**Project 1: How to measure telomere size in 3D?- Does KD of shelterins affect telomere size/compaction?**

**1. What we have done so far (status: December 2014):**

Setup of the method:

* Comparison and compaction of telomere sizes with STORM of Hela S and L (not transfected):

3 slides à 15 pictures each; will be compared to the Hela S and Hela L transfected with pSuper (10 different experiments à 15 pics each)

🡪 telomere size determination with this method is reliable (giving always the same results)

* Telomere length determination of Hela S and L using Southern Blot:

Comparison of sizes, distribution and ratio (S/L) we got by STORM with the ones on Southern Blot

* Tested usage of activation laser 405nm to increase blinking efficiency:

🡪Using activation shows smaller telomere size

* Tested taking 30000 frames instead of 10000 frames in order to get max. of blinks:

🡪30000 frames gives bigger telomere size, but we see the same effect with TRF2 KD, i.e. smaller telomeres in 10000 and 30000 frames

* Comparison of determination of telomere size when doing FISH and TRF1 IF in STORM in Hela L:

TRF1 IF telomere sizes are smaller than FISH-stained telomeres even though the antibody also adds size!

🡪staining problem?

Does KD of shelterins affect telomere size/compaction by STORM?

* Telomere size determination by STORM analysis of KD of TRF1, TRF2, TRF1/2 in Hela S and L (5 days selection):

🡪in Hela S: TRF2 KD: smaller telomeres; TRF1 KD: bigger telomeres; TRF1/2 KD: bigger ones

🡪in Hela L: TRF2 KD: smaller; TRF1 KD: bigger telomeres; TRF1/2 KD: tiny bit bigger as control

* Telomere size analysis by Southern Blot of KD of TRF1, TRF2, TRF1/2 in Hela S and L (5 days selection):

🡪compare the results from Southern with the STORM results (no size differences seen on Southern blot with KD of shelterins!

**2. What we need to do:**

* **Kyle:**

1. compare telomere size, distribution and ratio of Southern blot data with STORM data of Hela S and L
2. determine and compare chromatin compaction (persistence length and packing ratio) for Hela S and L

🡪does telomere compaction change with size?

1. Compare the compaction/bending of telomeres with other chromatin compaction models

🡪on a scale from 0 (not compacted) to 1 (full compacted) where do the telomeres belong?

🡪how much compacted telomere stretch before it could bend?

1. Analyse data of TRF1, TRF2, TRF1/2 KD in Hela S and L (all 5 experiments)

🡪Does KD of shelterin components affect telomere size?

Yes!

🡪Does KD of shelterin components affect telomere compaction/bending?

1. Compare telomere length and distribution by Southern Blot and STORM in TRF1, TRF2, TRF1/2 KD in Hela S and L

🡪Trf1 and Trf2 KD do not change telomere size on Southern blot! (for STORM, Southern and ChIP!)

* **Us:**

1. Do heterochromatin ChIP (H3, H3K9me, me2, me3, IgG) in TRF1, TRF2, TRF1/2 KD in Hela S:

🡪TRF2 KD increases H3K9me3 in Hela S (not in Hela L) and shows smaller telomeres

🡪does TRF1 KD show less heterochromatin marks = bigger telomere size?

= is there a correlation between histone marks and physical size of the telomere?

1. Do heterochromatin ChIP (H3K9me3, H4Ac, HP1, gH2AX, IgG) in Hela S and L:

Are Hela S less compact because they have less H3K9me3 than Hela L?

**All STORM data given to Kyle:**

* **18\_06\_2014\_HelaS\_L\_KD\_SmchD1** *(External hard drive B)*

1 slide/condition, for each condition 15 pics

conditions: pSuper (mock control), pLVP041 (sh1-SmchD1), pLVP042 (sh2-SmchD1) in Hela S (short) and Hela L (long)

This is the same transfection and staining experiment as 19\_06\_2014,

* **19\_06\_2014\_HelaS\_L\_KD\_SmchD1** *(External hard drive B)*

1 slide/condition, for each condition 15 pics

conditions: pSuper (mock control), pLVP041 (sh1-SmchD1), pLVP042 (sh2-SmchD1) in Hela S (short) and Hela L (long)

* **30\_06\_2014\_HeLaS and L\_SMCHD1\_KD** *(External hard drive B)*

2 slide/condition (same transfection), for each condition 15 pics

conditions: pSuper (mock control), pLVP041 (sh1-SmchD1), pLVP042 (sh2-SmchD1) in Hela S (short) and Hela L (long)

* **11\_08\_2014\_HelaS\_L\_TRF2\_KD** *(External hard drive C)*

1 slide/condition, for each condition 15 pics

conditions: pSuper (mock control), pLVP041 (sh1-SmchD1), pLVP042 (sh2-SmchD1),TRF2-sh3 (TRF2 KD) in Hela S and Hela L.

🡪*filtered molecule lists need to be analyzed (until now only unfiltered analyzed!)*

* **20\_08\_2014\_HelaS\_L\_TRF2\_KD\_30000\_frames** (given on the 11.09)

1 slide/condition, for each condition 15 pics

conditions: pSuper (mock control), TRF2-sh3 (TRF2 KD) in Hela S and Hela L.

Here 30000 frames were taken instead of 10000 frames.

Mol lists are not filtered and filtered.

This is in part the same transfection experiment as: 11\_08\_2014\_HelaS\_L\_TRF2\_KD (here 10000 frames taken).

*🡪analyze filtered molecule lists*

* **13\_08\_2014\_HelaS\_L\_TRF2\_KD** *(External hard drive B)*

1 slide/condition, for each condition 15 pics

conditions: pSuper (mock control), pLVP041 (sh1-SmchD1), pLVP042 (sh2-SmchD1),TRF2-sh3 (TRF2 KD) in Hela S and Hela L.

🡪*filtered molecule lists need to be analyzed (until now only unfiltered analyzed!)*

* **18\_08\_2014\_HelaS\_L\_TRF2\_KD** *(External hard drive C)*

1 slide/condition, for each condition 15 pics

conditions: pSuper (mock control), TRF2-sh3 (TRF2 KD) in Hela S and Hela L.

Here I used the 405 nm laser as additional activation laser!!!!

Mol lists are not filtered and filtered.

This is in part the same transfection experiment as: 13\_08\_2014\_HelaS\_L\_TRF2\_KD (here no 405 laser was used in addition). The 18\_08\_2014 data needs to be compared to pSuper and TRF2-sh3 conditions of the 13\_08\_2014 data.

(question: does 405 nm activation laser change the results?)

*🡪analyze filtered molecule lists*

* **24\_08\_2014\_HeLaS\_SmchD1\_TRF2\_doubleKD\_FISH** (given on the 11.09)

1 slide/condition, for each condition 15 pics

conditions: pSuper (mock control), pLVP041 (sh1-SmchD1), pLVP042 (sh2-SmchD1), TRF2-sh3 (TRF2 KD), TRF2/041 (TRF2 and SmchD1 KD-sh1), TRF2/042 (TRF2 and SmchD1 KD-sh2) in Hela S

* **27\_08\_2014\_HelaS\_SmchD1\_Trf2\_doubleKD\_FISH** *(External hard drive B)*

1 slide/condition, for each condition 15 pics

conditions: pSuper (mock control), pLVP041 (sh1-SmchD1), pLVP042 (sh2-SmchD1), TRF2-sh3 (TRF2 KD), TRF2/041 (TRF2 and SmchD1 KD-sh1), TRF2/042 (TRF2 and SmchD1 KD-sh2) in Hela S

* **29\_08\_2014\_Hela\_L\_TRF1\_IF** *(External hard drive C)*

2 slides/ condition, 15 pictures/slide = 30 pictures for Hela L in TRF1 IF (immunofluorescence).

conditions: dilution of TRF1 antibody (488, LG) as 1/1000 and 1/2000; Mol lists are filtered.

This experiment needs to be compared to the FISH data of Hela L from experiment 12\_06\_2014\_HelaS\_L (question: are the telomere sizes and volume comparable between FISH and IF?)

* **08\_09\_2014\_HelaS\_L\_TRF1\_TRF2\_KD\_DAPI** *(External hard drive C)*

1 slide/condition, for each condition 8 pics

conditions: pSuper (mock control), TRF2-sh3 (TRF2 KD), TRF1-sh1 (TRF1 KD), TRF2/TRF1 (double KD TRF1/2) in Hela S and Hela L.

For this experiment 2 WF pictures were taken:

1. telomere Cy5
2. nucleus DAPI

to make sure that all the signals (telomeres) we get are in the nucleus.

Would need to be compared to the data from 09\_09\_2014 (no DAPI staining: questions: does DAPI staining influence the STORM acquisition?

* **09\_09\_2014\_HelaS\_L\_TRF1\_TRF2\_KD** (given next week)

1 slide/condition, for each condition 15 pics

conditions: pSuper (mock control), TRF2-sh3 (TRF2 KD), TRF1-sh1 (TRF1 KD), TRF2/TRF1 (double KD TRF1/2) in Hela S and Hela L.

Same transfection experiment as 08\_09\_2014, but not stained for DAPI.

* **21\_10\_2014\_HelaS\_L\_TRF1\_TRF2\_KD\_FISH** (disc C; given 27.10.14):

1 slide/condition, for each condition 15 pics

conditions: pSuper (mock control), TRF2-sh3 (TRF2 KD), TRF1-sh1 (TRF1 KD), TRF2/TRF1 (double KD TRF1/2) in Hela S and Hela L.

* **05\_11\_2014\_HelaS\_L\_TRF1\_TRF2\_KD\_FISH** (disc C; given 24.11.14): (hard drive C)

1 slide/condition, for each condition 15 pics

conditions: pSuper (mock control), TRF2-sh3 (TRF2 KD), TRF1-sh1 (TRF1 KD), TRF2/TRF1 (double KD TRF1/2) in Hela S and Hela L.

* **17\_11\_2014\_HelaS\_L\_TRF1\_TRF2\_KD\_FISH** (disc C; given 24.11.14): (hard drive C)

1 slide/condition, for each condition 15 pics

conditions: pSuper (mock control), TRF2-sh3 (TRF2 KD), TRF1-sh1 (TRF1 KD), TRF2/TRF1 (double KD TRF1/2) in Hela S and Hela L.

* **06\_11\_2014\_HelaS\_SmchD1\_Trf2\_doubleKD\_FISH** *(External hard drive C)*

1 slide/condition, for each condition 15 pics

conditions: pSuper (mock control), pLVP041 (sh1-SmchD1), pLVP042 (sh2-SmchD1), TRF2-sh3 (TRF2 KD), TRF2/041 (TRF2 and SmchD1 KD-sh1), TRF2/042 (TRF2 and SmchD1 KD-sh2) in Hela S

**Final results:**

* *For comparison of telomere FISH and TRF1 IF (immunofluorescence):*

Compare Hela L data from:

12\_06\_2014\_HelaS\_L\_FISH (compare only Hela L)

with

29\_08\_2014\_Hela\_L\_TRF1\_IF

* *For setting up the method:*

1. Does 405 nm activation laser change the results:

compare Hela S and L data from:

13\_08\_2014\_HelaS\_L\_TRF2\_KD (only pSuper and TRF2 KD)

18\_08\_2014\_HelaS\_L\_TRF2\_KD

2. Does acquisition of 30000 frames change the results:

compare Hela S and L data from:

11\_08\_2014\_HelaS\_L\_TRF2\_KD (only pSuper and TRF2 KD)

11\_08\_2014\_HelaS\_L\_TRF2\_KD\_30000\_frames

* *For SmchD1 KD affect in Hela S and L:*

pool data from:

18\_06\_2014\_HelaS\_L\_KD\_SmchD1

19\_06\_2014\_HelaS\_L\_KD\_SmchD1

30\_06\_2014\_HeLaS and L\_SMCHD1\_KD

29\_07\_2014\_HelaS\_L\_SmchD1\_KD

30\_07\_2014\_HelaS\_L\_SmchD1\_KD

11\_08\_2014\_HelaS\_L\_TRF2\_KD (only pSuper, pLVP041, pLVP042)

13\_08\_2014\_HelaS\_L\_TRF2\_KD (only pSuper, pLVP041, pLVP042)

* *For TRF2 KD affect in Hela S and L:*

pool data from:

11\_08\_2014\_HelaS\_L\_TRF2\_KD (only pSuper and TRF2 KD)

13\_08\_2014\_HelaS\_L\_TRF2\_KD (only pSuper and TRF2 KD)

*for just TRF2 KD affect in Hela S, pool data from:*

11\_08\_2014\_HelaS\_L\_TRF2\_KD (only pSuper and TRF2 KD)

13\_08\_2014\_HelaS\_L\_TRF2\_KD (only pSuper and TRF2 KD)

24\_08\_2014\_HeLaS\_SmchD1\_TRF2\_doubleKD\_FISH (only pSuper and TRF2 KD)

27\_08\_2014\_HeLaS\_SmchD1\_TRF2\_doubleKD\_FISH (only pSuper and TRF2 KD)

* *For affect of SmchD1 in TRF2 KD in Hela S:*

pool data from:

24\_08\_2014\_HeLaS\_SmchD1\_TRF2\_doubleKD\_FISH

27\_08\_2014\_HeLaS\_SmchD1\_TRF2\_doubleKD\_FISH

06\_11\_2014\_HelaS\_SmchD1\_Trf2\_doubleKD\_FISH

* *Questions: Does KD of major shelterin components (TRF1, TRF2) affect telomere size/volume?*

Pool data from:

08\_09\_2014\_HelaS\_L\_TRF1\_TRF2\_KD\_DAPI

09\_09\_2014\_HelaS\_L\_TRF1\_TRF2\_KD

21\_10\_2014\_HelaS\_L\_TRF1\_TRF2\_KD\_FISH

05\_11\_2014\_HelaS\_L\_TRF1\_TRF2\_KD\_FISH

17\_11\_2014\_HelaS\_L\_TRF1\_TRF2\_KD\_FISH

Please always analyze the data separately and if they all look the same you can pool them.

It would be great if on the html-page we would see each experiment separately as well as the pool!

**Paper Figures Outline:**

**Figure 1: Determination of 3D-telomere size; validation of method**

A: Model of STORM method

B: RG distribution of Hela S and L (wt = non-transfected)

C: mean RG of Hela S and L

D: Southern Blot of Hela S and L with distribution and mean

In the Supplementary or included in Fig 1:

Same analysis for Hela S and L pSuper (to state that there is no difference between wt and transfected cells)

**Figure 2: Determination of telomere compaction – Are longer telomeres more compact than short ones?**

A: comic of chromatin models 10 and 30 nm

B: parameter graph of Hela S and L in 1 graph with 10 and 30 nm fiber models marked

C: heterochromatin ChIP (H3K9me3, H4Ac, HP1gamma, gH2AX, IgG) in Hela S and L

In the Supplementary or included in Fig 2:

Same analysis for Hela S and L pSuper (to state that there is no difference between wt and transfected cells)

**Figure 3: KD of shelterins affects 3D-telomere size**

A: Western of shelterin KD in Hela S; qPCR for KD of TRF1 in Hela S (if no space, will go to Supplementary)

B: Southern Blot of shelterin KD in Hela S and telomere size distribution + mean values

C: RG distribution and mean values of shelterin KD in Hela S and Hela L

**Figure 4: Does telomere compaction change with KD of shelterin?**

A: parameter graph for Hela S and Hela L KD of shelterins

B: ChiP (H3K9me3, H4Ac, HP1alpha, gH2AX, IgG) heterochomatin in Hela S KD of shelterins