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DIAGNOSTIK GMBH

SuBiTo
THE SEQUENCING

Typing to the highest resolution

Ready Gene
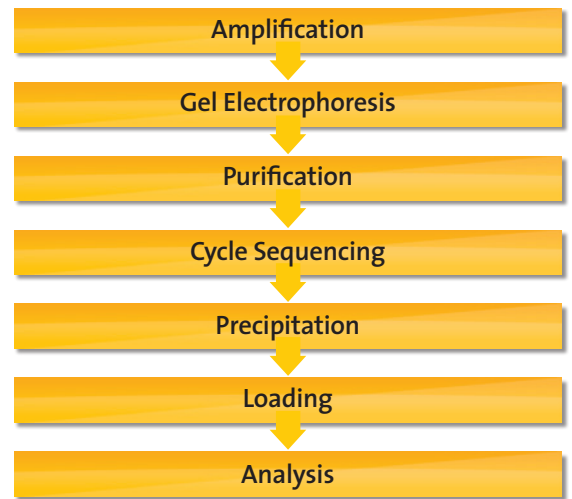
SuBiTo

FluoGene

Software

PCR & Electrophoresis

Ready Plate

DNA-Extraction

Life Science

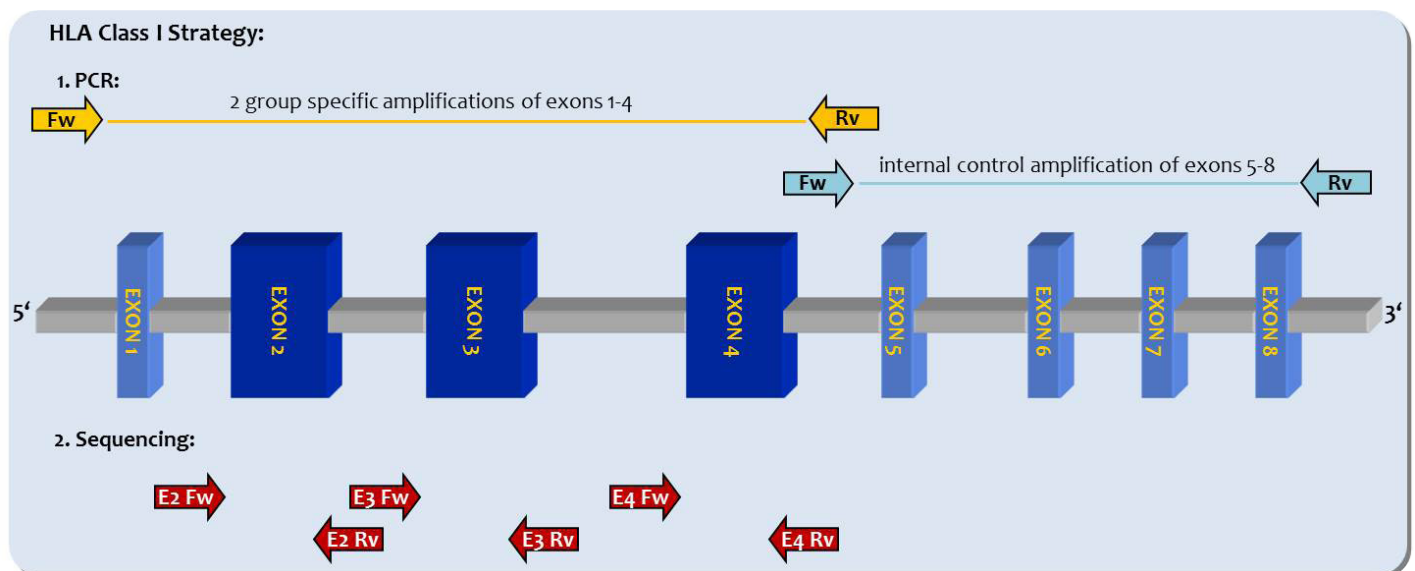# SAME SIMPLE AND UNIFORM SET UP FOR ALL HLA-SuBiTo KITS:

- To reduce ambiguities 2 amplifications are carried out in parallel in order to separate many heterozygous allele combinations in the first step

- Gel Electrophoresis

- Purification of the PCR products using ExoSAP-IT™

- 6 sequence reactions per HLA Class I Locus sequence

- 3-5 sequence reactions per HLA Class II Locus sequence

- The combination of forward and reverse reactions takes place according to positive PCR amplification

- Precipitation with IT-Prec-Buffer

- Loading of the capillary sequencer

- Analysis with HLA-HiType software

Amplification
Gel Electrophoresis
Purification
Cycle Sequencing
Precipitation
Loading
Analysis

NEW KIT IMPROVEMENTS SAVE YOU 3 HOURS ASSAY TIME:

- New chemistry on G1/G2 mixes enable shorter PCR profile for first PCR - takes now only 1 h 45 min

- Shorter protocol for Cycle Sequencing PCR takes now only 1 h 10 min
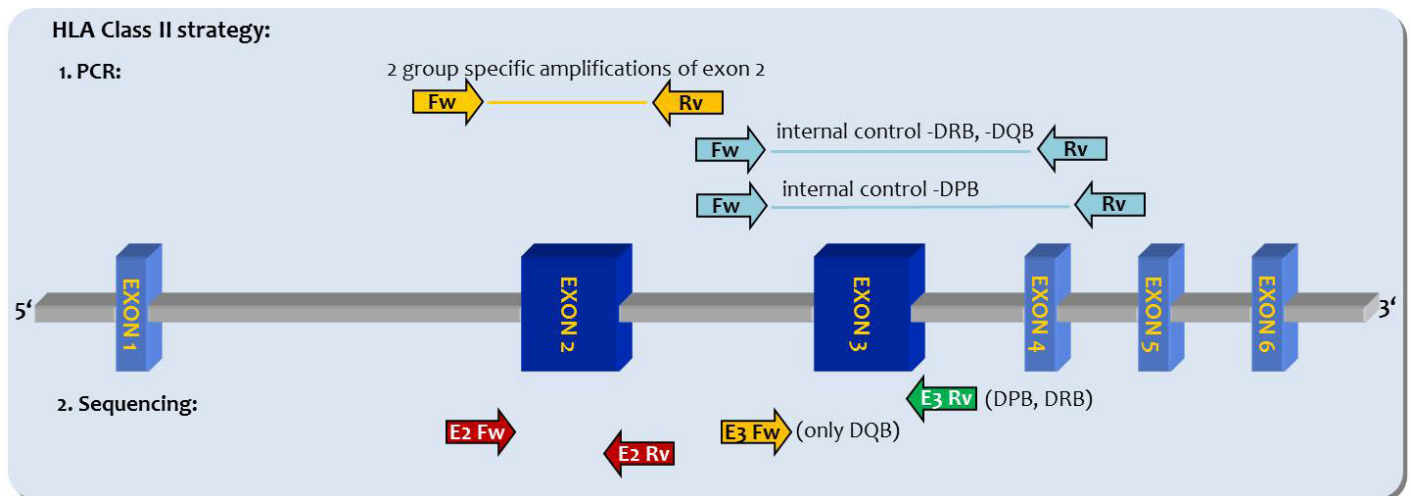
- Shorter precipitation protocol saves 30 minutes

CLASS I STRATEGY:

The strategy of 2 group specific amplifications is the ideal solution for high resolution as well as for high throughput. The amplifications are followed by 6 sequencing reactions for exon 2, 3 and 4.

Parallel amplification of exons 5 – 8 as an internal control provides the option of further sequence analysis.

CLASS II STRATEGY:

HLA Class II strategy:

1. PCR:

2 group specific amplifications of exon 2

Fw — Rv

internal control -DRB, -DQB

Fw — Rv

internal control -DPB

Fw — Rv

5' EXON 1 — EXON 2 — EXON 3 — EXON 4 — EXON 5 — EXON 6 — 3'

2. Sequencing:

E2 Fw — E2 Rv

E3 Fw (only DQB)

E3 Rv (DPB, DRB)

The group specific amplifications are followed by 2 sequencing reactions of exon 2 and 1 sequencing reaction of exon 3 .

Parallel amplification of exon 3 or exon 4 respectively as an internal control provides the option of further sequence analysis.

COMPLETE KIT CONTENTS:

- All components for amplification including IT-Taq Polymerase
- Exo-SAP-IT™ for purification of the PCR products
- Gene locus and exon-specific sequencing mixes incl. BigDye® chemicals

- IT-Prec Buffer for precipitation of the sequencing products
- 2 Second Level Sequencing Mixes for resolution of common ambiguities are directly included in the kit
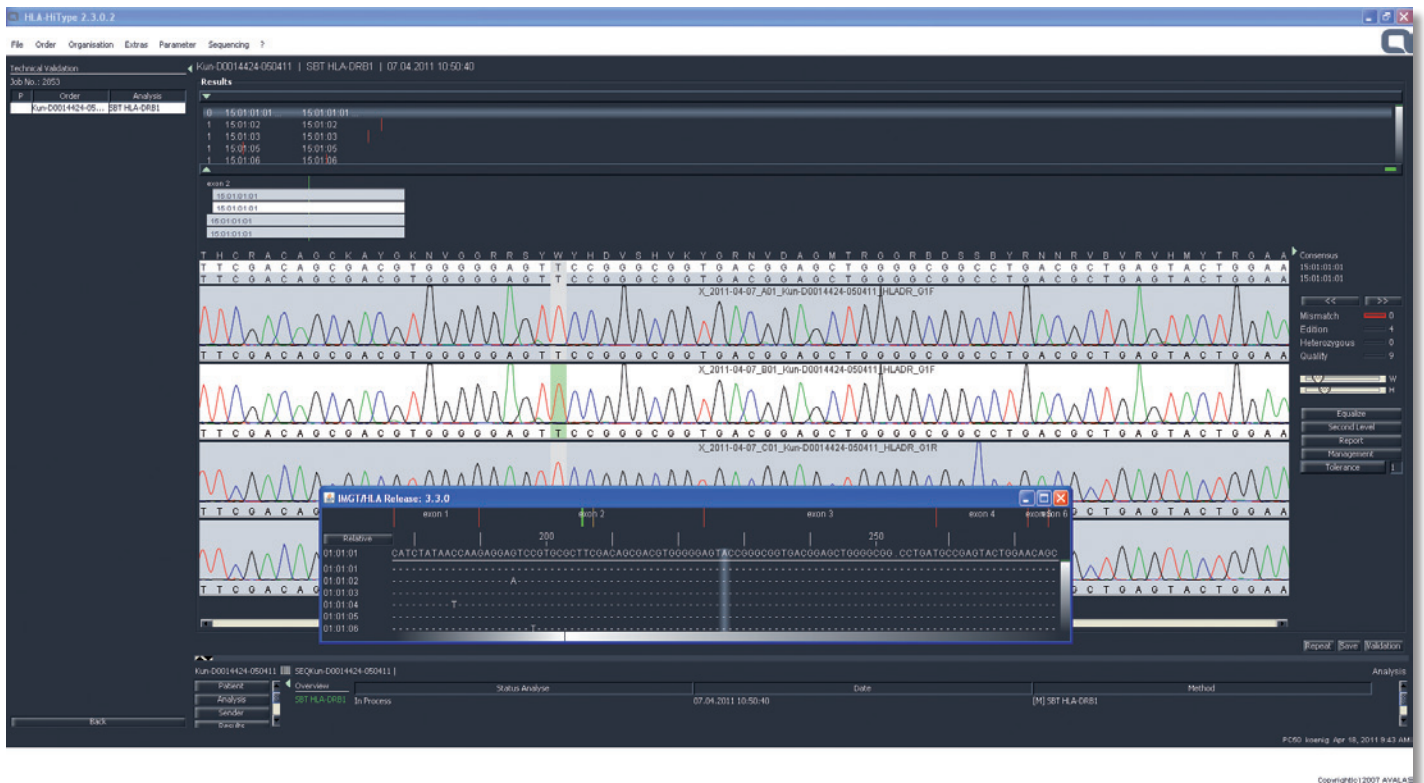
SECOND LEVEL SEQUENCING:

Due to the HLA-SuBiTo strategy of separating HLA allele groups by two initial amplifications, common cis-trans ambiguities are strongly reduced. However, in case the groups could not be separated, possible ambiguities can be resolved with the ready-to-use Second Level Sequencing Mixes using the existing PCR product of the standard kit.

There are a total of 31 different Second Level Sequencing Mixes available for HLA class I (10 for HLA-A, 11 for HLA-B and 12 for HLA-C) (some mixes are useable for several loci). For HLA-DRB1 we offer 9 Second Level Sequencing Mixes, 8 mixes for HLA-DQB1 and 4 mixes for HLA-DPB1. The powerful HLA-HiType Software proposes in the case of ambiguities the ideal Second Level Sequencing Mix for resolution.

ANALYSIS WITH HLA-HiType SOFTWARE:

- Integration of new nomenclature and automated translation in P- and G-groups as well as new NMDP-Codes

- Fully automated data management, no exhausting selection of *.ab1 Files necessary

- Automated recognition and dynamic display of hemi- and heterozygous sequences

- Several manual and automated editing possibilities

- Diverse alignment options, combined control of electropherogram and alignment

- High safety due to a defined 4 eyes principle (technical and medical validation)

- Complete history with a traceability of each step

HLA-SuBiTo KITS ARE AVAILABLE FOR 25 TYPINGS EACH:

ARTICLE NO.	HIGH RESOLUTION KITS	ARTICLE NO.	HIGH RESOLUTION KITS
002 090 025*	HLA-SuBiTo A high res	002 093 025*	HLA-SuBiTo DRB1 high res
002 091 025*	HLA-SuBiTo B high res	002 094 025*	HLA-SuBiTo DQB1 high res
002 092 025*	HLA-SuBiTo C high res	002 095 025*	HLA-SuBiTo DPB1 high res
002 096 025*	HLA-SuBiTo ABCDRDQ high res		

*All articles are also available without BDTs under the same article no. code with /1.

002 090 0SW	HLA HiType Software main licence
002 090 0AL	HLA HiType additional workstation licence

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