4/01/07
In vitro transcription translation

I. Make transcription/translation mix
   a. Add to 0.5ml tubes (**Tubes HpaII A and HhaI A**) in the order listed
      X H_2O
      2µl 5mM Methionine
      200ng DNA template (T7 HpaII cassette bio or T7 HhaI cassette bio)
      35 µL EcoPro T7 extract
      1.25µL 32mM SAM
      Total of 50 µl reaction
   b. Mix gently with pipette tip and incubate at 37°C for 60 min

Note: Make sure you keep the EcoPro T7 extract on ice until use.

II. Prepare 2 sets of beads to capture DNA from in vitro transcription/translation mix
   a. In 0.5ml tube (**Tube HpaII B and HhaI B**), incubate 0.5µg junk dna (T7 purN cassette) in 50µL of 1X B&Wbuffer for 30 minutes
   b. In **Tubes HpaII C and HhaI C** add 10µl Dynabeads and wash 3x with 20µL of 2X B&W buffer
   c. Resuspend in 50µL 2X B&W buffer
   d. Wash Tubes B 3X with 1X B&W buffer after incubation
   e. Add 50 µL of beads from Tubes C to Tubes B

III. Capture and purify DNA using beads
   a. Add EcoPro T7 mixes from Tubes A to beads in Tubes B
   b. Incubate for 40 min at room temperature
   c. Wash Tubes B 3X with 1X B&W buffer and 3X with 100 µL Digestion Buffer
      i. Digestion buffer is 1X NEB buffer 1 for digestion with HpaII
   d. Resuspend beads in 200µL digestion buffer

IV. Digest cassettes with HpaII and PCR
   a. Add 5µL HpaII, mix and digest for 3 hours at 37°C
   b. Remove digestion supernatant and save
   c. Wash beads 3X with 200 µL 1X B&W buffer and 3X with 50 µL PCR wash (Page 1)
   d. PCR beads, plus 10µl of the digestion supernatant and PCR stock (Page 1)
   e. Also PCR controls as before (Page 2)
   f. Check PCR on 1% agarose gel