

ENTWICKLUNG DES LIGATIONSDETEKTIONSREACTION (LDR)-TAQMAN  
ASSAY: EINE NEUE SNP-GENOTYPISIERUNGSMETHODE

Dissertation zur Erlangung des akademischen Grades des  
Doktors der Naturwissenschaften (Dr. rer. nat.)

Eingereicht im Fachbereich Biologie, Chemie, Pharmazie  
der Freien Universität Berlin

vorgelegt von

TATIANA A. BORODINA  
aus Petropavlovsk, Kazachstan

Februar, 2005

1. Gutachter: Prof. Dr. H. Lehrach
  2. Gutachter: Prof. Dr. V. Erdmann
- Disputation am 12.10.2005

DEVELOPMENT OF THE LIGATION DETECTION REACTION (LDR)-TAQMAN  
ASSAY: A NOVEL SNP-GENOTYPING METHOD

TATIANA A. BORODINA

February, 2005

## Acknowledgements

I am very grateful to Dr. Aleksey Soldatov for excellent supervising during my research in his group at the Max-Planck-Institute, for numerous discussions and for the critical reading of this thesis.

I thank Prof. Dr. H. Lehrach for intense interest in this work, helpful discussions, and friendly working atmosphere in his department.

I thank Prof. Dr. V. Erdmann for supervising me during my PhD study and evaluating this thesis.

I thank Prof. Dr. T. Altmann for providing *Arabidopsis thaliana* plant and DNA material for this work, for giving the access to the MASC SNP database, and for helpful cooperation.

I thank Dr. O. Torjek for cooperation in estimating the reliability of the *A. thaliana* genotyping kit.

I thank Prof. Dr. N. Yankovsky for providing human DNA samples and for helpful cooperation.

I thank Dr. S. Borinskaya and Dr. J. Kozhekbaeva for cooperation in estimating the reliability of the human genotyping kit.

I thank S. Shoichert for critically reading this thesis and correcting errors in English.

I thank A. Dahl for translating the Summary into German.

I thank my friends for countenance.

I thank my family for understanding and support.

# CONTENTS

<b>ABBREVIATIONS .....</b>	<b>6</b>
<b>INTRODUCTION .....</b>	<b>7</b>
SINGLE NUCLEOTIDE POLYMORPHISM – DEFINITION AND APPLICATIONS.....	7
<i>What are SNPs?</i> .....	7
<i>SNP maps</i> .....	8
<i>Technology development for SNP detection</i> .....	9
CURRENT STATE IN THE FIELD OF SNP DETECTION .....	9
<i>Hybridization-based methods</i> .....	10
Microarrays .....	10
DASH .....	12
Homogenous assays .....	14
<i>Enzymatic methods</i> .....	17
SNP discrimination by polymerase .....	17
Allele-specific PCR.....	17
Primer extension.....	19
Primer extension on microarrays.....	19
Homogenous primer extension assays .....	21
Primer extension with MALDI-TOF detection .....	22
Pyrosequencing .....	23
SNP discrimination by ligase .....	25
Invasive cleavage .....	29
Site-specific cleavage .....	31
<i>Chemical ligation</i> .....	31
<b>MATERIALS AND METHODS.....</b>	<b>34</b>
MATERIALS .....	34
<i>Oligonucleotides</i> .....	35
<i>DNA samples</i> .....	35
<i>SNP loci</i> .....	35
METHODS .....	35
<i>Universal oligonucleotides</i> .....	35
<i>Design of locus-specific oligonucleotides</i> .....	36
<i>Preparation of detector oligonucleotides</i> .....	37
<i>Ligation detection reaction (LDR) – TaqMan SNP detection</i> .....	38
"One tube – one locus" procedure.....	38
"One tube – many loci" procedure .....	39
Determination of minimal concentrations of DOs .....	39
Influence of PEG on the hybridization of DOs .....	39
<i>Hot start Taq polymerase preparation</i> .....	40
Purification of Taq polymerase .....	40
Determination of Taq polymerase activity.....	41
Preparation of hot start Taq polymerase .....	42
Estimation of the efficiency of formaldehyde inactivation .....	42
<i>Pfu DNA Ligase preparation</i> .....	43
Purification of Pfu DNA ligase .....	43
Estimation of Pfu DNA Ligase activity .....	44
<i>T4 DNA Ligase preparation</i> .....	44
<i>Genomic DNA purification</i> .....	44

Plant Genomic DNA purification using CTAB buffer .....	44
Plant Genomic DNA purification using homemade silica spin-columns. ....	45
Genomic DNA purification from saliva. ....	46
<b>RESULTS AND DISCUSSION .....</b>	<b>48</b>
BACKGROUND.....	48
LDR-TAQMAN SNP DETECTION METHOD.....	50
<i>The structure of detector oligonucleotides</i> .....	51
<i>The "one tube – one locus" procedure (Figure 13)</i> .....	53
<i>The "one tube – many loci" procedure (Figure 14)</i> .....	55
<i>Discussion of the LDR-TaqMan protocol</i> .....	58
LDR-TAQMAN GENOTYPING KITS .....	64
<i>Preparation of enzymes and Detector oligonucleotides</i> .....	64
Homemade Pfu DNA ligase .....	65
Homemade hot start Taq DNA polymerase.....	65
Plastic consumables .....	66
Plant and human genomic DNA isolation using homemade spin columns .....	66
Ligation-based synthesis of Detector Oligonucleotides (DOs) .....	66
<i>Arabidopsis thaliana SNP kit</i> .....	69
<i>Human SNP kit</i> .....	74
<b>SUMMARY .....</b>	<b>78</b>
<b>ZUSAMMENFASSUNG .....</b>	<b>79</b>
<b>REFERENCES.....</b>	<b>80</b>
<b>SUPPLEMENTS.....</b>	<b>97</b>
<b>CURRICULUM VITAE.....</b>	<b>119</b>

## **ABBREVIATIONS**

AFLP – amplification fragment length polymorphism

bp – base pair

DO – detector oligonucleotide

ELISA – enzyme-linked immunosorbent assay

FRET – fluorescence resonance energy transfer

LBS – ligation-based synthesis

LDR – ligation detection reaction

MALDI-TOF - matrix assisted laser desorption/ionization – time of flight

MALDI – matrix assisted laser desorption/ionisation

nt – nucleotide

PCR – polymerase chain reaction

PEG – polyethyleneglycole

RFLP – restriction fragment length polymorphism

RT – room temperature

SNP – single nucleotide polymorphism