

NXTGNT development of next generation molecular diagnostics and epigenetic profiling

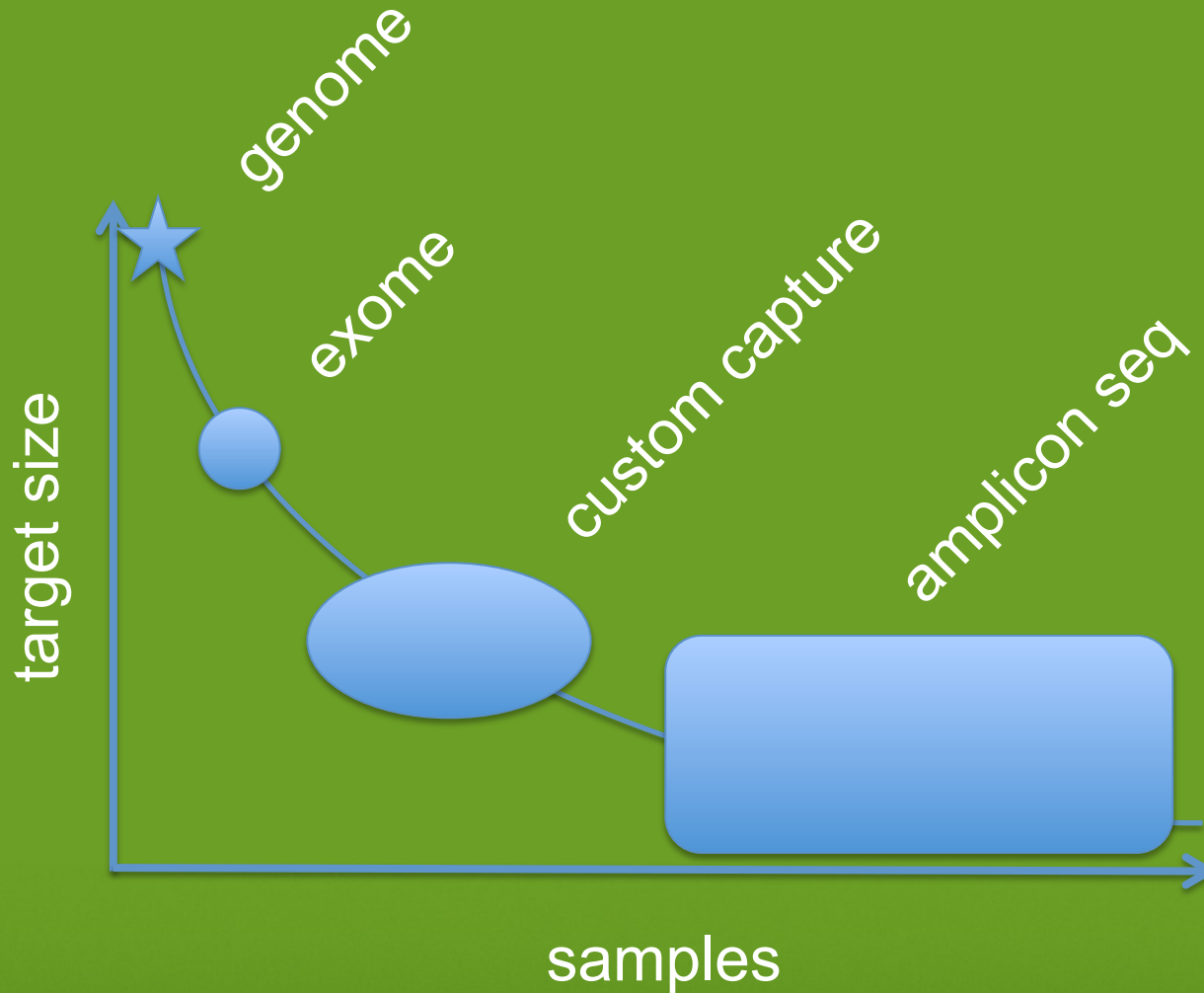
Jan Hellemans, PhD

NXTGNT



Knowledge for growth - 20110505

NGS resequencing options



Advantages of amplicon seq

- smallest shift for genetic labs
- flexibility
 - # samples
 - # regions
 - combinations
- lowest drop out & more equal coverage
- possibility to fix by repetition
- ready for Sanger confirmation

Amplicon seq workflows

- long read sequencer
 - fusion primers
 - adaptor ligation
 - two-round PCR (amplicon – sample specific)
- short read sequencers
 - concatenation > fragmentation > shotgun seq

Case 1 – hereditary deafness

- 40 genes for AR deafness
- first screening set
 - 15 most prevalent genes
 - 646 amplicons

Case 1 – hereditary deafness

Gene	Number of exons	Number of mutations worldwide	Number of homopolymer repeats in CDS	Function in hearing process
<i>GJB2</i>	2	> 220	0	ion homeostasis
<i>SLC26A4</i>	21	43	7	ion homeostasis
<i>MYO15A</i>	66	28	8	hair bundle, motor protein
<i>OTOF</i>	48	26	4	exocytose at auditory ribbon synapse
<i>CDH23</i>	69	21	4	hair bundle, adhesion protein
<i>TMC1</i>	24	20	4	unknown function
<i>TMPRSS3</i>	13	16	3	unknown function
<i>TECTA</i>	23	10	2	extracellular matrix protein
<i>TRIOBP</i>	24	9	7	hair bundle, cytoskeletal formation
<i>TMIE</i>	4	8	0	unknown function
<i>PJVK</i>	7	7	3	signaling of hair cells and neurons
<i>ESPN</i>	13	6	1	hair bundle, cytoskeletal formation
<i>PCDH15</i>	33	5	8	hair bundle, adhesion protein
<i>ESRRB</i>	12	5	1	transcription factor
<i>MYO7A</i>	49	5	5	hair bundle, motor proteins

Case 1 – hereditary deafness

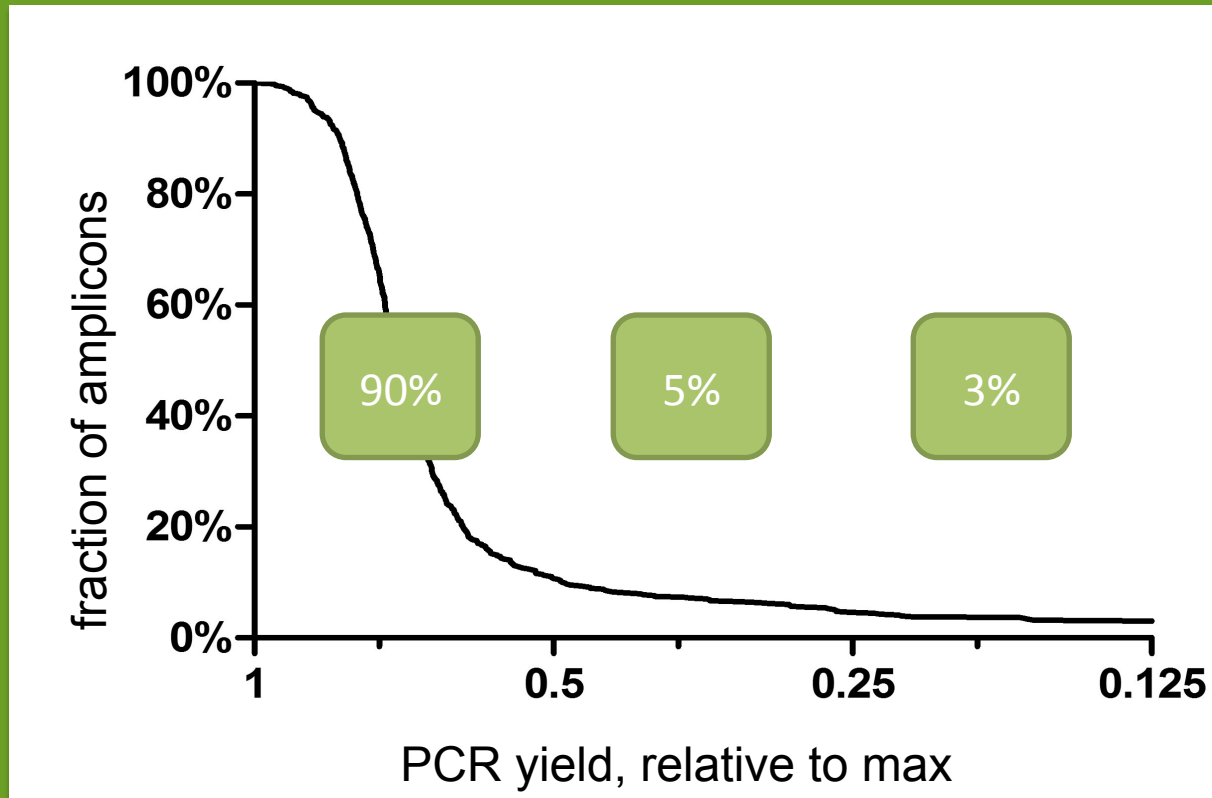
- 40 genes for AR deafness
- first screening set
 - 15 most prevalent genes
 - 646 amplicons
- custom primer design pipeline
 - all exons from all transcript variants
 - universal amplification protocol
 - primers are SNP & secondary structure free
 - fixed max length

Case 1 – hereditary deafness

- 40 genes for AR deafness
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- custom primer design pipeline

available as a service
including wet-lab validation & redesign

Case 1 – hereditary deafness



uniform amplification conditions resulting in high yield homogeneity & equal coverage

Case 1 – hereditary deafness

patient	gene	status	nucleotide	protein	cov > 30	status
1	CDH23	linkage	c.5542 G>T	Asp>Tyr	95.3%	new homozygous missense mutation
2	CDH23	linkage	c.5542 G>T	Asp>Tyr	94.6%	new homozygous missense mutation
3	OTOF	unknown	c.2317C>T	Arg>Cys	97.2%	compound heterozygous variants detected
	OTOF		c.4936C>T	Pro>Ser	97.2%	
4	TMC1	pos control			91.5%	known heterozygous variant confirmed in donor splice site intron 7
5	OTOF	linkage	c.3263 T>C	Leu>Pro	86.8%	new homozygous missense mutation

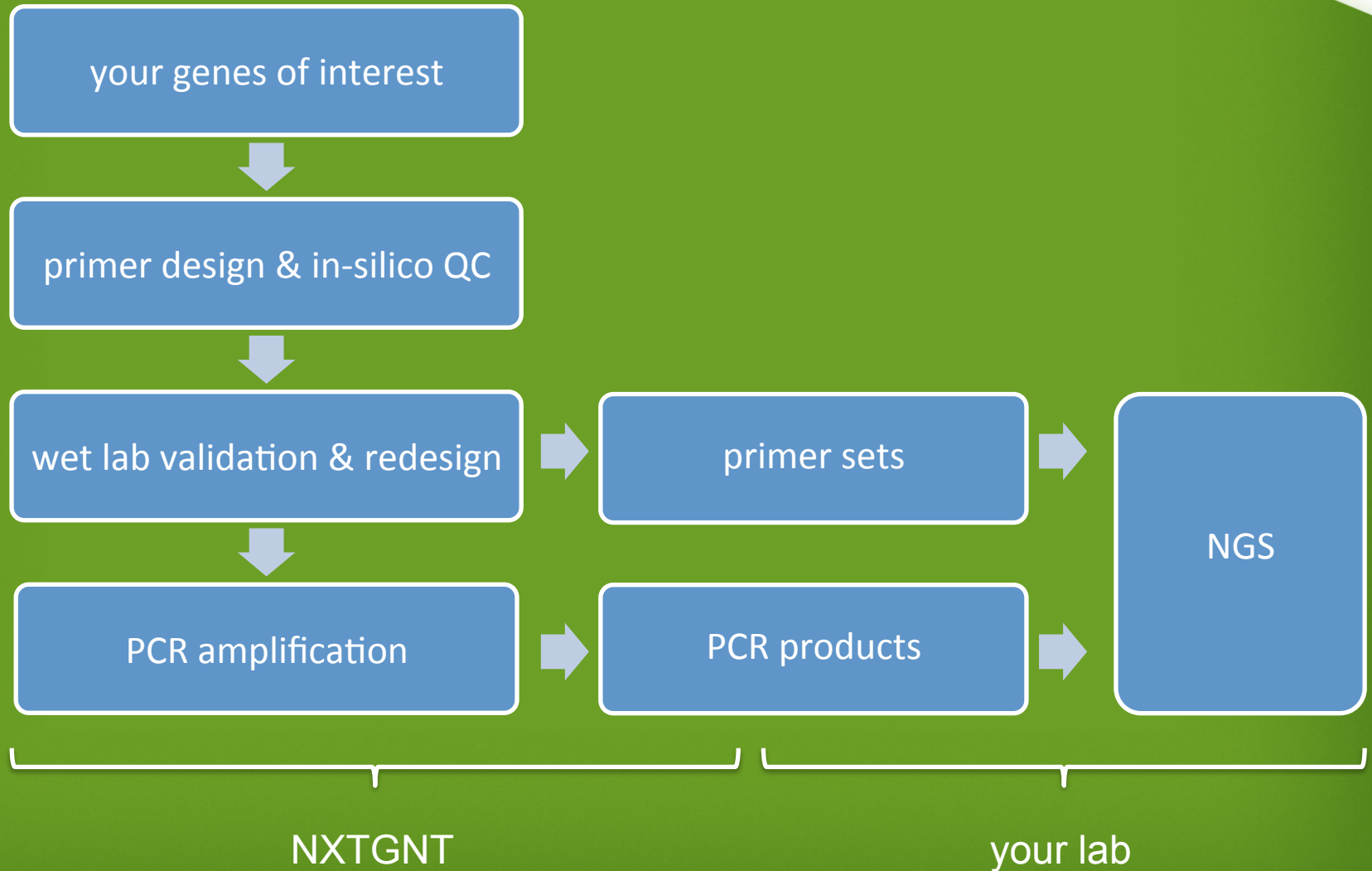
Case 2

- BRCA1 – BRCA2
 - De Leeneer et al, Hum Mutat. 2011
 - 123 samples
 - GS-FLX sequencing
 - sensitivity: >98%, 100% for substitutions
 - specificity: >90% (hompolymers!)

Case 3

- FBN – TGBFR1 – TGBR2
 - Baetens et al, Hum Mutat. 2011
 - 127 patients reported + 150 screened in 2 runs (GS-FLX)
 - mutation pick up rate: 92%, sensitivity: 100%
 - specificity: 98%

Your case



NGMD calculator

Guidelines

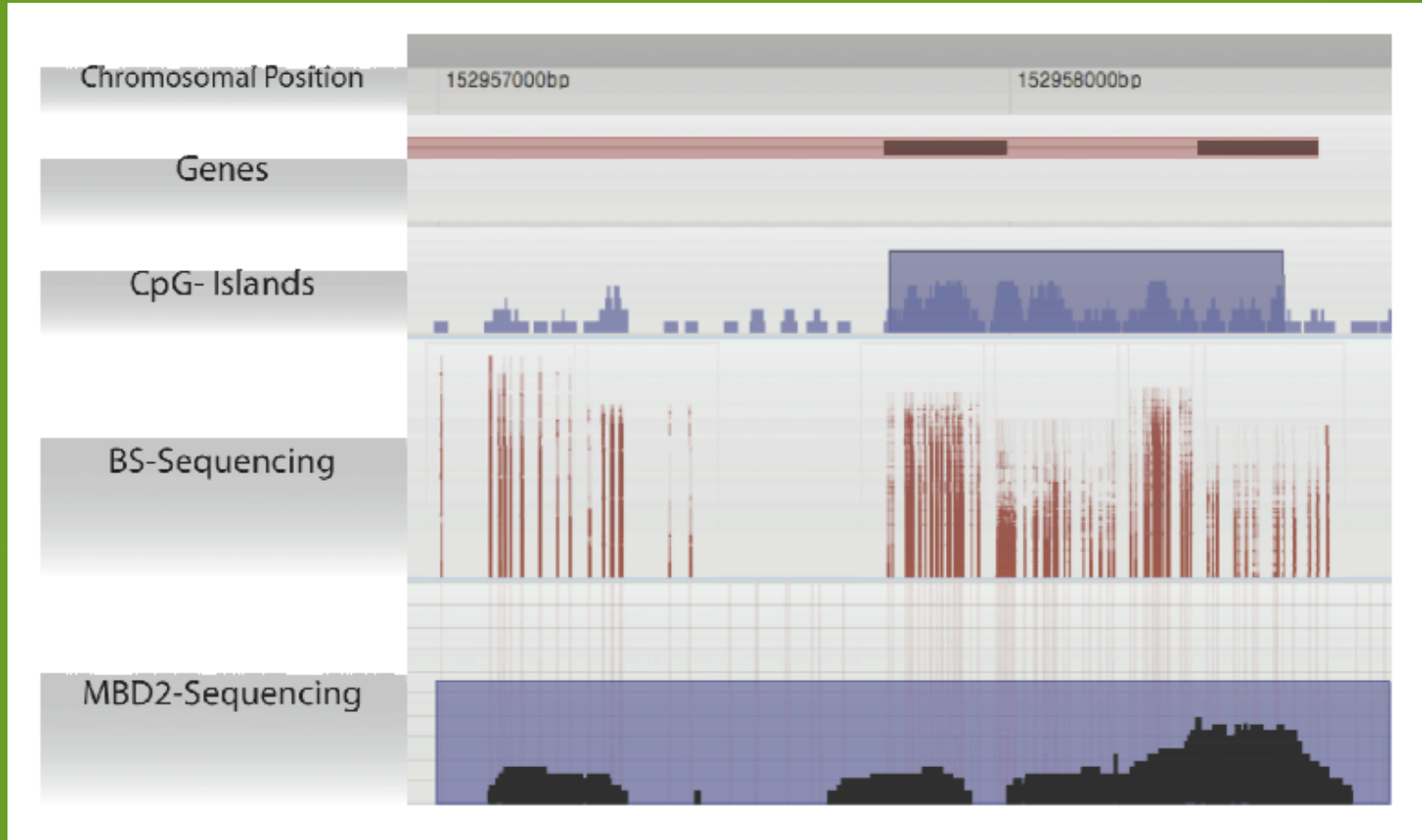
- 1) provide requirements in green boxes below
- 2) spread correction factor can be determined in first sheet
- 3) results are indicated in bold

required power to detect variants:	99.900%
threshold for sequence error filtering:	25%
required minimum coverage per SAC (MC):	38
spread correction factor (F, e.g. F90):	2.2134
required average coverage per SAC (AC):	85
instrument read count specifications:	1,100,000
read count correction:	80%
expected number of reads:	880,000
number of amplicons per sample:	111
number of samples in screening:	93

epigenetic profiling

- Established MBD2 enrichment workflow yielding a large collection of reference epigenomes
- Next Generation Bioinformatics
 - Integrating/leveraging different datasources (CGH, SNP, RNAseq, ...)
 - Intuitive query engine/export capabilities
 - Data visualisation tool H2G2 developed in collaboration with Genohm

epigenetic profiling



Integration of datasources revealing the methylome
(> 1 million methylated regions)

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- Current fields of interest:
 - Oncology: Long standing relationship with MDxHealth (MDxH has a unique validation methodology MSP, needed for final clinical application)
 - Stem cell research: collaboration with ReGenesys
 - Pronounced interest in many other disease areas and (re)newed interest in plant epigenomics

Acknowledgements

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