4.3 Protocol for wheat germ cell-free translation

Essentially the same set up as for RRL described earlier. The components are:

1. Transcript.
2. Master mix consisting of buffer, energy mix, nuclease treated wheat germ extract, creatine kinase, RNase inhibitor, and tRNA.

The key difference between the RRL and WG master mixes is, of course, WG rather than RRL extract. Since RRL extract is desalted into H2O or simple Tris buffers, you do not have to worry about ion compensation as you vary the amount of extract per translation. That is, you can cut down the RRL from 47% of translation volume typically, to lower levels with an essentially linear response of translation. In WG however, since centrifuge desalting was over G-25 equilibrated in buffer containing 5 mM Mg, you carry different amounts of Mg into the final translation reaction as you vary the amount of WG extract. Since the optimum amount of wheat germ extract to use varies from prep to prep you need to determine the optimum amount of WG extract for translation with each WG extract prep (e.g. 10, 15, 20, 25 or 30% of total translation volume, typically). The catch is that for each different % of WG extract you have to use a different CB 10 X, since the ions carried with the WG will vary with the fraction of translation volume made up by WG extract. Moreover, a different set of compensation buffers need to be made for linked and unlinked translations (i.e. for transcription-linked vs native mRNA or purified transcript translations). Otherwise WG and RRL translations are set up essentially the same.

WG master mix for 10 uL reaction:

CB 10X 20% WG	1 uL
E mix	2
WG	2
tRNA	0.1
CK	0.1
RNasin	0.1
Water	3.2
Total	8.5 uL

Incubate 24øC for 1 h, transfer onto ice to stop reaction.