Evidence for CRISPR/Cas9 In-Vivo-Geneediting by PCR

2 DNA Extraction out of Bacteria

2.1 Label 3 screw cap tubes with S (for starter plate), C, D und your group number.

2.2 **Homogenize** InstaGene-Matrix by snipping and pipet 250 µl in each of the 3 labeled tubes.

Pay attention all 3 tubes should contain a similar amount of InstaGene-perls.

2.3 Pick with an inoculation loop a blue colony from the KIX/ARA-starter plate.
Swirl the loop in screw cap tube S and mix it for 10 seconds by snipping.

2.4 Pick with a sterile loop a blue colony from Plate C.
Swirl the loop in screw cap tube C and mix it for 10 seconds by snipping.

2.5 Pick with a sterile loop a white colony from plate D.
Swirl the loop in screw cap tube D and mix it for 10 seconds by snipping.

2.6 Incubate 3 screw cap tubes for 15 minutes at 56 °C.
Let tubes cool down for 2 minutes and mix it for 10 seconds by snipping.

2.7 Incubate 3 screw cap tubes for 8 minutes at 95 °C.
Let tubes cool down for 2 minutes and mix it for 10 seconds by snipping.

2.8 Centrifuge 3 tubes for 2 minutes at 12000 rounds per minute.

3. Preparation of PCR Samples.

3.1 Label 5 PCR-Tubes with: S, C, D, (+), (-).

3.2 Pipet in each PCR tube 10 µl Master Mix containing fresh added primer (MMP).

3.3 Pipet 10 µl of supernatant out of screw cap tubes S, C, D in the corresponding PCR-tubes.

3.4 Pipet 10 µl of positive-control-DNA in (+)PCR tube.
Pipet 10 µl H_2O for negative-control in (-)PCR tube.

3.5 Mix 5 PCR-tubes by pipetting up and down and place them on ice until start of PCR.

4 Procedure of PCR

4.1 Place 5 PCR-tubes in the Cycler.

Pay attention that each tube is correct closed!

4.2 Conduct PCR by running the following temperature/time programm:

Step	Temp., °C	Time	Cycles
Initial denature	94	5 min	1x
Denature	94	30 sec	35x
Anneal	62	30 sec	
Extend	74	1 min	
Final extension	74	5 min	1x
Hold	12	–	1x

After PCR hold temperature of cycler at 12 °C till gele electrophoresis starts.

5 Gele-Electrophoresis

5.1 Prepare a 1 % agarose-gele in a total volume of 35 ml TAE-buffer (1x).

5.2 After cooling down of agarose solution to 45 °C (backhand test) add 3 µl GelRed. Mix by shaking and poor agarose solution into the gele-electrophoresis chamber.

5.3 After getting solid of the gele-electro-phoresis chamber is filled with 280 ml TAE-Puffer (1x) and pull the comb.

5.4 Add to each 5 PCR-samples 5 µl loading buffer and mix by pipetting up and down.

5.5 Pipet in the wells of the gele 10 µl like in the following table:

Spur	Probe
1	PCR Probe S
2	PCR Probe C
3	PCR Probe D
4	Positiv Kontrolle (+)
5	Negativ Kontrolle (-)
6	Marker (MWR)

5.6 Gele-elektrophoresis is done at 130 V (Duration approximately 15 minutes).

Task:
Evaluate band pattern in the gele and estimate *lac*Z gene status.