**Appendix I**

DNA transfection:

* + - 1. Procedure for transfection of a single glass-bottom dish:

DNA dilution:

Purified DNA (1 μg/μl) encoding for E-cadherin GFP 1 μg

P3000 reagent (this is provided together with Lipofectamine 3000) 3 μl

Opti-MEM 150 μl

Final volume 150 μl

Lipofectamine 3000 dilution:

Lipofectamine 3000 3 μl

Opti-MEM 150 μl

Final volume 150 μl

1. After dilutions are prepared, these are incubated for 5 min at room temperature and then mixed together and incubated for further 25 min under the same conditions.
2. Once the previous 25 min are finished, add the mixture drop-wise to the dishes containing the cells and incubate for 5 h in a cell incubator.
3. After the 5 h of incubation, change the media of the cells with 1.5 ml of DMEM supplemented with FBS and antibiotics.
   * + 1. The amount of DNA and Lipofectamine described in this protocol has been tested in MCF-7 and Caco-2 epithelial cell lines. However, these should be optimized for other cell line and/or plasmid (if different from the ones described in this protocol). In our experience 2 to 5 μl of lipofectamine 3000 and 0.5 to 2 μg of DNA would give a good range to optimization of transfection conditions.
       2. Although here we described the use of cells overexpressing GFP-tagged constructs, this might not be the best ideal scenario. Overexpression of junctional proteins like MRLC might have associated overexpression artefacts if expression levels are not kept at a level that does not cause a change in the physiology or morphology of cells. Approaches to circumvent any overexpression artefact are to express GFP-tagged constructs in cells that does not express the corresponding endogenous protein for example by RNAi using dual promoter lentiviral vectors (See Bio-protocol e937) or by genetically tagging the endogenous gene using genome-editing tools like CRISPR. We believe this last approach is the best possible one at the moment but we understand this could not be achievable in every laboratory.