The Plan

Easy part:

1. Trip to Plzeň, we must obtain the pYD plasmid and EBY100 cells.
2. We must also order g-blocks of streptavidin, anti-EpCAMand anti-cMycantibodies(everything with built in restriction sites, RE both for pYD1 plasmid integration and BioBrick assembly).

pYD plasmid:

1. Preparation of pYD plasmid solution.
2. Transformation of DH5alpha cells with electroporation.
3. Selection of transformants on Ampplates.
4. Miniprep.
5. Restriction test of the aquired plasmid for confirmation.
6. Gel with the result of restriction test and uncut plasmid.
7. Gel purification of pYD plasmid.

EBY100 cells:

1. Smear the EBY100 cells on minimal medium containing Leu, Trp.
2. Stamp test on minimal medium w/o AA.
3. Selection of EBY100 cells and preparation of glycerol stocks.
4. Grow EBY100 cells for electroporation.

G-Blocks:

1. PCR of g-blocks? (depends on conc. of the solution we get)
2. Gel purification of the PCR product.
3. Gel for confirmation.

Final construct:

1. Restriction of pYD and G-blocks(BamHI and NheI RE).
2. Ligation.
3. Gel for confirmation of succesful incorporation of g-block.
4. Gel purification of the three plasmids.
5. Transformation of DH5alpha cells and selection on Amp.
6. Miniprep.
7. Gel purification of plasmids.

Transformation of the EBY100 cells:

1. Electroporation of EBY100 with final constructs.
2. Selection on minimal medium containing only Leu.

Test for correct protein expression and MF experiments:

1. We must get fluorescent antibodies against the displayed proteins. There will be a HA-tag on every one of them. So FITC-coupled HA-antibodies should solve the problem.
2. Experiments on microfluidics chip – Pavel Fikar.