1. Prepare a solution of the target (collagen or fibronectin) as indicated in the tables bellow:

	Collagen:
1	9.5 µl in 65.5 µl AcACH 0.2M + 1.5 ml $NaHCO_3$
2	9.5 µl in AcACH + 1.5 ml 35mM $NaHCO_3$/Na_2CO_3 15mM

	Fibronectin:
1	9.5 µl + 1.5 ml $NaHCO_3$
2	9.5 + 1.5 ml 35mM $NaHCO_3$/Na_2CO_3 15mM

2 Add 1.5 ml of this solution to each well of a six well plate and swirl repeatedly until the surface is completely wet.

3. To coat plates with target, incubate overnight at 4°C with gentle agitation in a humidified container (a sealable plastic box lined with damp paper towels). Store plates at 4°C in humidified container until needed.

Next day:

4. Inoculate 20 ml of LB medium in a 250-ml Erlenmeyer flask **(do not use a 50-ml conical tube)** with ER2738. Incubate at 37°C with vigorous shaking; carefully, monitor the 20-ml culture so that it does not grow beyond early-log phase (OD600 0.01–0.05).

5. Pour off the coating solution from each plate and firmly slap it face down onto a clean paper towel to remove residual solution. Fill each plate or well completely with blocking buffer.Incubate for at least 1 hour at 4°C.

6. Discard the blocking solution as in step 5. Wash each plate rapidly 6 times with TBST (TBS + 0.1% [v/v] Tween-20). Coat the bottom and sides of the plate or well by swirling, pour off the solution, and slap the plate face down on a clean paper towel each time. Work quickly to avoid drying out the plates.

7. Dilute a 100-fold representation of the library stock with 1 ml of TBST. Pipette onto coated plate and rock gently for 60 minutes at room temperature.

8. Discard nonbinding phage by pouring off and slapping plate face-down onto a clean paper towel.

9. Wash plates 10 times with TBST as in step 6. Use a clean section of paper towel each time to prevent cross-contamination.

10. Elute bound phage with 1 ml of a general buffer for nonspecific disruption of binding interactions: 0.2 M Glycine-HCl (pH 2.2), 1 mg/ml BSA. Rock gently for 20 minutes, pipette eluate into a microcentrifuge tube, and neutralize with 150 µl of 1 M Tris-HCl, pH 9.1.

11. Titer a small amount (~1 µl) of the eluate as described in General M13 Methods (page 8).

Next day

12. Amplify the rest of the eluate by adding the eluate to the 20-ml ER2738 culture from Step 4 (should be early-log at this point) and incubating with vigorous shaking for 4.5 hours at 37°C.

Note: The remaining eluate can be stored overnight at 4°C at this point, if preferred, and amplified the next day. In this case, inoculate 10 ml of LB+Tet with ER2738 and incubate with shaking overnight at 37°C. The next day, dilute the overnight culture 1:100 in 20 ml of LB in a

*250-ml Erlenmeyer flask **(do not use a 50 ml conical tube)** and add the unamplified eluate. Incubate with vigorous shaking for 4.5 hours at 37°C and proceed to Step 13.*

The eluate was stored at 4 C overnight and amplified on the next day as suggested on the note.

13. Transfer the culture to a centrifuge tube and spin for 10 minutes at 12,000 g at 4°C. Transfer the supernatant to a fresh tube and re-spin (discard the pellet).

14. Transfer the upper 80% of the supernatant to a fresh tube and add to it 1/6 volume of 20% PEG/2.5 M NaCl. Allow the phage to precipitate at 4°C overnight.

Next day

15. Spin the PEG precipitation at 12,000 g for 15 minutes at 4°C. Decant and discard the supernatant, re-spin the tube briefly, and remove residual supernatant with a pipette. The phage pellet should be a white finger print sized smear on the side of the tube.

16. Suspend the pellet in 1 ml of TBS.Transfer the suspension to a microcentrifuge tube and spin at maximum (14,000 rpm) for 5 minutes at 4°C to pellet residual cells.

17. Transfer the supernatant to a fresh microcentrifuge tube and reprecipitate by adding 1/6 volume of 20% PEG/2.5 M NaCl. Incubate on ice for 60 minutes. Microcentrifuge at 14,000 rpm for 10 minutes at 4°C, discard the supernatant, re-spin briefly, and remove residual supernatant with a micropipet.

18. Suspend the pellet in 200 µl of TBS. Microcentrifuge for 1 minute to pellet any remaining insoluble material. Transfer the supernatant to a fresh tube .This is the amplified eluate.

19. Titer the amplified eluate as described in General M13 Methods (page 8) on LB/IPTG/Xgal plates. The eluate can be stored for up to 3 weeks at 4°C. For long-term storage, add an equal volume of sterile glycerol and store at –20° C.

20. Coat a plate or well for the second round of panning as in Steps 1–3 above.