

Strip & Reprobe protocol (PVDF membranes)

1. Reactivate PVDF in MeOH
2. Stripping buffer: 62.5 mM Tris pH 6.8, 2% SDS, 100 mM BME

$$\begin{array}{ll} 20\% \text{ SDS (x)} = 75 \text{ mL (2\%)} & \rightarrow 7.5 \text{ mL} \\ 2\text{M Tris (x)} = 75 \text{ mL (62.5 mM)} & \rightarrow 2.343 \text{ mL} \\ 380 \text{ ul BME/ 50mL} & \rightarrow 570 \text{ ul} \\ & \underline{64.587 \text{ mL H}_2\text{O}} \\ & 75 \text{ mL} \end{array}$$

3. Incubate 60C x 20min
4. Wash 2 x 10 min in PBST.
5. Rinse in PBS
6. Block in 5% milk/PBS, begin Western blotting protocol from the blocking step.