# RAPID AND RELIABLE ASSAYS FOR INHERITANCE DISEASE DETECTION IN RUSSIAN POPULATION OF HOLSTEIN CATTLE

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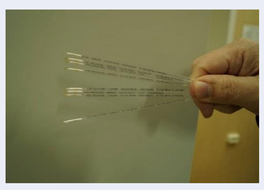
## Procedure for detection of inherited recessive diseases

Sample preparation

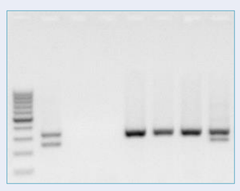


DNA extraction

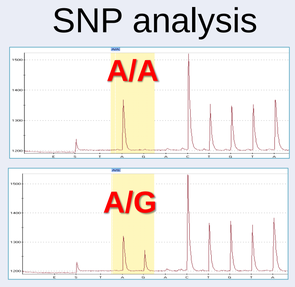
PCR amplification of target DNA

Pyrosequencing

Gel electrophoresis of PCR products



SNP analysis




Conclusion for recessive diseases

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

### 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

### Allele-specific PCR

**Pro**

- rapid and easy (compared to Sanger method)
- low cost
- great capacity to discriminate alleles in cases of extended deletions, insertions, inversions et cetera.

**Contra**

- using for detection only known variations
- difficult design of PCR technique for SNP's and short INDEL's

## Results

| variant phenotype | chromosome | gene | c. or n. | p. |
|---|---|---|---|---|
| **brachyspina syndrome (BY)** | 21 | FANCI | 526-64495_526-67824del | p.Val876Leufs26X |
| **cholesterol deficiency (CDH)** | 11 | APOB | 1.3 kb insertion of a transposable LTR element | p. Gly135ValfsX10 |
| **factor XI deficiency (FXID)** | 27 | FX1 | 870insC> ATATGTGCAGAATATA | p.Phe290LeuTyrValGlnAsnIle |
| **bovine leucocyte adhesion deficiency (BLAD)** | 1 | ITGB2 | 383A>C | p.Val128Ala |
| **complex vertebral malformation (CVM)** | 3 | SLC35A3 | 538G>T | p.Val180Phe |
| **deficiency of uridine monophosphate synthase enzyme (DUMPS)** | 1 | UMPS | 1213C>T | p.Arg405X |
| **bovine citrullinemia (CIT)** | 11 | ASS1 | 256C>T | p.Arg86X |

| inherited disease | assay | total (Holstein) | of them carriers |
|---|---|---|---|
| **BY** | PCR | 256 | **3** |
| **CDH** | PCR | 256 | **15** |
| **FXID** | PCR | 256 | **3** |
| **BLAD** | Pyrosequencing | 256 | **2** |
| **CVM** | Pyrosequencing | 256 | **7** |
| **DUMPS** | Pyrosequencing | 256 | **-** |
| **CIT** | Pyrosequencing | 256 | **-** |

## Conclusions

We developed sensitive, rapid and robust PCR and PCR-pyrosequencing assays for detection of the above disorders in Holstein cattle population. The assays are able to discriminate a wild-type and defective alleles, so that carriers and affected animals can be easily distinguished.

A total of 256 imported and local Holstein bulls were tested. Fifteen HCD, seven CVM, three BY, three FXID and two BLAD-carriers were identified, corresponding to heterozygote frequencies of 6%; 3%; 1%, 1% and 0,8% respectively. No BC and DUMPS-carriers were identified. Relatively high prevalence of HCD-carriers in the Russian livestock are explained by the fact that the HCD-associated mutation was reported only in 2016 while DUMPS, BLAD, CVM, BC mutations have been known for about twenty years.

Last decade routine testing of sperm and heifers allowed to gradually eradicate the deleterious alleles from the Holstein population. The high frequency of the HCD-allele in cattle shows that implementation of HCD-testing of bull sperm is necessary.

## Acknowledgement

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