***Sample Collection***

*(Before* [*Radioactive Assay Test*](#30j0zll)*)*

1.) Collect 15-20 test tubes that will fit into a centrifuge. Label them according to the people that you sample. Be sure to note the age of the person, if possible.

2.) After finding a large sample size to work from (use of 15-20 people should be plenty), collect the cheek cells via use of a buccal scrape.

* *Buccal Scrape*: Collection of cheek cells as the sample materials
* Use approximately 3-4 scrapes to allow for plenty of cells to be gathered.

3.) Fill the test tube approximately \* up with a sterile saline solution.

4.) Place the collected cells into the appropriate test tubes and make sure as many cells come off the scraper as is possible.

5.) Stopper the test tubes completely for a run in the centrifuge.

6.) Place the test tubes into the centrifuge and cover the machinery. ·

* ***WARNING***: Centrifuges are *very* dangerous if you leave the cover open!! ***NEVER*** put anything into the machine while it is running and **always** close the cover (if there is one) **before** starting any runs!!!

7.) Centrifuge the mixture for approximately 2 minutes. This will allow for the cheek cells to concentrate at the bottom of the test tubes. After centrifuging the mixtures, remove the test tubes.

8.) Drain the solution to leave approximately 1 cc of fluid within the tube. Allowing some of the solution to be in the test tube will ensure that the cells will not dry out.

9.) Cover the test tube again tightly with Parafilm M or a stopper.

10.) Refrigerate the test tubes until ready for use in assay. Freeze the samples if the assay will not occur until a week or later.

***Radioactive Assay Testing***

*(As Outlined by Geron Corporation)*

The [Geron Corporation](http://www.geron.com/) is to be given credit to allow me to use this procedure for the assay used in testing telomere length and [telomerase](http://docs.google.com/TERMS.HTML#telomerase) activity in a person's cells.

The following is a *summary* of the procedure used at Geron; it will **not** contain all the preparatory steps (such as the preparation of the lysis solution) prior to the radioactive assay:

1.) The Telomeric Sequence Motif Abundance (TSMA) assay is run from using 200,000 (2x105) cells from a single source (in this case, I used cheek cells).

2.) Run a standard whole-cell lysate through dot-blot assay filters with the immobilized DNA. The sample is dot-blotted to a positively charged nylon membrane.

* A *lysate* is prepared from a fixed number of cells; the DNA in that lysate is immobilized onto a nylon filter by dot-blotting
* Each blot filter should also have a standard, as in all experiments.
* Each sample is tested in quadruplet
* Each "dot" contains 30x103 cells.

3.) Probing of DNA

* A. Use a microsatellite specific probe (CAC)8, which is 5'-end labeled with [33P]. This specific probe is intended to quantitate the amount of total DNA in each sample for the purpose of normalization.
* B. Abundance of DNA in each sample is measured using ImageQuant(tm) software.
* C. A second probe is used by hybridizing the DNA with a [32P]-(C3TA2)n riboprobe or [32P]-(C3TA2)4 DNA oligonucleotide. Each of these probes are specific for (anti-sense to) the telomeric repeat sequence.
* D. To calculate the abundance of the [telomeric sequence](http://docs.google.com/TERMS.HTML#telomere) motif, normalize the hybridization signal from the probe to the signal obtained from the microsatellite probe.
* E. Direct comparisons of data can be made from different filter plates by normalizing TSMA data for the samples should be normalized to TSMA value of the standard, which is tested in parallel on each blot.

***Data***

*Average Telomeric Signal Motif (TSM) vs. Std. Deviation P*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ***Sample #*** | ***Avg TSM*** | ***Std. Deviation P*** | ***Sample #*** | ***Avg TSM*** | ***Std. Deviation P*** |
| 1 | 2.637 | 0.138 | 13 | 1.632 | - |
| 2 | 1.393 | 0.133 | 14 | 1.229 | 0.117 |
| 3 | 1.242 | 0.101 | 15 | 2.672 | - |
| 4 | 1.202 | 0.071 | 16 | 1.474 | - |
| 5 | 1.927 | - | 17 | 2.087 | - |
| 6 | 1.389 | - | 18 | 1.586 | - |
| 7 | 1.010 | 0.130 | 19 | 1.133 | - |
| 8 | 1.471 | 0.118 | 20 | 3.070 | - |
| 9 | 1.456 | - | 21 | 2.497 | - |
| 10 | 1.307 | 0.110 | 22 | 1.791 | - |
| 11 | 1.268 | 0.159 | 23 | 1.528 | - |
| 12 | 1.746 | - | 24 | 1.793 |  |
| ***Control*** | ***1.721*** | ***0.130*** |  |  |  |

The above graph displays the data that was obtained. Until we are able to individually compare the assay, we cannot be 100% certain that the data is accurate for the samples collected. However, it can be assumed that if the [prediction](http://docs.google.com/HYPO.HTML) is correct, then the peaks shown are from those individuals that had a higher repetition of telomeres, and possibly more [telomerase](http://docs.google.com/TERMS.HTML) in their cells.