HLA typing, FA diagnosis and Gel Electrophoresis

Acknowledgements
The activity itself is based on information found in
V. Verlinsky et al, 2001, Preimplantation Diagnosis for Fanconi Anemia Combined
with HLA Matching, JAMA: 285, 3130-3133. and
S. Grewal et al, 2004, Successful hematopoietic stem cell transplantation for Fanconi
anemia from an unaffected HLA-genotype-identical sibling selected using
preimplantation genetic diagnosis, Blood: 3, 1147-1151
Note: the Nash family is not identified in the above papers, so although we discussing
the case of the Nash family in today’s workshop, the information may not be that of
the Nash family.

Introduction
In this activity you will use a simulated form of gel electrophoresis to analyze the HLA
type and FANCC genes of one cell from several embryos that were created in vitro.
This activity is based on a real medical case, real genotypes, and real embryos.

Background
A family wanted to have a second child who would not have FA, and who would be
an HLA match for the first child. This would allow them to use the cord blood
following birth as a source of cells for a bone marrow transplant for their first child.

In vitro fertilization was performed that resulted in 14 embryos to be screened. At the
8-cell stage, a single cell was removed from each of the embryos. Then, two PCR
reactions were performed on each sample, one to examine the alleles present at the
FANCC locus, and the other to determine the HLA type of the embryo. For 3 embryos,
no PCR product was obtained for one of the two reactions (doctors were unable to
determine either FA status or HLA type on the embryo because the reaction didn’t
work). You will look at the results from the remaining 11 embryos.
The genotypes of members of the family are shown below.

**FANCC PCR**

The FANCC mutation carried by both parents in this case is the same; ivs4+4. To test for ivs4+4, a PCR reaction was performed to amplify a portion of exon and intron 4 from the FANCC gene. The primers were designed such that a Scal site would be generated in the product from the normal allele but not the ivs4+4 allele. Following PCR amplification, the PCR product was digested with Scal and run on a gel.
The results of this analysis on the mother (M), father (P) and existing child (S) are shown below.

HLA type PCR
PCR primers were designed that would amplify only the specific HLA alleles desired. 4 sets of primers were used; one each for HLA-B44, HLA-B35, HLA-A2, HLA-A26. If the embryo had this HLA type, a product would be produced. If the embryo did not have this HLA type, no product would be observed.

The results of this analysis on the mother (M), father (P) and existing child (S) are shown below.

Note that no PCR was performed on the HLA-DR locus. Because the doctors had only one cell from each embryo to work with as a source of DNA for PCR they were not able to run an additional HLA-DR test on that one cell. Instead, they counted on the very low likelihood of cross over between the HLA-A and HLA-DR. Once the embryo was implanted and growing they did chorionic villus sampling (a sample was taken from the placenta at 10-13 weeks of pregnancy), and confirmed the HLA-DR type at this time (as well as repeating the earlier PCR reactions).
Procedure
You are given samples of DNA representing several individuals:
1) The mother (labeled M)
2) The father (labeled D)
3) The affected child (labeled AC)
4) One cell from 2 or 3 embryos per gel.
   As a class you will run all of the embryos to be tested (numbered 1 – 11)

You performed a PCR analysis on these samples from two loci
The FANCC locus (labeled FA)
The HLA locus (labeled HLA)

♦ Set up two gels and gel boxes according to the instructions for Gel Electrophoresis.
♦ Load the gels with placing all the FA samples on one gel and all the HLA samples on the other gel.
♦ Label your gels so that you know which sample is in each well.
♦ Run the gels at 100 volts for approximately 20 minutes.

Sketch the results from both gels below.

FA Results

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HLA results

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Questions
Will any of the embryos you tested develop FA? If yes, which one(s)?

Will any of the embryos carry FA? If yes, which one(s)?

Are any of the embryos an HLA match for the affected child? If yes, which one(s)?

Are any of the embryos both FA-free and an HLA match for the affected child? If yes, which one(s)?

Why are there three bands on the HLA type of the mother compared to only two on the HLA type of the father?

Be prepared to share your results with the class
Note: Embryo 2 on these gels (numbered embryo 3 in the paper and preceding power point presentation) was chosen by doctors as FA-free and an HLA match. This embryo was implanted and resulted in a healthy baby (now 6 years old). The cord blood was saved and used to successfully transplant the affected child, who is now 11.