Molecular biology of partial D and weak D

Implications for Blood Bank Practice

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2002

Rhesus molecular biology

- Introduction
  - Structure of *RH* genes and Rh proteins
- partial D (D category)
- weak D
- Rh negative and *RHD* heterozygosity
- Rh pos. units in Rh neg. donor pool

Relevance of Rhesus

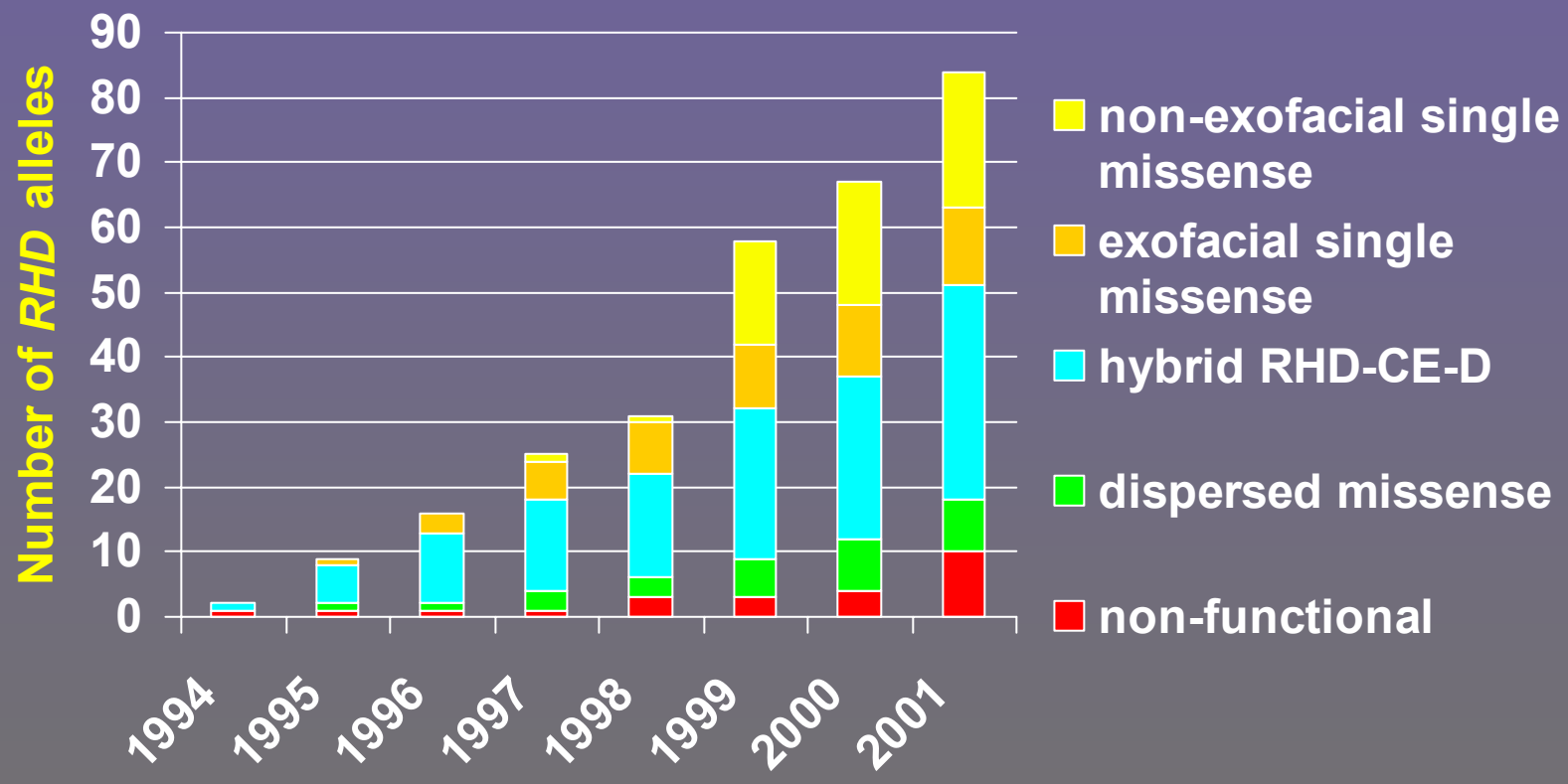
Protein

- Most important blood group system encoded by proteins
- Major cause of HDN
- Group of Rhesus-like proteins are major constituents of RBC membrane

Gene

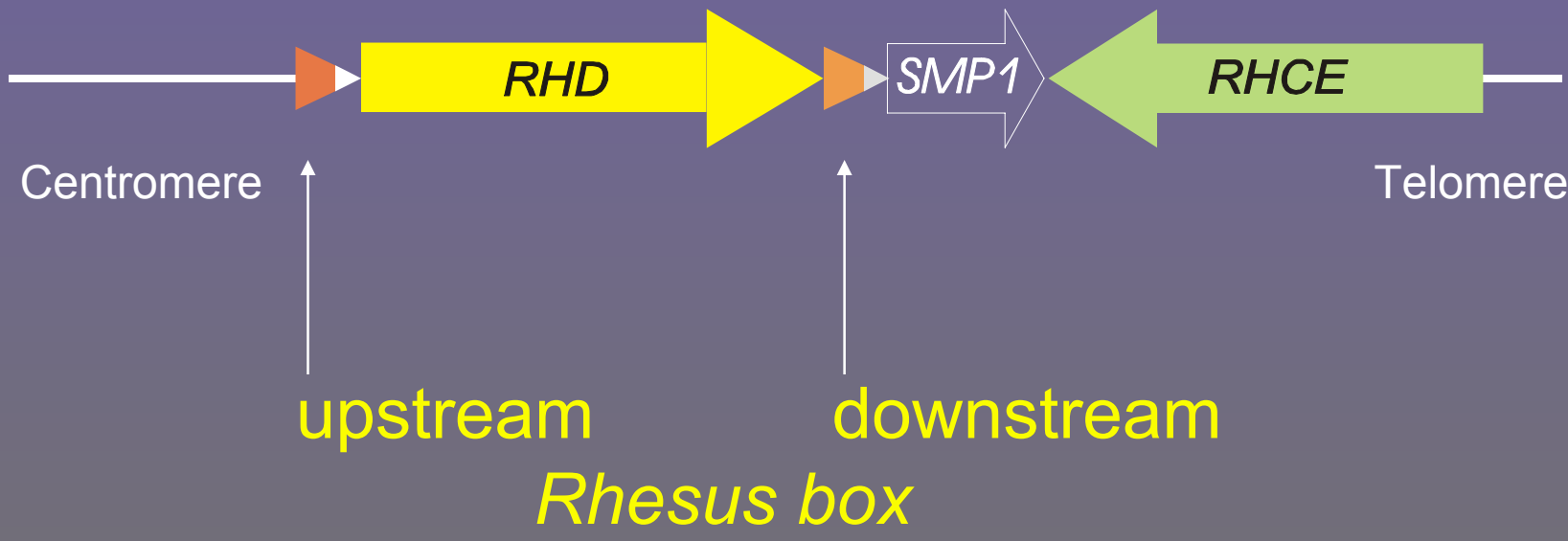
- Most polymorph blood group system known
- Gene cluster: duplication and deletion
- Multiple gene con-versions and mutations
- Complex model system for genotyping

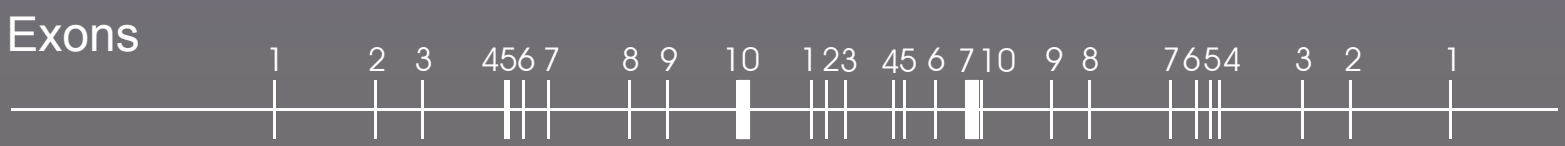
RHD alleles by type of molecular variation



adapted from: The RhesusBase
http://www.uni-ulm.de/~fwagner/RH/RB/

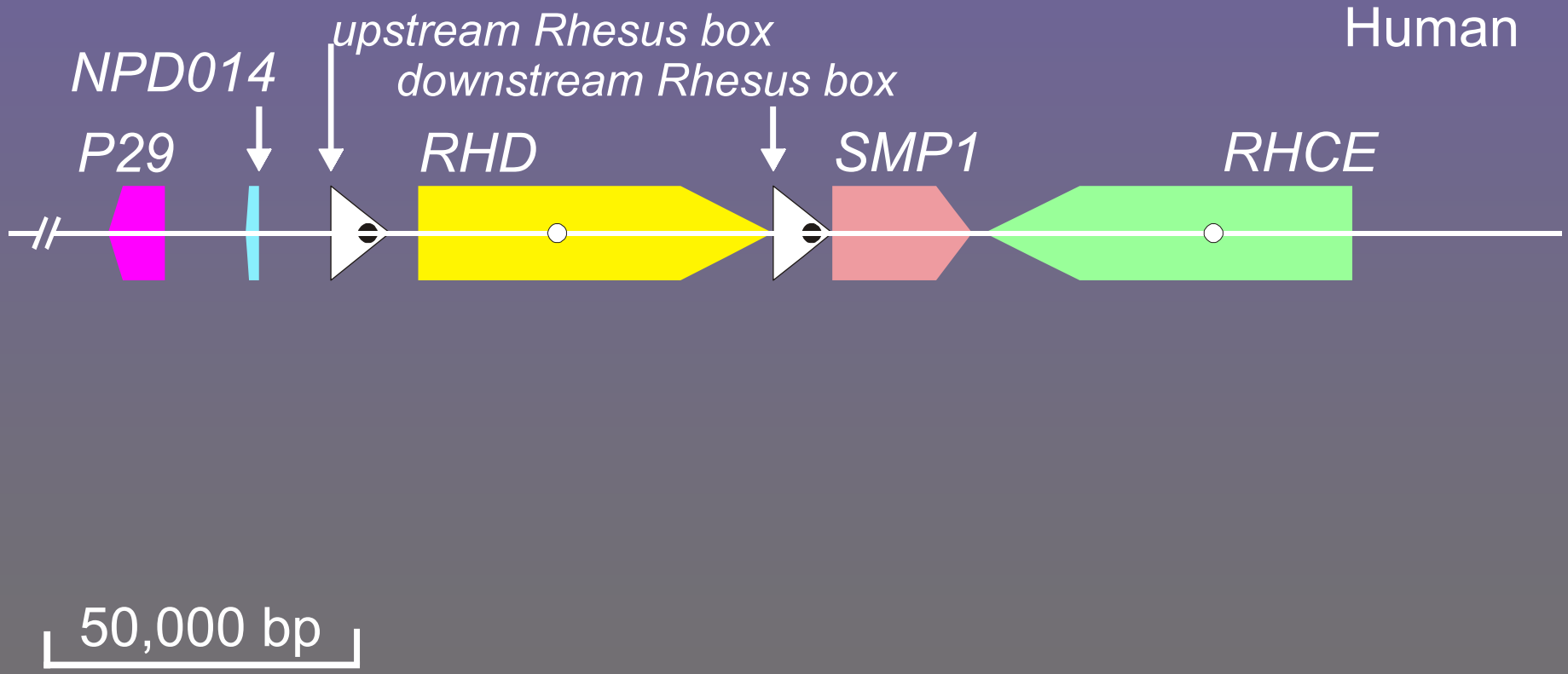
Molecular structure of *RH* gene locus



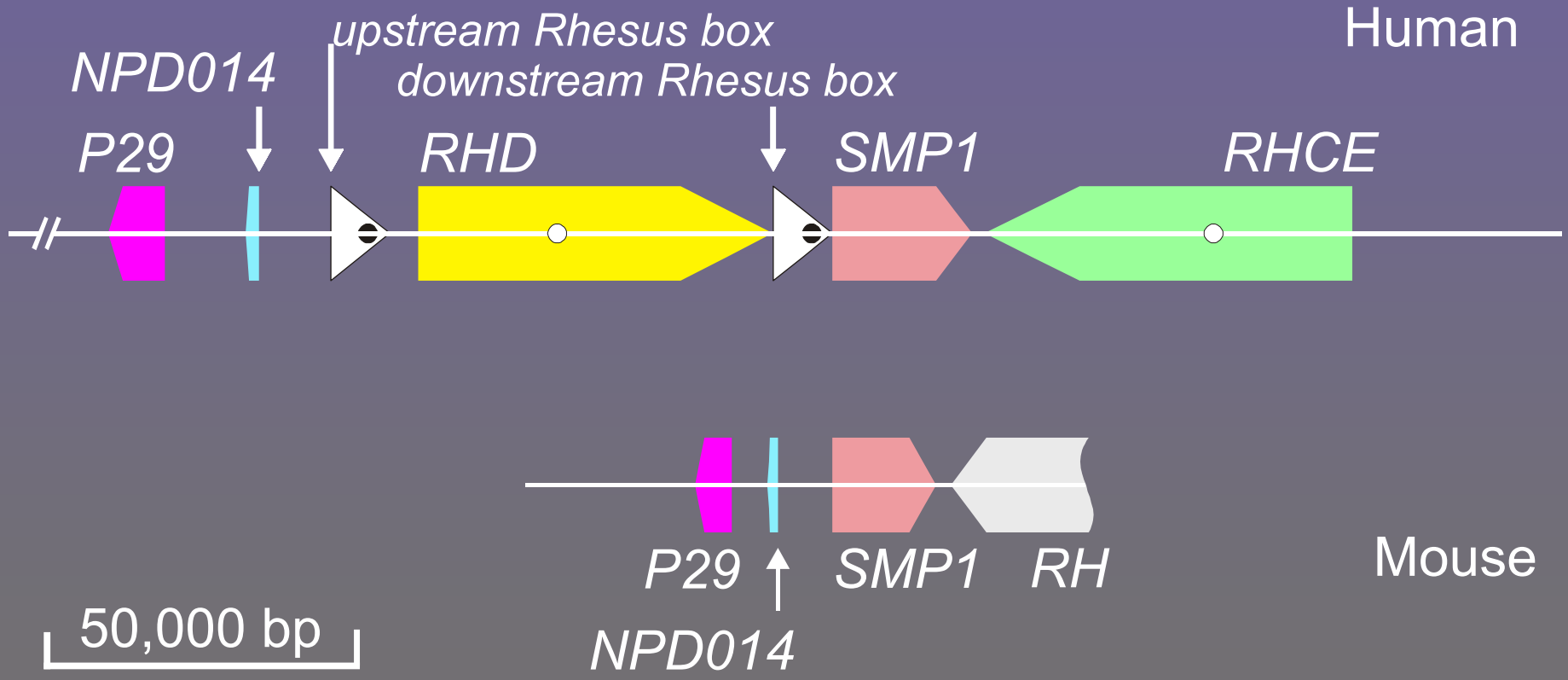


Blood 95(2000)2272

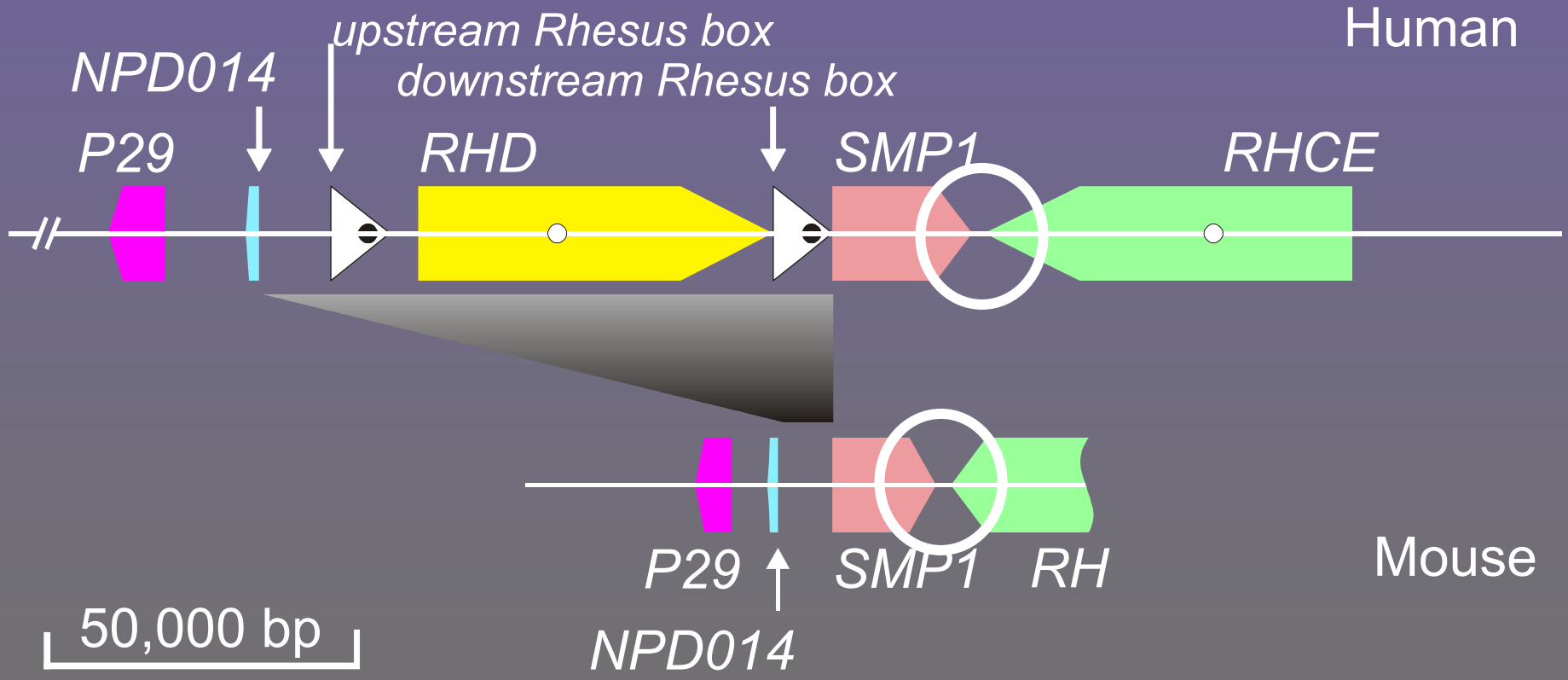
Extended molecular structure of *RH* gene locus



Human *RH* locus compared to mouse genome project data

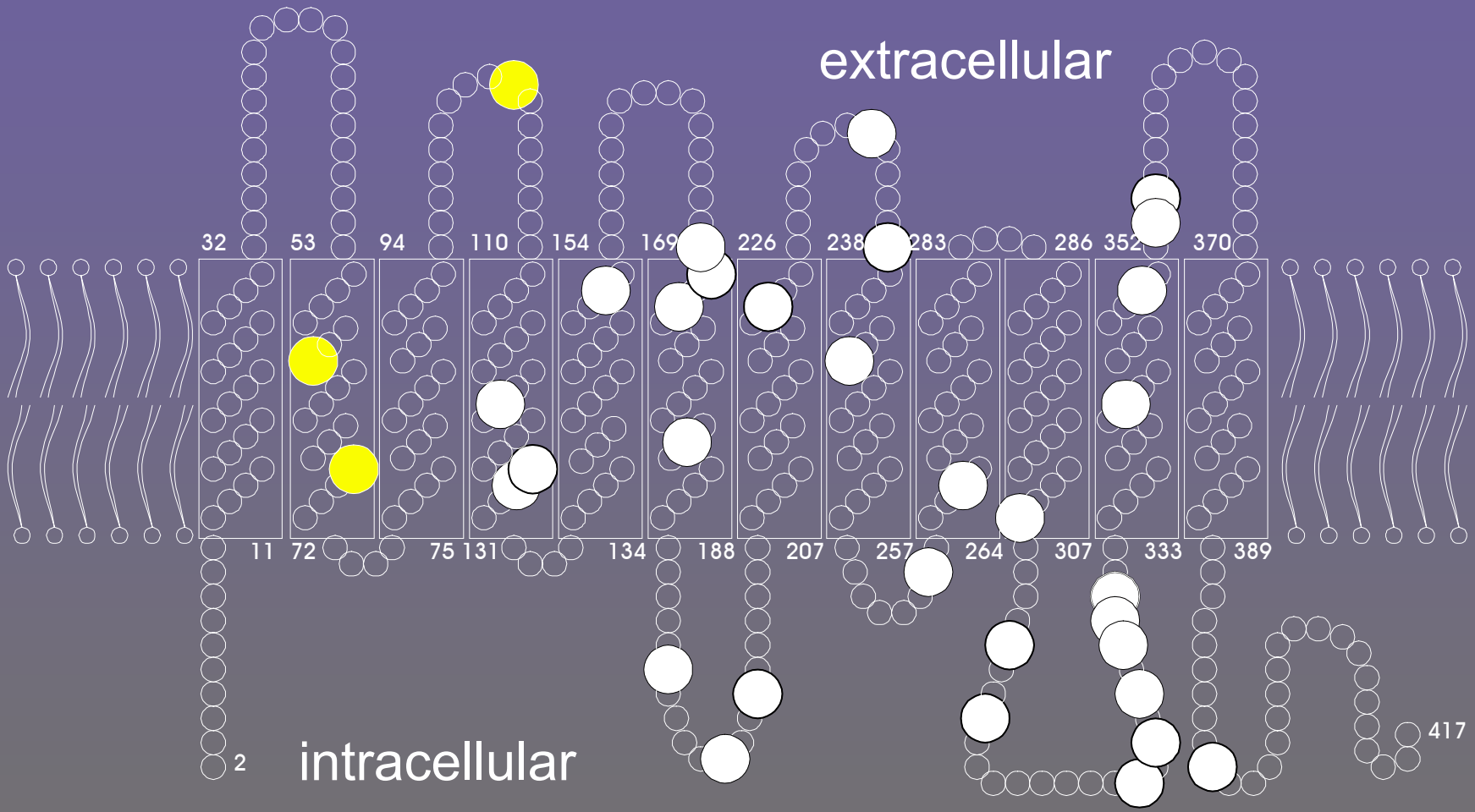


RHCE: ancestral position
RHD is the duplicated gene



Blood 99(2002)2272

Differences between RhD and RhCE

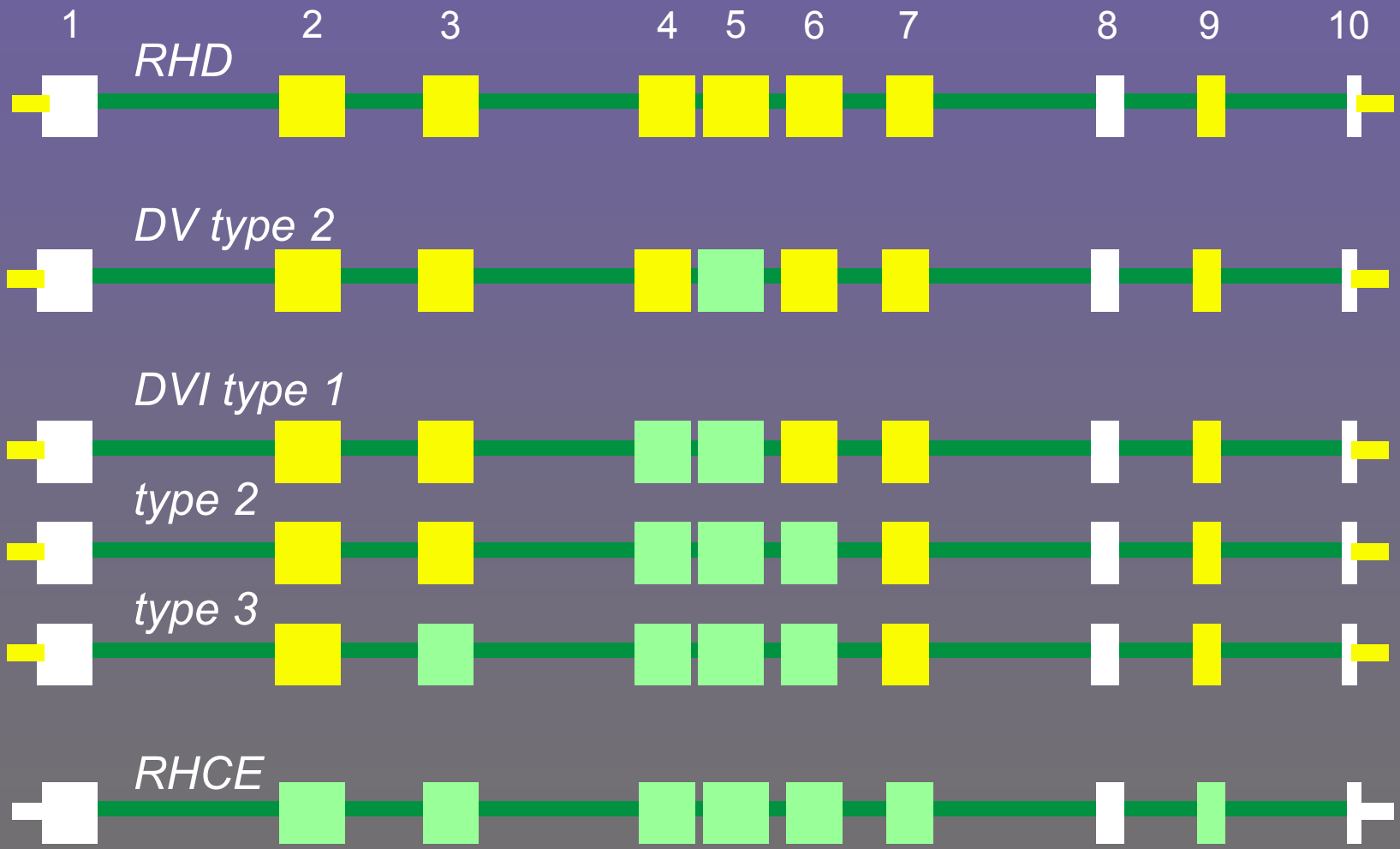


Clin Lab 48(2002)53

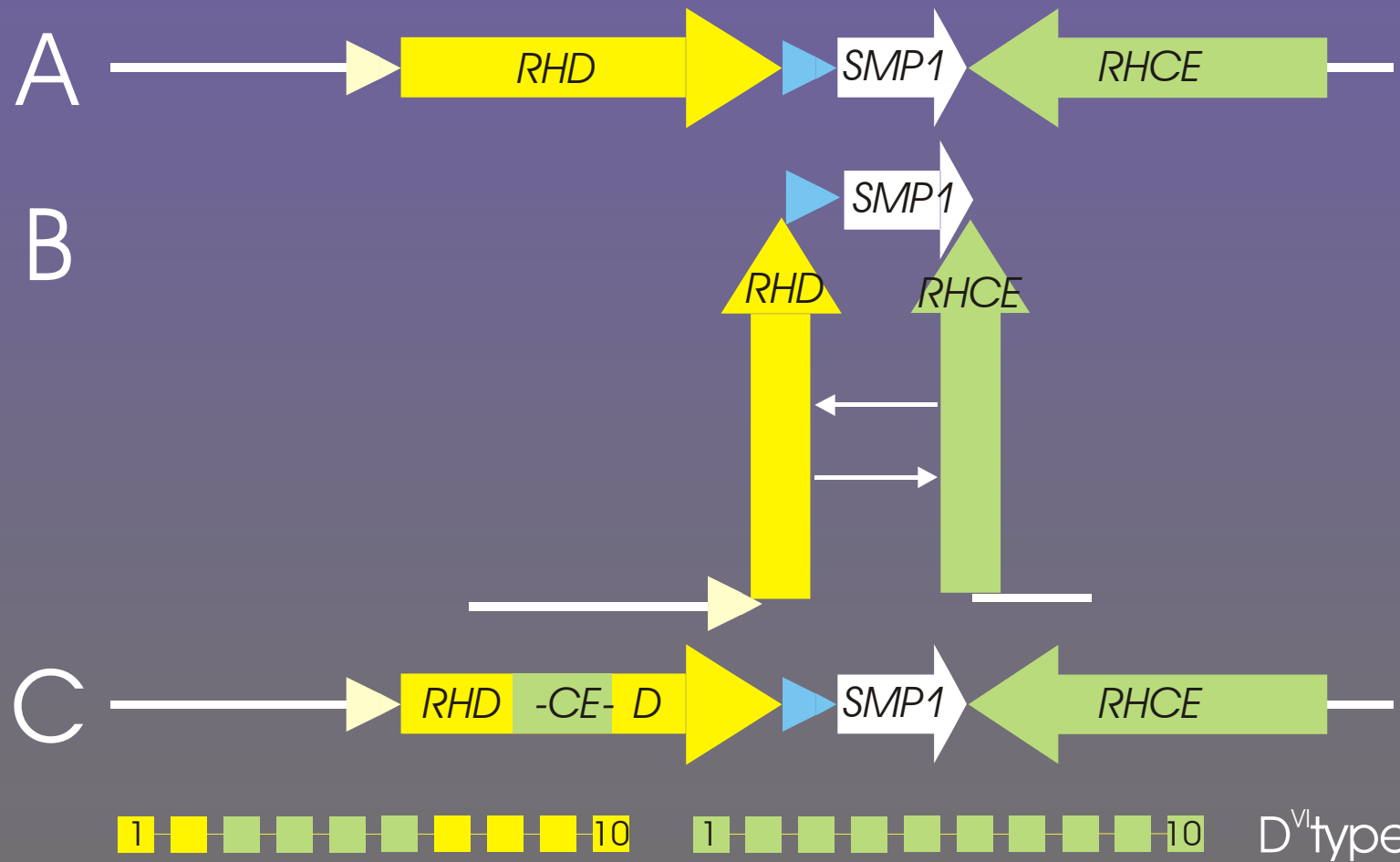
Rhesus molecular biology

- Introduction
- partial D (D category)
  - *RHD/RHCE* hybrid alleles
    - Typically causing „D categories“ – subset of partial D
- weak D
- Rh negative and *RHD* heterozygosity
- Rh pos. units in Rh neg. donor pool

Examples for *RHD-CE-D* hybrid alleles

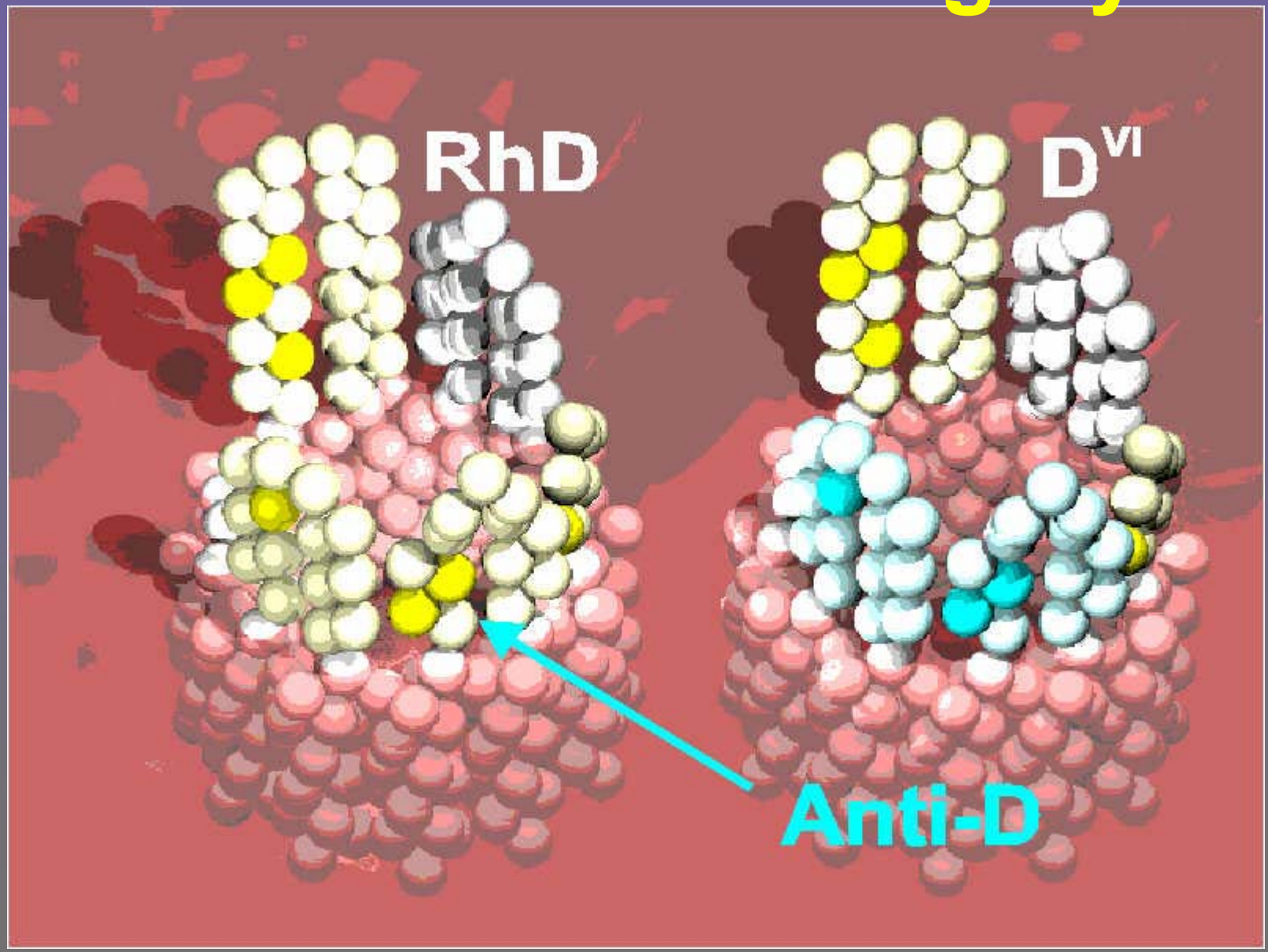


Mechanism of gene conversion



BMC Genet 2(2001)10

D category VI



Frequency of aberrant *RHD*

Aberrant RhD	minimal frequency *	anti-D
DVII	1:900	uncommon
DVI	1:6,800	common
DIV	1:10,000	variable
DV	1:30,000	variable
DIII-like	1:30,000	uncommon
DFR	1:60,000	uncommon
R_0^{Har} (Rh33)	< 1:60,000	uncommon

* Means, serologic screen of > 60,000 donors

Transfus Clin Biol 3(1996)10s

Implications for Blood Bank Practice Current D typing in Europe differs between patients and blood donors

- patients, pregnant women and newborns
  - two IgM monoclonal type that do not detect D category VI
  - no antiglobulin test
    - DVI is deliberately typed Rh negative
- donors
  - suitable polyclonal or oligoclonal reagents in indirect antiglobulin test (e.g. gel test)
    - DVI, all partial D and very weak D are typed Rh positive

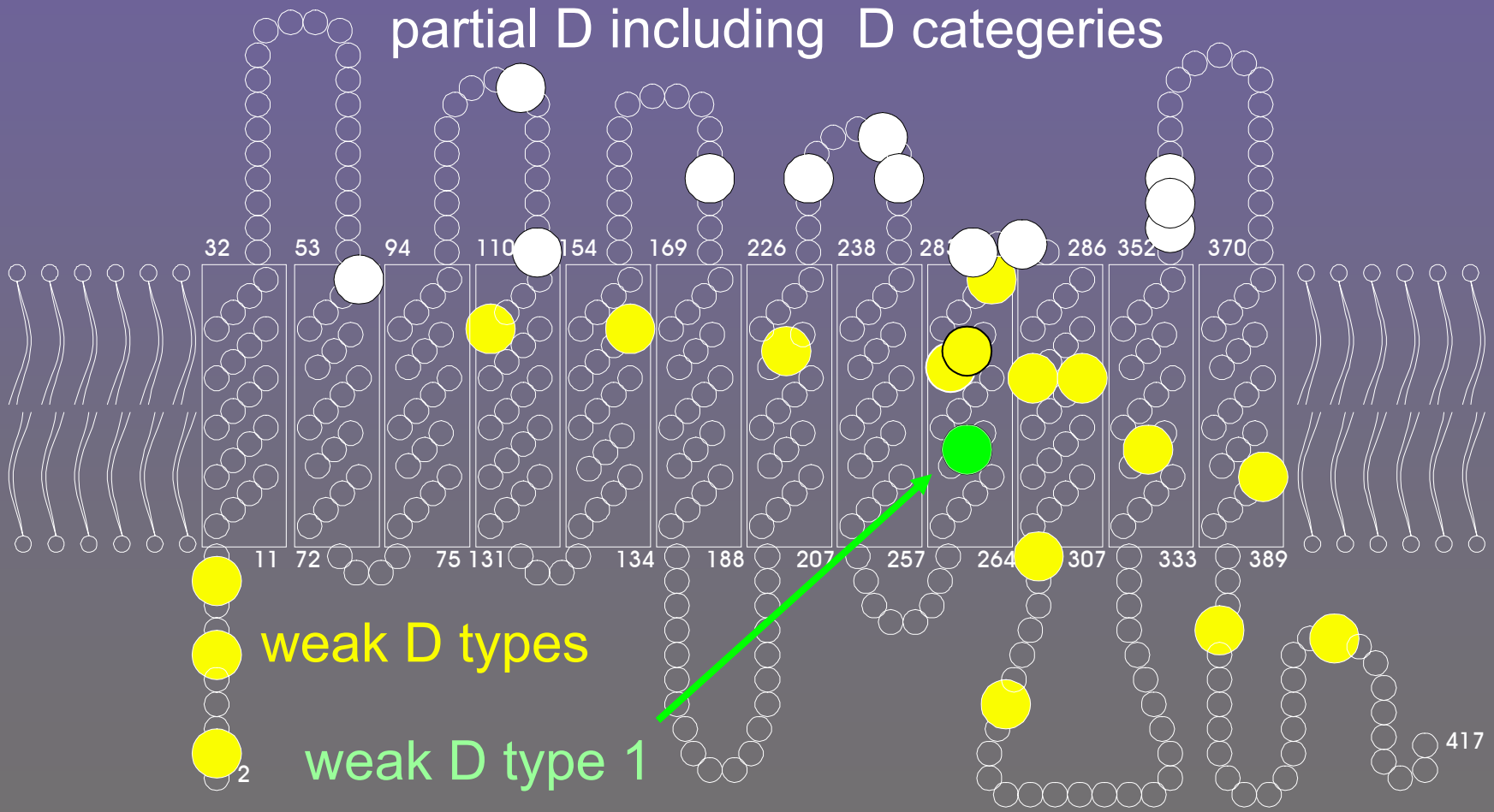
Blood 91(1998)2166

German (since 1996), UK & Dutch guidelines

Rhesus molecular biology

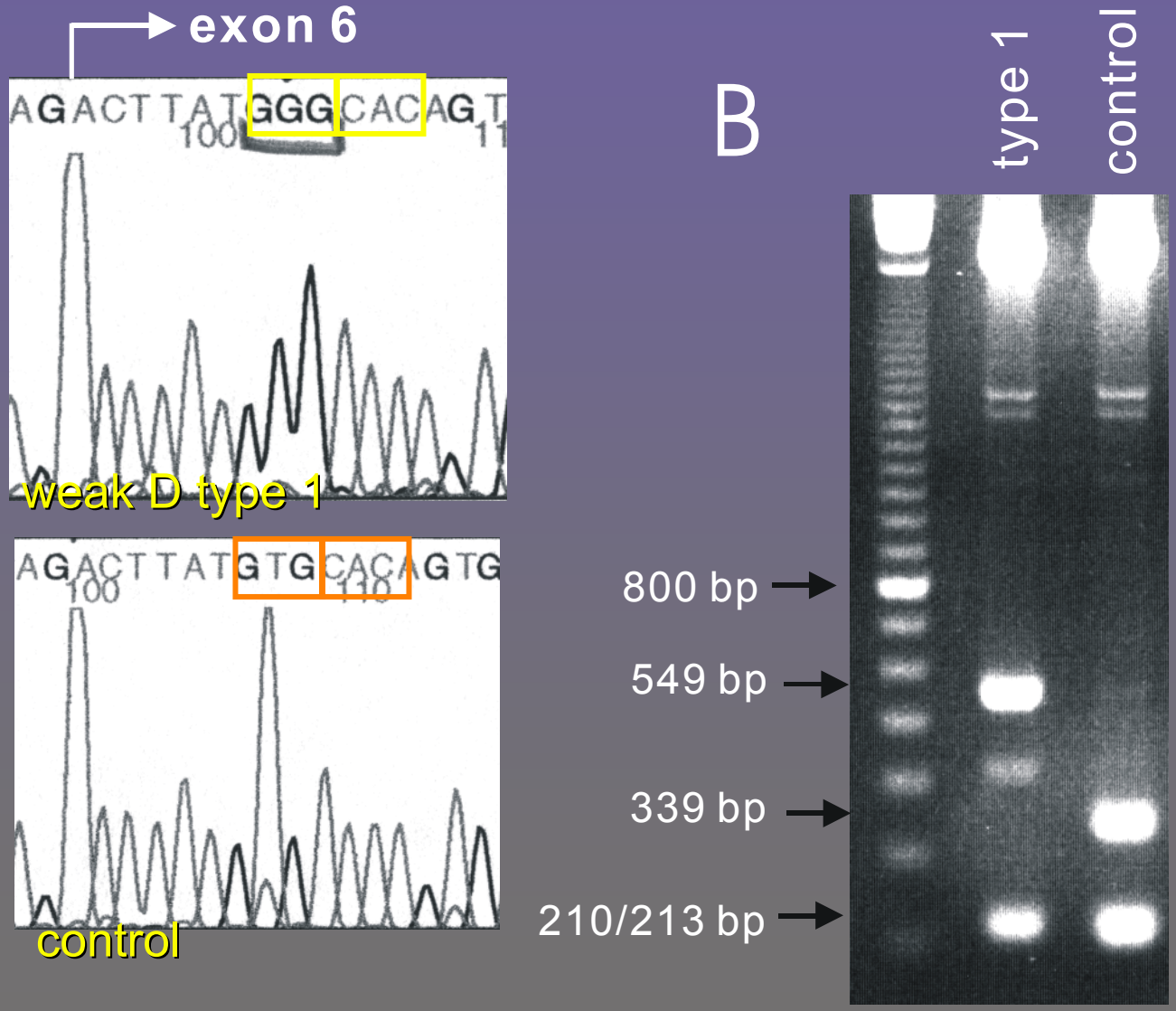
- Introduction
- partial D (D category)
  - Missense mutations
    - If exofacial, typically causing partial D other than D categories
- weak D
  - Missense mutations
    - If non-exofacial, causing weak D types
- Rh negative and *RHD* heterozygosity
- Rh pos. units in Rh neg. donor pool

Aberrant *RHD* with single mutations

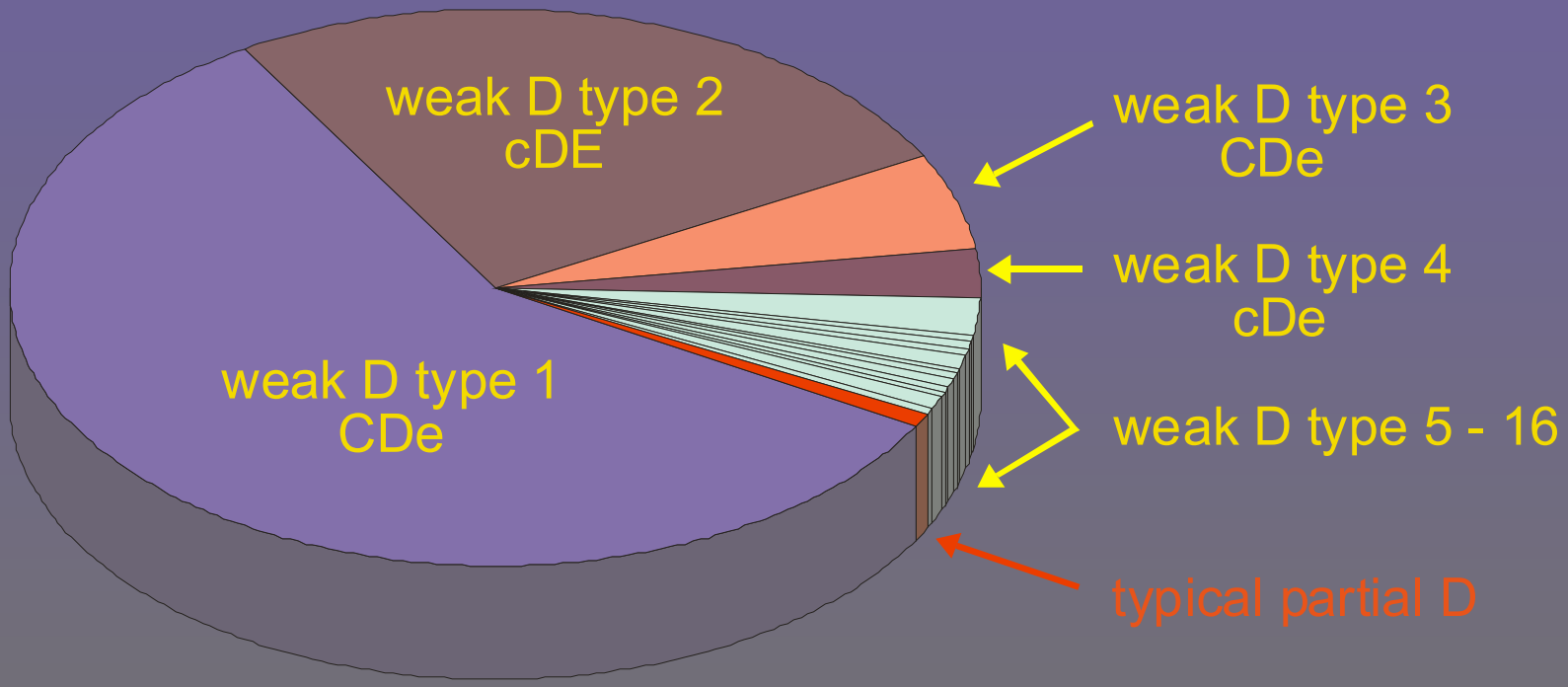


Blood 93(1999)385

weak D type 1


exon 6
A
AGACTTATGGGCACAGT
100
weak D type 1
AGACTTATGTGCACAGTG
100
control
B
type 1
control
800 bp
549 bp
339 bp
210/213 bp

Distribution of weak D types



among 272 weak D samples excluding DVI
Blood 93(1999)385

Quality assurance

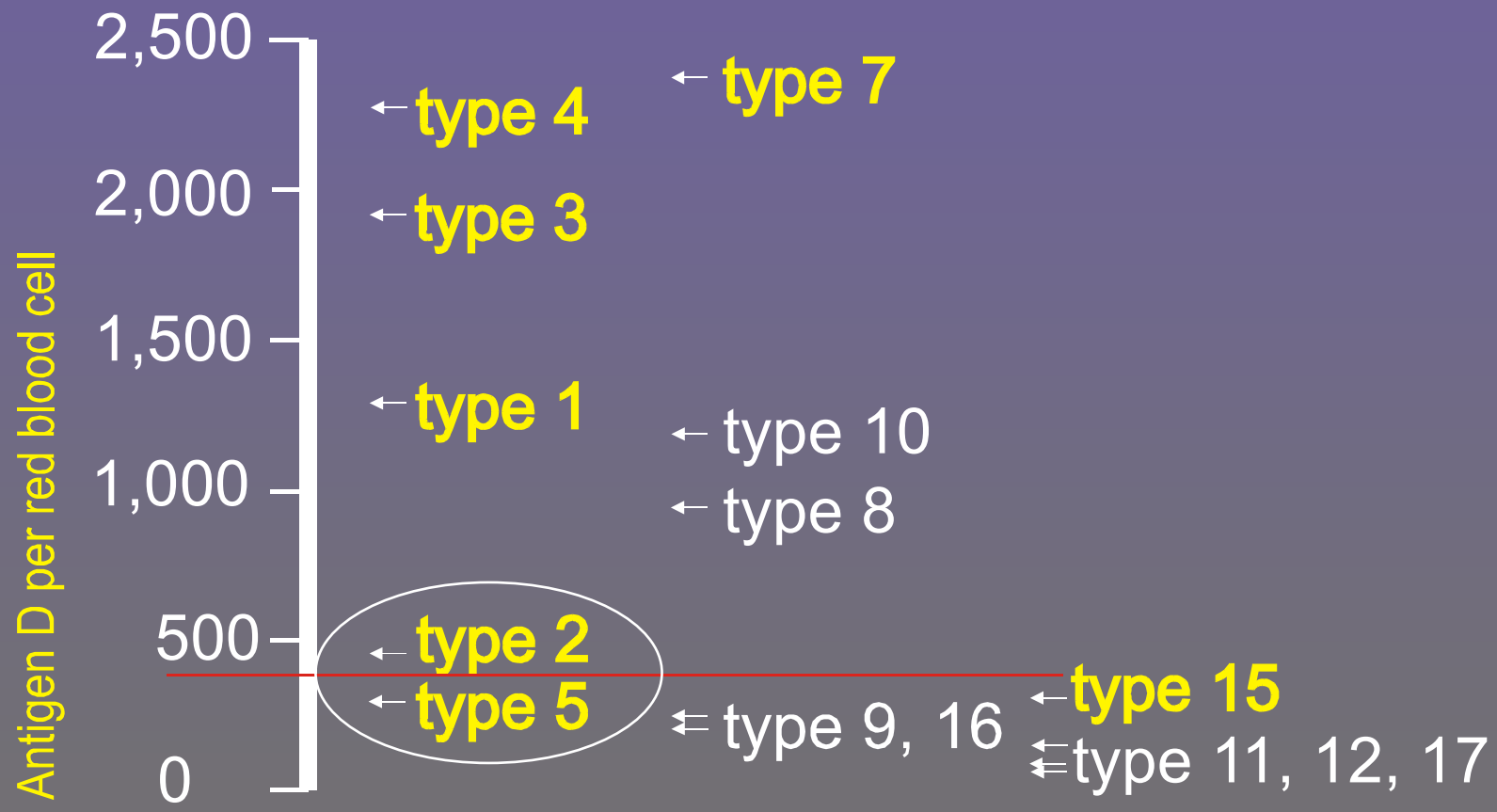
- D pos. proband with anti-D
- Registry since 1998
- 60 submissions confirmed
- 13 international submissions
- Several new partial D, like DNB, DOL, DAU-3
- 20 weak D samples:
  - Allo-anti-D among weak D type 4.2 (DAR) & type 15 only
  - No allo-anti-D among prevalent weak D types: they all carried auto-anti-D

http://www.uni-ulm.de/~wflegel/RH/RIR/ accessed Oct 2002

Implications for Blood Bank Practice Does knowledge of partial D and weak D status serve a clinically useful purpose?

- Carriers of most partial D and some weak D types can be anti-D immunized:
  - D typing should avoid their being transfused with Rhesus positive blood
- Carriers of most weak D types cannot be anti-D immunized:
  - transfuse with Rhesus positive blood
  - avoid common practice of wasting Rh neg. blood

Antigen densities of weak D types



Implications for Blood Bank Practice Quality control by molecularly defined weak D types

- weak D type 2
  - preferred for quality assurance (sensitivity) of anti-D sera and of D typing methods
- weak D types of lower antigen density, like type 5 or type 15
  - useful for precise cut-off definition and control
- applicable to
  - D typing methods in clinical lab
  - D typing kits by manufacturers

Blood 95(2000)2706 & Transfusion 40(2000)433

Rhesus molecular biology

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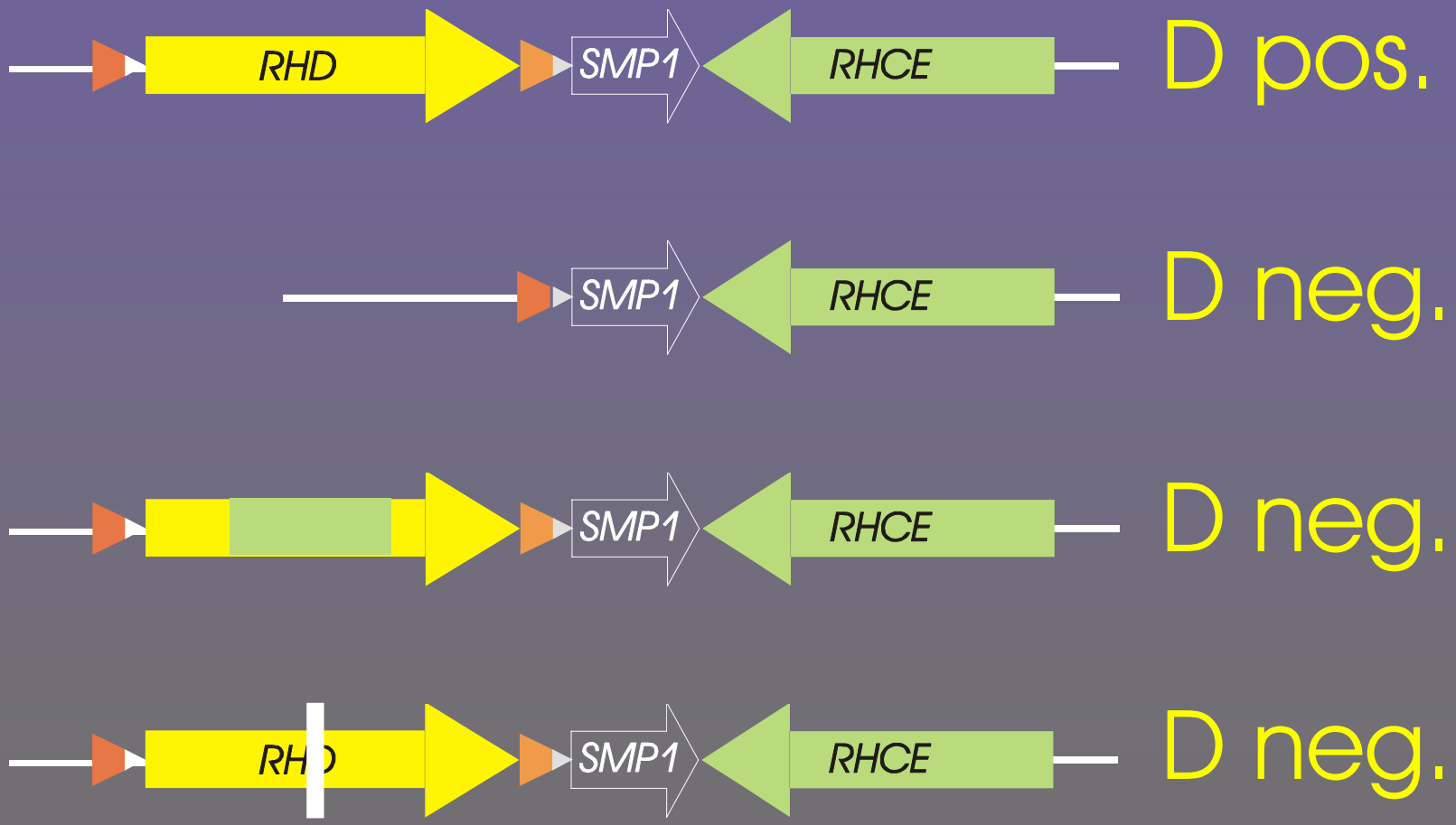
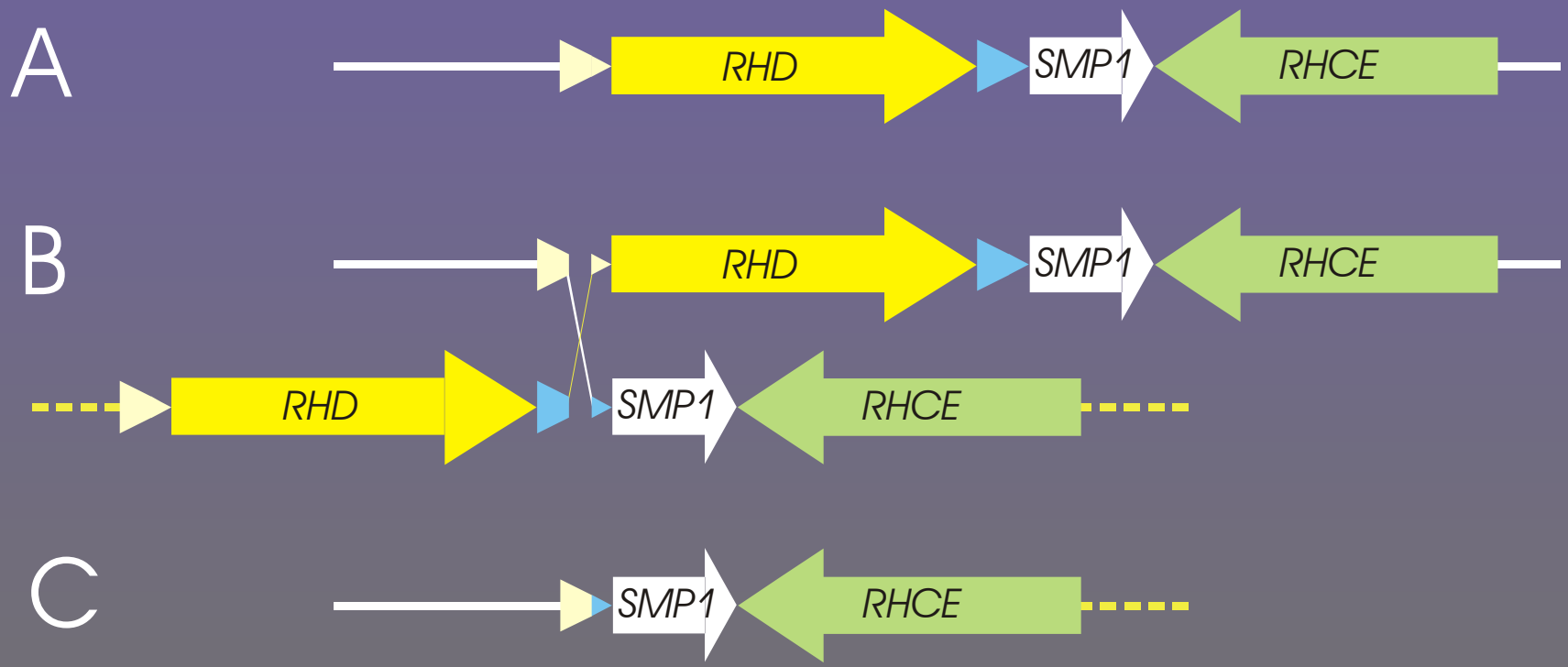
- weak D
- Rh negative and *RHD* heterozygosity
- Rh pos. units in Rh neg. donor pool

D positive and D negative

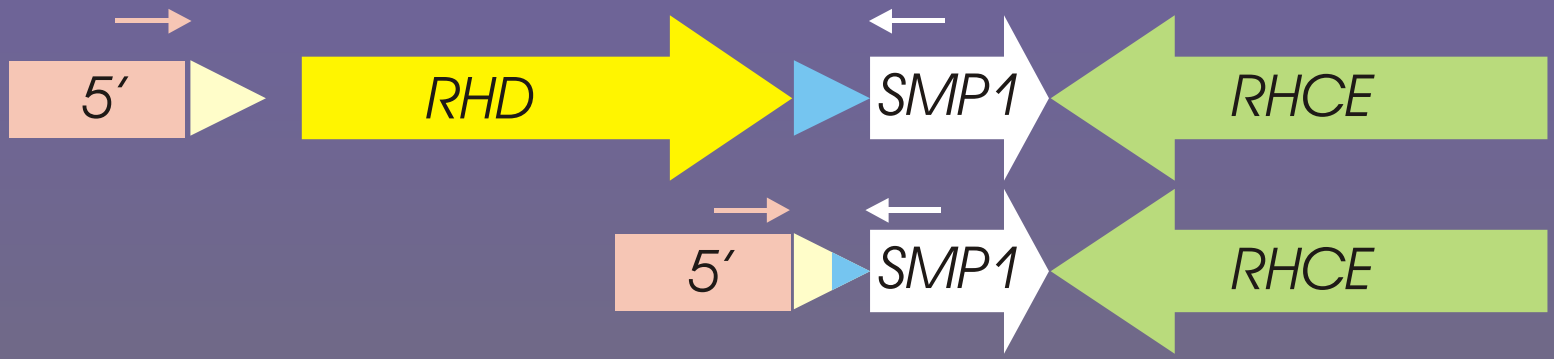


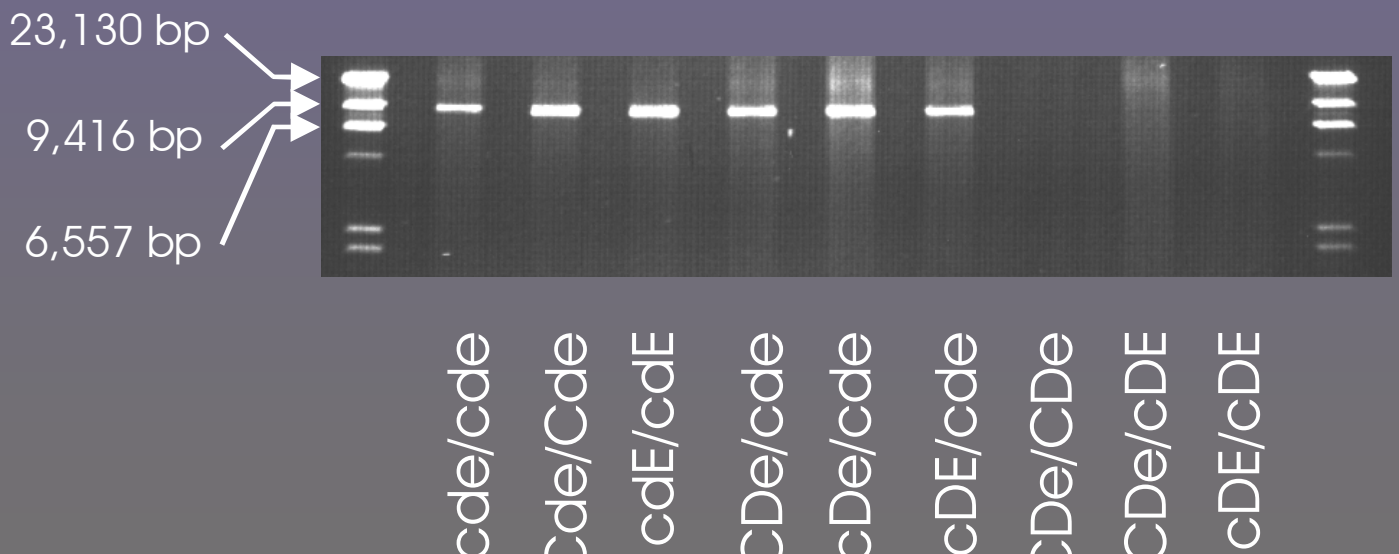
RHD deletion



Blood 95(2000)2272

Detection of *RHD* hetero-zygous status in fathers



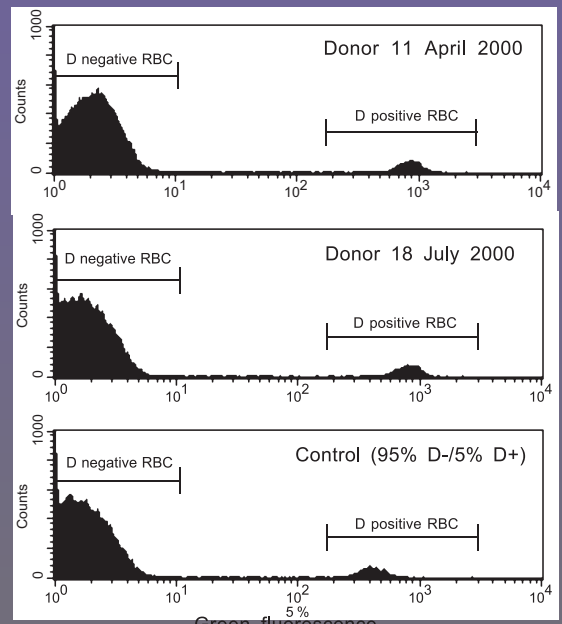


Rhesus molecular biology

- Introduction
- partial D (D category)
- weak D
- Rh negative and *RHD* heterozygosity
- Rh pos. units in Rh neg. donor pool

D+/- chimera causing anti-D immunizations

- Donor carries two RBC populations:
  - 95% Rh neg.
  - 5% Rh pos.
- total of 13 donations
  - caused anti-D in the latest 2 eligible transfusion recipients



A

Marker	Events	% Total
All	20000	100.00
D negative RBC	18618	93.09
D positive RBC	1162	5.81

B

Marker	Events	% Total
All	20000	100.00
D negative RBC	18726	93.63
D positive RBC	1190	5.95

C

Marker	Events	% Total
All	20000	100.00
D negative RBC	18857	94.28
D positive RBC	1042	5.21

BMC Genet 2(2001)10

Implications for Blood Bank Practice Quality control by molecular typing of serologic Rh neg. donors

- among 8,442 Rh neg. donors
  - 1 donor with D+/- chimerism
  - 4 donors with very weak positive antigen D
- If representative
  - about 1 anti-D immunization per 50,000 donations (4 per 200,000 donations in 1 year)
  - cost-efficiency of molecular typing would be proven
- Established practice since 1-1-2002

Implications for Blood Bank Practice
Why *RHD* genotyping?

- Superior sensitivity
  - uncover (many?) weak D in the “Rhesus negative“ donor pool of blood centers
- Superior specificity
  - as genotyping becomes routine, clinical implications of known and new *RHD* alleles will be recognized

DNB: a partial D with anti-D frequent in Central Europe

Population	Phenotype frequency
Swiss (Lugano)	1:292
Swiss (Bern)	1:538
German (Ulm)	1:1,644
Dane (Aarhus)	< 1:798 *

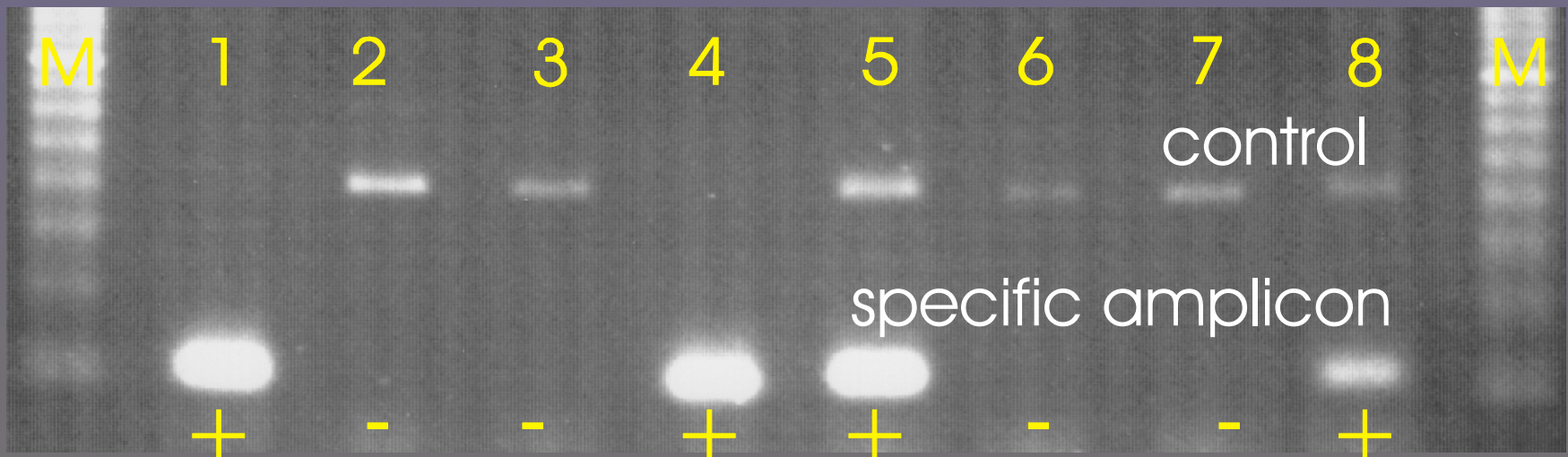
* upper limit of 95% confidence interval (Poisson distribution)

most frequent partial D known so far

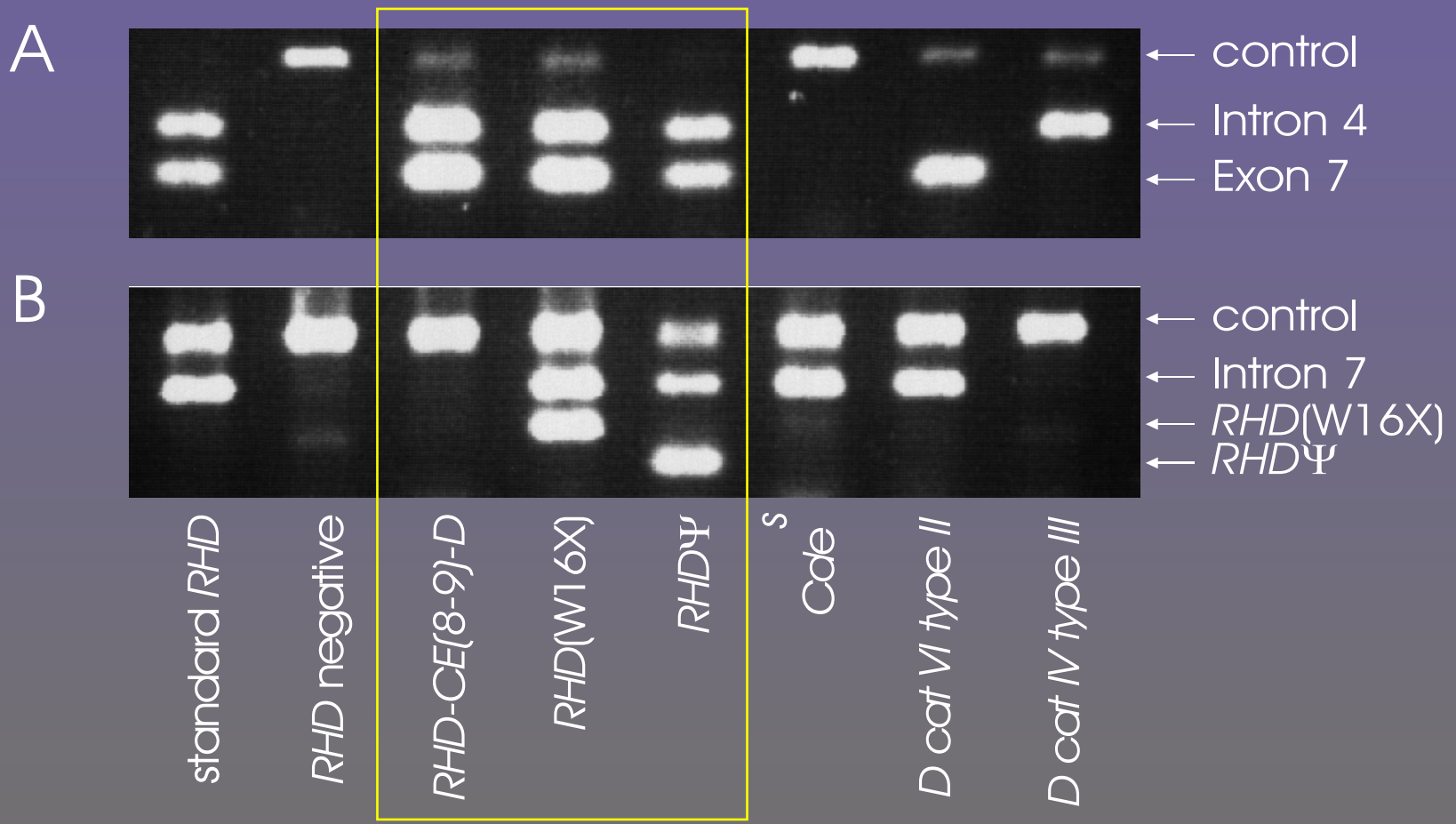
Blood 100(2002)2253

Routine application of PCR

DNA isolation	30 min
PCR set up	10 min/45 min
PCR (cycling)	90 min
Gel separation	30 min
Gel dyeing	30 min
Evaluation	10 min
	3.5 – 4 h



Recommended *RHD* PCR



positive predictive value > 0.9999

Specificity of *RHD* genotyping: expected rates of false positives in published assays

PCR strategy	Rate of false positives	Positive predictive value of positive result	Polymorphism tested (n)
Exon 10 only	1:1,276	0.999216	1
Intron 4/exon 7	1:4,081	0.999755	2
Intron 4/exon 7/*RHDΨ*	1:4,700	0.999787	3
Exons 3, 4, 5, 6, 7, 9	1:6,051	0.999835	6
Exons 2, 3, 4, 5, 6, 7, 9, 10	1:6,051	0.999835	8
Intron 4/exon 7/ intron7/W16X/*RHDΨ*	1:12,533	0.999920	5

Established indications for blood group genotyping

- First choice in prenatal diagnosis
  - from amniotic fluid or trophoblastic cells
  - from mother‘s peripheral blood
- Poly-transfused patients
  - if standard serology failed
- Auto- and allo-immunohemolytic anemia
  - if standard serology failed
- *RHD* genotyping in fathers
- weak D types and other aberrant *RH* alleles
  - for decision on anti-D prophylaxis and anti-D prophylaxis

Transfus Med 8(1998)281 & Vox Sang 78 suppl 2(2000)109

Implication for Blood Bank Practice

- Type *patients* with two monoclonal anti D that do not react with DVI
  - no antiglobulin, but sensitive methods
  - no slide tests for Rh neg.!
  - patients, including pregnant women & newborns
- Type *donors* with oligoclonal anti-D
  - plus antiglobulin *and* sensitive methods
- Use weak D for quality assurance
  - molecularly defined weak D type 2 . . .

Implication for Blood Bank Practice

. . .

- Transfuse weak D with Rh positive blood
  - don‘t waste Rh negative blood
- Transfuse DVI with Rh negative blood
  - and other partial D, if known
- Utilize *RH* genotyping for established applications

Current problems in *Rhesus*

- Molecular biology
  - Frequency and types of aberrant *RH* haplotypes in various populations
  - 3D structure of RhD
  - Composition of Rh complex
- Clinical aspects
  - Immunization caused by partial D, weak D & D+/- chimera
  - Immunization in recipients carrying partial D or weak D
- Rendering genotyping practical & cost-efficient in the routine lab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