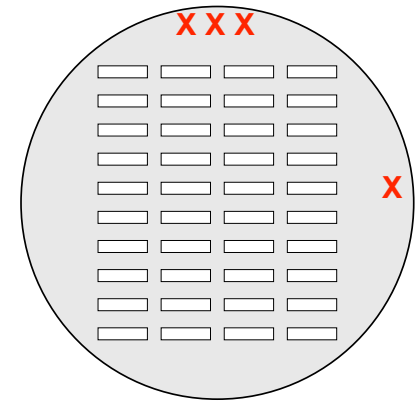


COLONY LIFT

COLONY LIFT

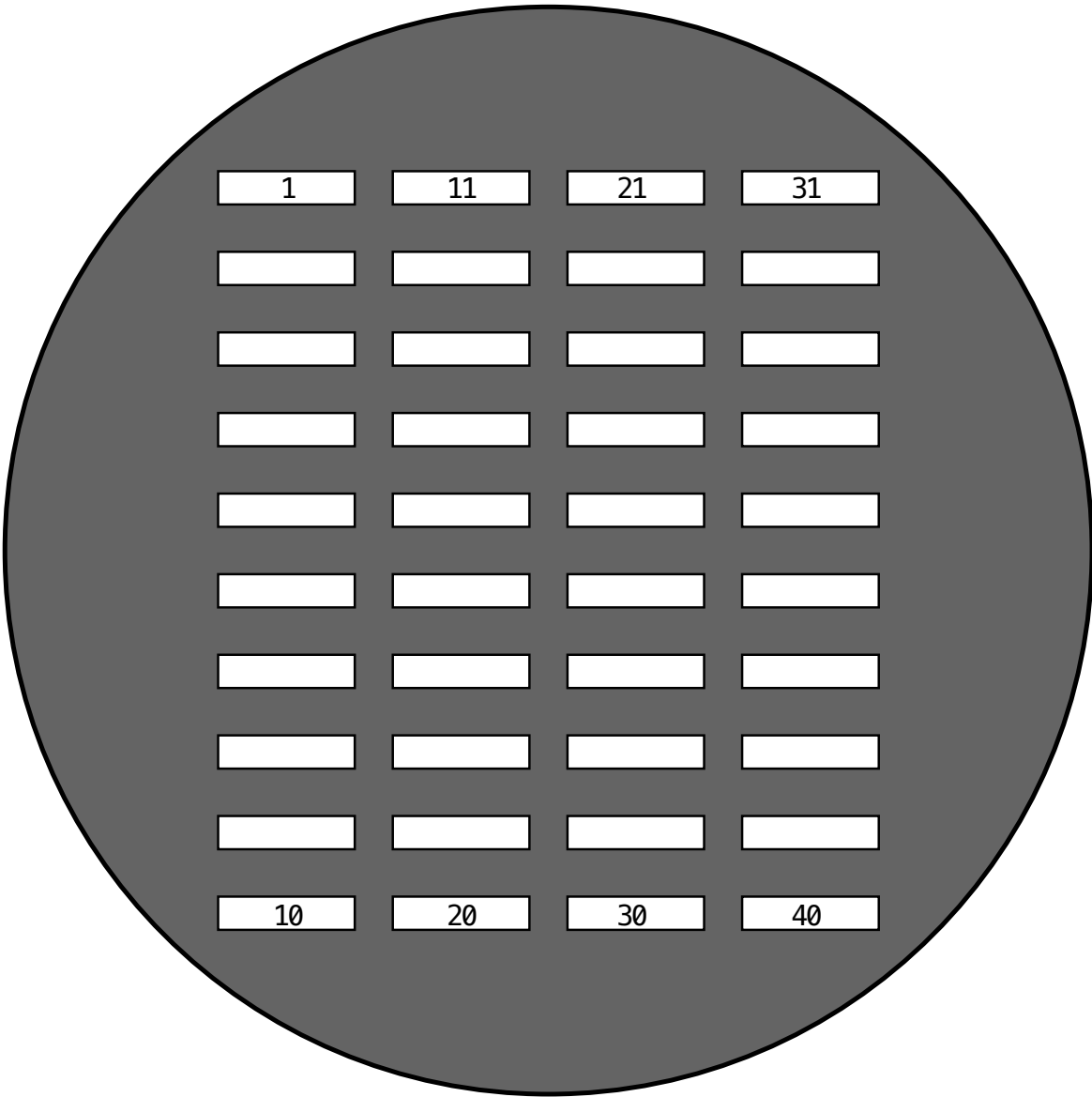


- ▶ Patch out colonies in 4x10 grid (**4x5 grid patches for *H. med***)
- ▶ Use plates that are not wet, and with discrete colonies or patches
 - ▶ **For *H. med*, incubate patched colonies for only 24 hours.**
- ▶ Bend the 82 mm filter and roll onto surface of plate from centre outwards
 - ▶ Mark position asymmetrically on filter and plate using needle, as above
 - ▶ Alternatively, write on filter with ballpoint pen beforehand
- ▶ Leave filter on **plate** for **5 minutes** until wet (filter turns dark)
 - ▶ Handle filters with forceps once wet
 - ▶ **Two forceps may be necessary with *H. med***
- ▶ Place colony side up on blotting paper soaked in **10% SDS**
 - ▶ Paper should be as damp as possible, but not so wet that liquid pools
 - ▶ Leave for **>20 minutes** until colonies are slimy (**less time for *H. med***)

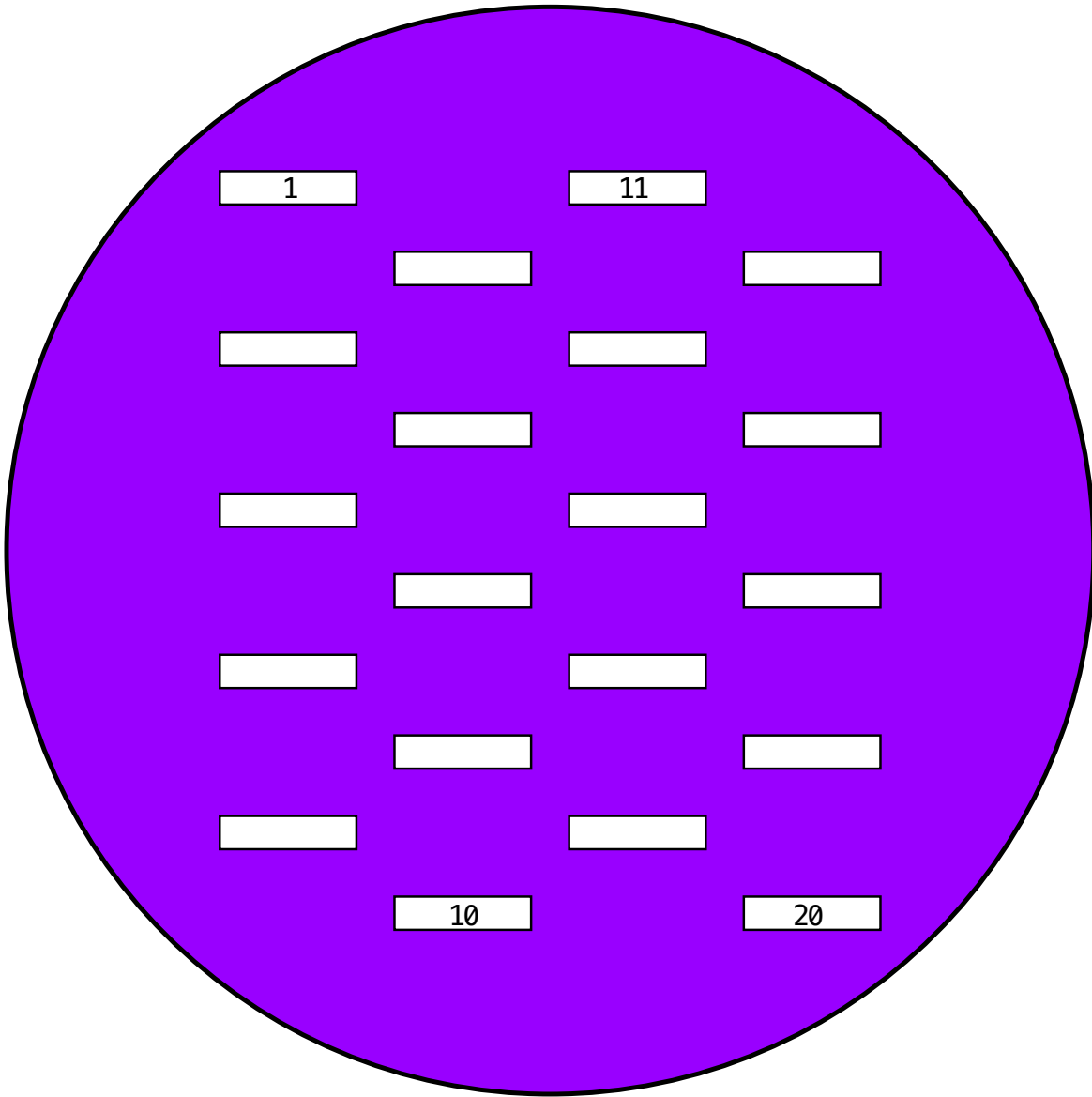
COLONY LIFT

- ▶ Transfer colony side up to paper soaked in [denaturing solution](#) (1.5 M NaCl, 0.5 M NaOH)
 - ▶ Leave for **>20 minutes** until colonies are blurry with clear edges
- ▶ Transfer to paper soaked in [neutralising solution](#) (1.5 M NaCl, 0.5 M Tris.HCl, 1 mM EDTA)
 - ▶ Leave for **>5 minutes**
- ▶ **Repeat** with fresh paper and [neutralising solution](#), again for **>5 minutes**
- ▶ Wash filter in about ~100 ml [2X SSPE](#) for **5 seconds** (submerge, do not agitate)
- ▶ Transfer to dry paper, repeat, and allow to **air dry completely** until patches are flat
 - ▶ If pressed for time, put in plate drying oven for 5 minutes
- ▶ [UV-crosslink](#) DNA at "Auto setting" (reading of 1200 = $1200 \text{ J/m}^2 = 120 \text{ mJ/cm}^2$)
 - ▶ Prehyb and hyb as usual

COLONY LIFT



H. vol 4x10 grid



H. med 4x5 grid

DENATURING SOLUTION

- ▶ 87.66 g NaCl
- ▶ 20 g NaOH
- ▶ make up to 1 L with dH₂O

NEUTRALISING SOLUTION

- ▶ 87.66 g NaCl
- ▶ 500 ml 1M Tris.HCl pH7.5
- ▶ 2 ml 0.5M EDTA pH8.0
- ▶ make up to 1 L with dH₂O

20X SSPE

- ▶ 175.3g NaCl
- ▶ 27.6g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$
- ▶ 7.4g EDTA (solid) or 40ml 0.5M EDTA (liquid)
- ▶ 800ml dH_2O
- ▶ Adjust pH to 7.4 with NaOH
- ▶ make up to 1L with dH_2O
- ▶ Autoclave

