Some Reflection on Gel NMR Sample Preparation

The information presented below is intended to be used as a guide in developing your procedure and not as a set of specific instructions. A technique needs to be developed by the user that works best, and the first attempt will be a learning experience.

What stretch ratio can give the optimal size of RDC?
Version 6 to 4.2mm: works well with proteins like protein G.
Version 5.4 to 4.2mm: generally good for strong aligning proteins.
Version 5 to 4.2mm: generally good for proteins that are both large and elongated.
Experimentation with different stretch ratios may be necessary to determine which one is best for the samples at hand.

Allow 2-3 days for complete sample preparation.

Some gel solutions may be basic and/or contain unreacted components which could affect the stability of the protein if it were added to the initial solution.
Approximately 250µl of gel solution would make a sample column height of about 20mm.

Assuming protein has not been added, seal the bottom of the Gel Chamber (parafilm) and fill with gel solution. Allow to polymerize for at least 1-2 hours. The seal (parafilm) can then be taken off the chamber.

Carefully remove the gel with the use of the Piston and an appropriate tool for pushing it through the chamber. Be careful not to scratch the inner surface of the chamber.

Dialyze the gel against deionized water overnight to remove unreacted chemicals.

While the gel is still soft, it may be cut to some appropriate length. Allow for the length of the Piston and for some air space between it and the surface of the gel.
As a guide line, the gel length should be about ⅓ the length of the Gel Chamber allowing room for an air space and the Piston. The air space will allow more of the gel to be expelled.

Dry overnight at room temperature or for some hours at an elevated temperature (40-45^{o}C). The gel will become somewhat rigid and smaller in diameter.

Place the “dried” gel back into the Gel Chamber, seal the bottom (parafilm) and add protein until the gel swells to the full diameter of the chamber. Let it sit for 24 hours or longer to allow the protein to diffuse in.

Remove any excess protein that has not been diffused into the gel.

Assemble the Gel Press and load the gel into the sample tube.
The Piston will only go as far as the top of the actual funnel area which may still contain some gel. With the Gel Chamber removed from the Funnel, the action of an auto-pipet may be used to apply enough air pressure to expel the remaining gel.

Seal the bottom of the sample tube by gently pressing the Gel End Plug in to place, in such a manner as to avoid trapped air bubbles (the Extraction Rod is used only to remove the Gel End Plug from the sample tube).

Insert the Gel Top Plug on top of the gel column with the Support Rod.

Ideally, the sample column should be centered in the receiver coil area. It may take a few trials to find the optimum sample position for this application in the probe.

Some references:
Bax, A. Tjandra, N. (1997) J. Biomol. NMR, **10**, 289-292
Chou, J.J., Li, S., Klee, C.B. and Bax, A. (2001) Nat. Struct. Biol., **8**, 990-997
Chou, J.J., Gaemers, S., Howder, B., Louis, J.M. and Bax, A. (2001) J. Biomol. NMR, **21**, 377-382
Tycko, R., Blanco, F.J. and Ishii, Y. (2000) J. Am. Chem. Soc., **122**, 9340-9341