G. GEL ELECTROPHORESIS

PCR and restriction enzyme digestion products are visualized by agarose gel electrophoresis and ethidium bromide fluorescence. Two types of agarose are used to prepare electrophoresis gels:

- agarose (Fisher Scientific, cat# BP1356)
- MetaPhor agarose (Fisher Scientific, cat# 50180)

Most gels are prepared with regular agarose, metaphor agarose is only used for gels with an agarose content of 2.5% or higher.

The content of agarose in the electrophoresis gel depends on **the size of PCR products** subjected to electrophoresis:

Size of PCR products	Agarose content
200 bp or larger	**1% gel**
between 150 bp and 200 bp	**1.5% gel**
smaller than 150 bp	**2% or 2.5% gel**

The content of agarose in the electrophoresis gel also depends on **size differences among restriction enzyme digestion products**:

Size differences of digestion products	Agarose content
150 bp or larger	**2% gel**
between 50 bp and 150 bp	**2.5% gel**
50 bp or smaller	**3% gel**

Note that 2.5% or 3% gels are prepared with both agarose and MetaPhor agarose combined in equal proportions. Gels whose agarose content is lower than 2.5% are solely made with agarose.

Zebrafish International Resource Center (ZIRC)
5274 University of Oregon
Eugene, OR 97403-5274, USA
Phone: 541-346-6028
Email: genotyping@zebrafish.org
Web: http://zebrafish.org

To prepare gels, agarose is dissolved in the 0.5 X solution of Tris borate with EDTA (TBE). The 0.5 X TBE working solution contains 44.5 mM Tris base, 44.5 mM boric acid and 1 mM EDTA. Typically, TBE is prepared as a 10 X stock solution that is subsequently diluted with water to obtain a 0.5 X working solution. To dissolve agarose in 0.5 X TBE, the solution is heated up and boiled for several minutes. After agarose has dissolved, the entire solution is cooled down and ethidium bromide is added. The final concentration of ethidium bromide in the gel is 0.5 μg/ml.

At ZIRC, gel electrophoresis is performed in a horiziontal electrophoresis system supplied by Fisher Scientific (cat# FB-SB-2025). The gels are typically run at the constant voltage of 100V. This voltage is decreased to 75V or more for gels with higher agarose content (i.e. 2%, 2.5% and 3% gels).

Zebrafish International Resource Center (ZIRC)
5274 University of Oregon
Eugene, OR 97403-5274, USA
Phone: 541-346-6028
Email: genotyping@zebrafish.org
Web: http://zebrafish.org