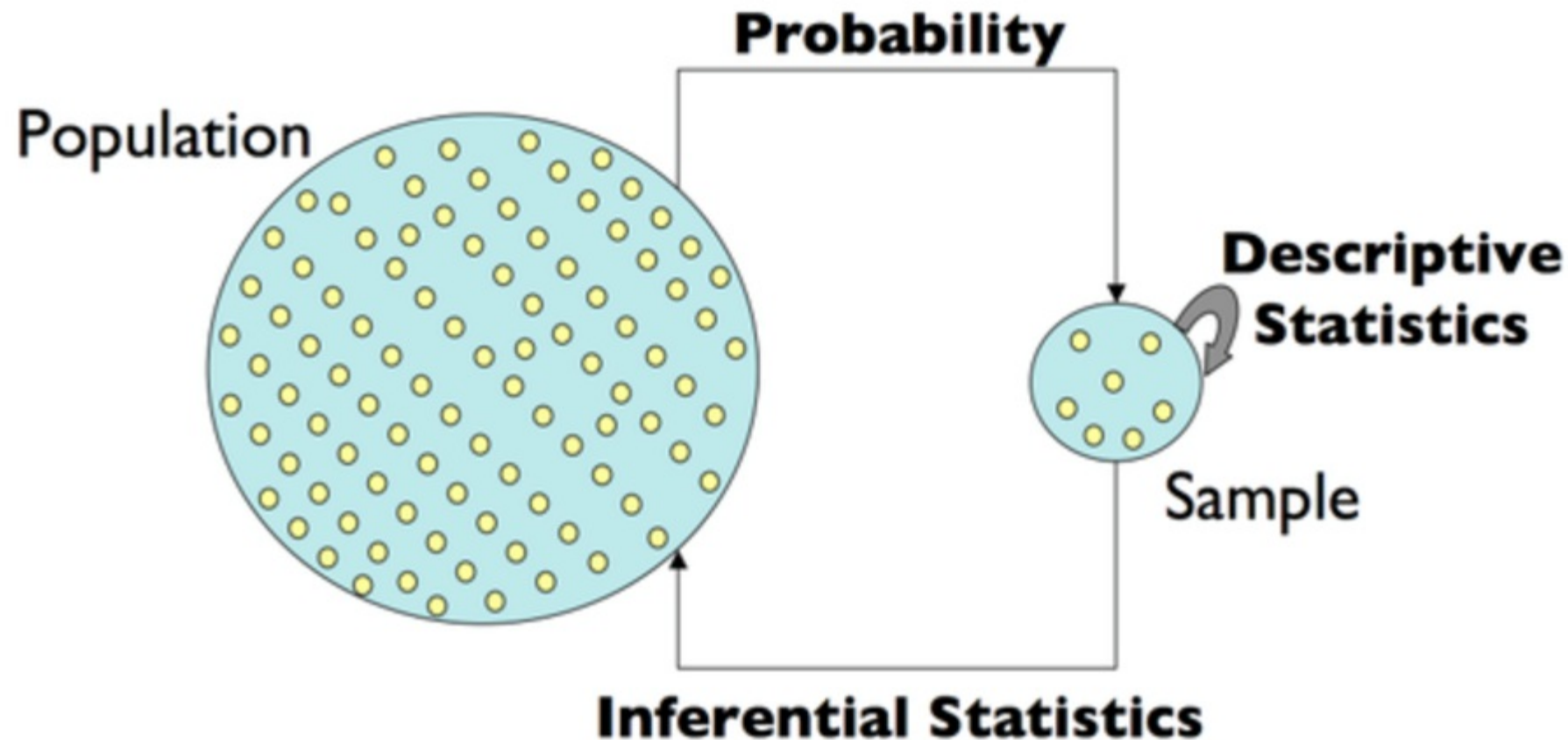# Statistical Methods for
# High Dimensional Biology
## STAT/BIOF/GSAT 540

## Lecture 5 – Two Group Comparisons

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### May 1, 2019

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# Statistical inference



We want to understand a **population** (e.g., gene behaviour) but we can only study a **random sample** from it.

Picture from Dr. Fowler, UW

# Hypothesis Testing in Genomics

## Targeting of GFP to newborn rods by Nrl promoter and temporal expression profiling of flow-sorted photoreceptors

Masayuki Akimoto*[†], Hong Cheng[‡], Dongxiao Zhu[§¶], Joseph A. Brzezinski[∥], Ritu Khanna*, Elena Filippova*, Edwin C. T. Oh[‡], Yuezhou Jing[¶], Jose-Luis Linares*, Matthew Brooks*, Sepideh Zareparsi*, Alan J. Mears*·**, Alfred Hero[§¶††‡‡], Tom Glaser[∥§§], and Anand Swaroop*[‡∥¶¶]

- Retina presents a model system for investigating **regulatory networks** underlying neuronal differentiation.

- **Nrl** transcription factor is known to be important for Rod development

- **What happens if you delete Nrl?**
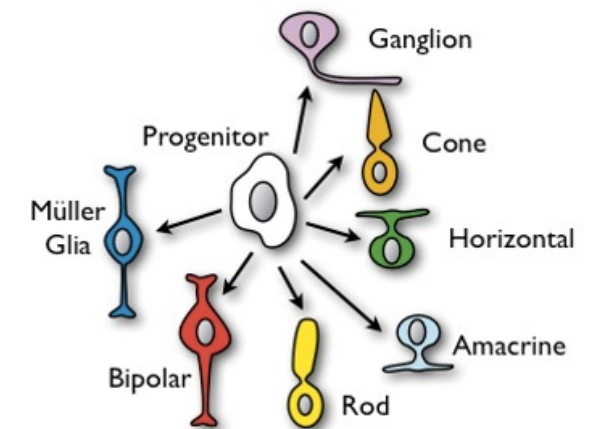
# Why a hypothesis test?

From paper: ". we hypothesized that Nrl is the ideal transcription factor to gain insights into gene expression changes ..."

> **Biological question:** Is the expression level of gene A affected by ablation of the Nrl gene in mice?

**Experimental Design:** we observe a random sample
(random sample of gene expressions from our experiment)

# Get Started

# Hello World

Install the **xaringan** package from Github:

```
devtools::install_github("yihui/xaringan")
```

You are recommended to use the RStudio IDE, but you do not have to.

- Create a new R Markdown document from the menu `File -> New File -> R Markdown -> From Template -> Ninja Presentation;`[1]

- Click the `Knit` button to compile it;

- or use the RStudio Addin[2] "Infinite Moon Reader" to live preview the slides (every time you update and save the Rmd document, the slides will be automatically reloaded in RStudio Viewer.

[1] ??????????????????????????????

[2] See #2 if you do not see the template or addin in RStudio.

You only live once!

# Hello Ninja

As a presentation ninja, you certainly should not be satisfied by the "Hello World" example. You need to understand more about two things:

1. The remark.js library;

2. The **xaringan** package;

Basically **xaringan** injected the chakra of R Markdown (minus Pandoc) into **remark.js**. The slides are rendered by remark.js in the web browser, and the Markdown source needed by remark.js is generated from R Markdown (**knitr**).

# remark.js

You can see an introduction of remark.js from its homepage. You should read the remark.js Wiki at least once to know how to

- create a new slide (Markdown syntax[*] and slide properties);

- format a slide (e.g. text alignment);

- configure the slideshow;

- and use the presentation (keyboard shortcuts).

It is important to be familiar with remark.js before you can understand the options in **xaringan**.

[*] It is different with Pandoc's Markdown! It is limited but should be enough for presentation purposes. Come on... You do not need a slide for the Table of Contents! Well, the Markdown support in remark.js may be improved in the future.

I was so happy to have discovered remark.js!

# Using xaringan

# xaringan

Provides an R Markdown output format `xaringan::moon_reader` as a wrapper for remark.js, and you can use it in the YAML metadata, e.g.

```
---
title: "A Cool Presentation"
output:
  xaringan::moon_reader:
    yolo: true
    nature:
      autoplay: 30000
---
```

See the help page `?xaringan::moon_reader` for all possible options that you can use.

# remark.js vs xaringan

Some differences between using remark.js (left) and using **xaringan** (right):

1. Start with a boilerplate HTML file;

2. Plain Markdown;

3. Write JavaScript to autoplay slides;

4. Manually configure MathJax;

5. Highlight code with *;

6. Edit Markdown source and refresh browser to see updated slides;

1. Start with an R Markdown document;

2. R Markdown (can embed R/other code chunks);

3. Provide an option `autoplay`;

4. MathJax just works;[*]

5. Highlight code with {{}};

6. The RStudio addin "Infinite Moon Reader" automatically refreshes slides on changes;

[*] Not really. See next page.

# Math Expressions

You can write LaTeX math expressions inside a pair of dollar signs, e.g. $\alpha+\beta$ renders $\alpha + \beta$. You can use the display style with double dollar signs:

`$$\bar{X}=\frac{1}{n}\sum_{i=1}^nx_i$$`

$$\bar{X} = \frac{1}{n}\sum_{i=1}^n X_i$$

Limitations:

1. The source code of a LaTeX math expression must be in one line, unless it is inside a pair of double dollar signs, in which case the starting $$ must appear in the very beginning of a line, followed immediately by a non-space character, and the ending $$ must be at the end of a line, led by a non-space character;

2. There should not be spaces after the opening $ or before the closing $.

3. Math does not work on the title slide (see #61 for a workaround).

# R Code

```
# a boring regression
fit = lm(dist ~ 1 + speed, data = cars)
coef(summary(fit))
```

```
#                 Estimate Std. Error    t value     Pr(>|t|)
# (Intercept) -17.579095  6.7584402 -2.601058 1.231882e-02
# speed         3.932409  0.4155128  9.463990 1.489836e-12
```
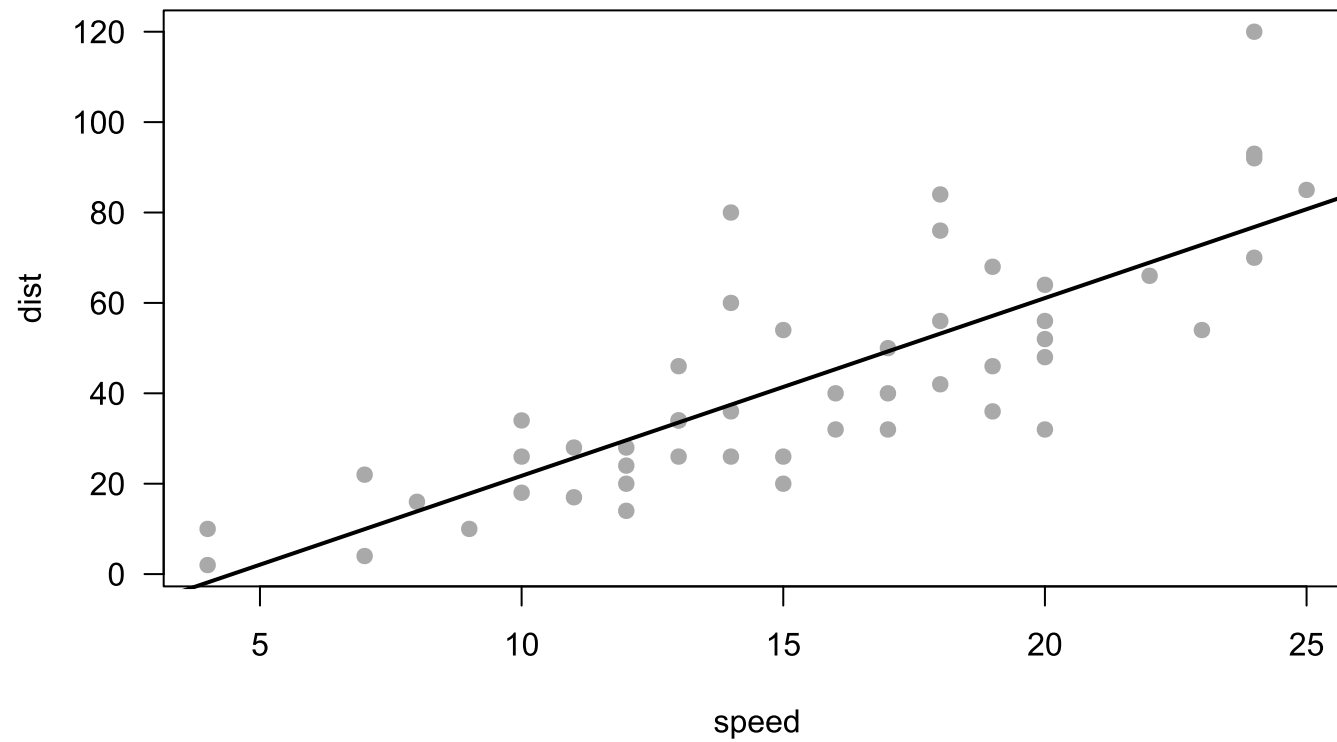
```
dojutsu = c('????????????', '??????', '????????????', '??????', '????????????', '????????????')
grep('???', dojutsu, value = TRUE)
```

```
# [1] "????????????" "??????"        "????????????" "??????"
# [5] "????????????" "????????????"
```

# R Plots

```
par(mar = c(4, 4, 1, .1))
plot(cars, pch = 19, col = 'darkgray', las = 1)
abline(fit, lwd = 2)
```

# Tables

If you want to generate a table, make sure it is in the HTML format (instead of Markdown or other formats), e.g.,

```
knitr::kable(head(iris), format = 'html')
```

| Sepal.Length | Sepal.Width | Petal.Length | Petal.Width | Species |
|---:|---:|---:|---:|---|
| 5.1 | 3.5 | 1.4 | 0.2 | setosa |
| 4.9 | 3.0 | 1.4 | 0.2 | setosa |
| 4.7 | 3.2 | 1.3 | 0.2 | setosa |
| 4.6 | 3.1 | 1.5 | 0.2 | setosa |
| 5.0 | 3.6 | 1.4 | 0.2 | setosa |
| 5.4 | 3.9 | 1.7 | 0.4 | setosa |

# HTML Widgets

I have not thoroughly tested HTML widgets against **xaringan**. Some may work well, and some may not. It is a little tricky.

Similarly, the Shiny mode (`runtime: shiny`) does not work. I might get these issues fixed in the future, but these are not of high priority to me. I never turn my presentation into a Shiny app. When I need to demonstrate more complicated examples, I just launch them separately. It is convenient to share slides with other people when they are plain HTML/JS applications.

See the next page for two HTML widgets.

```
library(leaflet)
leaflet() %>% addTiles() %>% setView(-93.65, 42.0285, zoom = 17)
```

```
DT::datatable(
  head(iris, 10),
  fillContainer = FALSE, options = list(pageLength = 8)
)
```

# Some Tips

- When you use the "Infinite Moon Reader" addin in RStudio, your R session will be blocked by default. You can click the red button on the right of the console to stop serving the slides, or use the *daemonized* mode so that it does not block your R session. To do the latter, you can set the option

```
options(servr.daemon = TRUE)
```

  in your current R session, or in `~/.Rprofile` so that it is applied to all future R sessions. I do the latter by myself.

  To know more about the web server, see the **servr** package.

- Do not forget to try the `yolo` option of `xaringan::moon_reader`.

```
output:
  xaringan::moon_reader:
    yolo: true
```

# Some Tips

- Slides can be automatically played if you set the `autoplay` option under `nature`, e.g. go to the next slide every 30 seconds in a lightning talk:

```
output:
  xaringan::moon_reader:
    nature:
      autoplay: 30000
```

- A countdown timer can be added to every page of the slides using the `countdown` option under `nature`, e.g. if you want to spend one minute on every page when you give the talk, you can set:

```
output:
  xaringan::moon_reader:
    nature:
      countdown: 60000
```

Then you will see a timer counting down from `01:00`, to `00:59`, `00:58`, ... When the time is out, the timer will continue but the time turns red.

# Some Tips

- The title slide is created automatically by **xaringan**, but it is just another remark.js slide added before your other slides.

  The title slide is set to `class: center, middle, inverse, title-slide` by default. You can change the classes applied to the title slide with the `titleSlideClass` option of `nature` (`title-slide` is always applied).

  ```
  output:
    xaringan::moon_reader:
      nature:
        titleSlideClass: [top, left, inverse]
  ```

- If you'd like to create your own title slide, disable **xaringan**'s title slide with the `seal = FALSE` option of `moon_reader`.

  ```
  output:
    xaringan::moon_reader:
      seal: false
  ```

# Some Tips

- There are several ways to build incremental slides. See this presentation for examples.

- The option `highlightLines: true` of `nature` will highlight code lines that start with `*`, or are wrapped in `{{ }}`, or have trailing comments `#<<`;

```
output:
  xaringan::moon_reader:
    nature:
      highlightLines: true
```

See examples on the next page.

# Some Tips

An example using a leading *:

```r
if (TRUE) {
* message("Very important!")
}
```

Output:

```
if (TRUE) {
  message("Very important!")
}
```

This is invalid R code, so it is a plain fenced code block that is not executed.

An example using {{}}:

```{r tidy=FALSE}
if (TRUE) {
{{ message("Very important!") }}
}
```

Output:

```
if (TRUE) {
  message("Very important!")
}
```

```
## Very important!
```

It is valid R code so you can run it. Note that {{}} can wrap an R expression of multiple lines.

# Some Tips

An example of using the trailing comment **#<<** to highlight lines:

```
```{r tidy=FALSE}
library(ggplot2)
ggplot(mtcars) +
  aes(mpg, disp) +
  geom_point() +    #<<
  geom_smooth()     #<<
```
```

Output:

```
library(ggplot2)
ggplot(mtcars) +
  aes(mpg, disp) +
  geom_point() +
  geom_smooth()
```

# Some Tips

When you enable line-highlighting, you can also use the chunk option `highlight.output` to highlight specific lines of the text output from a code chunk. For example, `highlight.output = TRUE` means highlighting all lines, and `highlight.output = c(1, 3)` means highlighting the first and third line.

```r
```{r, highlight.output=c(1, 3)}
head(iris)
```
```

```
##   Sepal.Length Sepal.Width Petal.Length Petal.Width Species
## 1          5.1         3.5          1.4         0.2  setosa
## 2          4.9         3.0          1.4         0.2  setosa
## 3          4.7         3.2          1.3         0.2  setosa
## 4          4.6         3.1          1.5         0.2  setosa
## 5          5.0         3.6          1.4         0.2  setosa
## 6          5.4         3.9          1.7         0.4  setosa
```

Question: what does `highlight.output = c(TRUE, FALSE)` mean? (Hint: think about R's recycling of vectors)

# Some Tips

- To make slides work offline, you need to download a copy of remark.js in advance, because **xaringan** uses the online version by default (see the help page `?xaringan::moon_reader`).

- You can use `xaringan::summon_remark()` to download the latest or a specified version of remark.js. By default, it is downloaded to `libs/remark-latest.min.js`.

- Then change the `chakra` option in YAML to point to this file, e.g.

```
output:
  xaringan::moon_reader:
    chakra: libs/remark-latest.min.js
```

- If you used Google fonts in slides (the default theme uses *Yanone Kaffeesatz*, *Droid Serif*, and *Source Code Pro*), they won't work offline unless you download or install them locally. The Heroku app google-webfonts-helper can help you download fonts and generate the necessary CSS.

# Macros

- remark.js allows users to define custom macros (JS functions) that can be applied to Markdown text using the syntax `![:macroName arg1, arg2, ...]` or `![:macroName arg1, arg2, ...](this)`. For example, before remark.js initializes the slides, you can define a macro named `scale`:

```
remark.macros.scale = function (percentage) {
  var url = this;
  return '<img src="' + url + '" style="width: ' + percentage + '" />';
};
```

Then the Markdown text

```
![:scale 50%](image.jpg)
```

will be translated to

```
<img src="image.jpg" style="width: 50%" />
```

# Macros (continued)

- To insert macros in **xaringan** slides, you can use the option `beforeInit` under the option `nature`, e.g.,

```
output:
  xaringan::moon_reader:
    nature:
      beforeInit: "macros.js"
```

  You save your remark.js macros in the file `macros.js`.

- The `beforeInit` option can be used to insert arbitrary JS code before `remark.create()`. Inserting macros is just one of its possible applications.

# CSS

Among all options in `xaringan::moon_reader`, the most challenging but perhaps also the most rewarding one is `css`, because it allows you to customize the appearance of your slides using any CSS rules or hacks you know.

You can see the default CSS file here. You can completely replace it with your own CSS files, or define new rules to override the default. See the help page `?xaringan::moon_reader` for more information.

# CSS

For example, suppose you want to change the font for code from the default "Source Code Pro" to "Ubuntu Mono". You can create a CSS file named, say, `ubuntu-mono.css`:

```css
@import url(https://fonts.googleapis.com/css?family=Ubuntu+Mono:400,700,400italic);

.remark-code, .remark-inline-code { font-family: 'Ubuntu Mono'; }
```

Then set the `css` option in the YAML metadata:

```yaml
output:
  xaringan::moon_reader:
    css: ["default", "ubuntu-mono.css"]
```

Here I assume `ubuntu-mono.css` is under the same directory as your Rmd.

See yihui/xaringan#83 for an example of using the Fira Code font, which supports ligatures in program code.

# Themes

Don't want to learn CSS? Okay, you can use some user-contributed themes. A theme typically consists of two CSS files `foo.css` and `foo-fonts.css`, where `foo` is the theme name. Below are some existing themes:

```
names(xaringan:::list_css())
```

```
##  [1] "chocolate-fonts"  "chocolate"     "default-fonts"
##  [4] "default"          "duke-blue"     "fc-fonts"
##  [7] "fc"               "hygge-duke"    "hygge"
## [10] "kunoichi"         "lucy-fonts"    "lucy"
## [13] "metropolis-fonts" "metropolis"    "middlebury-fonts"
## [16] "middlebury"       "ninjutsu"      "rladies-fonts"
## [19] "rladies"          "robot-fonts"   "robot"
## [22] "rutgers-fonts"    "rutgers"       "shinobi"
## [25] "tamu-fonts"       "tamu"          "uo-fonts"
## [28] "uo"               "uol-fonts"     "uol"
```
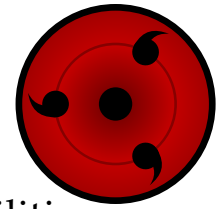
# Themes

To use a theme, you can specify the `css` option as an array of CSS filenames (without the `.css` extensions), e.g.,

```
output:
  xaringan::moon_reader:
    css: [default, metropolis, metropolis-fonts]
```

If you want to contribute a theme to **xaringan**, please read this blog post.

Naruto

# Sharingan

The R package name **xaringan** was derived[1] from **Sharingan**, a dojutsu in the Japanese anime *Naruto* with two abilities:

- the "Eye of Insight"

- the "Eye of Hypnotism"

I think a presentation is basically a way to communicate insights to the audience, and a great presentation may even "hypnotize" the audience.[2,3]

[1] In Chinese, the pronounciation of *X* is *Sh* /??/ (as in *shrimp*). Now you should have a better idea of how to pronounce my last name *Xie*.

[2] By comparison, bad presentations only put the audience to sleep.

[3] Personally I find that setting background images for slides is a killer feature of remark.js. It is an effective way to bring visual impact into your presentations.

# Naruto terminology

The **xaringan** package borrowed a few terms from Naruto, such as

- Sharingan (?????????; the package name)

- The moon reader (??????; an attractive R Markdown output format)

- Chakra (?????????; the path to the remark.js library, which is the power to drive the presentation)

- Nature transformation (????????????; transform the chakra by setting different options)

- The infinite moon reader (????????????; start a local web server to continuously serve your slides)

- The summoning technique (download remark.js from the web)

You can click the links to know more about them if you want. The jutsu "Moon Reader" may seem a little evil, but that does not mean your slides are evil.

# Hand seals (???)

Press h or ? to see the possible ninjutsu you can use in remark.js.
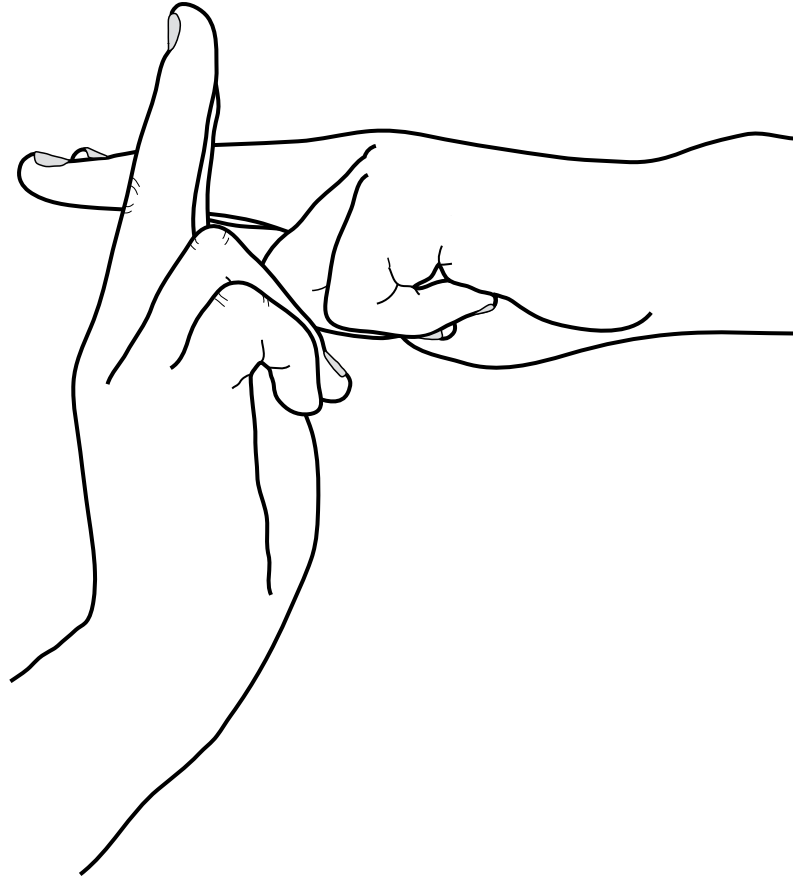
# Thanks!

Slides created via the R package **xaringan**.

The chakra comes from remark.js, **knitr**, and R Markdown.