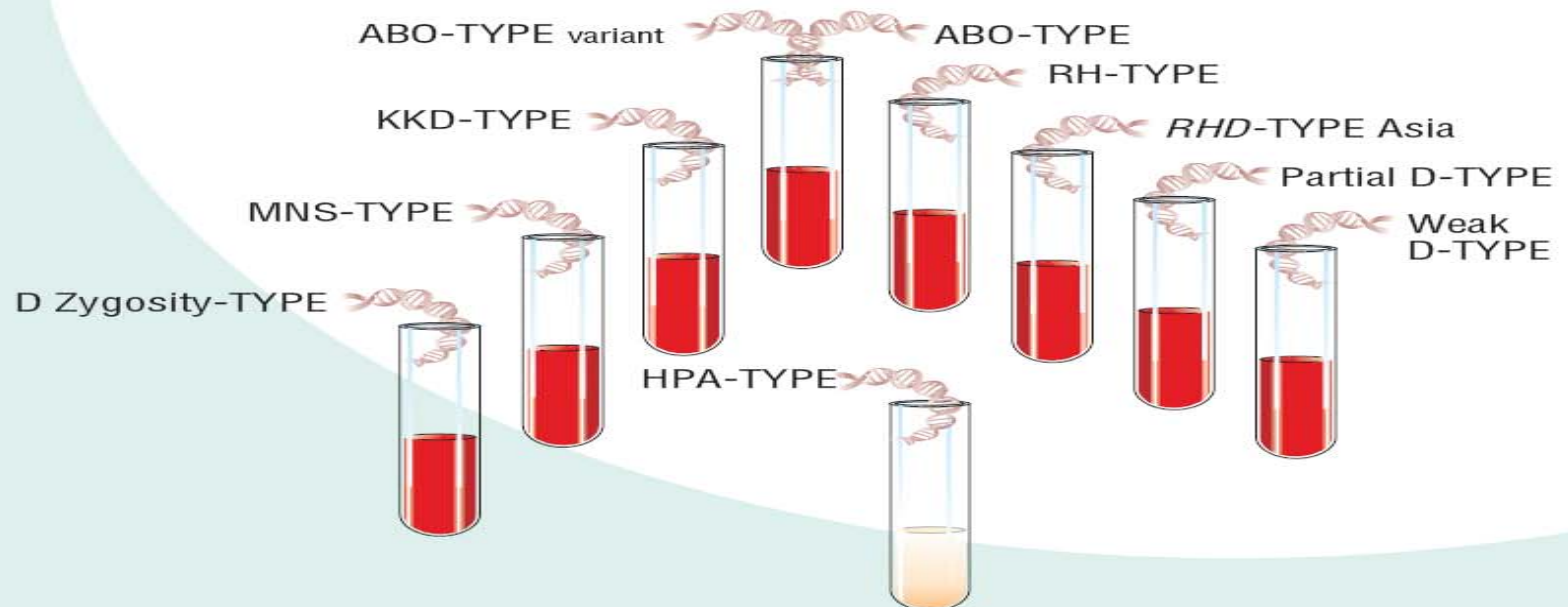


# BAGene

SSP kits for determination of

- **ABO blood groups**
- **RH types**
- **Kell, Kidd, Duffy systems**
- **MNS system**
- **HPA specificities**

on a molecular genetic basis

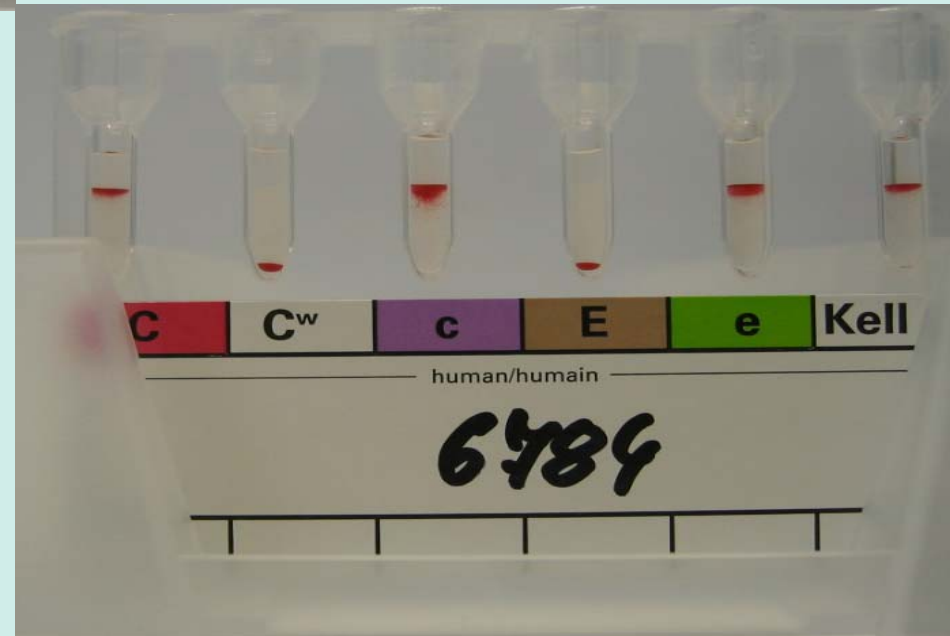
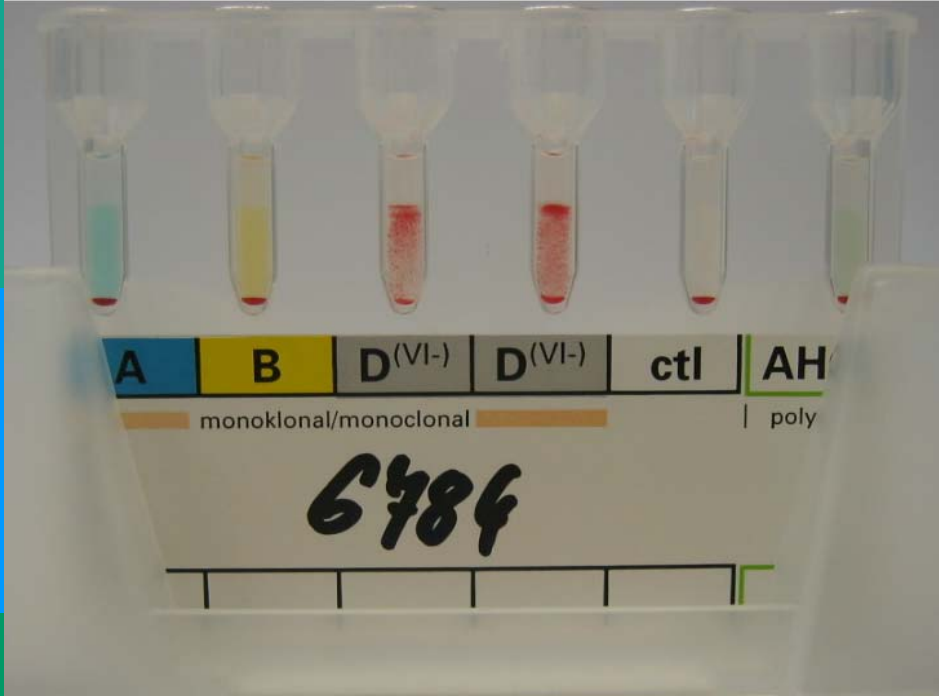


# Application

- **Genotype multi-transfused recipients**
- **Genotype patients after ABO-incompatible bone marrow transplantation**
- **Determine *RHD* zygosity of partners of alloimmunized D-negative women before pregnancies**
- **Genotype Rh D negative donors with C or E in order to exclude the presence of the *RHD* gene and thus preventing anti-D alloimmunization of recipients caused by hidden Rh D variants in RBC units**
- **Identify genotype in case of weakly expressed Rh D (e.g. DEL) in donors**
- **Confirm weak D genotype in recipients in order to avoid the donation of Rh D negative blood units**
- **Quality control of serological methods**
- **External Quality Assurance trials**

Result according to  
Transfusion guideline

Rhesus D positive  
CcD.ee Kell positive





**C**    **c**    **D**    **E**    **e**    **ctl**

human/humain

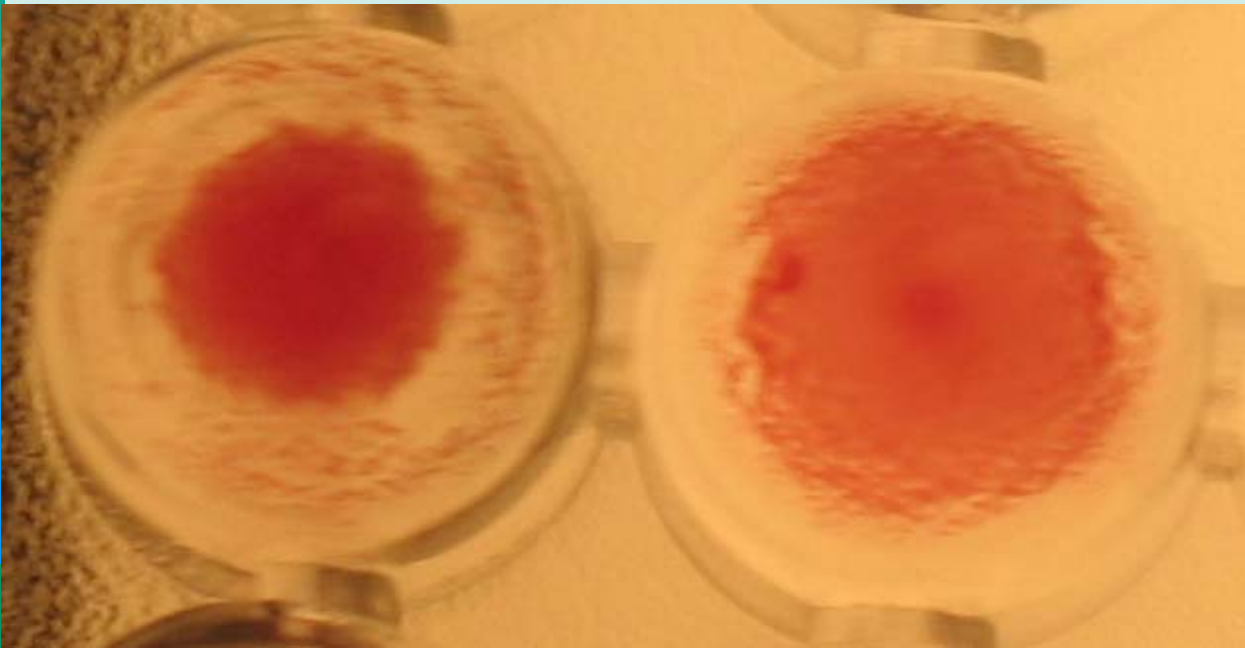
E000 206 10.95

M    9031-6784

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microwell plate  
antisera  
dilution 1:5

agglutination  
strength (1) -1



tube test  
immediate spin

questionable  
agglutination  
with monoclonal  
Anti-D



9034-5777

# Weak D-TYPE

Worksheet und Auswertetabelle / Worksheet and Evaluation diagram

Reaktions-Nr. / Reaction No.	1	2	3	4	5	6	7	8
PCR-Produkt (Größe in bp) PCR product (size in bp)	150	126	165	101	130	112	198	153
weak D Allele / weak D alleles								
weak D type 1	+	-	-	-	-	-	-	-
weak D type 2	-	+	-	-	-	-	-	-
weak D type 3	-	-	+	-	-	-	-	-
weak D type 4.0, 4.1	-	-	-	+	-	-	-	-
weak D type 4.2, DAR	-	-	-	+	+	-	-	-
weak D type 5	-	-	-	-	-	+	-	-
weak D type 11 (haplotype cDe)	-	-	-	-	-	-	+	-
RHD(M295I) (haplotype CD <sub>e</sub> e)	-	-	-	-	-	-	+	-
weak D type 15	-	-	-	-	-	-	-	+
RHD pos. oder / or RHD neg.	-	-	-	-	-	-	-	-

Ergebnis Result	1	2	3	4	5	6	7	8	Genotyp Genotype

Proben-ID / Sample-ID: 11 9031 6784

Name: \_\_\_\_\_

Geb.-Datum / Birthdate: \_\_\_\_\_

Ergebnis / Result: Dw typ 1

Datum / Date: 22.10.04

Unterschrift / Signature: Löwmeier P.



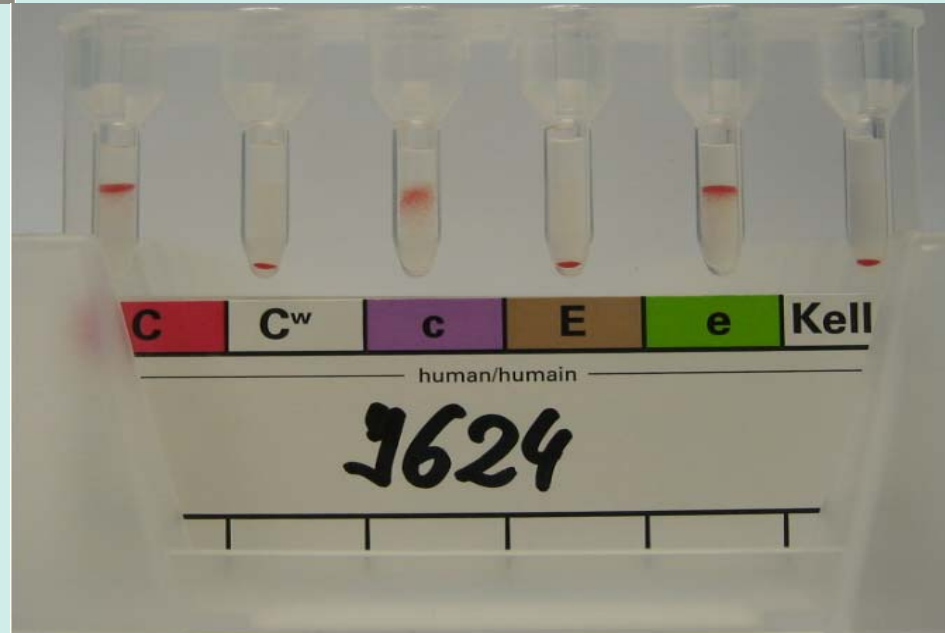
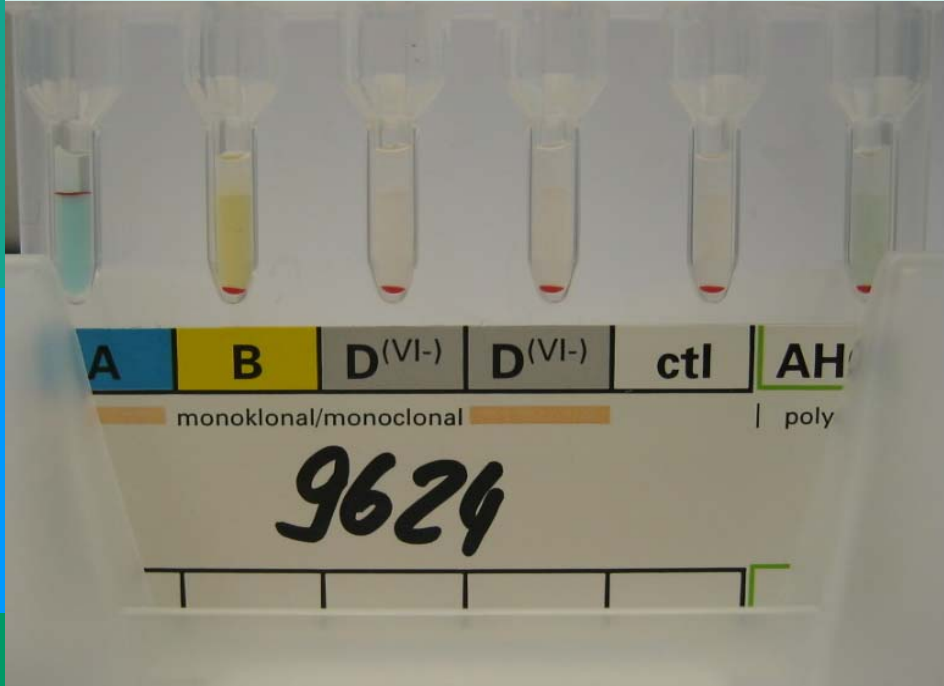
Final result  
weak D type 1

Rhesus prophylaxis  
not required

Transfusion of D  
positive red blood  
cells is possible

Result according to  
Transfusion guideline

Rhesus D negative,  
Ccddee Kell negative







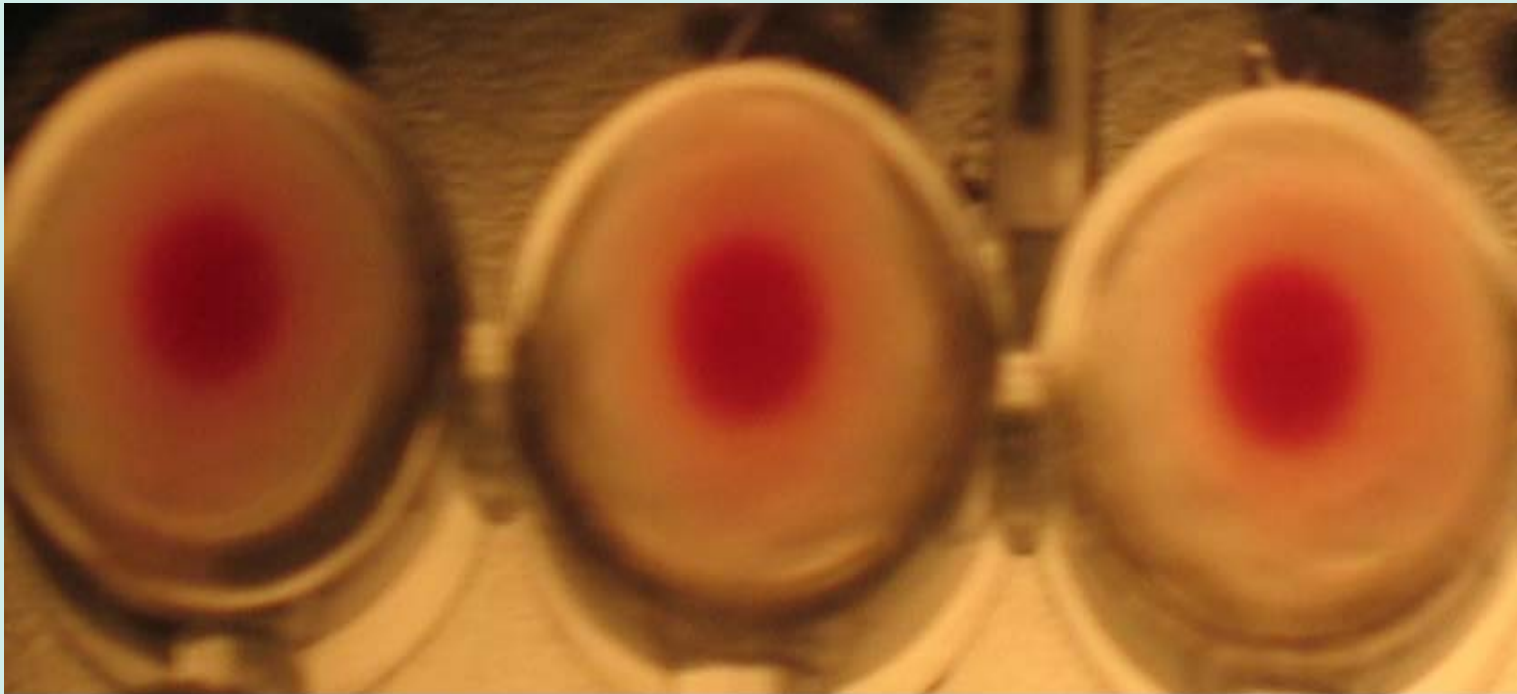
**C**   **c**   **D**   **E**   **e**   **ctl**

human/humain

E000 206 10.95

627-9624

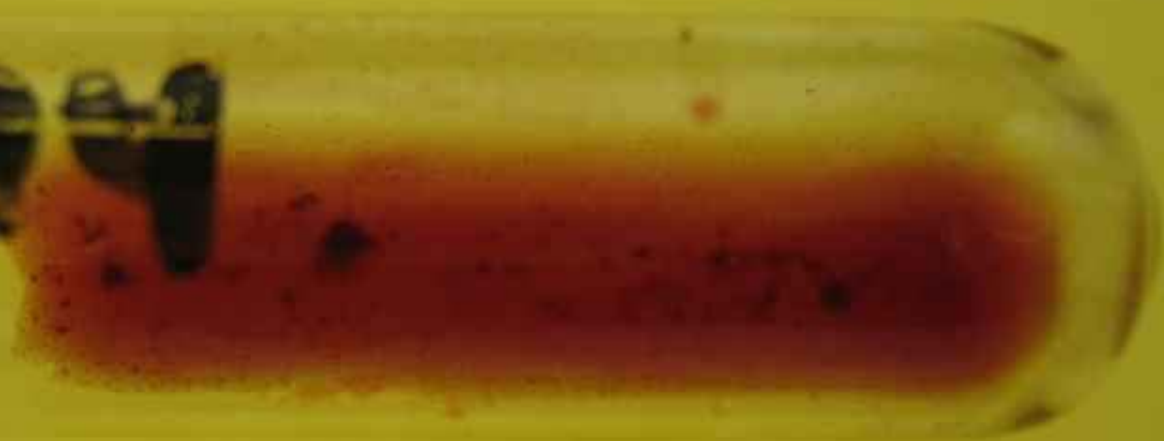
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microwell plate  
antisera dilution 1:5

negative reaction

9624



# Weak D-TYPE

## Worksheet und Auswertetabelle / Worksheet and Evaluation diagram

Reaktions-Nr. / Reaction No.	1	2	3	4	5	6	7	8
PCR-Produkt (Größe in bp) PCR product (size in bp)	150	126	165	101	130	112	198	153
weak D Allele / weak D alleles								
weak D type 1	+	-	-	-	-	-	-	-
weak D type 2	-	+	-	-	-	-	-	-
weak D type 3	-	-	+	-	-	-	-	-
weak D type 4.0, 4.1	-	-	-	+	-	-	-	-
weak D type 4.2, DAR	-	-	-	+	+	-	-	-
weak D type 5	-	-	-	-	-	+	-	-
weak D type 11 (haplotype cDe)	-	-	-	-	-	-	+	-
RHD(M295I) (haplotype CD <sub>e</sub> )	-	-	-	-	-	-	+	-
weak D type 15	-	-	-	-	-	-	-	+
RHD pos. oder / or RHD neg.	-	-	-	-	-	-	-	-

Ergebnis Result	1	2	3	4	5	6	7	8	Genotyp Genotype

Proben-ID / Sample-ID: 0627 9624

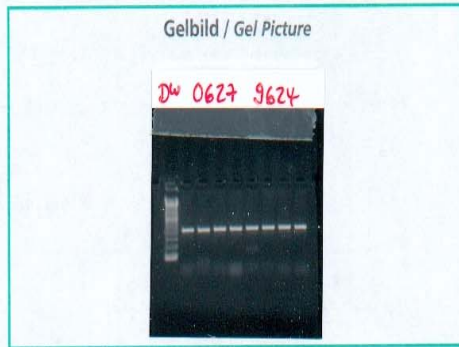
Name: /

Geb.-Datum / Birthdate: /

Ergebnis / Result: => D, partial

Datum / Date: 25.10.04

Unterschrift / Signature: Krämer



# Partial D-TYPE



## Worksheet und Auswertetabelle / Worksheet and Evaluation diagram

Reaktions-Nr. / Reaction No.	1	2	3	4	5	6	7	8	9	10	11
PCR-Produkt (Größe in bp) PCR product (size in bp)	146	118	135	132	132	120	166	117	140	107	113
RHD Exons	D <sub>2</sub>	D <sub>3</sub>	D <sub>4</sub>	D <sub>5</sub>	D <sub>6</sub>	D <sub>7</sub>	D <sub>2</sub>	D <sub>7</sub>	D <sub>8</sub>	D <sub>6</sub>	D <sub>9</sub>
Phänotypen / Phenotypes											
Standard RHD											
D	+	+	+	+	+	+	-	-	-	-	-
RHD Varianten / RHD Variants											
D cat. IIIa, IIIc, III type IV <sup>n.t.</sup>	+	-	+	+	+	+	-	-	-	-	-
D cat. IIIb <sup>n.t.</sup>	-	+	+	+	+	+	-	-	-	-	-
D cat. IVa <sup>n.t.</sup>	+	-	+	+	+	-	-	-	-	-	-
D cat. IVb, IVb(I), IV type IV <sup>n.t.</sup>	+	+	+	+	+	-	-	-	-	-	-
D cat. IV type III	+	+	+	+	-	-	-	-	-	-	-
D cat. Va, Va-like, Va-associated	+	+	+	-	+	+	-	-	-	-	-
D cat. VI type I	+	+	-	-	+	+	-	-	-	-	-
D cat. VI type II	+	+	-	-	-	+	-	-	-	-	-
D cat. VI type III	+	-	-	-	-	+	-	-	-	-	-
D cat. VI type IV <sup>n.t.</sup>	-	-	-	-	+	+	-	-	-	-	-
D cat. VII	+	+	+	+	+	+	+	-	-	-	-
DAR	+	+	+	+	+	+	-	-	-	-	-
DAU	+	+	+	+	+	+	-	-	+	-	-
DBT	+	+	+	-	-	-	-	-	-	-	-
DFR	+	+	-	+	+	+	-	-	-	-	-
DHMi	+	+	+	+	+	+	-	-	-	+	-
DHMij <sup>n.t.</sup>	+	-	-	-	+	+	-	-	-	-	-
DNB	+	+	+	+	+	+	-	+	-	-	-
ROHar (Rh33)	-	-	-	+	-	-	-	-	-	-	-
D cat. II, DCS, DFW, DHR, DIM, DNU <sup>n.t.</sup>	+	+	+	+	+	+	-	-	-	-	-
Rh D <sup>n</sup> (RHD(K409K))	+	+	+	+	+	+	-	-	-	-	+

n.t. = not tested currently

Ergebnis Result	1	2	3	4	5	6	7	8	9	10	11	Phänotyp Phenotype	Genotyp Genotype

Proben-ID / Sample-ID: 0627 9624

Name: /

Geb.-Datum / Birthdate: /

Ergebnis / Result: D, VI

Datum / Date: 26.10.04

Unterschrift / Signature: Krämer



# Weak D-TYPE

Worksheet und Auswertetabelle / Worksheet and Evaluation diagram

Reaktions-Nr. / Reaction No.
PCR-Produkt (Größe in bp) / PCR product (size in bp)
weak D type 1
weak D type 2
weak D type 3
weak D type 4.0, 4.1
weak D type 4.2, DAR
weak D type 5
weak D type 11 (haplotype RHD(M295I) (haplotype
weak D type 15
RHD pos. oder / or RHD

**Final result: D category VI type II**

**Rhesus prophylaxis required**

**Transfusion of D negative red blood cells is required**

Ergebnis Result	1	2	3	4	5	6	7	8	Genotyp Genotype

# Partial D-TYPE

Worksheet und Auswertetabelle / Worksheet and Evaluation diagram

Reaktions-Nr. / Reaction No.	1	2	3	4	5	6	7	8	9	10	11
PCR-Produkt (Größe in bp) / PCR product (size in bp)	146	118	135	132	132	120	166	117	140	107	113

ROHar (Rh33)	-	-	-	+	-	-	-	-	-	-	-
D cat. II, DCS, DFW, DHR, DIM, DNU n.t.	+	+	+	+	+	+	-	-	-	-	-
Rh D <sup>II</sup> (RHD(K409K))	+	+	+	+	+	+	-	-	-	-	+

n.t. = not tested currently

Ergebnis Result	1	2	3	4	5	6	7	8	9	10	11	Phänotyp Phenotype	Genotyp Genotype

Proben-ID / Sample-ID: 0627 9624

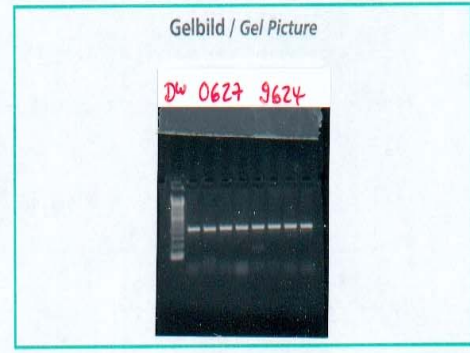
Name: \_\_\_\_\_

Geb.-Datum / Birthdate: \_\_\_\_\_

Ergebnis / Result: => D partial

Datum / Date: 25.10.04

Unterschrift / Signature: *Gesäuer*



Proben-ID / Sample-ID: 0627 9624

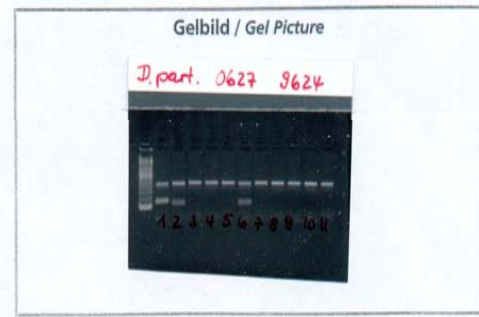
Name: \_\_\_\_\_

Geb.-Datum / Birthdate: \_\_\_\_\_

Ergebnis / Result: D VI

Datum / Date: 26.10.04

Unterschrift / Signature: *Gesäuer*



# Contents of our BAGene SSP kits

- PCR plates or strips with prealiquoted, dried and colored reaction mixes containing allele specific primers, internal control primers (specific for the HGH gene) and nucleotides.
- 10 x PCR buffer
- PCR strip caps
- Worksheets and Evaluation Diagrams
- Instructions for Use

# Evaluation of Results - RHD-RHCE genotyping

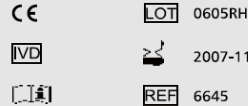


Biologische  
Analyse-System  
GmbH

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Telefon: 0 64 04/925-0 · Fax: 0 64 04/925-250  
www.bag-germany.com  
info@bag-germany.com

Auftragsannahme / Ordering:  
Telefon: 0 64 04/925-450  
Fax: 0 64 04/925-460  
verkauf@bag-germany.com

Customer Service:  
Telefon: 0 64 04/925-125  
Fax: 0 64 04/925-421  
service@bag-germany.com



**BAGe**  
RH-TYPE

## Worksheet und Auswertetabelle / Worksheet and Evaluation diagram

Reaktions-Nr. / Reaction No.	1	2	3	4	5	6	7	8	9	10	11	12	13
PCR-Produkt/e / PCR products (Größe in bp/size in bp)	224 123	390 248 154	140	113	113	198	143	215	162	145	157	155	181
Reaktionsmuster / Reaction pattern	D <sub>A14</sub> D <sub>7</sub>	D <sub>7</sub> RHD(W16X) RHD Ψ	D <sub>A</sub> RHD Ψ	D <sub>A</sub> RHD (K409K) neg.	D <sub>A</sub> RHD (K409K) neg.	D <sub>A</sub> RHD (M295I)	D <sub>A</sub> RHD (IVS3+1G>A)	Cde <sup>S</sup>	C <sup>CW</sup>	c	E	e	C <sup>W</sup>
Phänotyp Phenotype													
Genotyp Genotype													
Standard RHD/RHCE Allele/s													
D-positive	+	+	+	+	+	+	+	+	+	+	+	+	+
D-negative	-	-	-	-	-	-	-	-	-	-	-	-	-
C									+	-	-	-	-
C <sup>W</sup>									+	-	-	-	+
c									-	+	-	-	-
E									-	-	+	-	-
e									-	-	-	+	-
Größe der internen PCR-Kontrolle in Reaktion Nr. 2: 659 bp, in allen anderen Reaktionen: 434 bp (HGH) Size of internal PCR control in reaction no. 2: 659 bp, in all the other reactions: 434 bp (HGH)													
RHD-Varianten / RHD variants													
D-positive	RHD / RHD(K409K)	+	+	+	+	+	+	-	-	-	-	-	-
D <sub>A</sub>	RHD(K409K)	+	+	+	+	-	+	-	-	-	-	-	-
D-positive	RHD / RHD(M295I)	+	+	+	+	-	+	-	-	-	-	-	-
D <sub>A</sub>	RHD(M295I)	+	+	+	+	-	+	-	-	-	-	-	-
D-positive	RHD / RHD(IVS3+1G>A)	+	+	+	+	-	+	-	-	-	-	-	-
D <sub>A</sub>	RHD(IVS3+1G>A)	+	+	+	+	-	+	-	-	-	-	-	-
D-positive	RHD / RHD Ψ	+	+	+	+	-	+	-	-	-	-	-	-
D-negative	RHD Ψ	+	+	+	+	-	+	-	-	-	-	-	-
D-negative	RHD-CE(8-9)-D	+	-	+	-	-	-	-	-	-	-	-	-
D-negative	RHD(W16X)	+	+	+	+	-	+	-	-	-	-	-	-
D-positive	RHD / Cde <sup>S</sup>	+	+	+	+	-	+	-	-	-	-	-	+
D-negative	Cde <sup>S</sup> / Cde <sup>S</sup> ; Cde <sup>S</sup> / d	-	+	-	+	-	+	-	-	-	-	+	-
Beispiele / Examples													
ccddee		-	-	-	-	-	-	-	-	+	+	-	-
Ccddee		-	-	-	-	-	-	-	-	+	+	-	+
CcD.Ee		+	+	+	+	-	-	-	-	+	+	+	-
ccD.EE		+	+	+	+	-	-	-	-	+	+	+	-
CCD.ee		+	+	+	+	-	-	-	-	+	-	+	+
CC <sup>W</sup> D.Ee		+	+	+	+	-	-	-	-	+	-	+	+
D cat. VI		+	+	-	+	-	-	-	-	-	-	-	-
D cat. IV type III		+	-	+	+	-	-	-	-	-	-	-	-
DFR		+	+	-	+	-	-	-	-	-	-	-	-

Fehlende Banden in den Reaktionen Nr. 1 - 4 können auf RHD psi oder auf Partial D-Gene hinweisen.  
In Gegenwart von Cde<sup>S</sup> kommt es zum Ausfall der Reaktion Nr. 9.  
Missing bands in reaction no. 1 - 4 may either indicate RHD psi or partial D genes.  
In presence of Cde<sup>S</sup> reaction no. 9 is missing.

Ergebnis Result	1	2	3	4	5	6	7	8	9	10	11	12	13	Phänotyp Phenotype	Genotyp Genotype

Proben-ID / Sample-ID:  
Probenname / Name:  
Geb.-Datum / Born:  
Ergebnis / Result:  
Datum / Date:  
Unterschrift / Signature:

Gelbild / Gel Picture

VER 05/2006

## Kurzanleitung / Short Instructions

- Die gewünschte Anzahl **BAGe**-Platten/-Streifen aus dem Gefrierschrank (-20...-80°C) nehmen und den 10 x PCR-Puffer bei Raumtemperatur auftauen.  
Remove the required number of **BAGe**-plates/-strips from -20...-80°C and thaw the 10 x PCR-buffer.
- Der erste Reaktionsmix ist markiert (PCR-Streifen: schwarzer Strich um das erste Reaktionsgefäß, PCR-Platten: schwarzer Punkt oberhalb des ersten Reaktionsgefäßes).  
The first reaction mix is marked (PCR-strips: black line around the first reaction tube, PCR-plates: black dot above the first reaction tube).
- Den Mastermix, bestehend aus 10 x PCR-Puffer, DNA-Lösung, Taq-Polymerase und Aqua dest. zusammenpipettieren und gründlich vortexen. Die verschiedenen **BAGe** DNA-SSP Kits werden mit dem gleichen Mastermix angesetzt und sind daher miteinander kombinierbar.  
Pipet the mastermix, consisting of 10 x PCR-buffer, DNA-solution, Taq-Polymerase and aqua dest. and mix well. The different **BAGe** DNA-SSP Kits do all work with the same mastermix and can therefore be combined.

## Zusammensetzung des Mastermixes in Abhängigkeit von der Anzahl der Reaktionsmische Composition of the Mastermix depending on the number of reaction mixes

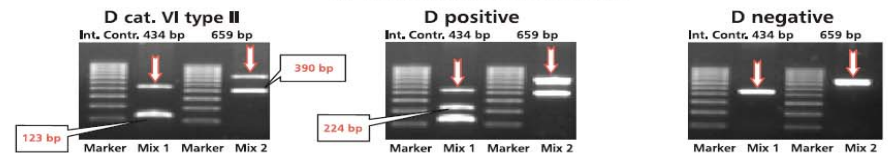
No. of mixes	DNA-sol. (50-100 ng/µl)	Aqua dest.	10 x PCR-buffer	Taq-Polymerase (5 U/µl)	total volume app.	
1	1	8	1	0,08	10	µl
2	2	16	2	0,2	20	µl
6*	7	50	7	0,5	65	µl
7	9	70	9	0,7	90	µl
8	10	80	10	0,8	100	µl
9	11	88	11	0,9	110	µl
10	12	96	12	1,0	120	µl
11	13	104	13	1,0	130	µl
12	14	112	14	1,1	140	µl
13	16	128	16	1,3	160	µl
14	17	136	17	1,4	170	µl
15	18	144	18	1,4	180	µl
16	19	152	19	1,5	190	µl

Bei abweichenden DNA-Konzentrationen sind die Mengen von DNA und Wasser entsprechend zu variieren.  
For different DNA concentrations, the quantities of DNA and water must be varied accordingly.

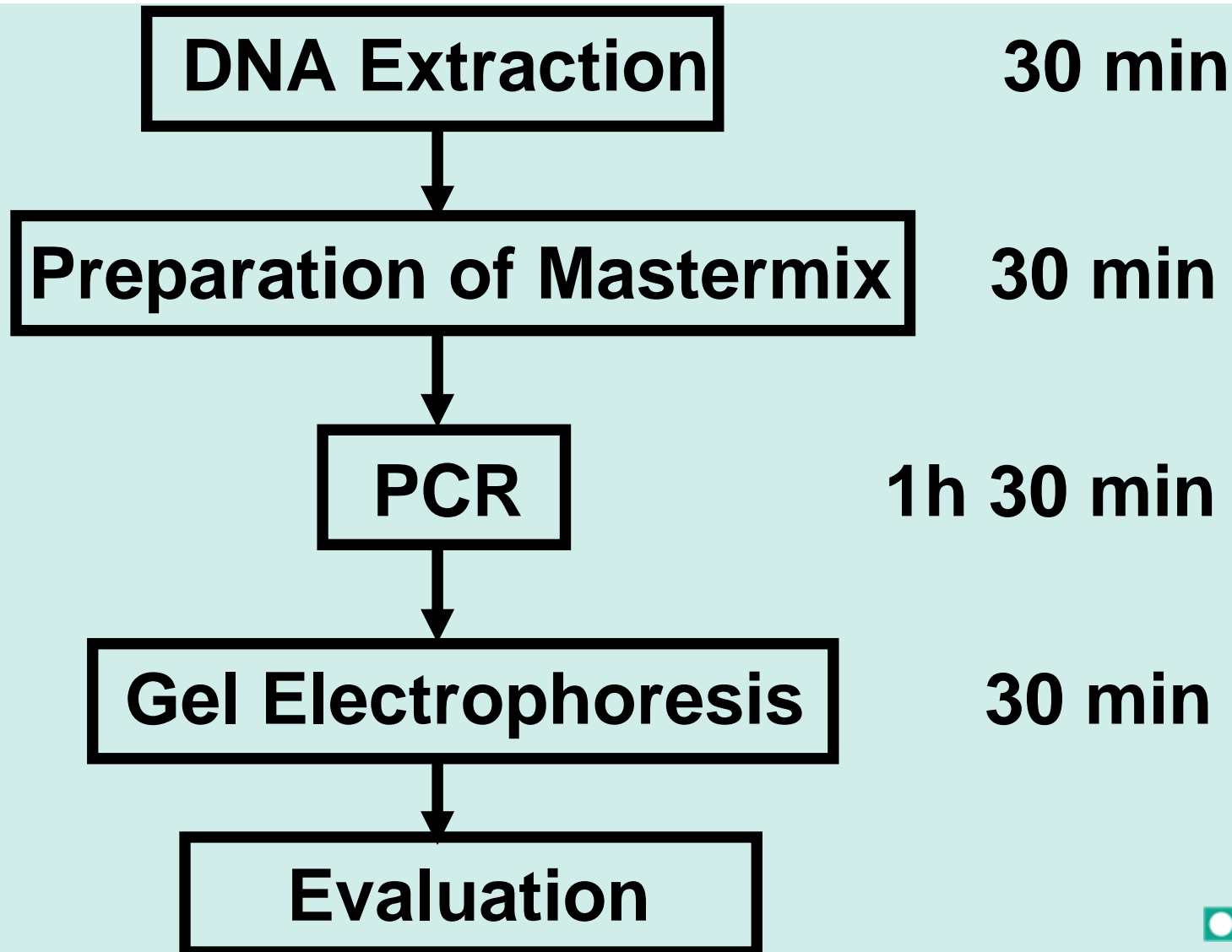
- \* Mastermix für 6 Reaktionsmische wird wegen des geringen Volumens Taq-Polymerase als Mindestansatz empfohlen.  
Minimum preparation of mastermix for 6 reaction mixes is recommended due to the small volume of Taq-Polymerase.
- Nach dem Vortexen werden von diesem Gemisch umgehend je 10 µl zu den angetrockneten Reaktionsmischen pipettiert.  
After vortexing add 10 µl of this mixture immediately to the dried reaction mixes.
  - Die Reaktionsgefäße werden mit den dafür vorgesehenen Deckeln **dicht** verschlossen. Die Platten/Streifen werden leicht bewegt, um das Pellet auf dem Gefäßboden etwas anzulösen. Die gesamte Reaktionslösung soll sich im Gefäßboden befinden.  
Tightly close the tubes with the respective strip caps. Slightly move the plates/strips to dissolve the pellet at the bottom of the tube. All PCR-solution should settle on the bottom of the tube.
  - Nach der PCR und Auftrennung der Amplifikate im Gel erfolgt die Auswertung.  
After PCR and separation of the amplicons in the gel, the evaluation can be carried out.
  - Alle **BAGe** Reagenzien sind nach Gebrauch wieder bei -20...-80°C zu lagern.  
Return all **BAGe** reagents to -20...-80°C after use.

➔ Ablesen der Ergebnisse in Pfeilrichtung / Read the results in direction of the arrow

## Beispiel: Gelbilder der Reaktionen 1 und 2 Example: gel pictures of reaction 1 and 2



# Test Procedure - Workflow



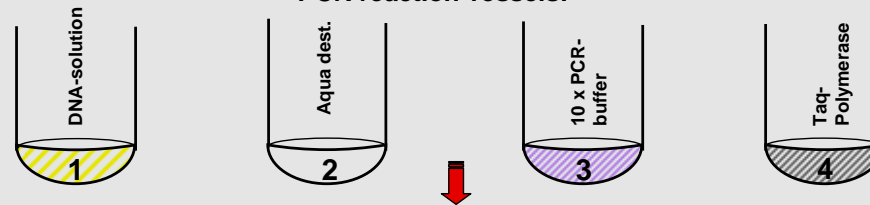


# Preparation of the PCR reaction mixes

## BAGene DNA-SSP Kits

### Pipetting Chart

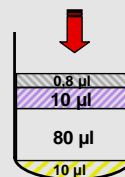
PCR reaction vessels:



Preparation of the mastermix depending on the number of reaction mixes

	No. of mixes	DNA-solution (50-100 ng/μl)	Aqua dest.	10 x PCR-buffer	Taq-Polymerase (5 U/μl)	total volume, app.	
<b>D Zygosity-TYPE, MNS-TYPE</b> <b>preparation for 6 mixes</b> →	6	7	50	7	0,5	65	μl
<b>RHD-TYPE Asia - 7 mixes</b> →	7	9	70	9	0,7	90	μl
<b>ABO-, KKD-, or Weak D-TYPE - 8 mixes</b> →	8	10	80	10	0,8	100	μl
<b>HPA-TYPE - 12 mixes</b> →	12	14	112	14	1,1	140	μl
<b>RH-TYPE - 13 mixes</b> →	13	16	128	16	1,3	160	μl
<b>ABO-TYPE variant or Partial D-TYPE - 16 mixes</b> →	16	19	152	19	1,5	190	μl

reaction mix  
e.g. ABO-TYPE, KKD-TYPE or  
Weak D-TYPE

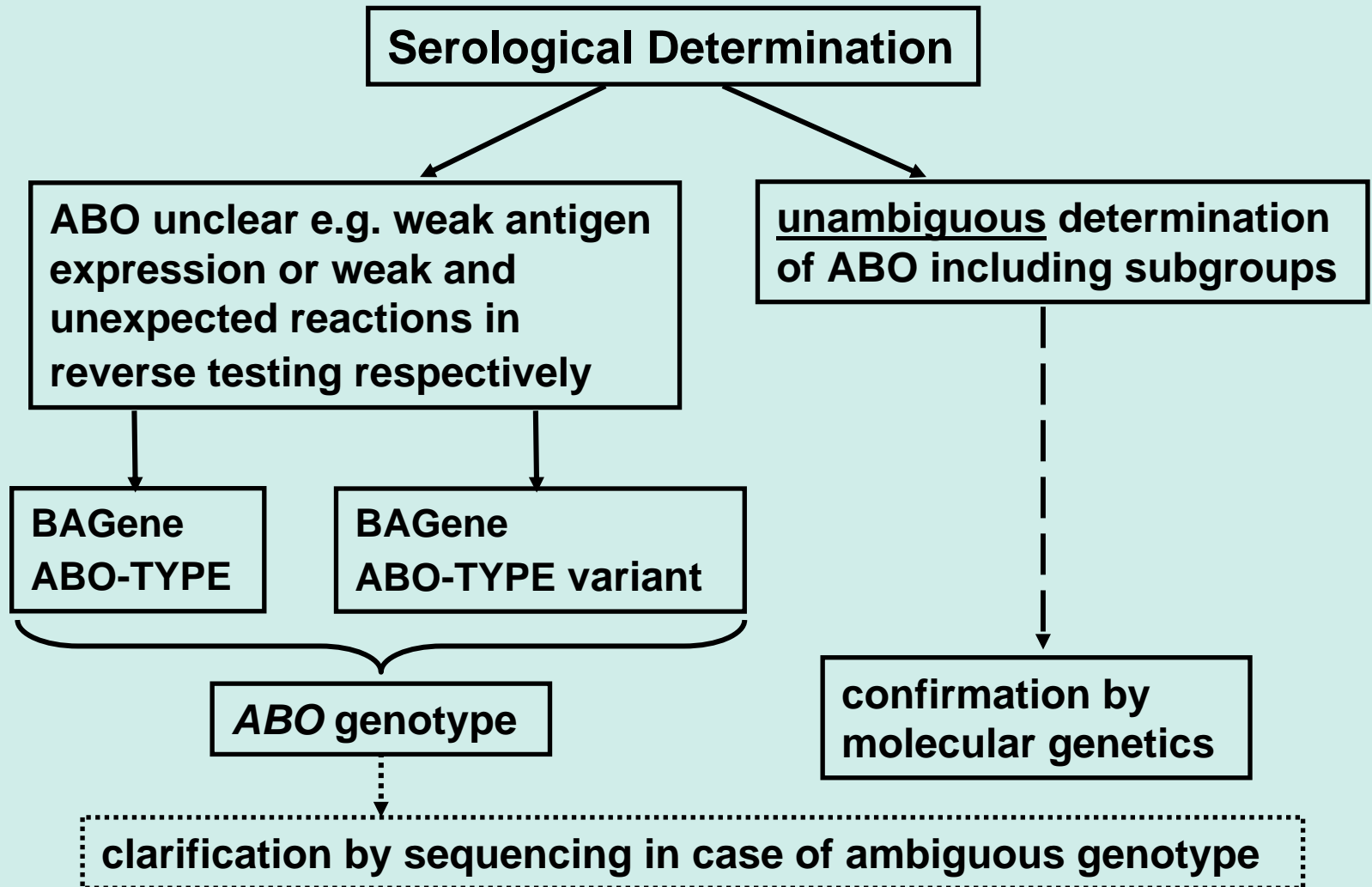


mix well (vortex)

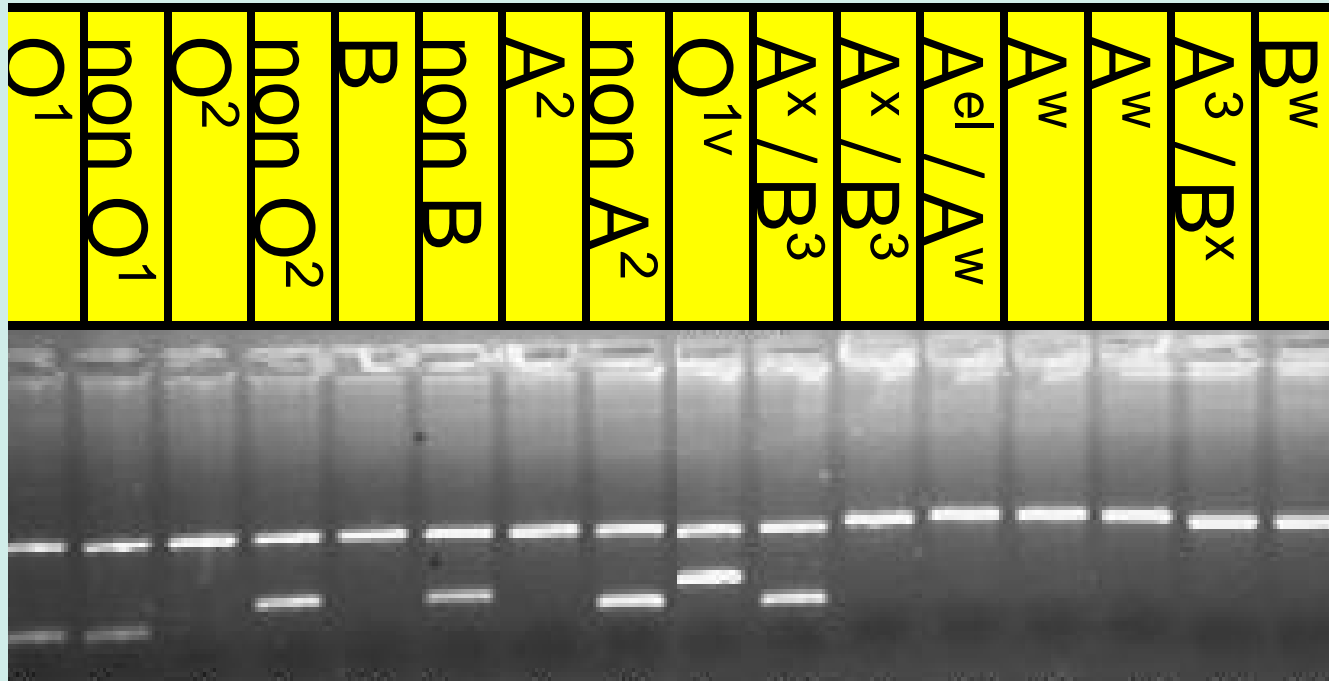
add 10 μl to each well of the PCR-strips/-plates

# Investigation Strategy

## 🔥 ABO blood group typing



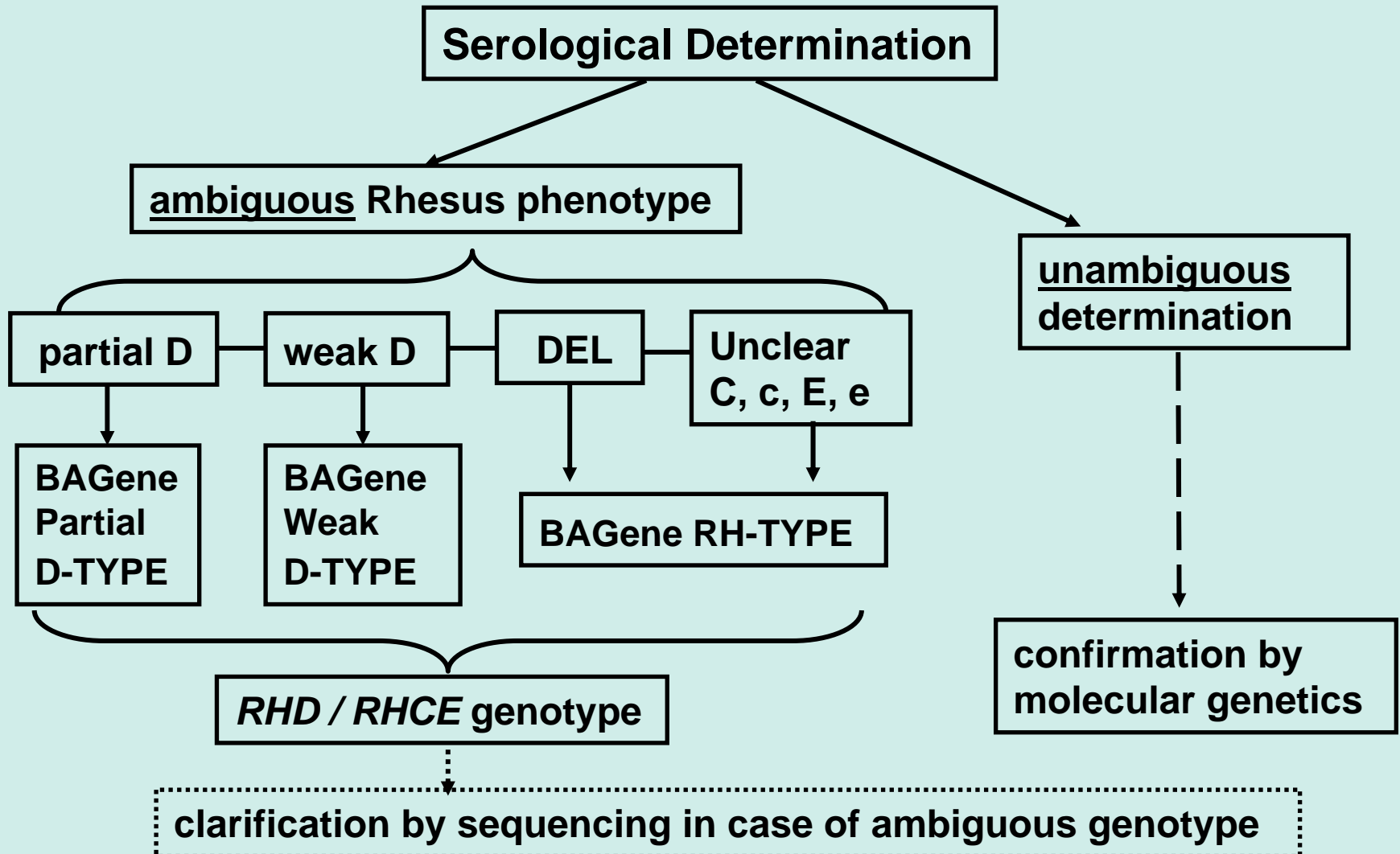
# ABO Genotyping



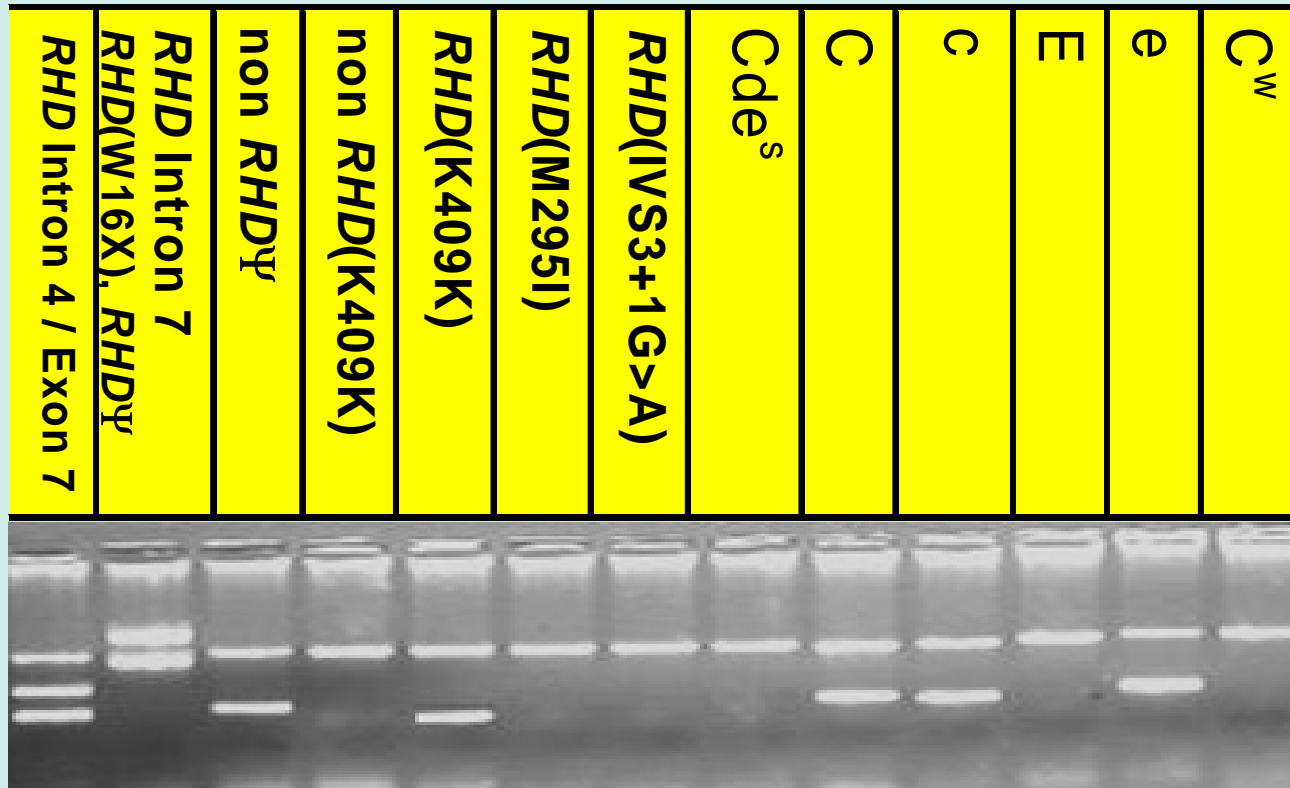
**BAGene ABO-TYPE variant genotype  $O^{1v}A^x$**

# Investigation Strategy

## 🔥 Rhesus typing



# RHD, RHCE Genotyping



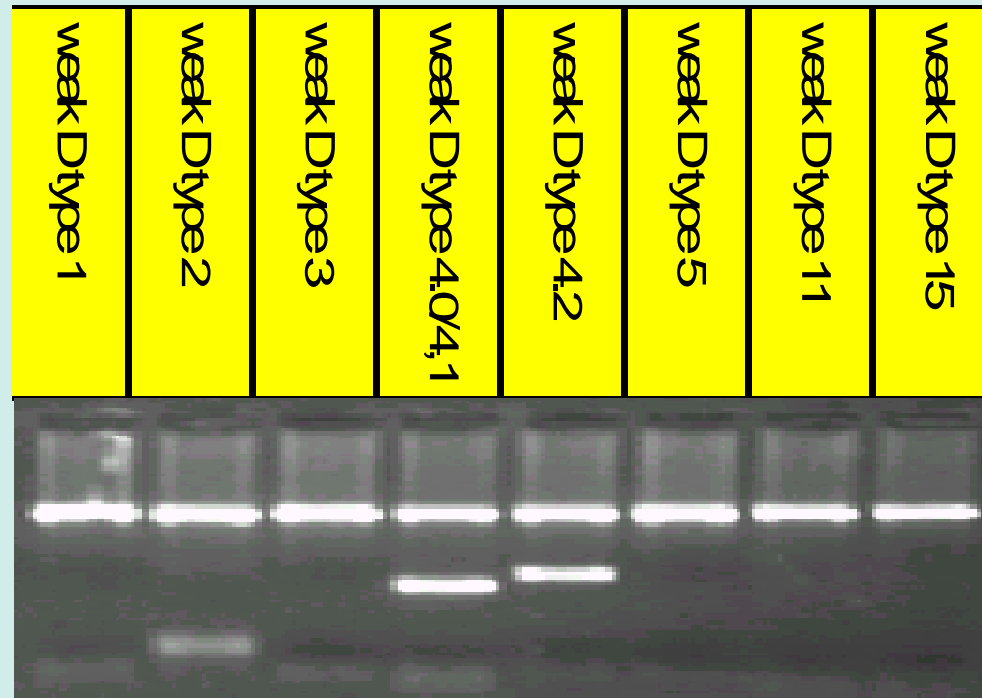
BAGene RH-TYPE genotype *RHD(K409K) Ccee*

# Genotyping partial D



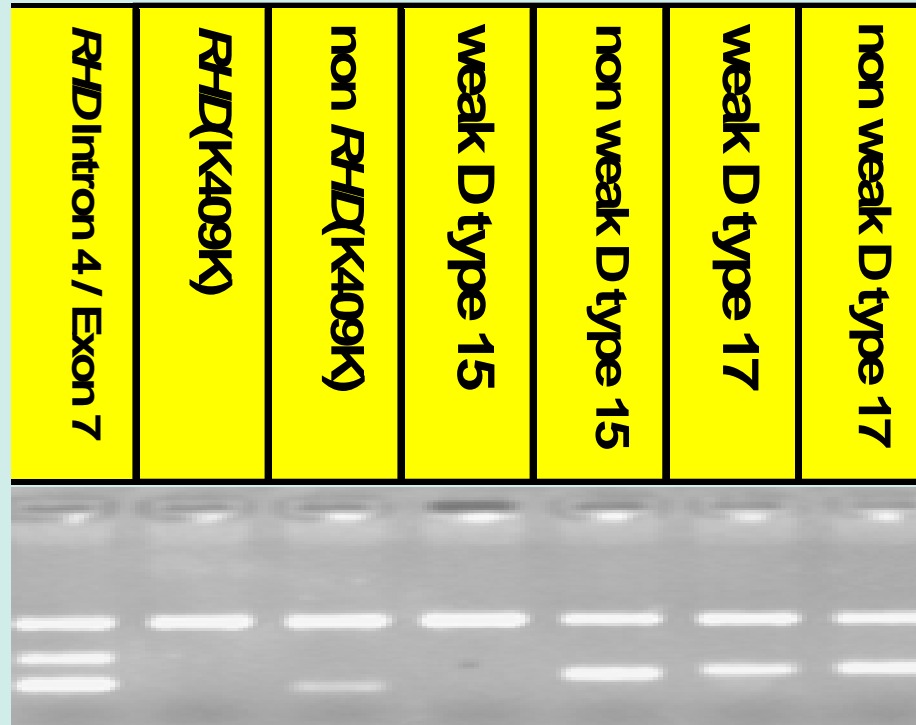
**BAGene Partial D-TYPE genotype *D cat. VI type II***

# Genotyping weak D



**BAGene Weak D-TYPE genotype *weak D type 4.2***

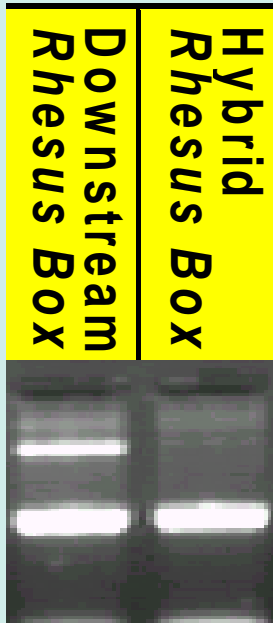
# *RHD* Genotyping „Asia“



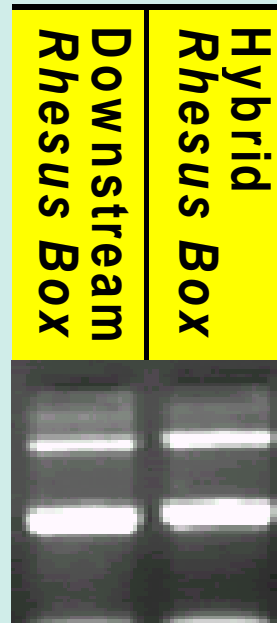
BAGene *RHD*-TYPE Asia genotype *RHD* positive / weak D type 17



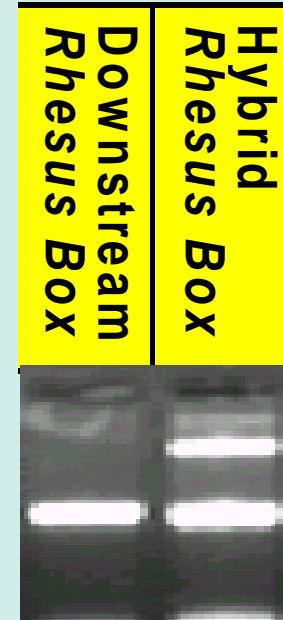
# RHD Zygosity



**DD**



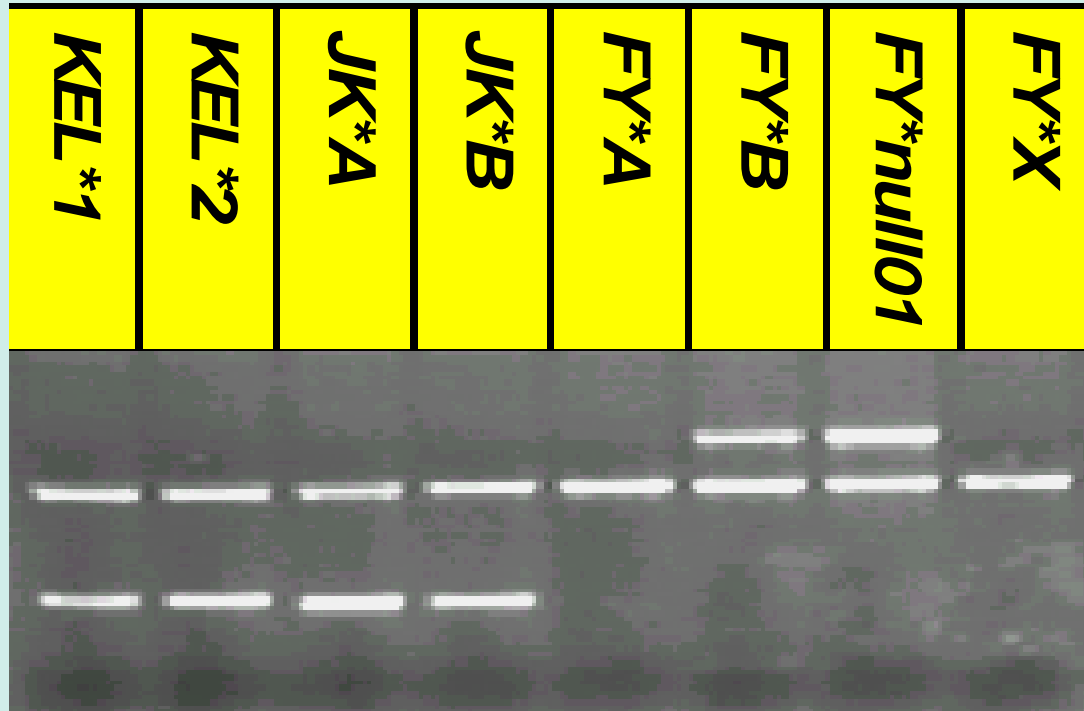
**Dd**



**dd**

**BAGene D Zygosity-TYPE**

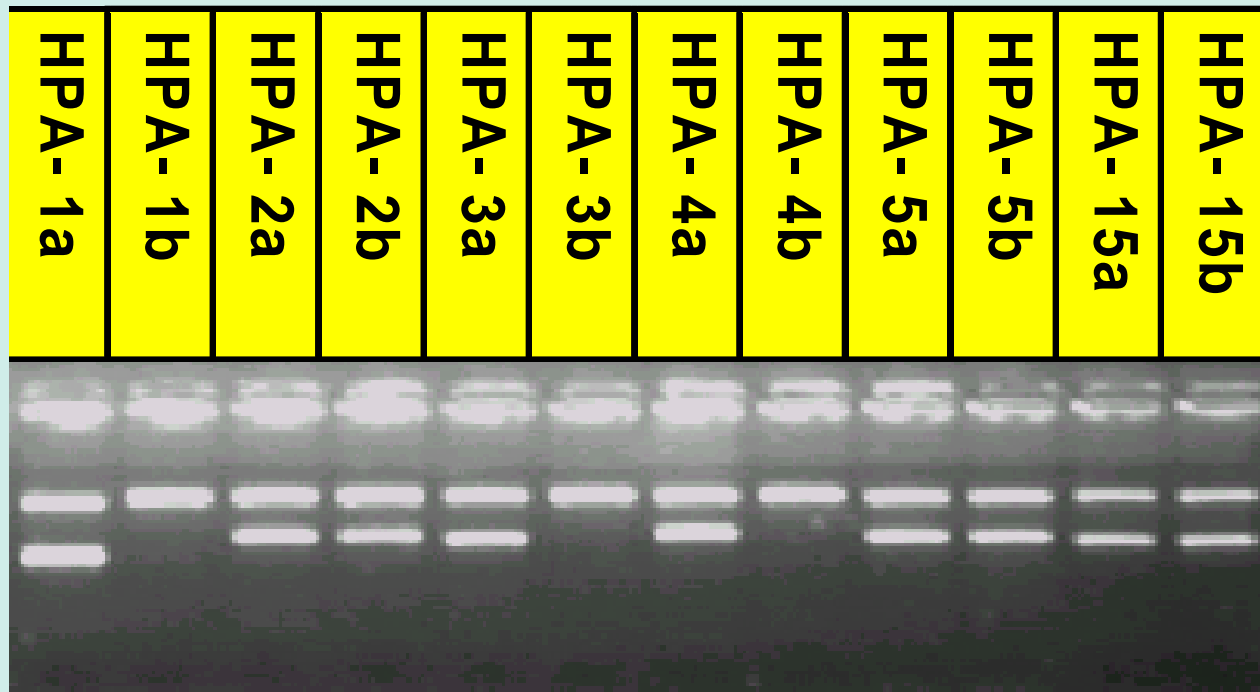
# Genotyping *KEL*, *JK*, *FY*



**BAGene KKD-TYPE**

**genotype *KEL*\*1/*KEL*\*2; *JK*\*A/*JK*\*B; *FY*\*B/*FY*\*null01**

# Genotyping *HPA*



**BAGene HPA-TYPE**

**genotype *HPA-1a/a; 2a/b; 3a/a; 4a/a; 5a/b; 15 a/b***

# Genotyping *MNS*



**BAGene MNS-TYPE  
genotype NSs**

# Recently published, presented soon

- 🔴 **Molecular genetic blood group typing by the use of PCR-SSP technique**

*M Prager, BAG Health Care, Lich, Germany*

presented at the FDA Workshop on Molecular Methods in Immunohematology

Bethesda 25 – 26 Sept 2006

published in Transfusion 2007;47:54S-59S.

- 🔴 **ABO genotyping for diagnosis of unusual ABO blood groups:**

**A Comparative Study in German Blood Donor Centers**

*M Prager, EA Scharberg, FF Wagner, J Burkhart, A Seltsam*

Presentation at 40. Annual Meeting of the German Society for Transfusion Medicine and Immunohematology (DGTI) September 18-21, 2007