PYROSEQUENCING TECHNOLOGY FOR INHERITED DISEASE DETECTION IN RUSSIAN CATTLE POPULATION

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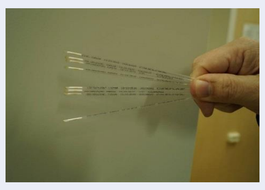
Procedure for detection of inherited recessive disease

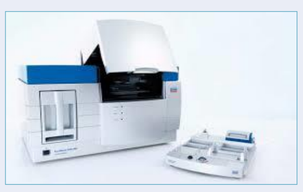
Sample preparation



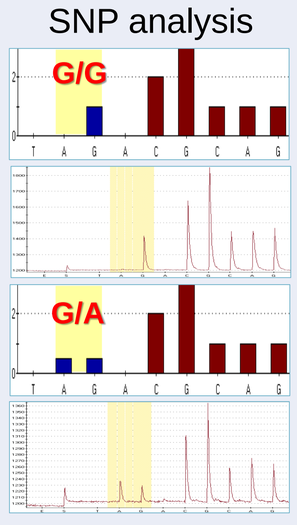
DNA extraction

PCR amplification of target gene

Pyrosequencing



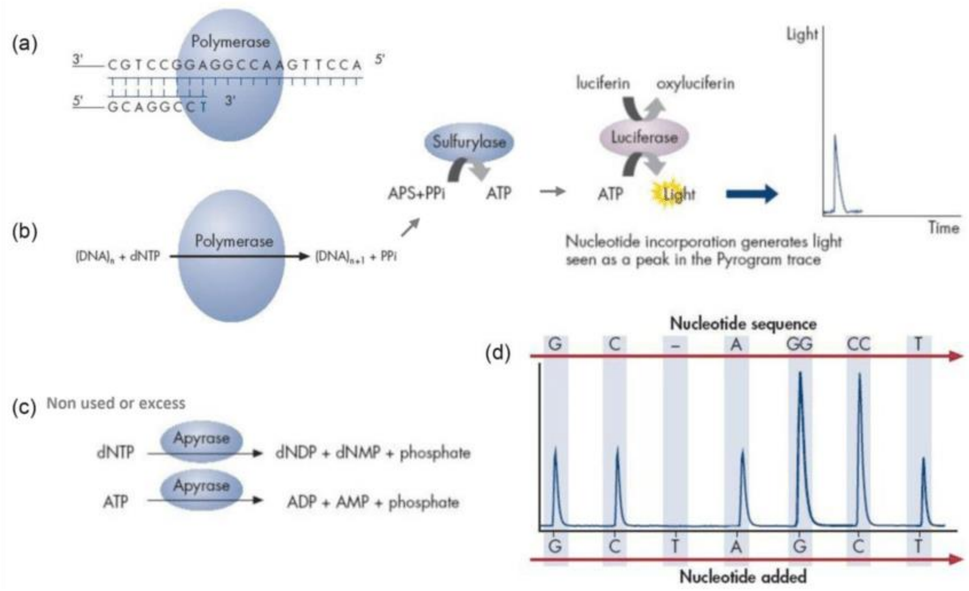
SNP analysis



Conclusion for recessive diseases

N / N – clear
N / m – carrier
m / m - affected

The pyrosequencing reaction cascade



Pyrosequencing

Pro

- rapid method
- less complex comparing with Sanger sequencing
- easy to discriminate alleles
- analysis up to 96 samples simultaneously

Contra

- size of fragments analyzed (10-20 bp)
- for known mutations only
- for SNP and short indels only
- expensive

Typical problems in pyrosequencing

- SNP in primer-annealing sites may cause false negative results due to an unequal contribution of alleles to signal => re-design primers
- Unexpected peaks in reference region of target DNA (unknown nucleotide polymorphisms) => exploration of polymorphisms of target DNA
- Unspecific peaks because of nucleotide sequence signals from self-annealed primers => using short target DNA fragment, re-design primers

Results

breed	variant phenotype	chromosome	gene	c. or n.	p.
Brown Swiss	spinal muscular atrophy	24	KDSR	490C>T	p.Ala164Thr
	spinal dysmyelination	11	SPAST	560G>A	p.Arg560Glu
	Wiwer	4	PNPLA8	1703G>A	p.Ser568Asn
	abortion due to haplotype BH2	19	TUBD1	757T>C	p.His210Arg
Aberdeen Angus	mannosidosis alpha	7	MAN2B1	961T>C	p.Phe321Leu
	developmental duplications	26	NHLRC2	932T>C	p.Val311Ala
	dwarfism	6	PRKG2	2032C>T	p.Arg678X

breed	inherited recessive disease	total tested	of them carriers
Brown Swiss	spinal muscular atrophy (SMA)	62	3
	spinal dysmyelination (SDM)		-
	Wiwer (W)		-
	abortion due to haplotype BH2		-
Aberdeen Angus	mannosidosis alpha (MA-α)	49	-
	developmental duplications (DD)		4
	dwarfism (DW)		-

Conclusions

Pyrosequencing is the one of the most appropriate techniques to discriminate a wild-type and defective alleles associated with single-nucleotide polymorphisms (SNP). Sensitive, rapid and robust pyrosequencing based tests have been developed for the detection of the above disorders in Brown Swiss and Aberdeen Angus cattle populations. Exclusion of sperm carriers defective alleles from breeding work will reduce the prevalence of these anomalies in the cattle population.

Acknowledgement

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