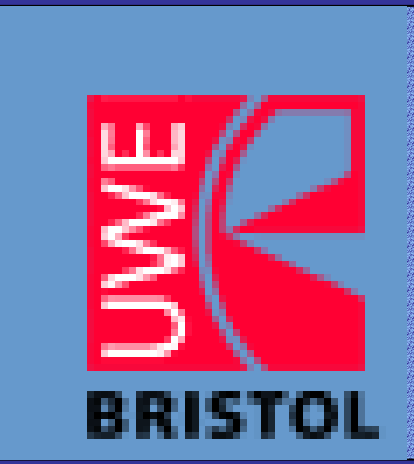


The Bloodgen project : Platform Technology for Mass Scale Genotyping of Blood Groups and Beyond

Prof. Neil D. Avent and Bloodgen consortium members

DGTI Symposium SY1 Friday 24th September 2004

Bloodgen visions

- To provide platform technologies for Genotyping for all major blood group alleles – Gene chip and fluoro-SSP
- To produce Commercial products at the conclusion of the project
- Full Genotyping of ALL Blood donors in the EU
- Reduce incidence of alloimmunisation by extensive use of electronic cross matching cf genotype

Quality of Life and Management of Living Resources

Blood Grouping and Genotyping: Improving patient safety and Blood Transfusion Compatibility

Acronym: **BloodGen**

Key Actions:
3: The Cell Factory
3.1: Improving the diagnostic and therapeutic arsenal for healthcare
3.1.1 The development of new diagnostics:

Submitted 2001, Funded 2002 and initiated 1st September 2003

QLK3-2003-01772

CLB (Sanquin)

Amsterdam, Netherlands

Rotterdam, Netherlands

Lund, Sweden

Dreieich, Germany

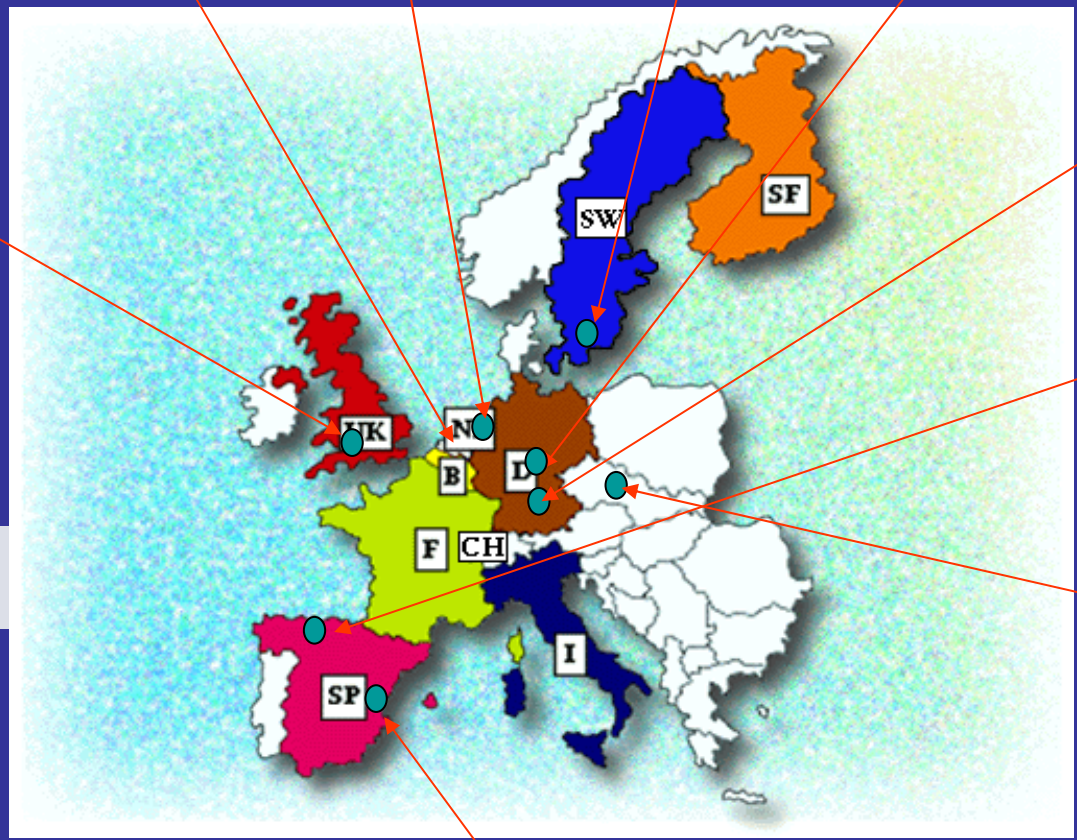
Bristol, UK (UWE and BITS)

Ulm, Germany

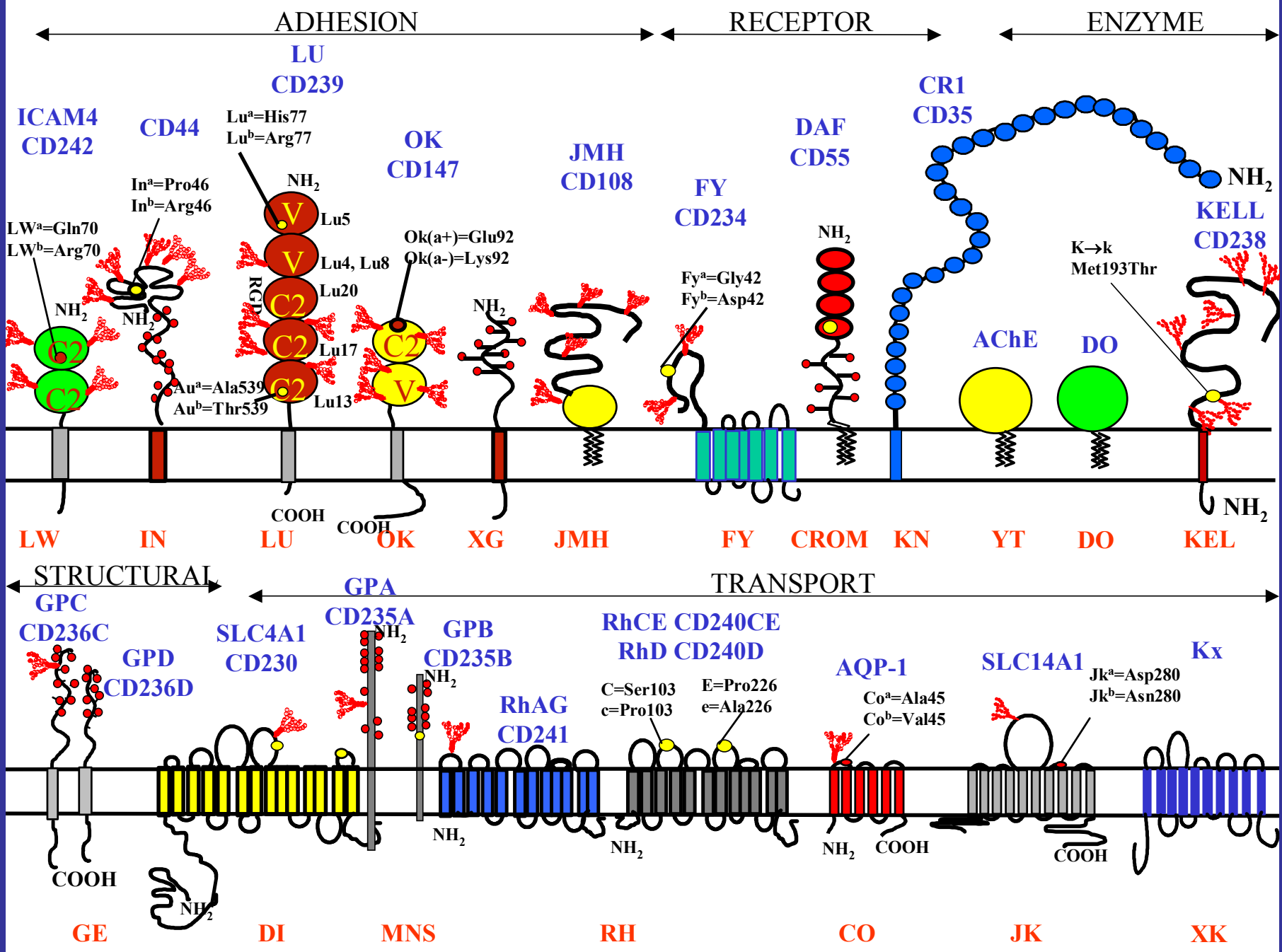
Derio, Spain

Prague, Czech Republic

Barcelona Spain



bloodgen
blood grouping & genotyping


ADHESION
RECEPTOR
ENZYME
LU
CD239
ICAM4
CD242
CD44
Lu^a=His77
Lu^b=Arg77
OK
CD147
JMH
CD108
FY
CD234
DAF
CD55
CR1
CD35
NH2
KELL
CD238
In^a=Pro46
In^b=Arg46
LW^a=Gln70
LW^b=Arg70
Ok(a+)=Glu92
Ok(a-)=Lys92
Fy^a=Gly42
Fy^b=Asp42
K→k
Met193Thr
AChE
DO
V
Lu5
Lu4, Lu8
Lu20
Lu17
Lu13
C2
RGD
Au^a=Ala539
Au^b=Thr539
COOH
LW
IN
LU
OK
XG
JMH
FY
CROM
KN
YT
DO
KEL
STRUCTURAL
TRANSPORT
GPC
CD236C
GPD
CD236D
SLC4A1
CD230
GPA
CD235A
GPB
CD235B
RhCE CD240CE
RhD CD240D
RhAG
CD241
AQP-1
SLC14A1
Kx
C=Ser103
c=Pro103
E=Pro226
e=Ala226
Co^a=Ala45
Co^b=Val45
Jk^a=Asp280
Jk^b=Asn280
GE
DI
MNS
RH
CO
JK
XK

Current usage of Blood Group Genotyping

- Fetuses at risk of HDNF
- Multi-transfused patients (e.g. sufferers of sickle cell disease)

Scheme for genotyping blood group antigens

DNA isolation

↓

PCR amplification of gene fragment carrying the polymorphic site using a multiplex (MPX) PCR

↓

Labelling/fragmentation of MPX PCR products

↓

Hybridisation on array

↓

Measurement of the fluorescence

↓

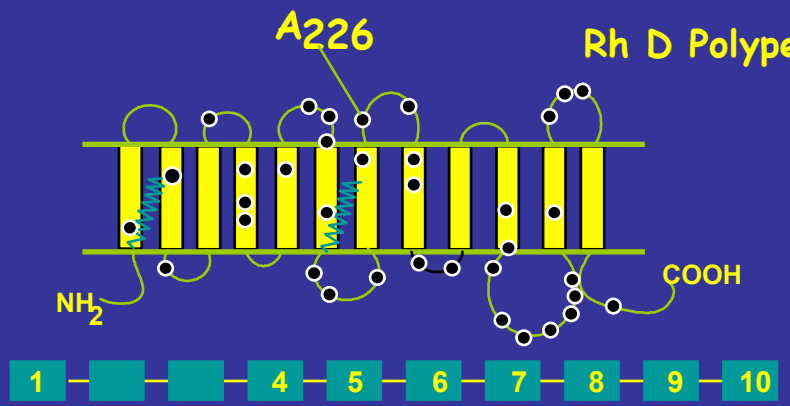
Interpretation of the signals: typing of the sample

Blood group (Symbol/Number)	Antigen involved	SNPs affecting critical amino acid(s)
ABO (ABO/001)	ABO	All common A, B and O alleles
RhD (RH/004)	RhD	9 RHD exons + 24 Mandatory alleles
RhCcEe (RH/004)	Rhc RhC *RHD* promotor C^w C^x RhE/Rhe VS VS/V	T307C Intron 2 RHCE r's gene A122G G106A C676G C733G G1006T (Gly336Cys)
Kell (KEL/006)	K/k Kp^a/Kp^b Kp^c Js^a/Js^b European Ko	T698C T961C G962A C1910T
Kidd (JK/009)	Jk^a/Jk^b Jk_{null}	G838A
Duffy (FY/008)	Fy^a/Fy^b FY-GATAmut Fy^x	G125A T-33C C265T
MNS (MNS/002)	M/N S/s	C59T , G71A , T72G T143C
Diego (DI/010)	Di^a/Di^b	T2561C

Deliverable 1: Designate blood group active SNPs

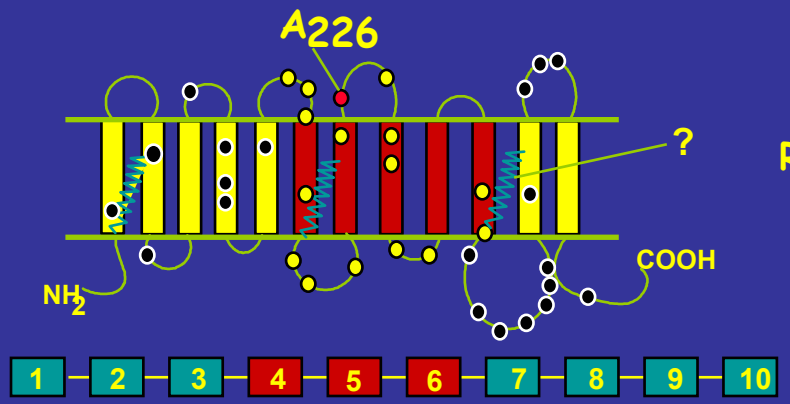
Vulnerable patient groups and Transfusion practice that would benefit most from Blood Group Genotyping

- Women of childbearing age
- Pregnant Women (to prevent alloimmunisation)
- Multi-transfused patients (e.g. sufferers of sickle cell disease)
- Prevention of alloimmunisation due to the Jk^a antigen
- D negative patients that may receive transfusions of variant Rh D incorrectly serotyped as D negative (e.g. DVI and DHAR)
- Panel cells (Fy(b-) identified as Fy^b weak)

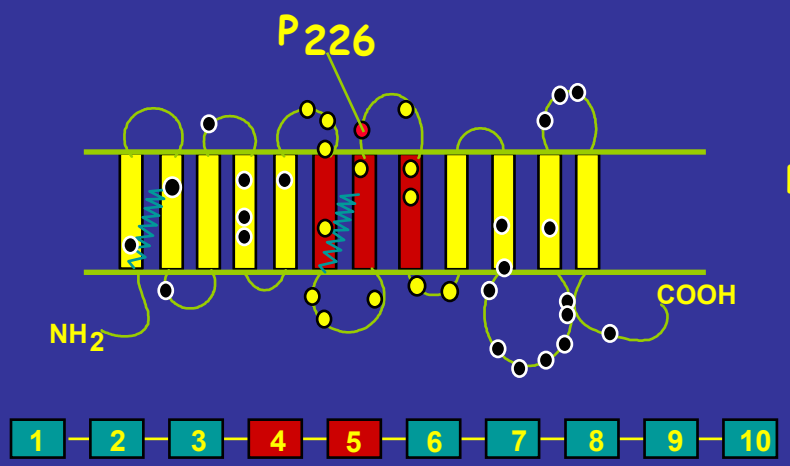


Potential D alloimmunisation by DVI phenotype Red Cells

Wild Type Rh D and hybrid $CD^{VI}e$ & $cD^{VI}E$ proteins



Rh $CD^{VI}e$ Polypeptide



Rh $cD^{VI}E$ Polypeptide

RHD derived exon

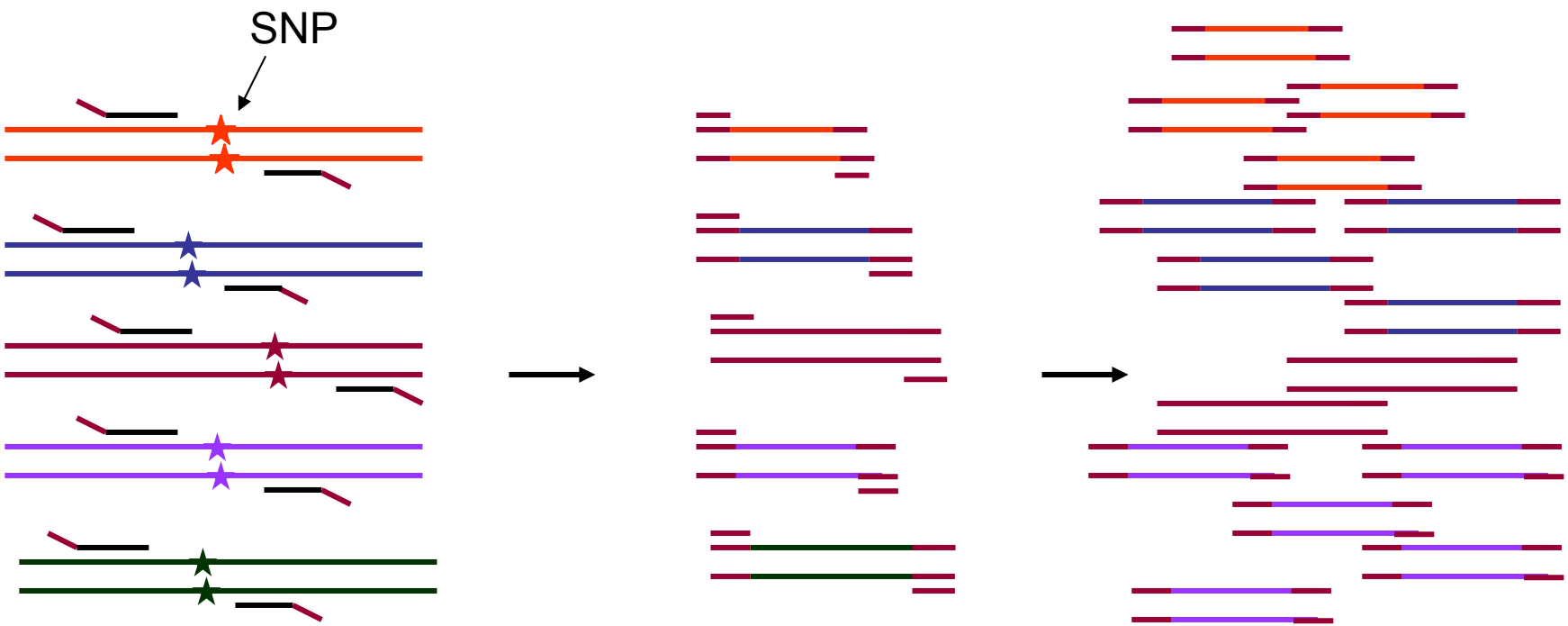
RHCE derived exon

Bloodgen Technical Approach I – Multiplex PCR

Aim to amplify polymorphic regions of all clinically significant blood group active genes

- List of clinically significant alleles "Mandatory" "Preferable" and "Optional" DELIVERABLE 1
- Develop 3 separate MPX PCRs 1. ABO, 2. RHD and 3. "the rest" (RHCE, KEL, FY, JK, DO, DI, MNS, CO)
- Develop Prototype Bloodgen chip
- Hybridise chip with 3 MPX reactions

Multiplex PCR using MAPH-primers



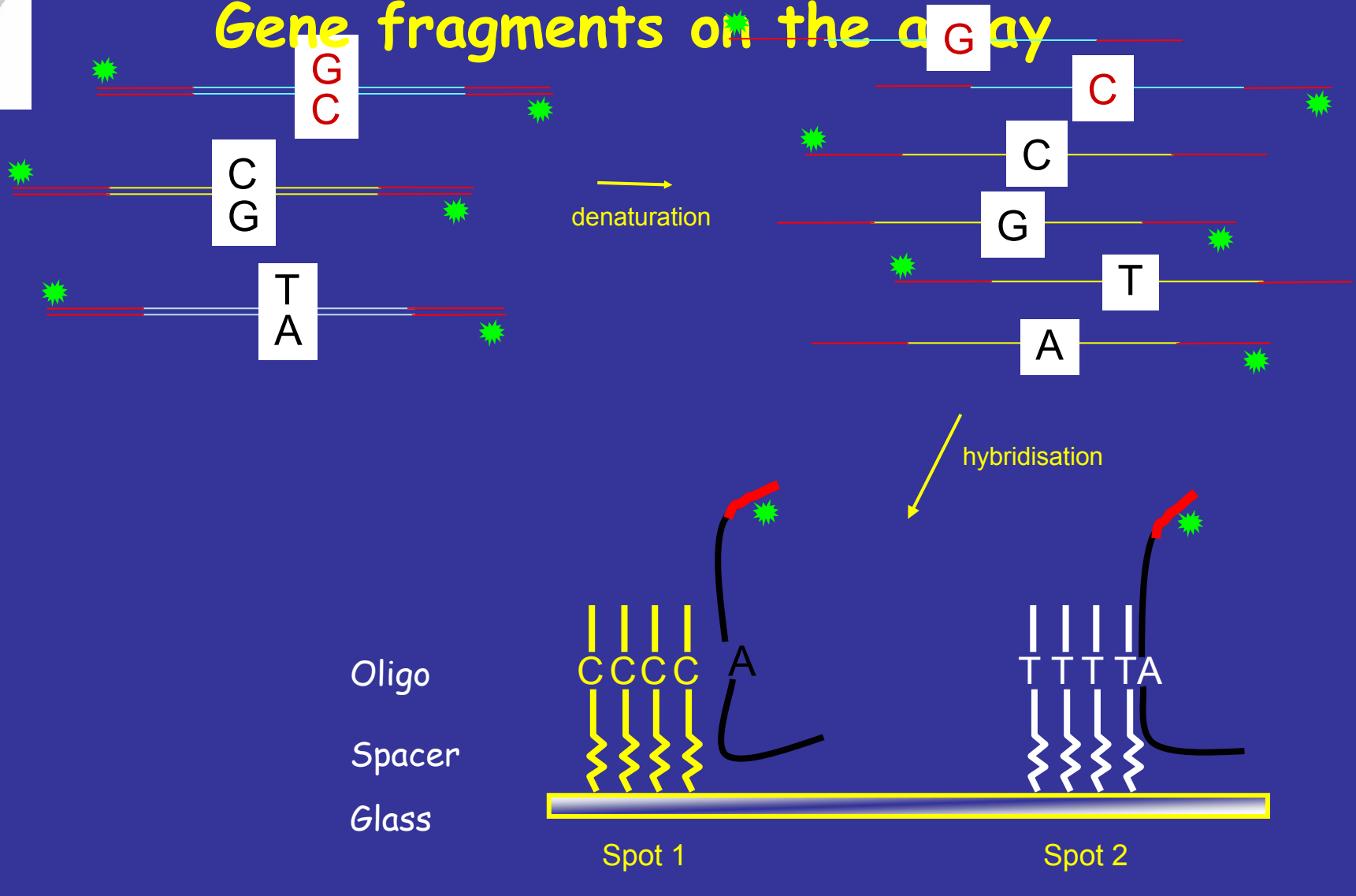
Different genes with gene specific primers

PCR products with MAPH-tags

Efficient amplification with MAPH-primers

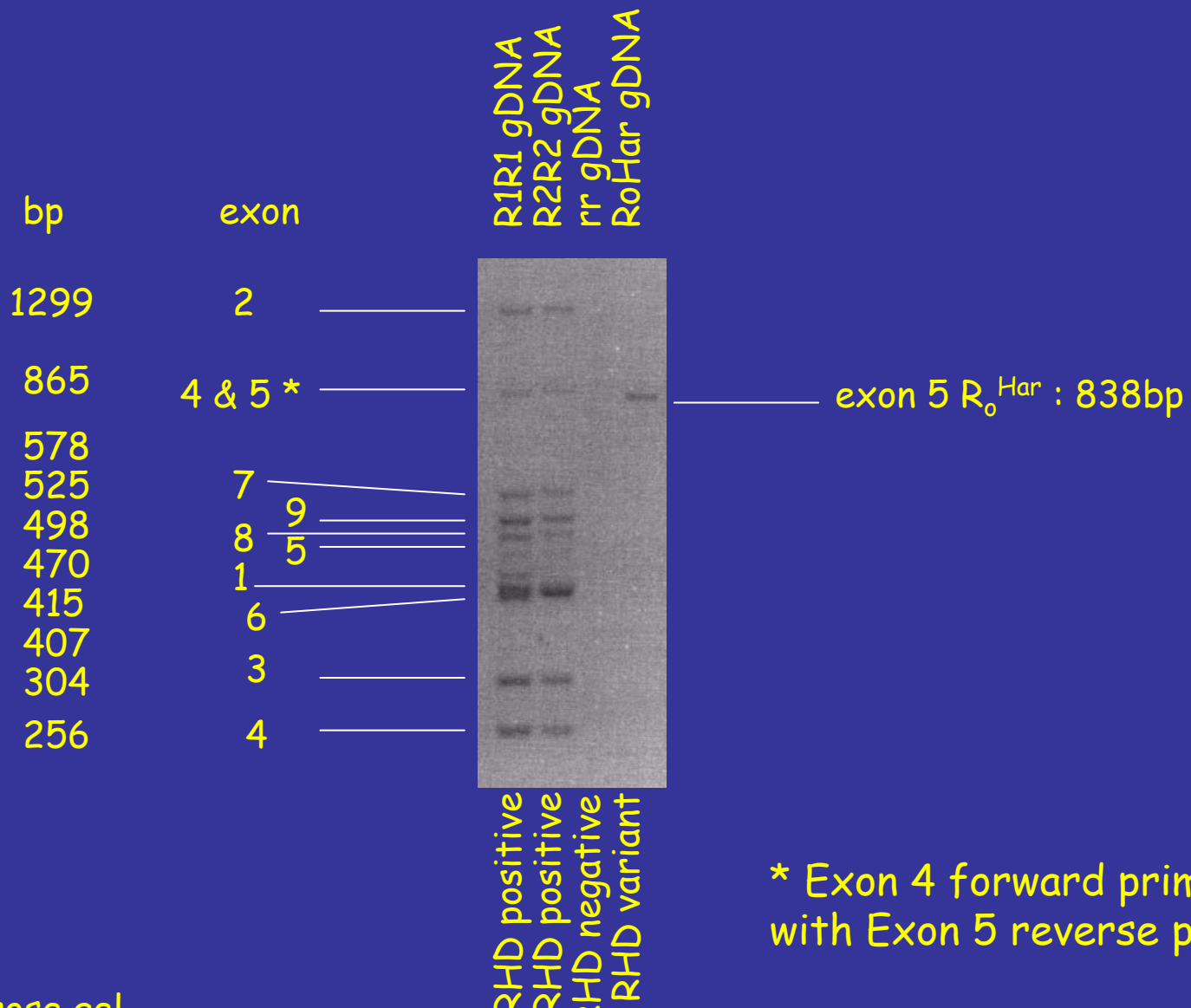
Slide courtesy Dr Masja de Haas, CLB

Gene fragments on the array



Slide courtesy Dr Masja de Haas, CLB

RHD MPX PCR Analysis I



* Exon 4 forward primer pairing with Exon 5 reverse primer

3% agarose gel

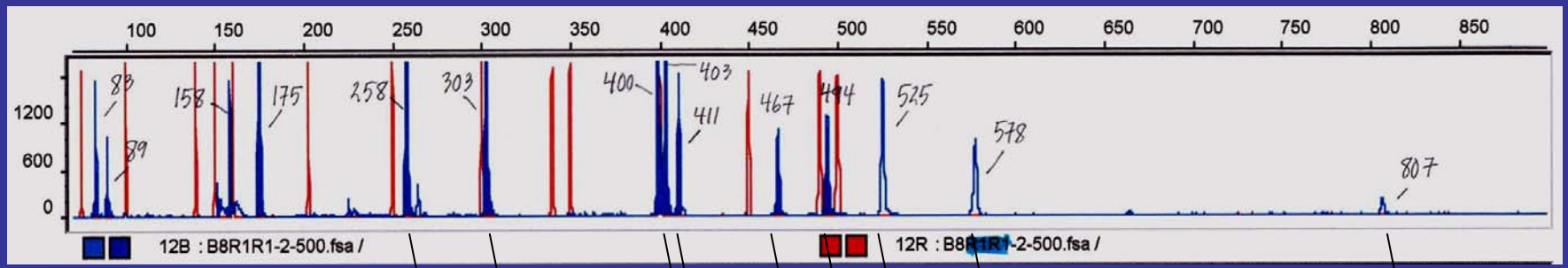
RHD MPX PCR Analysis II

Sequencing: individual exon PCR products (R_1R_1)

MPX PCR (R_2R_2)

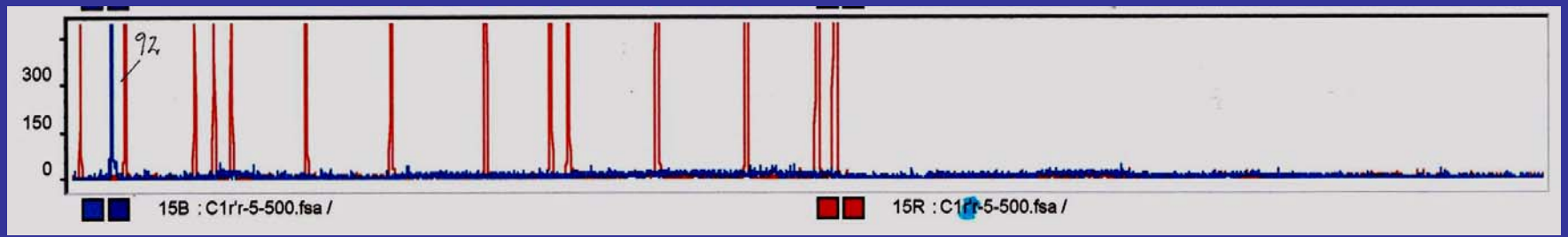
Fragment analysis (GeneScan – ABI capillary sequencer, Lund):

Dpos



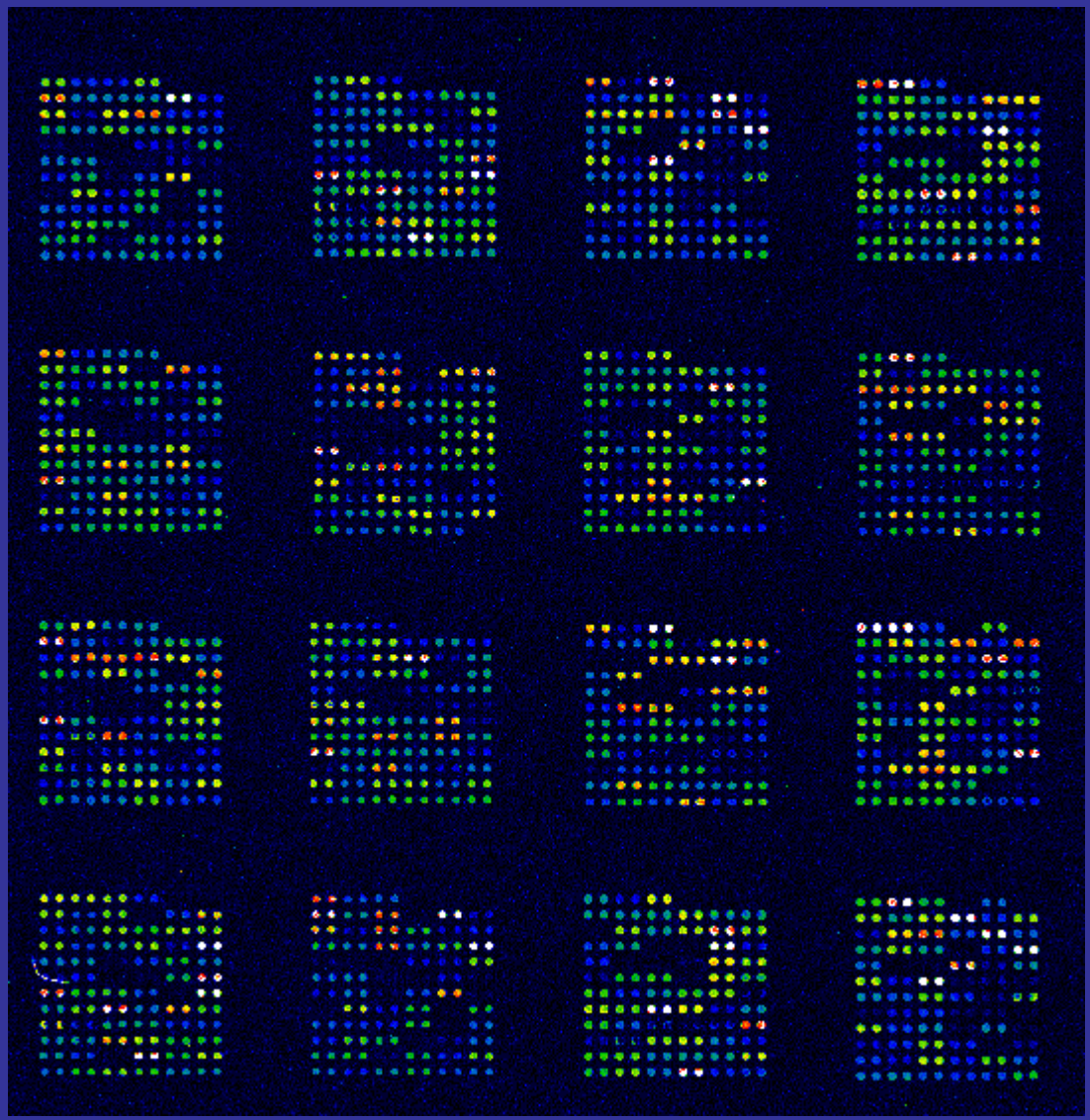
exon: 4 3 6 1 5 8 9 7 4 + 5

Dneg



Bloodgen Technical Approach II – MPX and hybridisation to chip

- MPX products labelled with Cy5
- Fragmented/labelled (biotin-dUTP+streptavidin Cy3) and then hybridised to chip using a Ventana Discovery hybridisation station
- Chips imaged using conventional scanner
- Alleles scored using software developed by Progenika



White= Strongest signal
Red
Orange
Yellow
Green
Blue= Weakest signal

Bloodgen Prototype gene chip

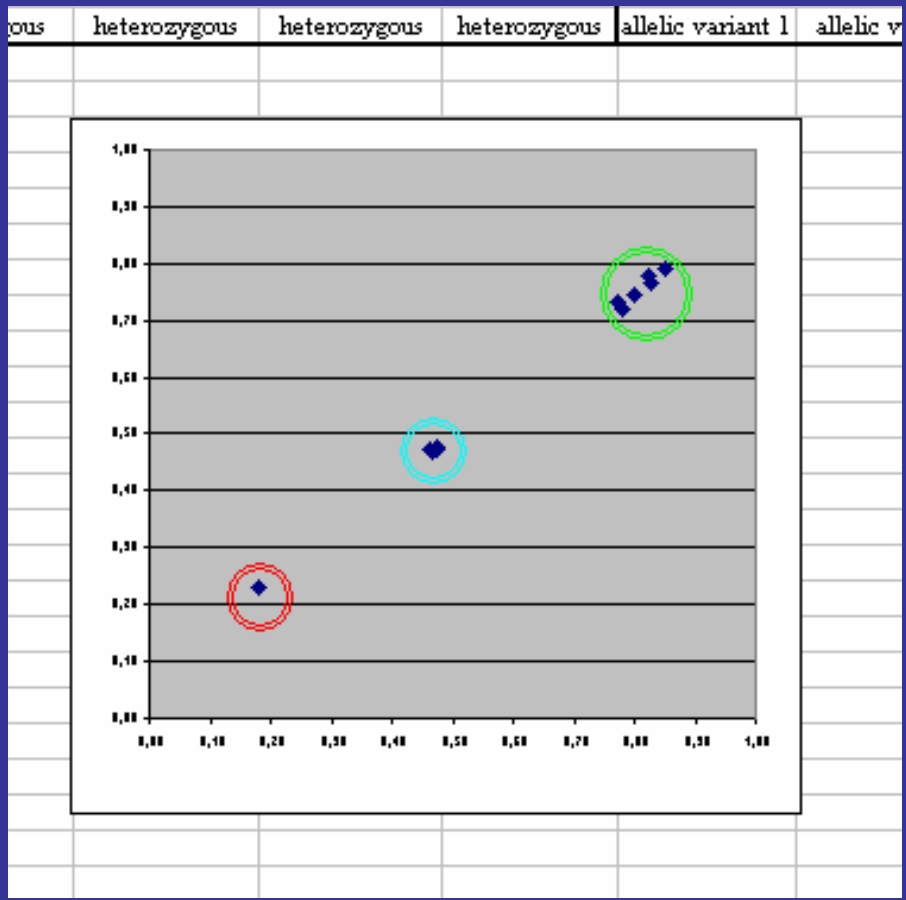
Bloodchip Specifications	
Number of Arrays	1
Number of Subgrids	32
Array Size	25 x 40 mm
Oligo Length	19-27 mer
SNPs	94
Background control	88 spots
Oligo replicates each mutation	40 spots
Total number of spots	4608

Allele Scoring process

Homozygote BB

Heterozygote AB

Homozygote AA



Bloodgen years 2+3

- Slight amendments to MPX design and to Chip oligonucleotides
- Test "Production" Bloodgen chip with a cohort of DNA samples obtained from fully serotyped donors
- Screened using a cohort of patient samples "blinded"
- Launch of the Bloodgen chip via dissemination event
- Analysis of new variants identified in the study
- Estimate of the population frequency in the EU of certain blood group alleles
- Commercially available BLOODGEN chip from Progenika AG

Fluoro Single Sequence Primer : BIOTEST AG

- Based on tested HLA genotyping platform
- Fluoro-SSP test in 96 well format
- Will be developed for medium throughput RH genotyping
- Product will be in the marketplace at the conclusion of the Bloodgen project.

Every revolutionary idea seems to evoke three stages of reaction.

They may be summed up by the phrases:
(1) It's completely impossible.
(2) It's possible, but it's not worth doing.
(3) I said it was a good idea all along.

Arthur C. Clarke