Prepare λ_HindIII Marker that include also 10X Loading Buffer

λ_HindIII Marker is provided at a concentration of 500 ng/µl, and we need a final concentration of stock of 100 ng/µl to upload 10 µl sample volume in a DNA Gel.

100 ng/µl * 10 µl = 1000 ng = 1 µg

Comparison of the intensity of the λ_HindIII Marker bands and plasmid band is made by Loading 1 µg of λ_HindIII Marker.

For instance, WT-RL3-D8 plasmid is about 4.5 kb in molecular weight, so that co-migrates with a 4.3 kb band from λ_HindIII Marker. However, NEVER do compare the intensity of your plasmid with the intensity of this band of 4.3 kb. The band comes from the cleavage of Lambda vector!!!!

The right column indicates the amount of ng/band. If the amount is about 150 ng/band, and you loaded up 5 µl plasmid per lane, that means that you have a concentration of 30 ng/µl in tube.

If the plasmid is 4.5 kb, that means 4.5*1000*660 (dsDNA) = about 2.97*10^6 Da per plasmid.
30 ng/µl is equivalent to 10 fM/µl of a 4.5 kb plasmid

Preparation of stock solution of 100 ng/µl Lambda_HindIII Marker in a volume of 200 µl:

40 µl λ_HindIII Marker (stock provided by the NEB is 500 ng/µl)
20 µl 10X DNA Loading Buffer
160 µl TE7.5 or TE8 Buffer or other buffer of minimal ionic strength

<u>For Materials/DNA Gels:</u>

Lambda DNA - Hind III Digest, New England BioLab, Cat#N3012S

Photo film: Polaroid 667