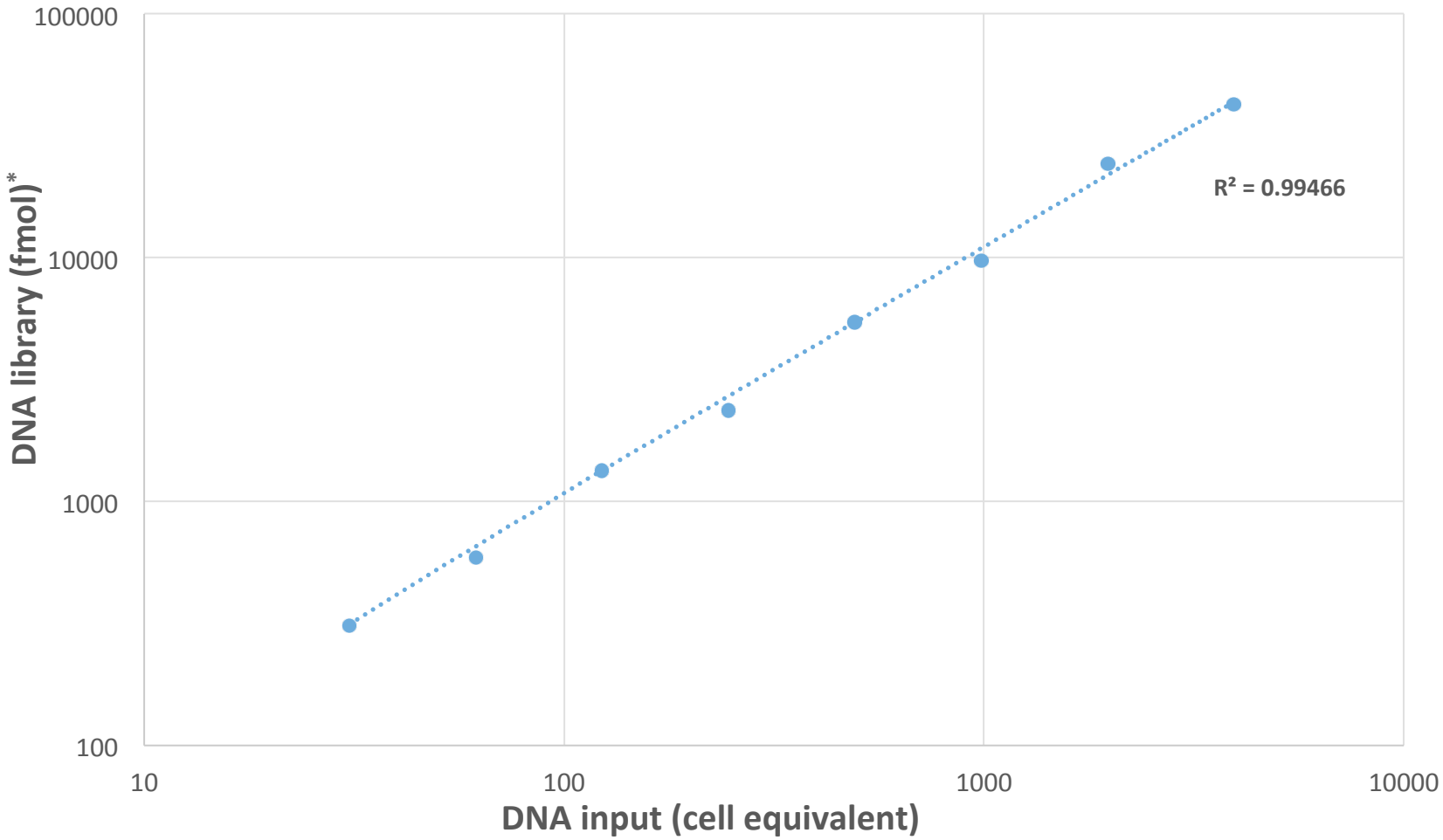
ULTRA II FS: DNA library yield v DNA input



Human genomic DNA of variable input was used to establish DNA library yields using Ultra II FS (normalised for 14 cycles of PCR; see graph). This standard curve of input v. yield was used to inform on the efficiency of genomic DNA extraction from laser-captured micordissection (LCM) material and guide protocol development for steps such as tissue embedding, sectioning, staining and subsequent cell lysis and DNA extraction.

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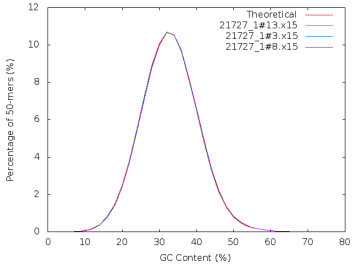
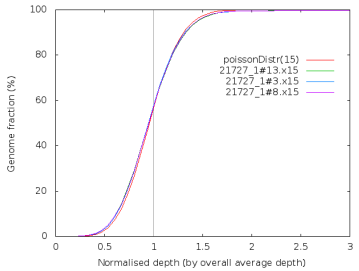
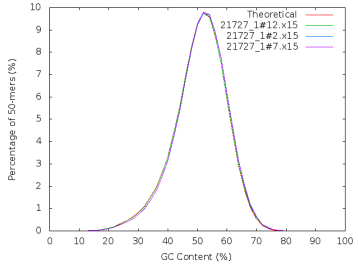
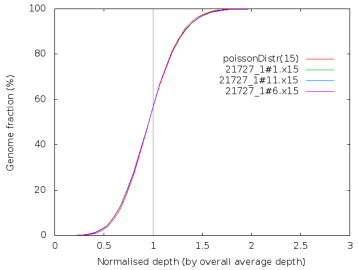
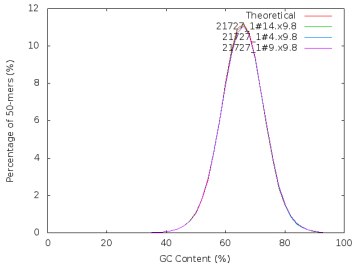
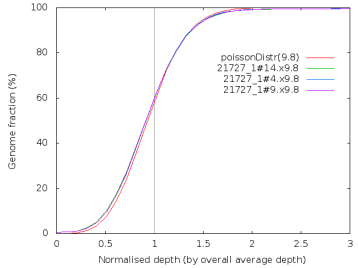
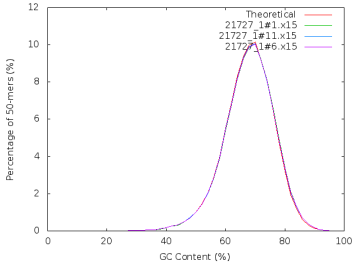
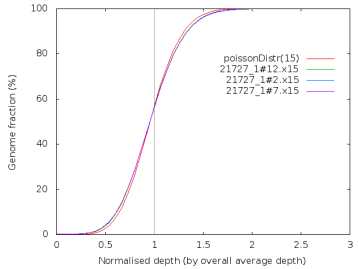
ULTRA II FS performance matches existing LC workflows for a broad range of genomes

	GC content	Genome coverage
Staphylococcus aureus		
Escherichia coli		
Mycobacterium		
Bordetella pertussis		

E. coli genomic DNA (50 ng) was subjected to automated DNA library construction using the Bravo platform (Agilent Technologies) and Ultra II FS reagents. Amplified libraries were purified and quantified prior to 100 base PE sequencing on the MiSeq platform (Illumina).

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