Tips for Western blot (Paul 2017):

1. In general, I like to load 12-20 l samples per well. Loading bias will be more pronounced if you load <10 l.
2. I personally prefer thick comb and small well.
3. Try to keep every sample with the same amount of glycerol, which influences the width of the band. When you need to re-run the samples based on the previous endogenous controls (which happens in most cases), adjust the samples with the same dilution of sample buffers. For examples, if the samples are in the Laemmli sample buffer with the final 2X concentration, dilute them with 2X sample buffer.
4. Clean up the gel residues when you remove the comb.
5. Rinse the wells with SDS running buffer with syringe & needle before loading the samples.
6. Use Gilson-type 20 l pipettor for sample loading.
7. Check the volume mark on the pipettor after you put on the tip (because the mark may move when punch the tip box).
8. Because the lysate is viscous, while the tip is still in the lysate, wait an extra, consistent time (for example, 2 sec) for each sample after you releasing the vacuum.
9. To load the sample, try to put the tip directly to the near-bottom of the well and start to load as soon as possible. Searching for the well will make you losing the samples (because the lysate has higher density).
10. Load the sample in the moderate speed.
11. After expelling the lysate, be slow when you pull the tip out of the well. Avoid the formation of the water channel of turbulence.
12. After transferring, check the whole amount of protein loading with Ponceau S (0.1% Ponceau S in 5% acetic acid) before blocking. It is simple, fast, and will not influence the result. It can also be used to judge if the endogenous control is appropriate for the experiment. Ponceau S, once prepared in the brown bottle, can be reused for multiple times.