Molecular Genetics of Blood Groups

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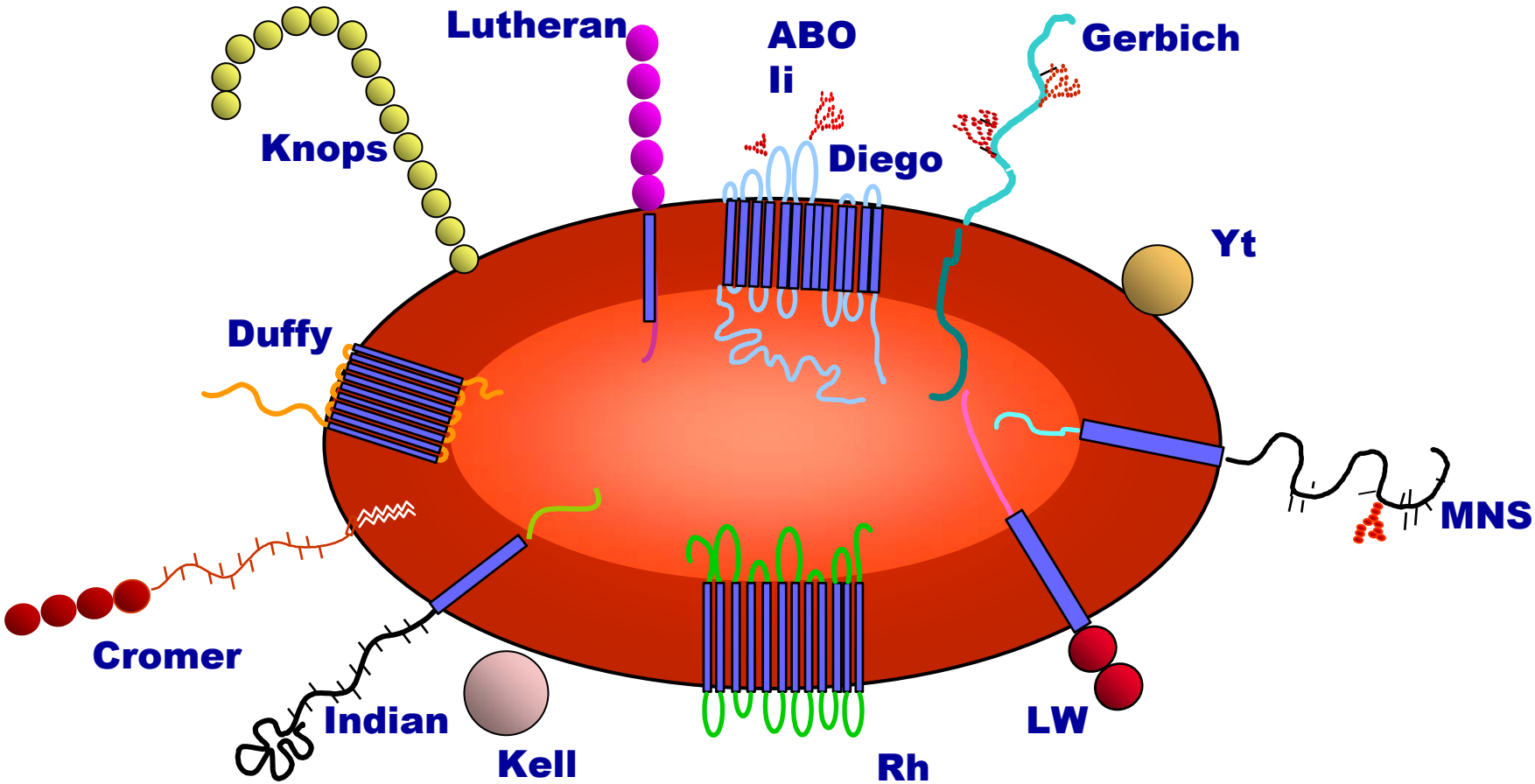
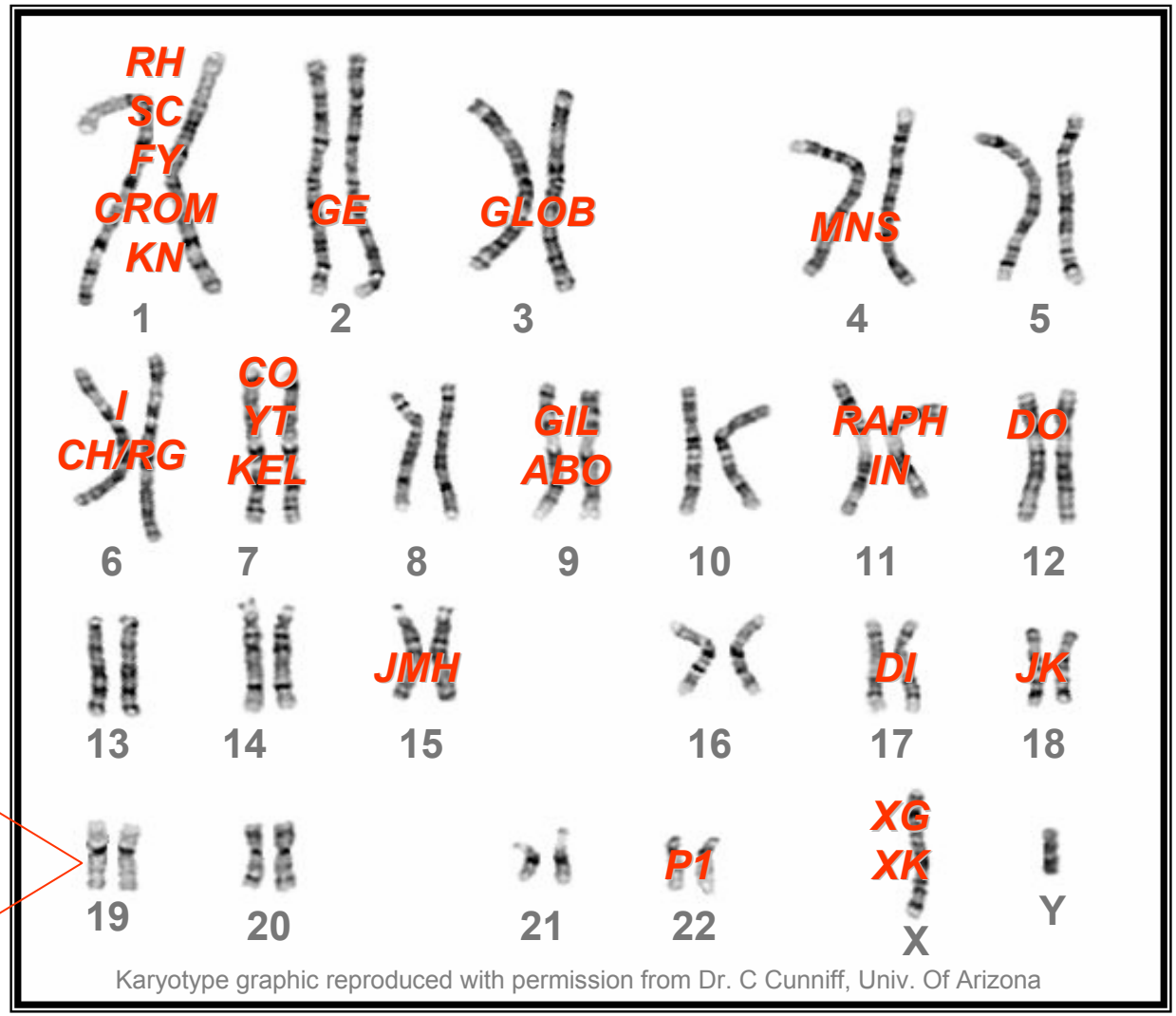
Blood Groups on the RBC



Slide courtesy of E. Sjöberg Wester

Blood Groups Are Inherited As The Products of Genes



Karyotype graphic reproduced with permission from Dr. C Cunniff, Univ. Of Arizona

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RBC Antigens are the Products of Genes

- Antigens carried on proteins are encoded directly by the gene, e.g. *RH, KEL, FY*
- Carbohydrate antigens are under the control of genes that encode glycosyltransferases, e.g. *ABO, P1, H*

Genetic Mechanisms That Generate Diversity

- Single nucleotide polymorphism (SNP)
  - Silent
  - Missense
  - Nonsense
- Insertions and deletions
- Crossover and recombination
- Gene conversion

Single Nucleotide Polymorphisms

SNPs occur every 100 to 300 bases

- **Silent SNPs do not alter the amino acid sequence**
- **Missense SNPs encode a change of one amino acid to another**
- **Nonsense SNPs cause the change of an encoded amino acid to a stop codon (TAA, TAG, TGA)**
- **SNPs in the conserved splice site sequences may cause altered splicing**

Useful database:

http://www.ncbi.nlm.nih.gov/SNP/index.html

SNP – Missense Mutations

A change of one nucleotide can alter the amino acid encoded
Example: S and s antigens on Glycophorin B

***GYPB* exon 4**

```
GA GAA ATG GGA CAA CTT GTC CAT CGT TTC ACT GTA CCA G S
   E   M   G   Q   L   V   H   R   F   T   V   P
GA GAA ACG GGA CAA CTT GTC CAT CGT TTC ACT GTA CCA G s
   E   T   G   Q   L   V   H   R   F   T   V   P
```

Most Blood Group Antigens Are the Result of SNPs

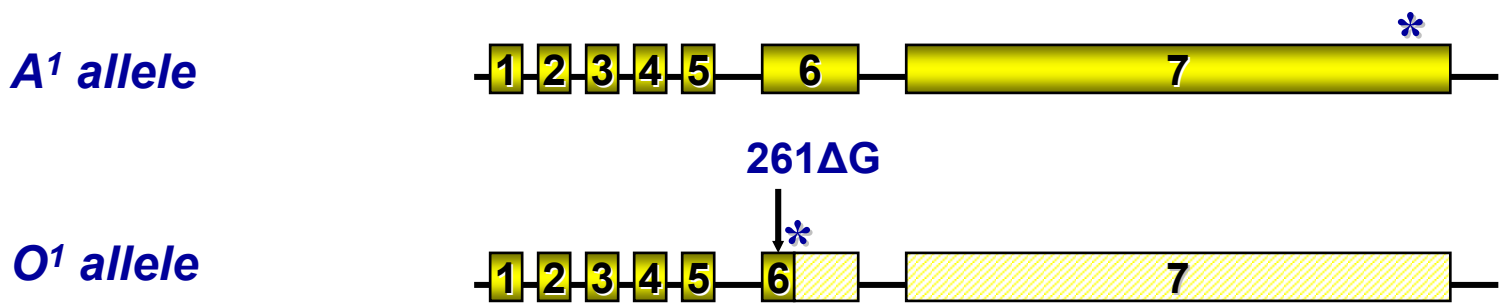
Common antigen pairs encoded by SNPs:

RH	**C/c, E/e**
MNS	**S/s**
Kell	**K/k, Kp^a/Kp^b, Js^a/Js^b**
Duffy	**Fy^a/Fy^b, (GATA)**
Kidd	**Jk^a/Jk^b**
Lutheran	**Lu^a/Lu^b**
Dombrock	**Do^a/Do^b**

Many other examples of high incidence and low incidence antigens

Genetic Mechanisms That Generate Diversity

- **Deletion/addition of nucleotides will alter the open reading frame**
  - **May result in generation of a premature stop codon e.g. *O* gene**

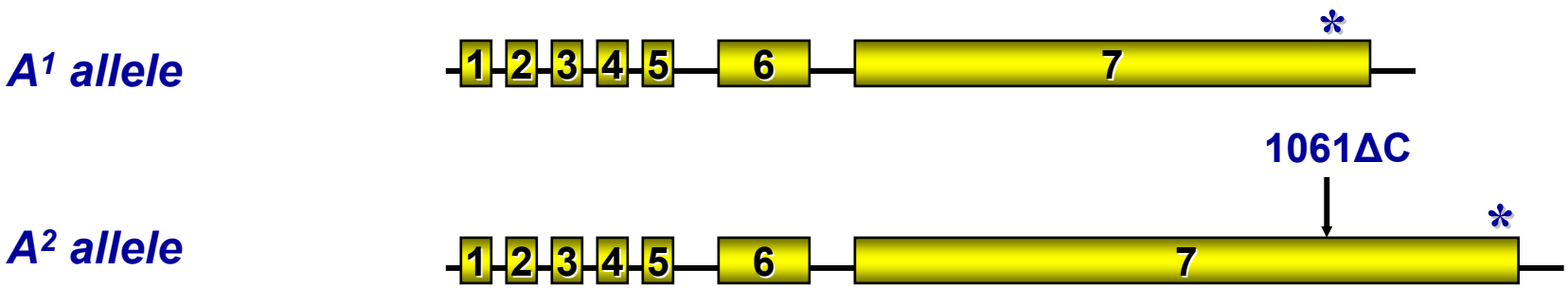


***O¹ allele* encodes an inactive protein of 117 amino acids**

Genetic Mechanisms That Generate Diversity

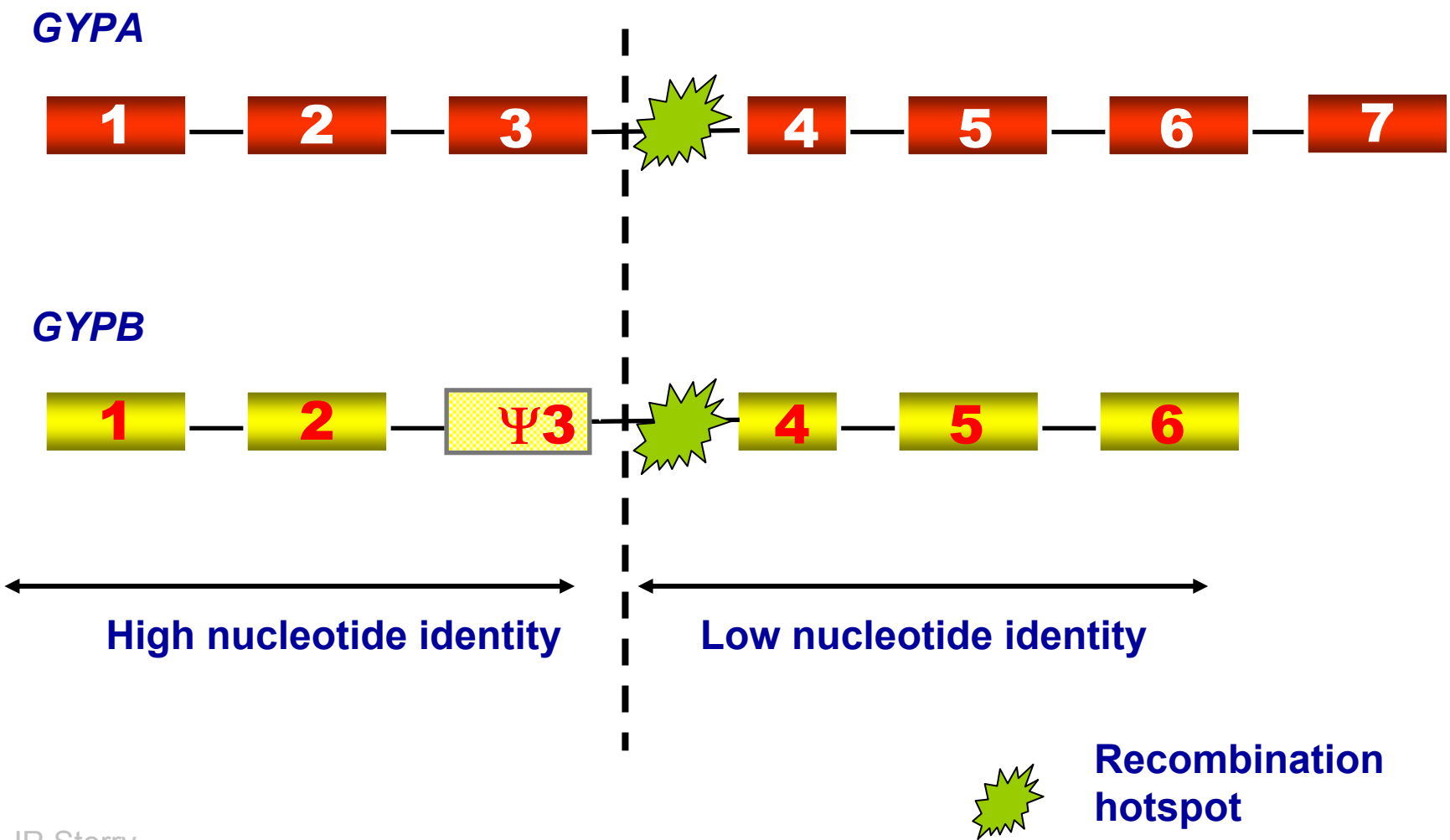
Deletion/addition of nucleotides will alter the open reading frame

- May result in a longer open reading frame e.g. *A²* gene



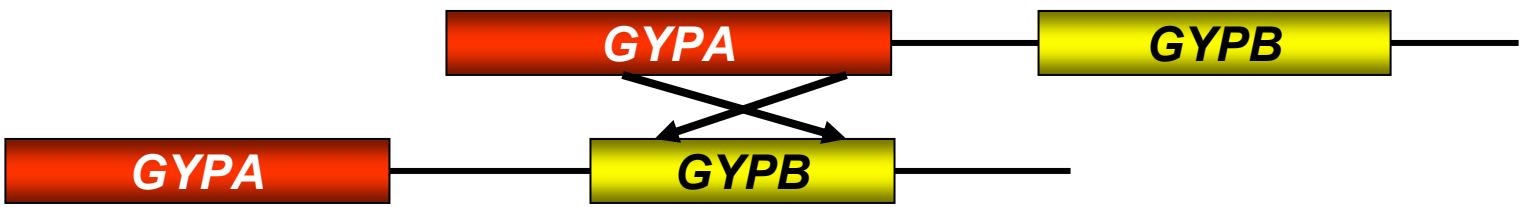
A2 transferase is 375 amino acids long compared with A1 transferase, which is 354 amino acids in length. The A2 enzyme is not as active.

Homology Between Genes Creates Diversity



Genetic Mechanisms That Generate Diversity

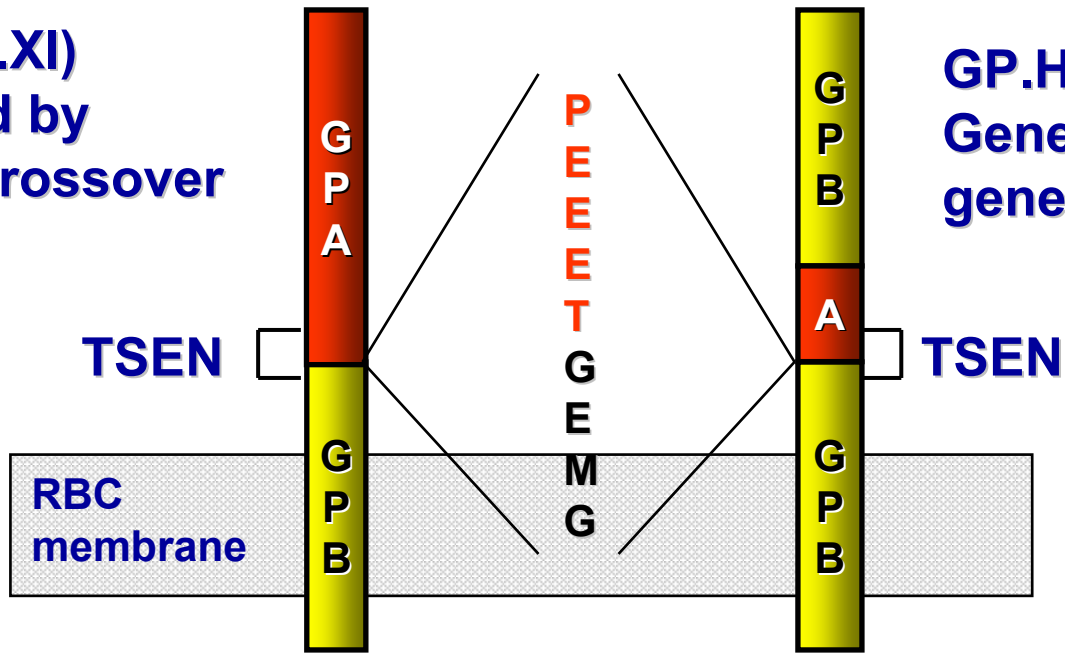
Genes with high sequence identity can misalign during meiosis
e.g. in the MNS system



Exchange of DNA by unequal crossover

Different Mechanisms Can Produce The Same Antigen

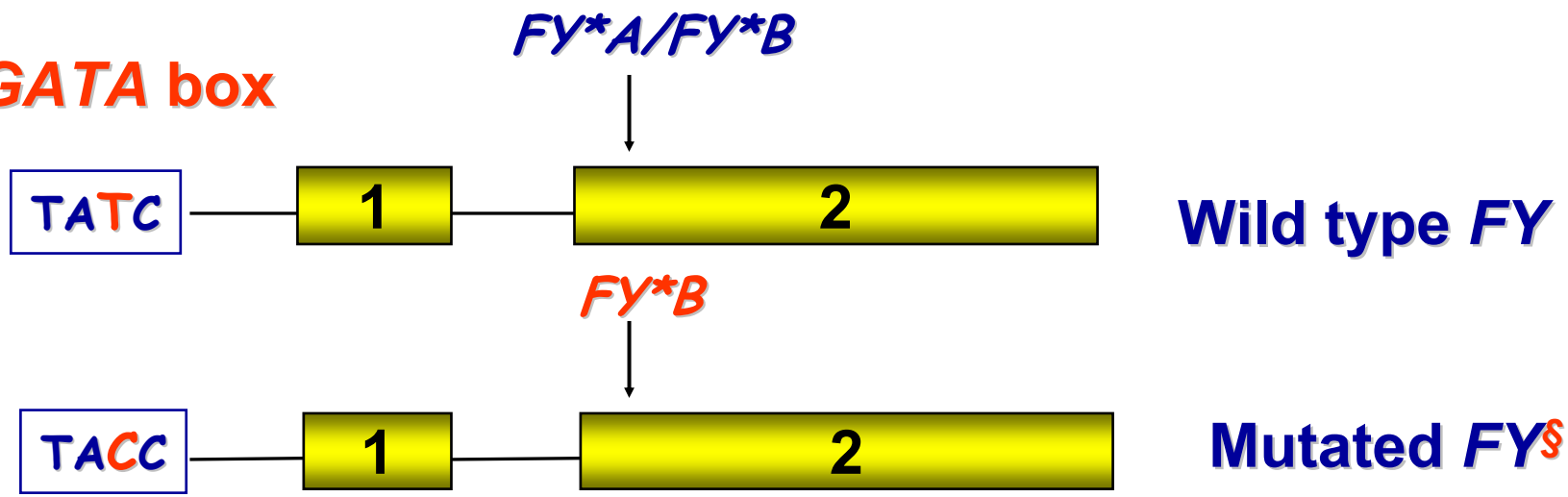
GP.JL (Mi.XI)
Generated by
unequal crossover



GP.Hop (Mi.IV)
Generated by
gene conversion

Genetic Mechanisms That Generate Diversity

Promoter Mutations Affect RBC Antigen Expression: T>C mutation in the *GATA* box prevents transcription of the *FY* gene



§In Fy(a–b–) persons of African descent, the FY gene encodes *FY*B*

Analysis of Blood Group Polymorphisms

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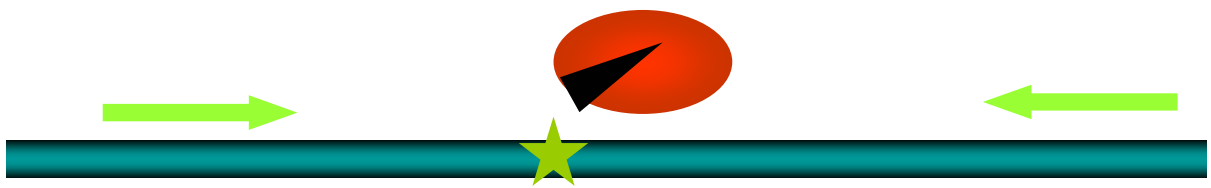
Different PCR strategies

- PCR with sequence- (or allele-) specific primers (PCR-SSP or PCR-ASP):

- PCR followed by restriction endonuclease digestion (PCR-RFLP):



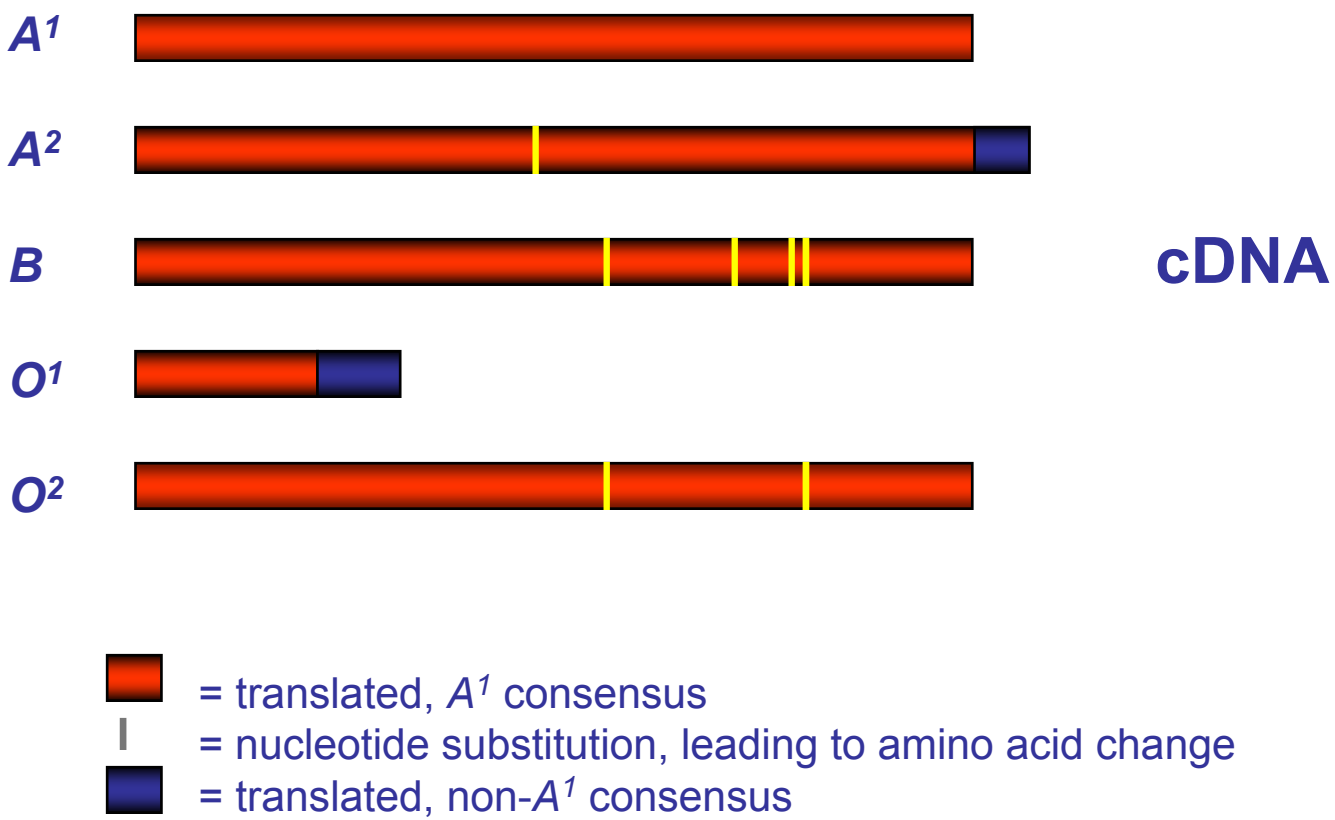
Possible reasons for *ABO* genotyping

- **Acquired weakness of A or B antigen expression**
  - ✓ *e.g.* in leukemia
- **Acquired A or B antigens**
  - ✓ *e.g.* gastrointestinal infection
- **Inherited weakness of A and/or B antigen expression**
  - ✓ *e.g.* A_3, B_x, cisAB…
- **Mixed field pattern due to transfusion or chimerism**

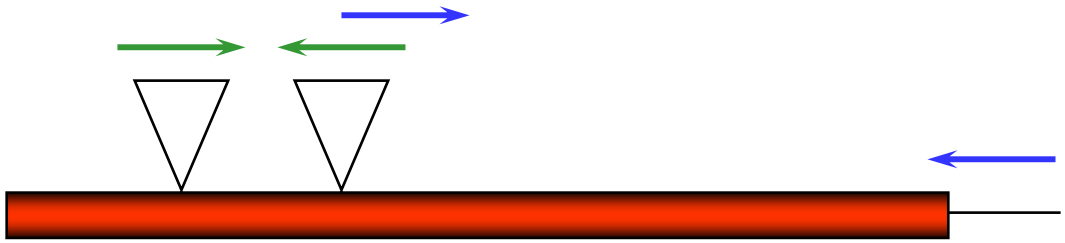
Possible reasons for *ABO* genotyping

- **Fetal blood group determination!**
  - ✓ **HDN**: At least 5 documented cases of hydrops fetalis due to ABO-antibodies reported since 1988. Ethnic/geographic variation.
  - ✓ **NAITP:** Samples from such a case have been referred to our lab
- **Confirmation of A_2 status in A_2 to O kidney transplant**

Major *ABO* alleles recognised in 1994

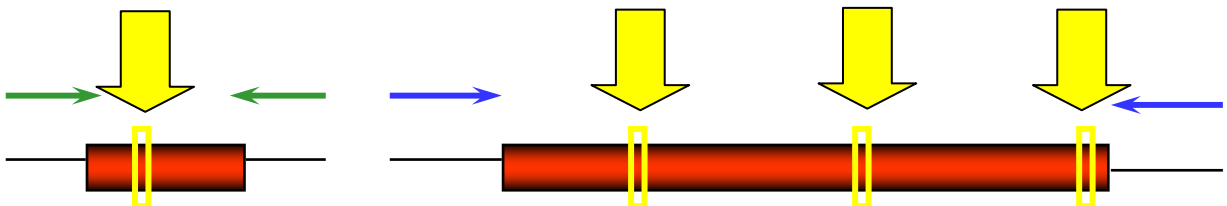


Duplex PCR-RFLP method for *ABO* genotyping



1. Multiplex PCR

Exon: 1-5 6 7

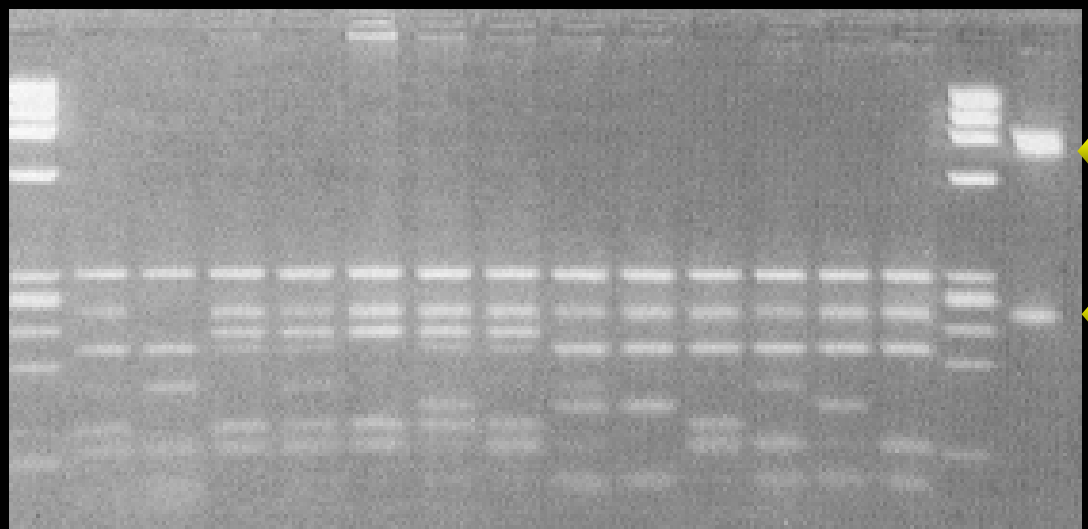


Size: 252 bp 843 bp

2. RFLP using *Kpn*I and *Hpa*II simultaneously

Olsson & Chester. Vox Sang 1995;69:242-7

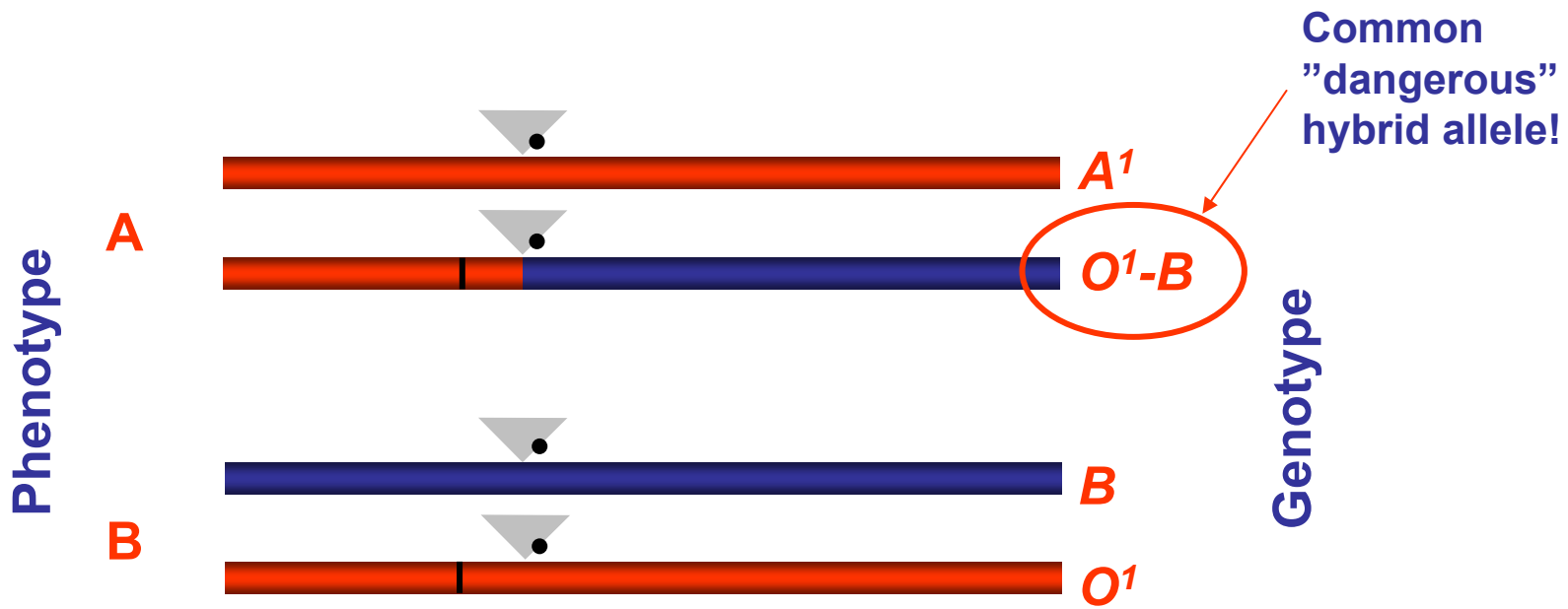
Duplex PCR-RFLP method for *ABO* genotyping



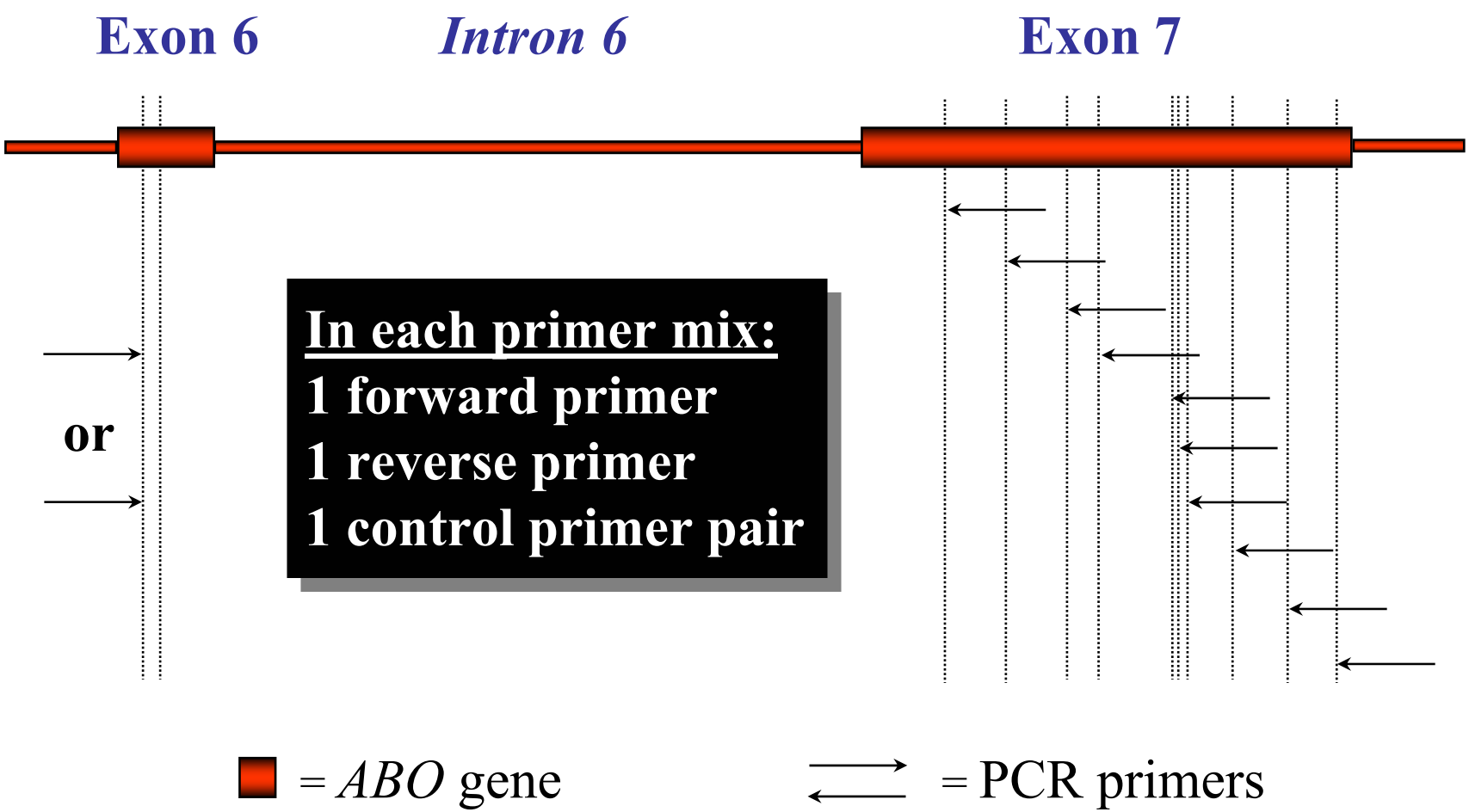
3. Electrophoresis in agarose gel
Resolution of >15 genotypes

Olsson & Chester. Vox Sang 1995;69:242-7

Identical genotyping patterns can result in completely different phenotypes



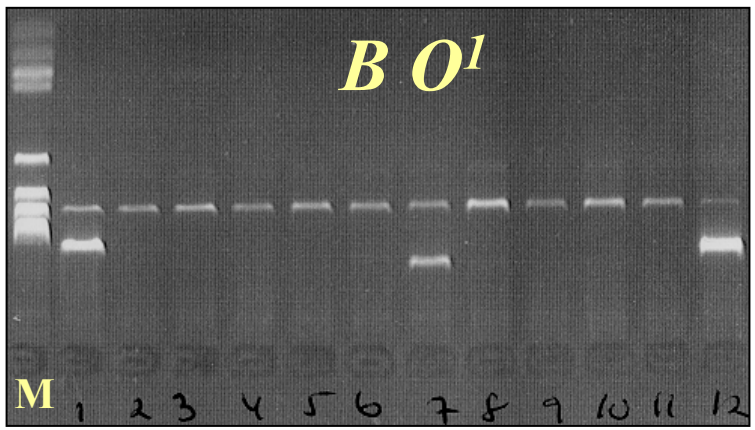
Hybrid-proof rapid PCR detection of common and rare *ABO* alleles

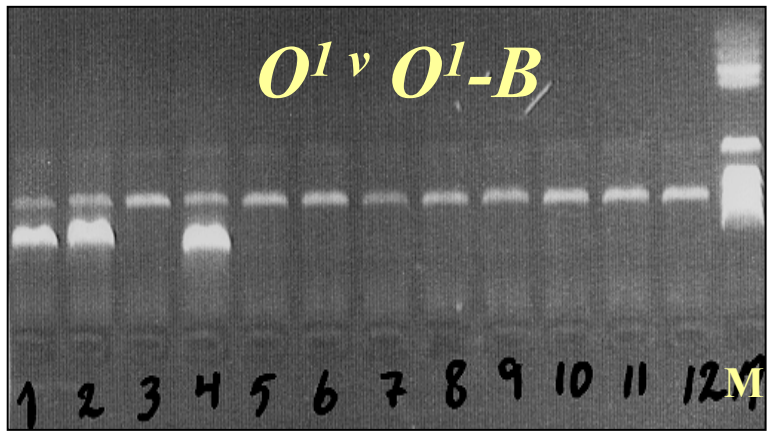


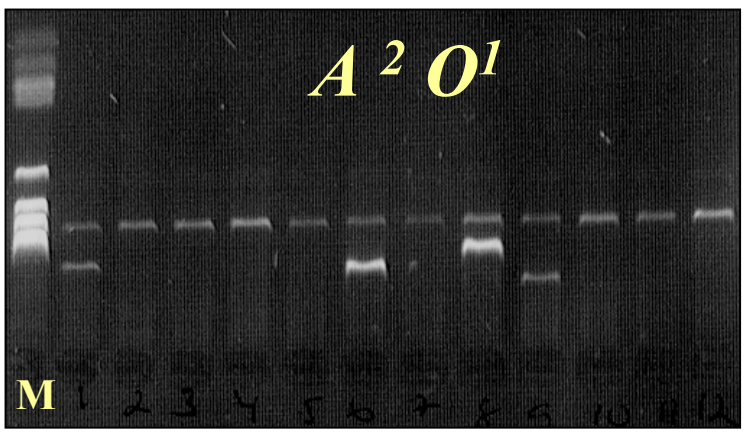
ABO PCR-ASP low-resolution typing across intron 6

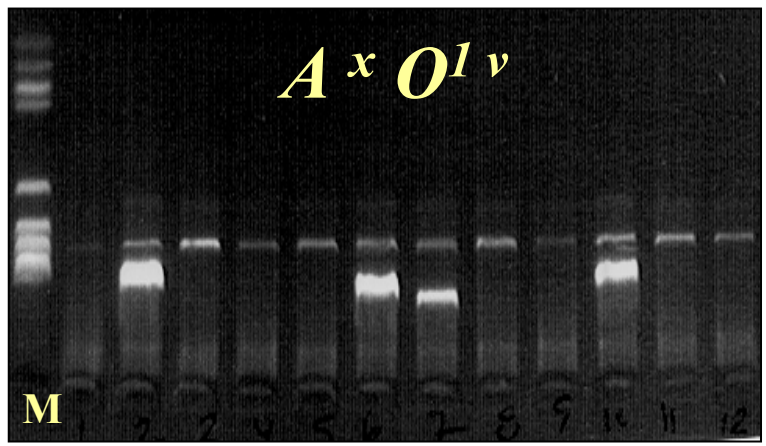
- **Fragment sizes: 1.3 - 1.9 kb**
- **12 primer mixes: 3 screen for rare *O* and A/B subgroup alleles**
- **Numerous genotypes based on all known alleles interpretable in <3 hours**
- **The following *ABO* alleles discriminated:**
  A^1, A^2, $A^{1(C467T)}$, A^{subgr}, B, B^{subgr}, $cisAB$, $B(A)$, O^1, O^{1v}, $O^{1(C467T)}$, O^1-B, O^1-A^2, O^2, O^3, O^4, O^5
- **FLEXIBILITY, i.e. primers detecting mutations in novel alleles can be added continuously**

ABO PCR-ASP gels



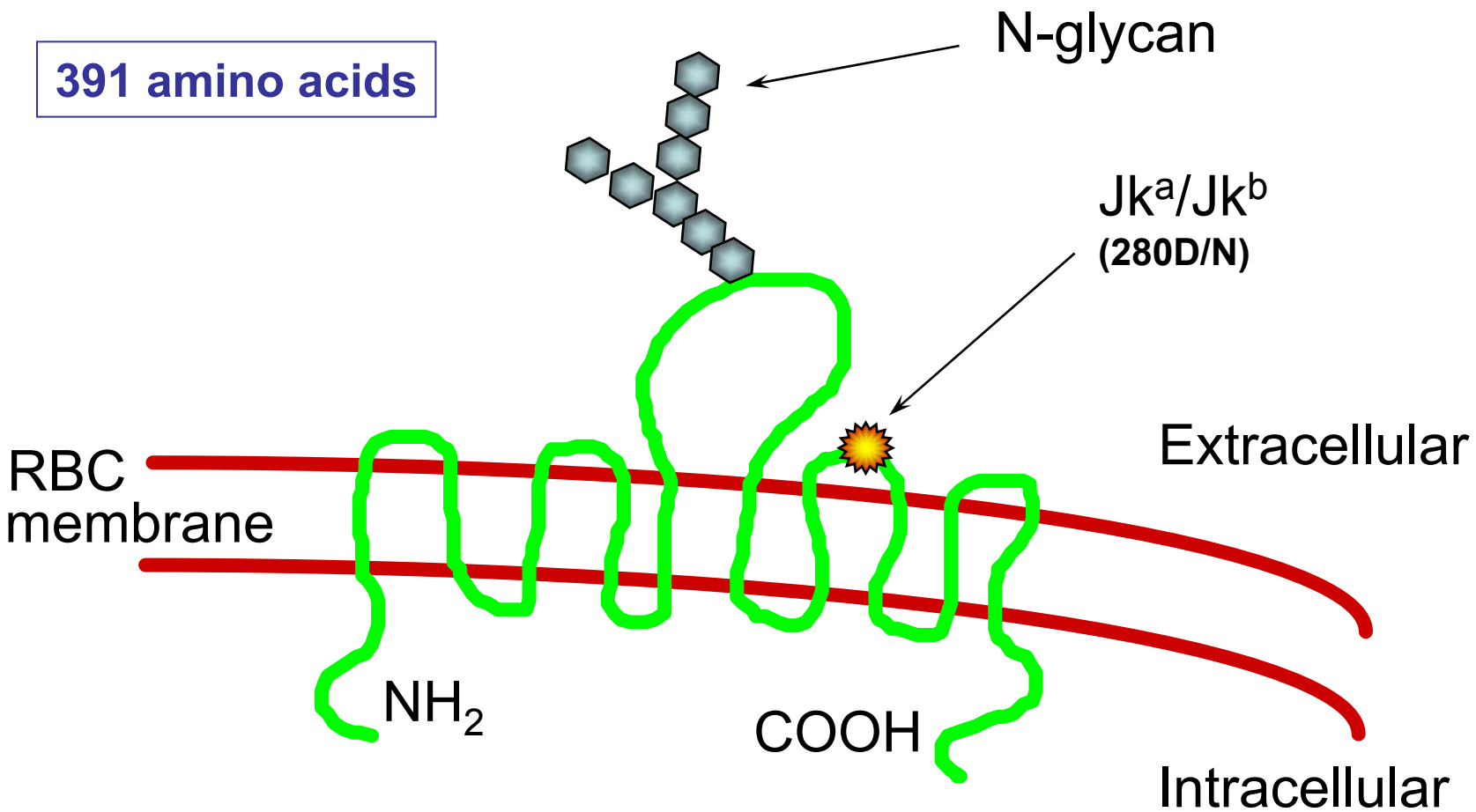




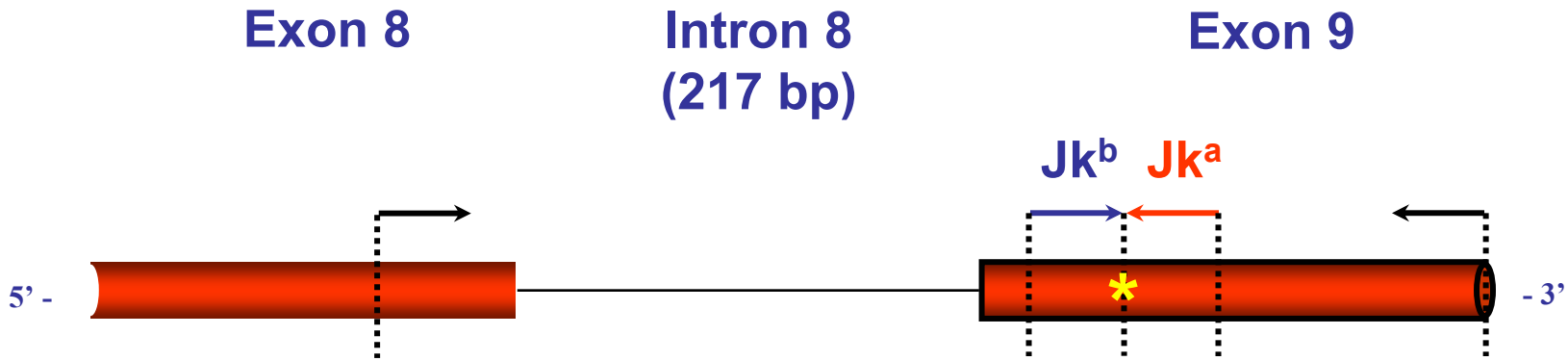


Olsson *et al. Transfusion* 1998; 39:3S

Schematic model of the JK glycoprotein

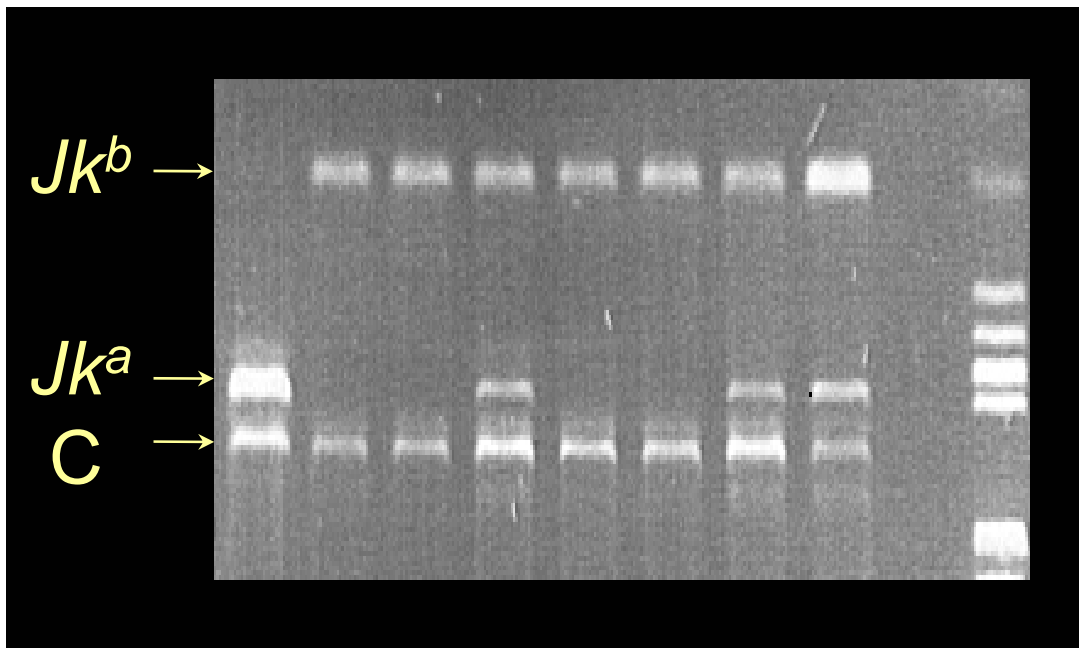


JK Genotyping



- **PCR-ASP**
- **Single PCR (10 uL)**
- **Validation:**
  - **119 samples**
  - **100% concordance**
- **Amniotic DNA OK**

***Irshaid NM, Thuresson B, Olsson ML. Br J Haematol* 1998;102:1010-14**



Current Genomic Testing for Blood Group Antigens

- Testing performed:
  - ✓Foetal DNA to predict foetal RBC phenotype
  - ✓Prediction of RBC phenotype in multi-transfused patients
  - ✓Resolution of serological discrepancies e.g. weak D, ABO subgroups
  - ✓Resolution of rare variants
- Mostly single/few samples per analysis

Can We "Type" Blood Donors by Genotyping?

WHY?

- Limited selection of antisera
- Scarcity of source material
  - ✓ Few immunized donors with potent antibodies for reagent manufacture
  - ✓ Zero risk climate eliminated immunization and boosting programs
  - ✓ Monoclonal antibodies not available for all antigens

Should We "Type" Blood Donors by Genotyping?

- Use of donor RBCs for in-house antibody detection and identification reagents
- Quality Assurance of Reagent Test RBCs
  - ✓Determination of single/double dose antigens for D, Fy^a, Fy^b

Requirements for Large Scale Genomic Typing

- Automated DNA extraction
- Potential for automated PCR set-up
- Rapid, automated post-PCR analysis of numerous blood group polymorphisms
- Closed system to prevent contamination
- Positive sample identification and data correlation

Potential Technology for High Throughput Genomic Typing

- Oligonucleotide Microarray
- High Performance Liquid Chromatography (HPLC)
- Matrix-Assisted Laser Desorption /Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF)
- Pyrosequencing

None of these techniques are automated *YET*

Blood Grouping and Genotyping

Improving Patient Safety and Blood Transfusion Compatibility

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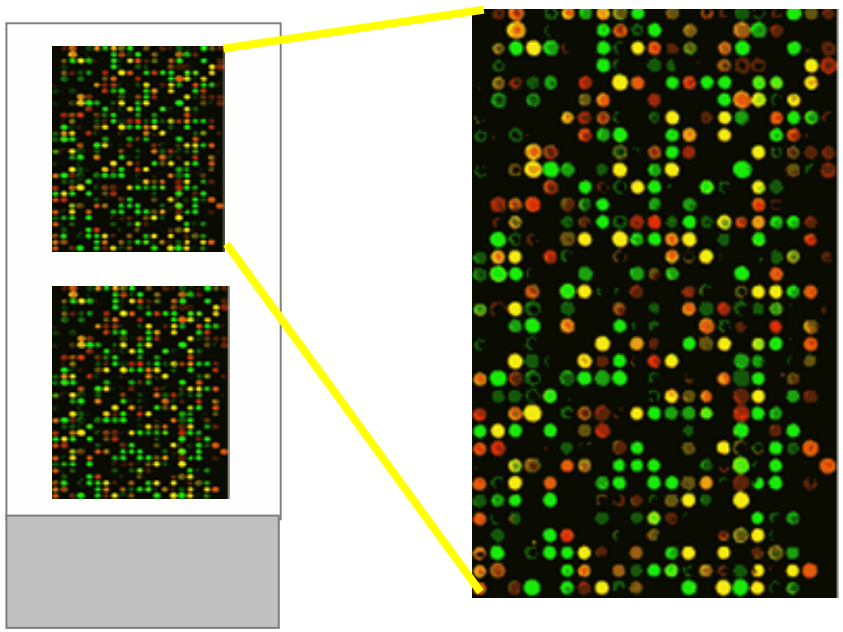
Technical Objectives

- To demonstrate novel, nucleic acid based diagnostic tests to reduce the instance of alloimmunization
- To provide an innovative approach to blood group genotyping on a large scale, which is easily extended to other alleles of clinical significance
- To provide a platform technology for future clinical approaches to genotyping

DNA Microarray Analysis

- Primarily used for looking at gene expression in normal and disease states, e.g.
  - ✓Haematologic malignancies
  - ✓Solid organ tumours
- Increasing use in SNP analysis
- Potentially automated

Principle of Microarray Analysis



Microscope slide spotted with specific oligonucleotides

- Multiplex PCR products are labeled with red or green fluorescent dye
- DNA is hybridised with synthetic oligonucleotide probes on slide
- Fluoresence measured by spectro-photometer
- Specific spots will fluoresce as the different DNAs bind
- Image is produced by the computer analysis program
- Comparison is made between test and control patterns

Challenges of Using Microarray for SNP Analysis

- Homologous genes are difficult to analyse:
  - ✓SNPS in *RHD* may be consensus sequence in *RHCE*
  - ✓Must be amplified in separate multiplexes
  - ✓Experimental procedure does not allow for identity of SNPs in *cis* or *trans*
- Data analysis is the biggest workload burden
  - ✓Analysis files may be Gigabytes in size!

Advantages of Microarrays

- Enormous potential to gather data on known alleles and to detect new mutations
- Automation potential although many manual steps currently
  - ✓Good platform for donor testing
  - ✓Could be used for testing a wide variety of phenotypic and genotypic differences

Conclusions

- 28 of 29 blood group genes have been identified
  - ✓ Blood group polymorphisms can be explained at the genetic level
- Blood group genes provide insights into gene processing and rearrangement
- Molecular analysis of blood group genes is clinically useful and has potential in the Blood Center
- Understanding of molecular basis permits exploration of protein function

Some Reviews from the BloodGen Group 1997-2004

1. Storry JR, Olsson ML. Genetic basis of blood group diversity. Brit J Haem, 2004;759-71
2. Daniels G. Molecular blood grouping.Vox Sang. 2004;87 Suppl1:63-6.
3. Storry JR. Molecular basis of erythrocyte blood group antigens and applications in transfusion medicine.Vox Sang. 2002;83 Suppl 1:81-4.
4. Northoff, Flegel WA. Genotyping and phenotyping: the two sides of the coin. Infusionsther Transfusionsmed 1999;26:5
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   Vox Sang 1998;74:275-84
6. Avent ND. Human erythrocyte antigen expression: its molecular bases. Brit J Biomed Sci 1997;54:16-37