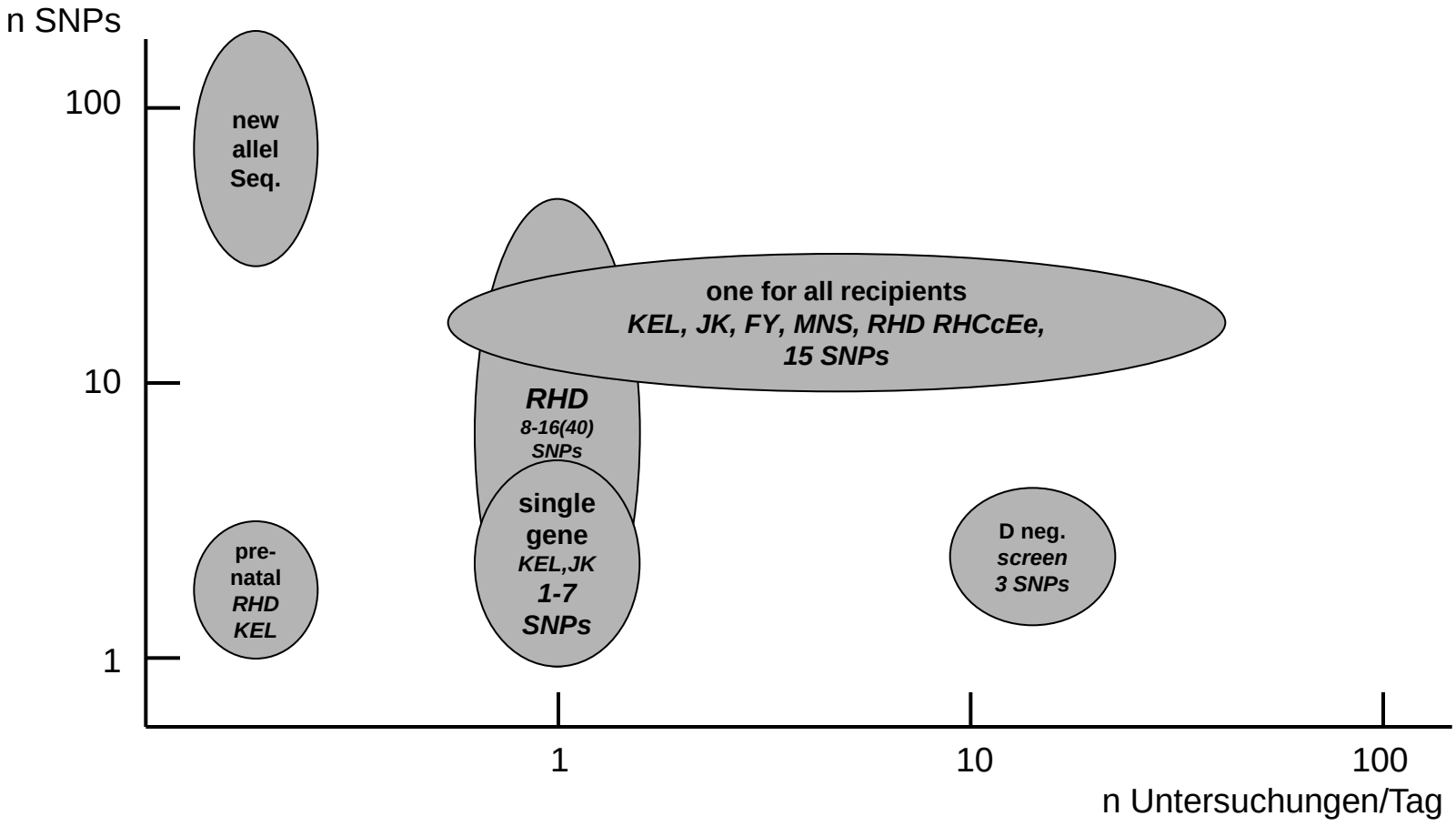

n SNPs
100
10
1
new allel Seq.
pre-natal RHD KEL
RHD 8-16(40) SNPs
single gene KEL,JK 1-7 SNPs
one for all recipients KEL, JK, FY, MNS, RHD RHCcEe, 15 SNPs
D neg. screen 3 SNPs
1
10
100
n Untersuchungen/Tag

Genetic diversity of KELnull and KELel: a nationwide Austrian survey

Günther F. Körmöczi, Thomas Wagner,* Christof Jungbauer, Maria Vadon, Norbert Ahrens, Willi Moll, Annelies Mühlbacher, Seyhan Özgül-Gülce, Thomas Kleinrath, Susanne Kilga-Nogler, Diether Schönitzer, and Christoph Gassner*

Volume 47, April 2007 **TRANSFUSION** 703

A novel *KEL*1,3* allele with weak Kell antigen exp confirming the cis-modifier effect of KEL3

Günther F. Körmöczi, Erwin A. Scharberg, and Christoph Gassne

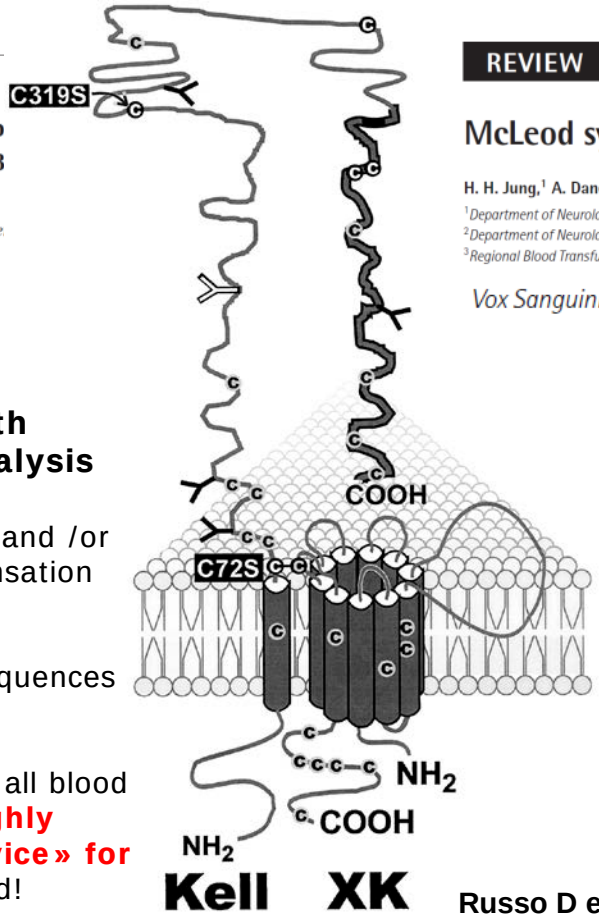
Volume 49, April 2009 **TRANSFUSION** 733

ACADEMIC
10 to 20 results per year with need for further molecular analysis

Usually laborious, involving sequencing and /or other methods. **UNPAYABLE**. Compensation partially via academic merit.

Findings **NEED TO BE PUBLISHED**. Sequences to be deposited in data-bases!

Consequence: No lab (?) is able to cover all blood groups. Prognosis: There will be **highly specialized labs with «academic service» for certain genes**, for «all» the world!



McLeod phenotype associated with a *XK* missense mutation without hematologic, neuromuscular, or cerebral involvement

Hans H. Jung, Martin Hergersberg, Marco Vogt, Jens Pahnke, Valerie Treyer, Benno Röthlisberger, Spyros S. Kollias, David Russo, and Beat M. Frey

928 **TRANSFUSION** Volume 43, July 2003

REVIEW

McLeod syndrome: a neurohaematological disorder

H. H. Jung,[1] A. Danek[2] & B. M. Frey[3]
[1]Department of Neurology, University Hospital Zürich, Zürich, Switzerland
[2]Department of Neurology, Hospital of the University of Munich Großhadern, Ludwig-Maximilians-Universität, Munich, Germany
[3]Regional Blood Transfusion Service, Swiss Red Cross, Zürich, Switzerland

Vox Sanguinis (2007) **93**, 112–121

Russo D et al. J. Biol. Chem. 1998;273:13950-13956

Number of D-A-CH donors and donations 2006-2009-(2011)

			first time donors (FTD)	number of all donors (D)	inhabitants (Mio)	donors per 1'000 inh.	repeat rate per year	sum EC	sum TC	n Dneg of all D (17,5%)	n Dneg of all FTD (17,5%)
D	Deutschland	2006	0.18	2'846'415	82.3	35	1.71	4'867'408	249'035	483'891	86'990
	Germany	2007	0.18	2'978'888	82.2	36	1.63	4'868'063	278'630	506'411	93'263
		2008	0.19	2'946'183	82.0	36	1.68	4'942'220	287'853	500'851	96'898
		2009	0.20	3'143'222	81.9	38	1.58	4'966'271	288'559	534'348	109'536
A	Österreich	2006	0.17	354'973	8.3	43	1.38	491'605	19'808	60'345	10'164
	Austria	2007	0.16	330'797	8.3	40	1.50	495'913	28'562	56'235	9'169
		2008	0.16	338'958	8.3	41	1.47	499'609	29'082	57'623	8'983
		2009	0.14	364'788	8.4	44	1.32	480'129	30'330	62'014	8'550
CH	Schweiz	2006	0.09	240'690	7.5	32	1.44	347'684	20'032	42'121	3'621
	Switzerland	2007	0.11	241'063	7.6	32	1.46	353'063	21'265	42'186	4'433
		2008	0.12	253'953	7.7	33	1.41	358'903	25'626	44'442	5'220
		2009	0.12	260'377	7.8	33	1.39	363'213	26'823	45'566	5'175
	BSD SRK AG Statistik	2010	0.11	233'313	7.9	30	1.61	376'360	31'776	40'830	4'470
	BSD SRK AG Statistik	2011	0.13	227'945	8.0	29	1.63	371'016	33'032	39'890	5'010
	Alle "D-A-CH"	2006	0.17	3'442'078	98.1	35	1.66	5'706'697	288'875	602'364	100'774
	all "D-A-CH"	2007	0.18	3'550'748	98.1	36	1.61	5'717'039	328'457	621'381	106'865
		2008	0.18	3'539'094	98.0	36	1.64	5'800'732	342'561	619'341	111'102
		2009	0.19	3'768'387	98.0	38	1.54	5'809'613	345'712	659'468	123'261

2006-2009, Prof. Dr. med. Barbara Blauhut, Linz, Austria

2010-2011, BSD SRK AG, yearly statistic

Why high-troughput molecular immunohematology?

Screen **donors with RARE blood groups**!

… almost all human blood group polymorphisms are known: Kp, Lu, Di, Wr, Yt, Co, Kn, Do, In, LW, Sc, Jr, Lan…

Do the "**dry-match**" = "***in silico* cross-match**"!

Applying molecular immunohematology discoveries to standards of practice in blood banks: now is the time.

Denomme GA, Flegel WA.
Department of Pathology & Laboratory Medicine, Mount Sinai Hospital, New York, USA.

Abstract

Lessons from more than 100 years of immunohematology exemplify that many critical discoveries were made serendipitously and their more rapid implementation could have benefited transfusion recipients and pregnancies. Constituents of blood that are not essential for the attempted therapeutic benefit of a transfusion are largely removed from today's blood products. We are now moving on to avoid unnecessary exposure to potentially harmful constituents of the therapeutically required cells, like blood group antigens that are foreign to the patient. Cost efficacy needs to be kept in mind but may eventually prove much better than anticipated, once hidden benefits are captured, as we show by examples from past immunohematologic developments. Here, we detail clinical applications for molecular immunohematology advances including "dry-matching" that will improve transfusion outcomes and argue for their widespread implementation by rapid timelines through standards of practice.

Reasoning for molecular blood group typing

blood

2009 114: 248-256
Prepublished online May 1, 2009;
doi:10.1182/blood-2008-11-146860

Red cell genotyping and the future of pretransfusion testing

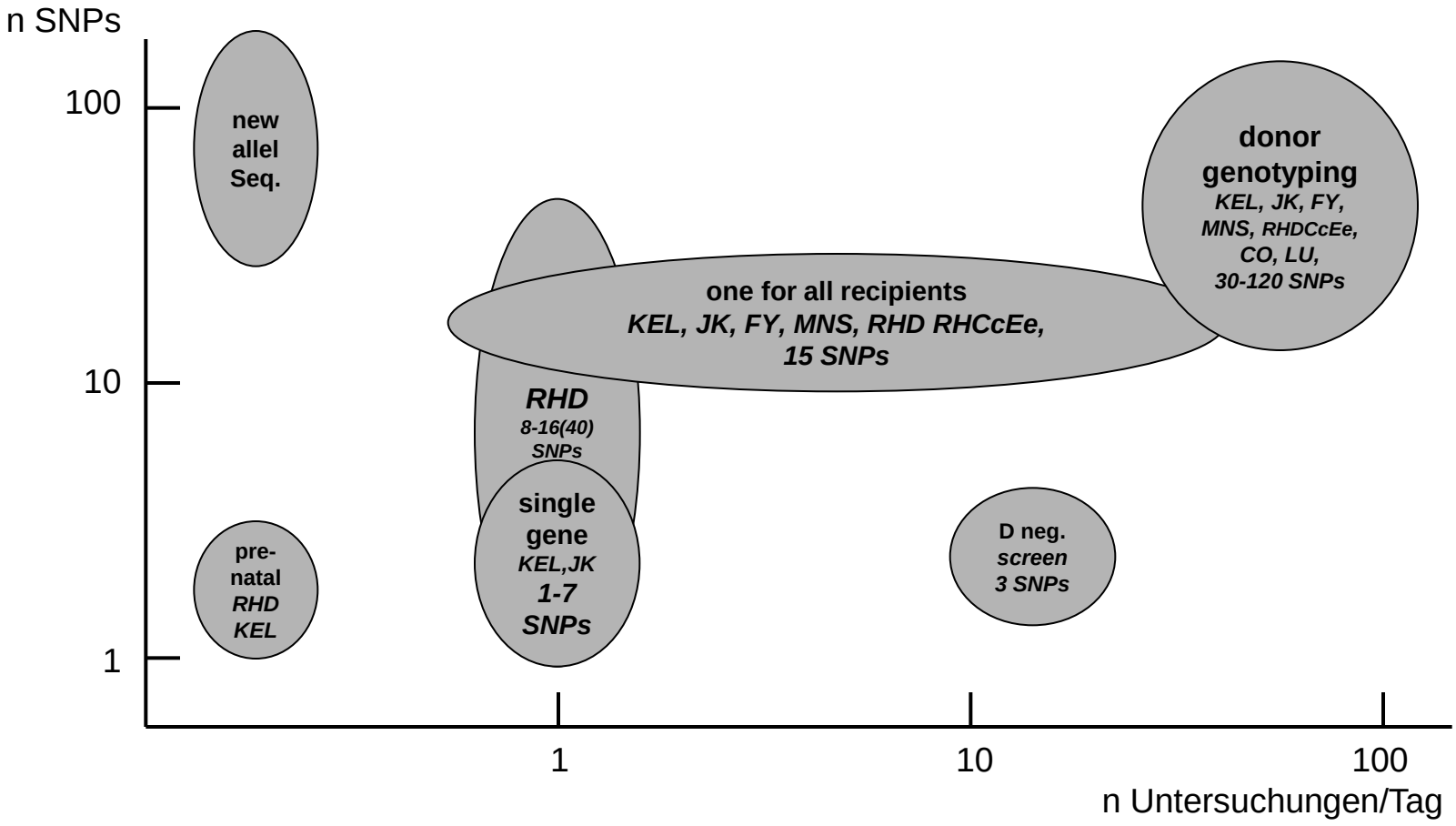
David J. Anstee

Table 1: Useful applications of red cell genotyping in transfusion medicine

- Fetal DNA typing
- Extensive blood group typing of donors for alloimmunized patients
- Determining the blood group of a recently transfused patient
- Screening blood donors to find rare blood group phenotypes
- Determining the frequency of blood group polymorphisms in a population
- Determining *RHD* zygosity for fathers of fetuses at risk for HDFN
- Blood group typing of patients with autoimmune hemolytic anemia

Christoph Gassner:

1. Routine relevant determination of variant types of blood groups (e.g. *RHD, DARC*)
2. Screening RhD negatives for *RHD* (null) genes
3. “Academic” analysis of blood group phenotypes


n SNPs
100
10
1
new allel Seq.
pre-natal RHD KEL
RHD 8-16(40) SNPs
single gene KEL,JK 1-7 SNPs
one for all recipients KEL, JK, FY, MNS, RHD RHCcEe, 15 SNPs
donor genotyping KEL, JK, FY, MNS, RHDCcEe, CO, LU, 30-120 SNPs
D neg. screen 3 SNPs
1
10
100
n Untersuchungen/Tag

Comercially available platforms for molecular blood group typing

platform	PCR-SSP, or ARMS	MALDI-TOF MS genotyping	Chip technology	Chip technology	Luminex technology (xMAP®)	Luminex technology (xMAP®)
product example	RBC Ready Gene	open	BLOODchip[ID]	BioArray BeadChip™	BLOODchip ID LINE (xMAP®)	LIFECODES RBC-R assay
company	Inno-Train, Kronberg i.T., Germany	SEQUENOM®, San Diego, USA	Grifols, Barcelona, Spain	Immucor, Norcross, USA	Grifols, Barcelona, Spain	Gen-Probe, San Diego, USA
commercial availability since	1998	(2013)	2008	~ 2009	~ 2010	2010
relevant early citation(s)	Gassner et al 1996 ABO, Gassner et al 1997 RHD	Henk Garritsen, Transfus Med Hemother. 2009;36(3):181-187.	Avent, Flegel, Olsson, Transfus Med Hemother. 2009;36(3):162-167.	Hashmi G, Shariff T, Transfusion. 2005 May;45(5):680-8.	Drago F, Karpasitou K, Poli F., Transfus Med Hemother. 2009;36(3):157-160.	Drago F, Karpasitou K, Poli F., Transfus Med Hemother. 2009;36(3):157-160.
performance specs						
tailoring of specificties ("unit")	RBC Ready Gene AB0, 8 rctns., 4SNPs	module *RH* broad panel (3 MPX)	138 genetic polymorphisms	HAE BeadChip™	ID|Core for 23 RBC antigens	RBC assay, 1 MPX, ~16 SNPs
	RBC Ready Gene CDE, 16 rctns., ~19 SNPs	module *RH* variant panel (2 MPX)	(including 12 SNPs for HPA)	RHCE BeadChip™	ID|Core+ for 33 RBC antigens	RBC-R assay, 1 MPX, ~14 SNPs
	RBC Ready Gene KKD, 8 rctns., 5 SNPs	module *KEL, JK, FY* (1 MPX)	(including ABO genotyping)	RHD BeadChip™	ID|HPA for 24 platelet antigens	.
	RBC Ready Gene MNS, 6 rctns., 4 SNPs	module *MNS (2 MPX)*	.	HPA BeadChip™	.	.
	RBC Ready Gene Rare ID, 16 rctns., 8 SNPs	module *RARE* blood group panel (2 MPX)	.	.	.	.
	and > 5 additional	module *HPNA (1 MPX)*	.	.	.	.
highest level of certification	CE self declared	in house validation in preparation	CE according to appendix II, list A (selected specificities)	CE according to appendix II, list A (some units)	n.a.	currently RUO, CE according to appendix II, list A in preparation
single specificity (blood group) typing possible	YES	(YES)	NO	(YES)	NO	NO
single sample testing	consists of 4 to 16 single PCR-SSPs per tailored specificity	consists of 1 to 5 MPX per module	consists of 1 MPX, analyzed on 1 individual chip	consists of 1 MPX, analyzed on 1 individual chip per tailored specificity	consists of 1 MPX, analyzed on 96-w MTP format	consists of 1 MPX, analyzed on 96-w MTP format
single sample testing (cost efficiency)	YES	NO (minimum of 96 MPX reactions in 96-w MTP format)	n.a.	n.a.	n.a.	1 sample, 1 unit (waste of sheath fluid, only)
ideal number of samples processed per run (cost efficiency)	e.g. 12 *KEL, JK, FY* genotypings, each consisting of 8 PCR-SSPs, processed in 96-w MTP format	384-w MTP format: 384 MPX reactions	24 samples	n.a.	96-w MTP format: 96 MPX reactions	96-w MTP format: 96 MPX reactions
post DNA-prep	2.5 h	10 h	10 h	6 h	5 h	4.5 h
Costs						
hardware-1 description	standard Agarose gel electrophoresis	384-w MTP format Sequenom Mass Spectrometer plus 2 X 96-w robots, plus 1 spotter	detailed instrument name n.a.	n.a.	detailed instrument name n.a.	Luminex® 100/200™
hardware-1 costs (in Euro)	neglible	~ 500.000,00	~ 105.000,00	~ 110.000,00	~ 40.000,00	~ 40.000,00
consumables example 1	RBC Ready Gene KKD, 8 rctns., 5 SNPs	module KEL, JK, FY (1 MPX)	BLOODchip[ID]	HAE BeadChip™	ID|Core for 23 RBC antigens	RBC assay, ~16 SNPs
consumables example 2	RBC Ready Gene MNS, 6 rctns., 4 SNPs	module MNS (2 MPX)	(including 12 SNPs for HPA)	.	ID|Core+ for 33 RBC antigens	RBC-R assay, ~14 SNPs
consumables example 3	RBC Ready Gene Rare ID, 16 rctns., 8 SNPs	module RARE blood group panel (2 MPX)	(including ABO genotyping)	.	ID|HPA for 12 HPA antigens	.
consumables per sample and sum of examples 1-3 (in Euro)	~ 75,00 to 90,00	~ 40,00 to 50,00	~ 185.00	~ 130.00	~ 85.00 to 98.00	~ 80.00 to 100.00

Matrix Assisted Laser Disorption Ionisation –
MALDI-

Time Of Flight
TOF

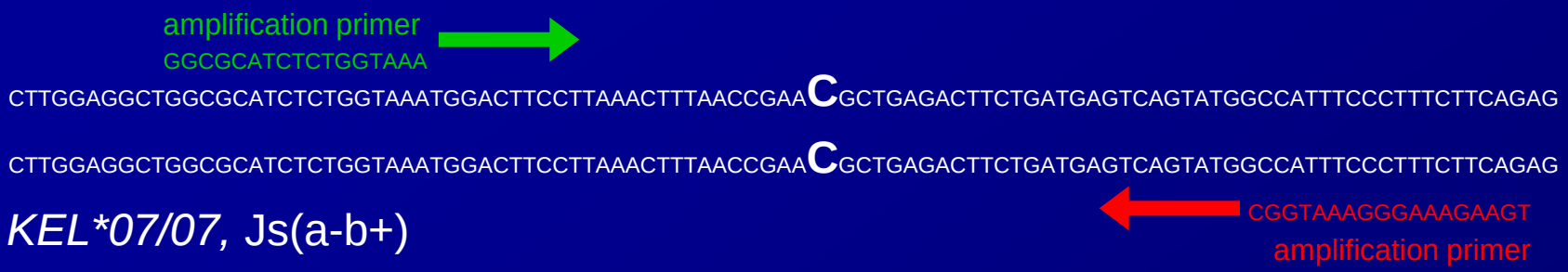
Mass Spectrometry
MS

GenoTyping
GT

- **technological principle**
- **hardware**
- **a Swiss project**
- **the modules**
- **current results**
- **conclusion**

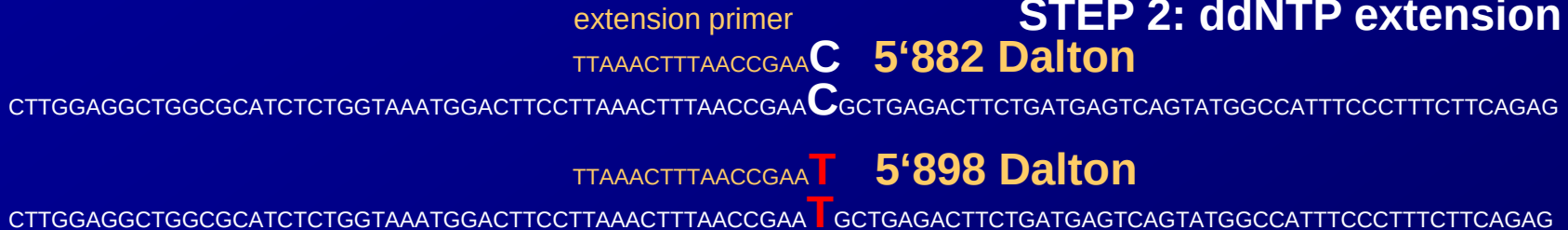
Matrix Assisted Laser Disorption Ionisation – Time Of Flight

MALDI-TOF principle on Js(a+b+), *KEL*01* I *KEL*02*



*KEL*07/07*, Js(a-b+)

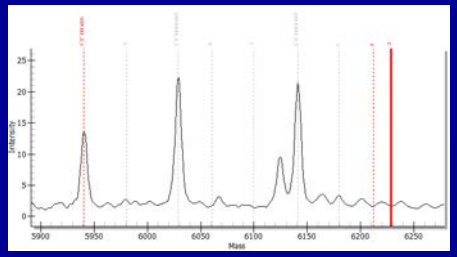
STEP 2: ddNTP extension



*KEL*06*/07, Js(a+b+)

*KEL*06/06*, Js(**a+b-**)

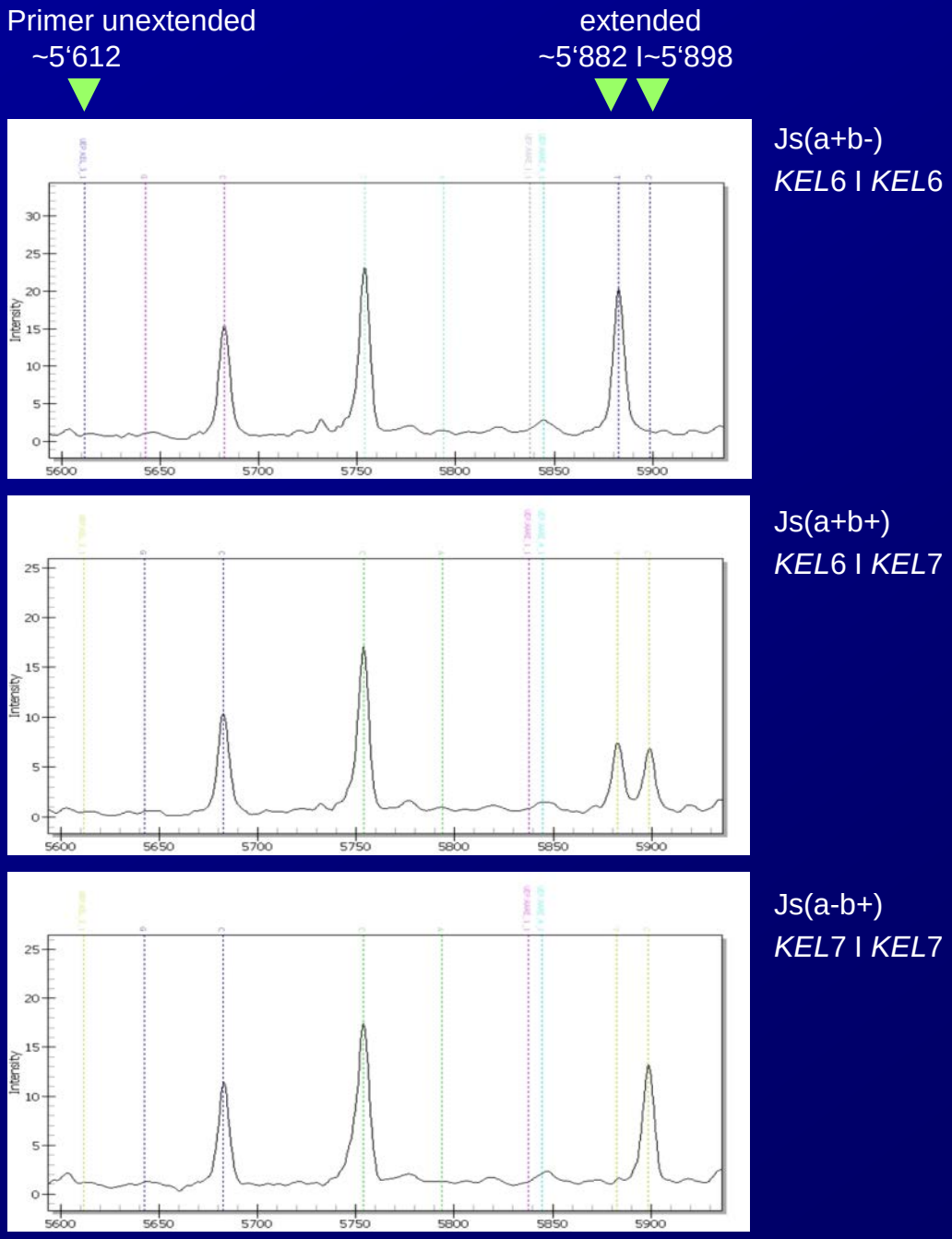
spectrogram of three different Js^{a}/Js^{b} (*KEL*06* I *KEL*07*) DNAs



MALDI-TOF mass spectrometry measures molecular weight (in Dalton) of extension primers (5‘612) plus 1 extended base.

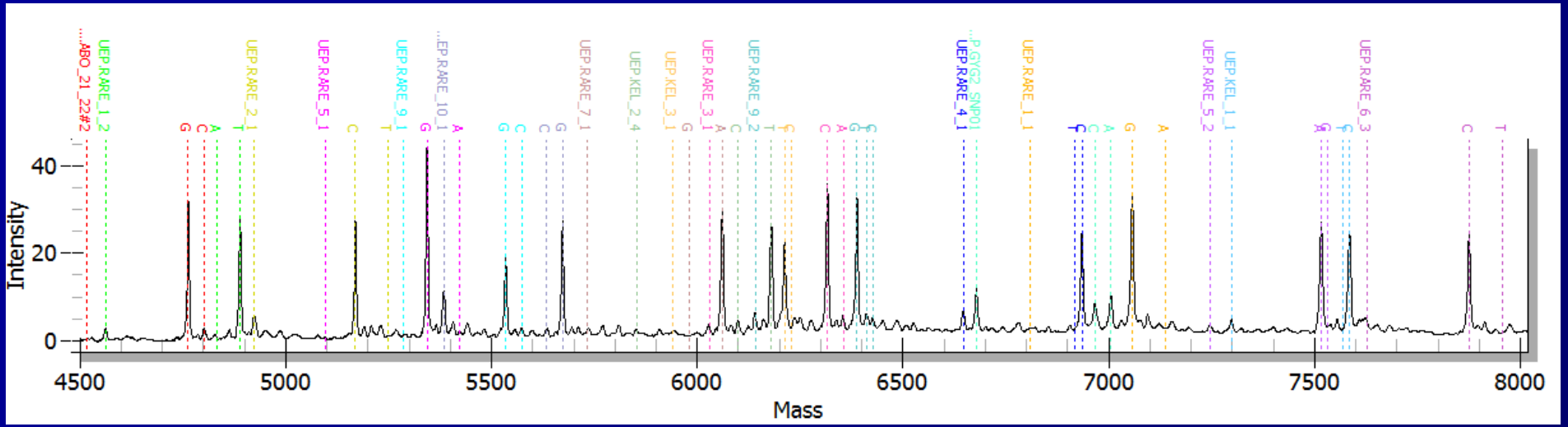
Heterozygous samples show 2 different molecular weights for those primers at about 5‘882 and 5‘898 Dalton (all indicated by arrow).

Other specific peaks are results of other bloodgroup SNPs, detected in multiplex in the same PCR.



Matrix Assisted Laser Disorption Ionisation – Time Of Flight

MALDI-TOF spectrogram of a "*RARE*" multiplex including 22 assays (=22 SNPs)



Kk, Kp, Suter, KEL11/17,
Lutheran, LU08/14, Auberger
Diego, Wright,
Cartwright,
Colton,
Knops, McCoy,
Dombrock, Holley, Joseph
Scianna,
Landsteiner-Wiener,
Cromer, Tc
Indian

Primer
unextended
~7'298

extended
~7'569 I~7'585

… 46 antigens

Matrix Assisted Laser Disorption Ionisation – **MALDI-**

Time Of Flight **TOF**

Mass Spectrometry **MS**

GenoTyping **GT**

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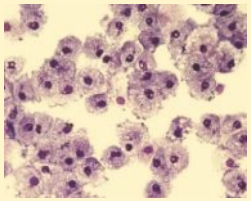
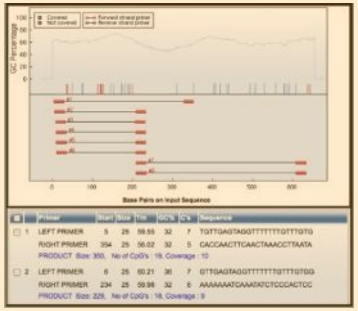
Typical MassARRAY ® Assay Workflow

Isolate
DNA or RNA



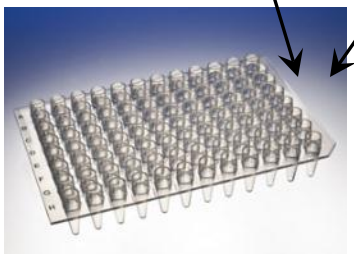
Obtain
primers

Design assays



Marker selection

~Days



PCR >> Single Base Ext.
or Cleavage chemistry

Transfer assay products to
SpectroCHIP ®

Read assay products on
Mass Spec; analyze data

~10
Hours

Matrix Assisted Laser Disorption Ionisation – **MALDI-**

Time Of Flight **TOF**

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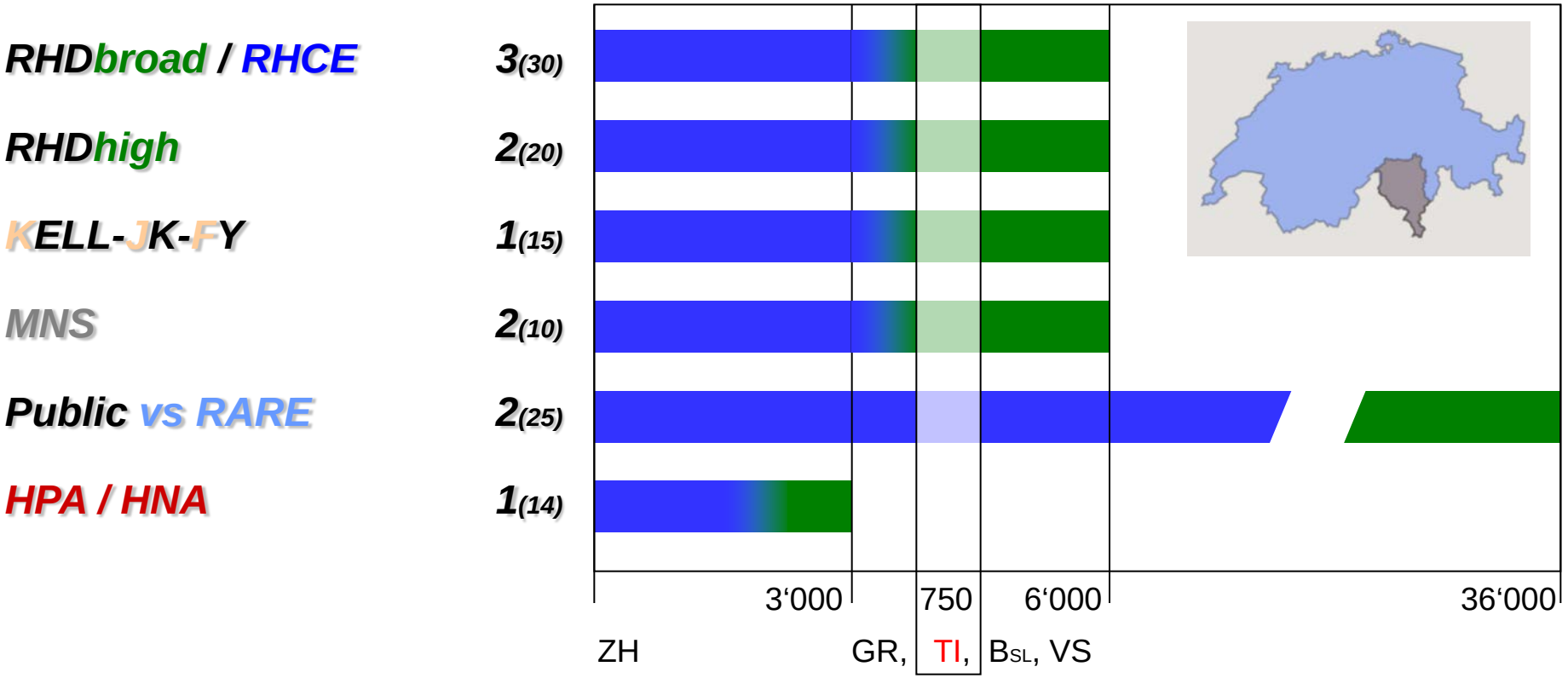
projects to come … participants are exemplary and not confirmed yet

Financial support granted by the Humanitarian Foundation of the Swiss Red Cross, Blutspende Zürich, Blutspende SRK Schweiz and Sequenom GmbH.



From 2011 for 3 years:

3‘000 DNAs for ***HPA/HNA***

36‘000 DNAs for ***Public vs Rare***

6‘000 DNAs for ***RHDCE, KEL, JK, FY, MNS***

50% (minimum) of all DNAs

will be from the **canton of Zurich**.

other 50% (maximum) from **other Swiss areas**.

Matrix Assisted Laser Disorption Ionisation – **MALDI-**

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Matrix Assisted Laser Disorption Ionisation – Time Of Flight

MALDI-TOF MS GT modules KEL-JK-FY, MNSs, “rare“

module name	genes	positions on genes, (trivial) (allele) names, additional information	n MPX	n SNPs	n alleles	n antigens
KEL-JK-FY			**1**	**15**	**21**	**19**
	KEL	K/k, Kp, Js, e.g. KEL(ISVS3+1g>a)null & 5 others, KEL(1719C>T)mod		9	11	10
	SLC14A1	Jk, JK(IVS5-1g>a)null & 2 others, JK(582C>G)		3	6	4
	DARC	Fy, -67T>C, FYX		3	4	5
	GYG2, AMXY	cross-ID-control		2	2	0
MNSs§	GYPA, GYPB, GYPE	**currently under development**	**2**	**11**	**15**	**16**
"rare antigens"			**2**	**22**	**34**	**46**
	KEL	K/k, Kp, Js, KEL11/17		4	5	8
	LU	Lu, LU8/14, Au		3	4	6
	Band 3	Di, Wr		2	3	4
	ACHE	Yt		1	2	2
	AQP1	Co		1	2	2
	CR-1	Kn, McC, Sl		3	4	6
	ART4	Do, Hy, Jo		3	4	6
	ICAM4	LW		1	2	2
	ERMAP	SC		1	2	2
	DAF	Cr, Tc		2	4	6
	CD44	In		1	2	2
	GYG2, AMXY, ABO	cross-ID-control, ABO positions: 261, 802, 803		5	6	0

Matrix Assisted Laser Disorption Ionisation – Time Of Flight

MALDI-TOF MS GT modules RH, HPA-HNA

module name	genes	positions on genes, (trivial) (allele) names, additional information	n MPX	n SNPs	n alleles	n antigens
RH	**RHCE, RHD**		**5**	**49**	**~79**	**28**
	RHC, RHc , RHCw	122, 201, 307, i2		4	3	4
	RHE, Rhe	676 generic and on RHCE only		2	2	2
	RHD exons	-132, i1+18, 455, 514, 787, 916, 968, i7-327, 1048, 1170, 1193, 1359		12	~45	2
	RHD categories & partials	VII, DFL, DOL, DVL-2, V(697A), weak type 4.0-3, 11, 15, DNB, DAU		11	11	18
	RHD weaks	1, 1.1, 2, 3, 5, 17		6	6	0
	RHD DELs	delA147, IVS3+1g>a, IVS3+2T>A, K409K, X418L		5	5	2
	RHD nulls	W16X, Dces type 1 & 2, RHD-CE(2-9)-D 2 subtypes, RHDpsi, Y401X		9	7	0
	GYG2, AMXY, ABO	cross-ID-control, ABO positions: 261, 803		5	5	0
HPNA	e.g. ITGB3, FCGR3b, SLC44A2		**1**	**13**	**25**	**23**
	HPA	HPA-1 to 6, 15		7	14	14
	HNA	HNA-1, 3 to 5		6	9	9
	AMXY	cross-ID-control		1	2	0
		redundancy of "rare" to "KEL-JK-FY"		-3	-4	-6
		total different blood (platelet, granulocyte) groups	**11**	**107**	**170**	**132**
		total different cross-ID-controls		**5**	**6**	n.a.

Matrix Assisted Laser Disorption Ionisation – **MALDI-**

Time Of Flight **TOF**

Mass Spectrometry **MS**

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Matrix Assisted Laser Disorption Ionisation – Time Of Flight

MALDI-TOF MS GT results on “rare typing“ 3.040 individuals

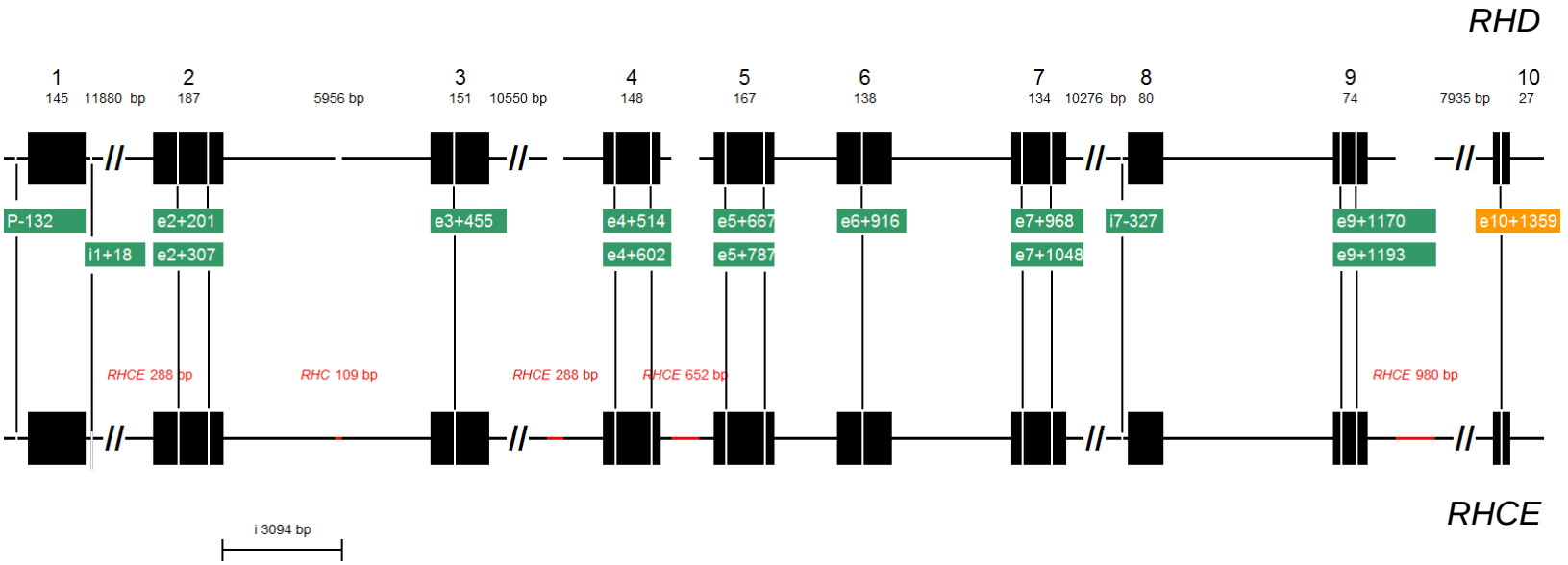
antigen	ISBT allele	observed "rare" allele frequency (%), MALDI-TOF MS	n observed "rare" homozygous carriers (in 3.040)	n expected "rare" homozygous carriers (in 3.040)	n expected "rare" homozygous carriers (in 36.000)	n expected "rare" homozygous carriers in Switzerland (8 Mio)
K	*KEL*01*	0.0395	4	4.7	56	
Kp[a]	*KEL*02.03*	0.0107	1	0.3	4	
Js[a]	*KEL*02.06*	0.0008	0	0.0	0	5.5
KEL17	*KEL*02.17*	0.0013	0	0.0	0	14.2
Lu[a]	*LU*01*	0.0370	5	4.1	49	
LU14	*LU*02.14*	0.0093	2	0.3	3	
LU19	*LU*02.19*	0.3182	313	303.9	3646	
Di[a]	*DI*01*	0.0002	0	0.0	0	0.2
Wr[a]	*DI*02.03*	0.0003	0	0.0	0	0.9
Yt[b]	*YT*02*	0.0648	14	12.6	151	
Co[b]	*CO*02*	0.0333	2	3.3	40	
Kn[b]	*KN*02*	0.0283	5	2.4	29	
McC[b]	*KN*01.06*	0.0003	0	0.0	0	0.9
Vil	*KN*01.07*	0.0018	0	0.0	0	26.7
Do[a]	*DO*01*	0.4061	487	495.8	5936	
Hy neg	*DO*02.–04*	0.0007	0	0.0	0	3.5
Jo[a] neg	*DO*01.–05*	0.0000	0	0.0	0	0.0
LW[b]	*LW*07*	0.0040	0	0.0	1	
SC:2	*SC*02*	0.0000	0	0.0	0	0.0
Cr[a] neg	*CROM*–01*	0.0002	0	0.0	0	0.2
Tc[b]	*CROM*01.03*	0.0002	0	0.0	0	0.2
Tc[c]	*CROM*01.04*	0.0008	0	0.0	0	5.6
In[a]	*IN*01*	0.0000	0	0.0	0	0.0

Matrix Assisted Laser Disorption Ionisation – Time Of Flight

MALDI-TOF MS results on HPA/HNA typing 1.520 individuals

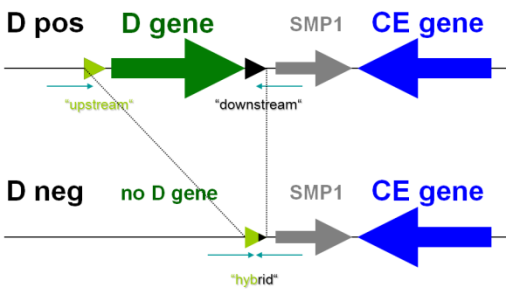
group system	allele	allele-frequency MALDI-TOF MS	allele-frequency Switzerland	allele-frequency Austria	allele-frequency Germany
HPA	1b	0.147	0.191	0.148	0.161
	2b	0.090	0.109	0.082	0.090
	3b	0.334	0.407	0.388	0.414
	4b	0.000	0.000	0.000	0.000
	5b	0.106	0.066	0.108	0.083
	15b	0.490	n.a.	0.500	n.a.
HNA	1b	0.621	n.a.	n.a.	0.601
	1c	0.033	n.a.	n.a.	0.008
	3b	0.207	n.a.	n.a.	0.256
	4b	0.117	n.a.	n.a.	0.092
	5b	0.276	n.a.	n.a.	0.269

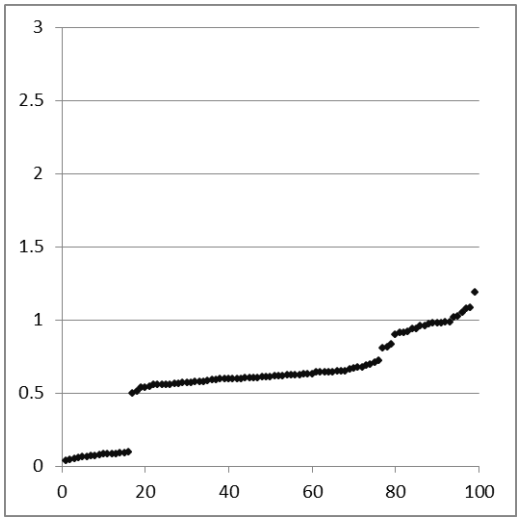
Specificities: *RHD:* zygosity is detectable…



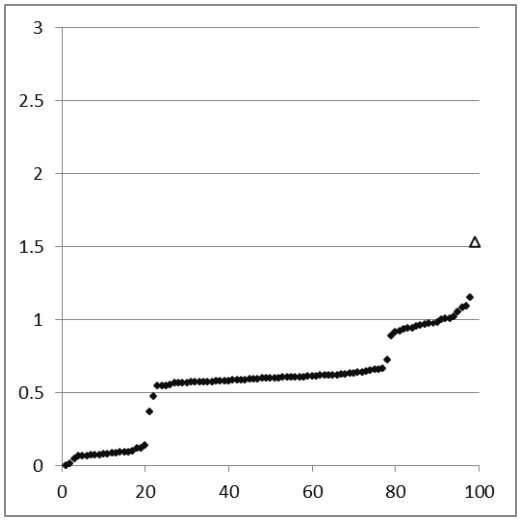
Matrix Assisted Laser Disorption Ionisation – Time Of Flight

MALDI-TOF MS GT results on *RHD* zygosity

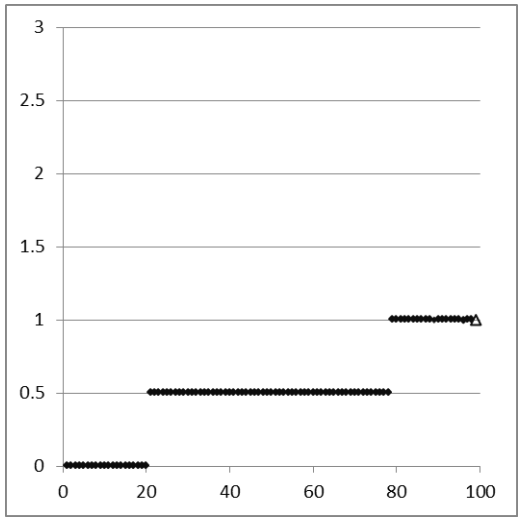




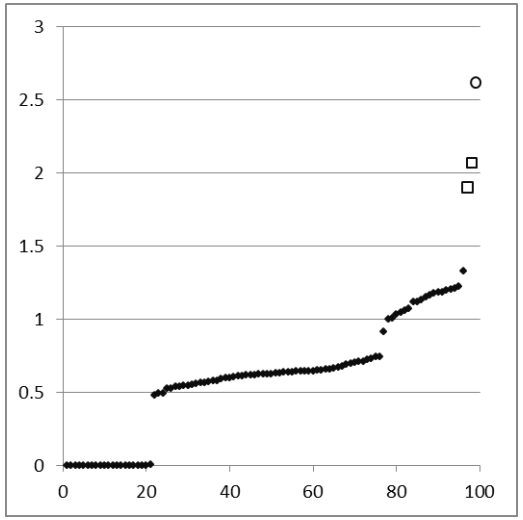
RH Intron 1,+18



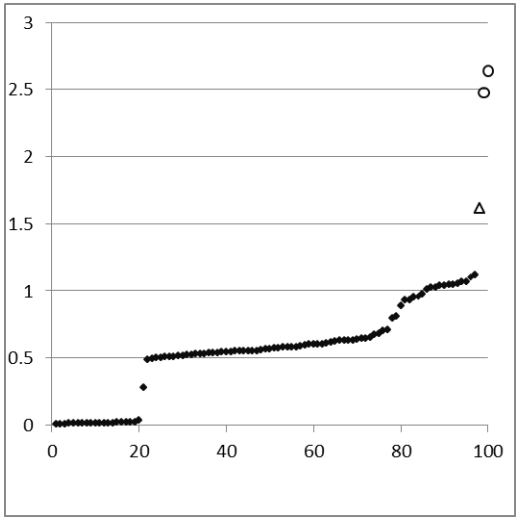
RH exon 3, 455



theoretically

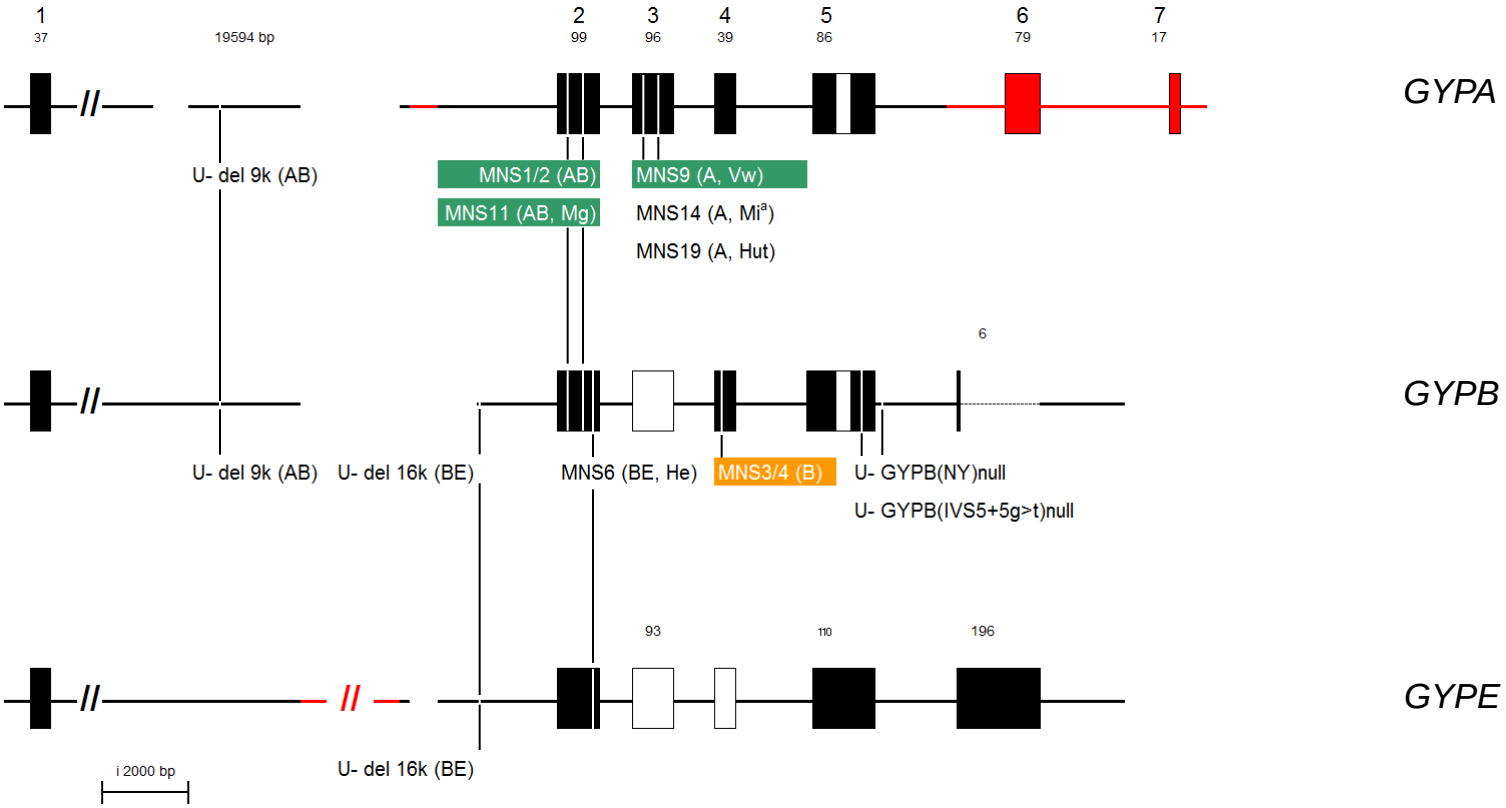


RH exon 6, 916



RH exon 9, 1170

Specificities: *MNS*, prototype 2



Matrix Assisted Laser Disorption Ionisation – **MALDI-**

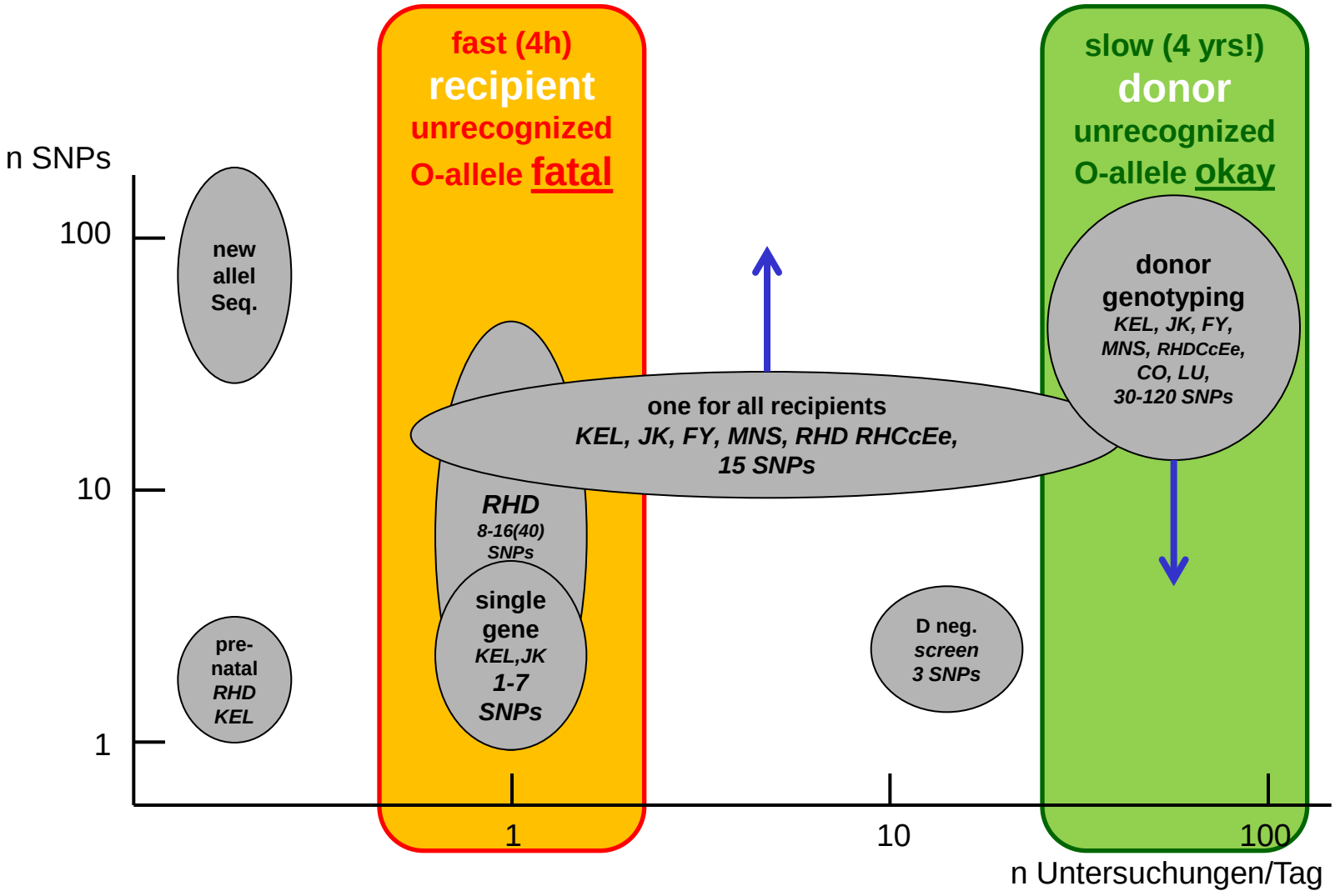
Time Of Flight **TOF**

Mass Spectrometry **MS**

GenoTyping **GT**

- technological principle
- hardware
- a Swiss project
- the modules
- current results
- **conclusion**

Methods & Indication I Resolution & Throughput



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