On Bead Digestion

Start with equal amounts of beads and material input

1) Do 4 buffer exchanges (enough to cover the beads plus about 100ul) with 50mM TEAB or ammonium bicarbonate. Shake for 20 minutes at 4C between each exchange.
2) At the 4th exchange add trypsin to the ammonium bicarbonate buffer (promega) at roughly a 1-100 ratio (for on bead digestions that is a sheer guess) and gently shake at room temperature overnight. Volume for this should be about 25ul over the beads (less than the first three exchanges where you were trying to wash out things that would interact with trypsin or MS).
3) The next morning remove and save the supernatant
4) Wash the beads with an additional 50 mM ammonium bicarbonate buffer just to cover.
5) Shake for 20 minutes.
6) Combine the supernatant from step 3 with the wash from step 4
    a. At this step freeze and send to UCDProteomics overnight. Blue Ice is fine, it doesn't have to be dry ice
7) Depending on experiment we will either load equal volumes or equal amounts of sample. To quantify we use Pierce Flourmetric peptide assay with three replicates / sample or standard.

*If you plan on doing TMT labeling you MUST use TEAB instead of Ammonium bicarbonate.

TEAB= Triethylamonium bicarbonate

Trypsin = https://www.promega.com/products/mass-spectrometry/trypsin/sequencing-grade-modified-trypsin/?tabset0=0

Other Things needed

- Something to shake your Eppendorf tubes like the Eppendorf thermomixer, but anything that shakes gently or rotates will do
- LC-MS/MS grade water to make buffers and ammonium bicarbonate needs to be made fresh for every experiment