

Making 1KB Ladder Working Solution (Note) Invitrogen 1KB DNA Ladder (1 $\mu\text{g}/\mu\text{l}$) is different than the 1KB “plus” ladder. We are using the older 1KB ladder in this lab.

The 1 Kb DNA Ladder is suitable for sizing linear double-stranded DNA fragments from 500 bp to 12 kb. The bands of the ladder each contain from 1 to 12 repeats of a 1018-bp DNA fragment. In addition to these 12 bands, the ladder contains vector DNA fragments that range from 75 to 1636 bp.

The DNA ladder is stored as a “1X Working ladder” solution is kept in the -20°C fridge. The ladder comes as a concentrated (1 $\mu\text{g}/\mu\text{l}$) without loading dye.

To make a working solution of 0.01 $\mu\text{g}/\mu\text{l}$, mix 5 μl of 1 $\mu\text{g}/\mu\text{l}$ ladder stock + 360 μl of 10mM Tris-Cl pH 8.5 (you can use leftover EB buffer from the Qiagen kits!) + 100 μl of 5x loading dye in a microfuge tube. Make sure the tube is labeled (1X 1Kb ladder), dated, and initialed, and put back in the “Gels” box.

When running a gel, if you use 10 μl of ladder that will be 0.1 $\mu\text{g}/\text{lane}$. Use this along with the ladder datasheet to estimate the quantity of your DNA relative to the ladder (for example, the bright 3kb band contains $\sim 200\text{ng}$ of DNA if you load 0.1 μg per lane of the ladder).

Comparison between 1 Kb Plus DNA Ladder and 1 Kb DNA Ladder. 0.9% agarose gel stained with ethidium bromide

