

# HISTO TYPE SSP Happy Packs

## Technical Report

### Influence of variations in DNA concentration on HISTO TYPE SSP results

#### Introduction

DNA concentration may have a major impact on the results of PCR techniques like HLA SSP typing and generally the DNA concentration should be adjusted to the specifications of a test. Sometimes however, the exact measurement of the DNA concentration is not possible or there may not be enough sample material available.

The robustness of the HISTO TYPE SSP Happy Packs including the validated Happy Taq Polymerase was evaluated with different DNA concentrations.

#### Material and Methods

Five DNAs with different HLA genotypes were diluted to different concentrations between 10 and 150 ng/μl (Table 1) and tested with the HISTO TYPE ABDR Happy Pack. The recommended concentration is 25-50 ng/μl.

#### Results

Except of one test with a concentration of 150 ng DNA/ μl all test were clearly interpretable. There were a few mixes with unspecific or weakly false positive reactions at high concentrations and only one mix for DRB1 reacted weaker than expected at a lower concentration. The results are summarized in Table 1.

Sample	<u>DNA 1</u>	<u>DNA 2</u>
HLA type	A*02,*68; B*07,*44; DRB1*13,*15	A*02,*11; B*35,*40; DRB1*07,*13
10 ng/μl	Ok	Ok
35 ng/μl	Ok	Ok
75 ng/μl	B: Mix 4: very weak false positive *	Ok
100 ng/μl	B: Mix 4: weak false positive*	A: Mix 2: 595 bp band lost, smaller one still there DR: Mix 5 unspecific reaction
150 ng/μl	B: Mix 4: false positive*	A: Mix 2: 595 bp band lost, smaller one still there B: Mix 41: very weak unspecific reaction DR: Mix 8: double band (correct positive)

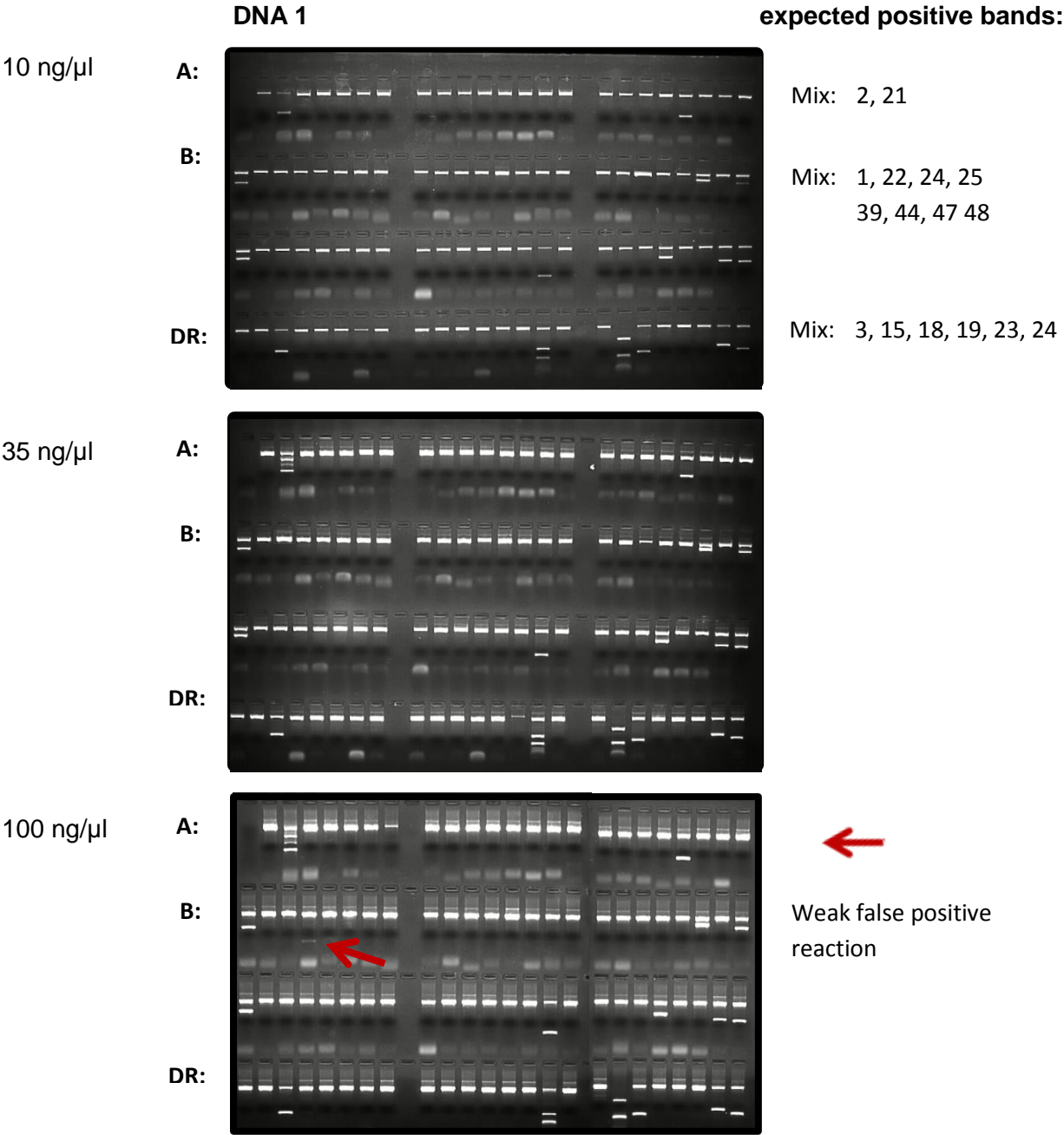
\* might be a cross reaction of B\*07 with one of the rare alleles B\*07:02:03, \*07:05:04 or 07:56:01 that are reacting positive with Mix 4

Sample	<u>DNA 3</u>	<u>DNA 4</u>	<u>DNA 5</u>
HLA type	A*03,*11; B*07,*35; DRB1*01,*15	A*01,*01; B*08,*37; DRB1*03,*04	A*01,*31; B*08,*40; DRB1*07,*08
10 ng/μl	Ok	DR: Mix 7 weak	Ok
35 ng/μl	Ok	Ok	Ok
75 ng/μl	Ok	Ok	Ok
100 ng/μl	Ok	Ok	Ok
150 ng/μl	Ok	Ok	Ok

**Table 1:** Summary of the results with the five DNAs at different concentrations

Green	all bands as expected
Yellow	slight deviations from the expected band, but result interpretable
Red	result unclear

For illustration purposes three gel images for sample DNA 1 are shown below.



**Conclusion**

The HISTO TYPE SSP kits can be used with a wide range of DNA concentrations even in the presence of a few unspecific, weak false positive or weak reactions. DNA concentrations between 10 ng/μl and 100 ng/μl generally give clear results. Taken together these results show that the HISTO TYPE SSP kits are a very robust and reliable test for HLA typing.