Linux: Beyond the Basics

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Bioinformatics and Research Computing
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http://barc.wi.mit.edu/hot_topics/

Whitehead Institute

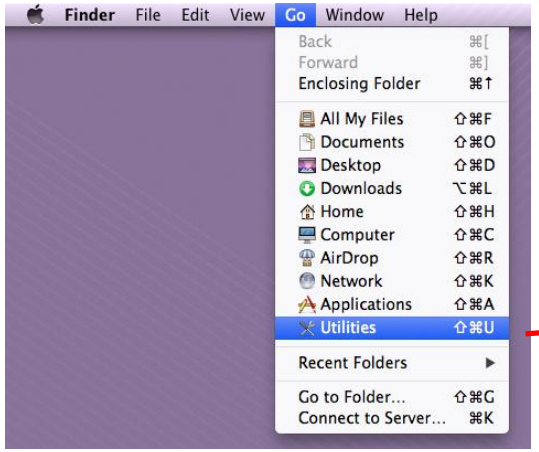
Logging in to our Linux server

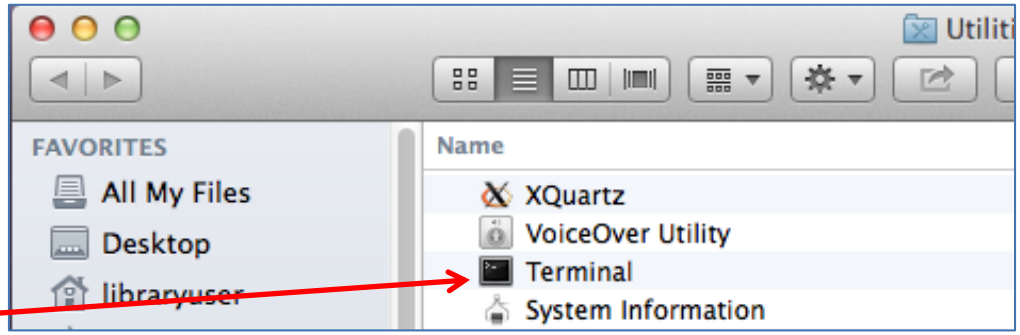
- Our main Unix/Linux server is called tak
- Request a tak account:
  http://bioinfo.wi.mit.edu/bio/software/unix/bioinfoaccount.php

- Connecting to tak:
  http://bioinfo.wi.mit.edu/bio/software/unix/serverConnect.php

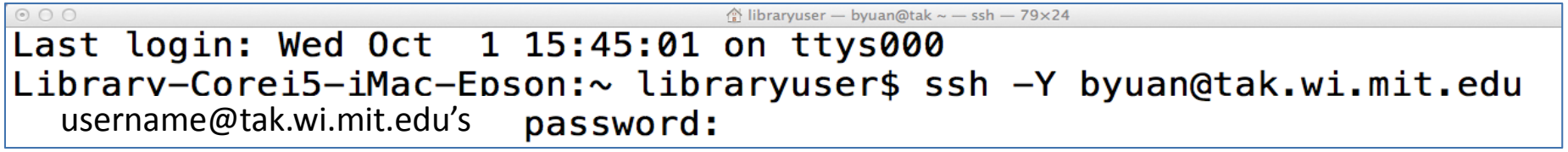
  - Windows:
    - MobaXterm
  - Mac
    - Access through Terminal

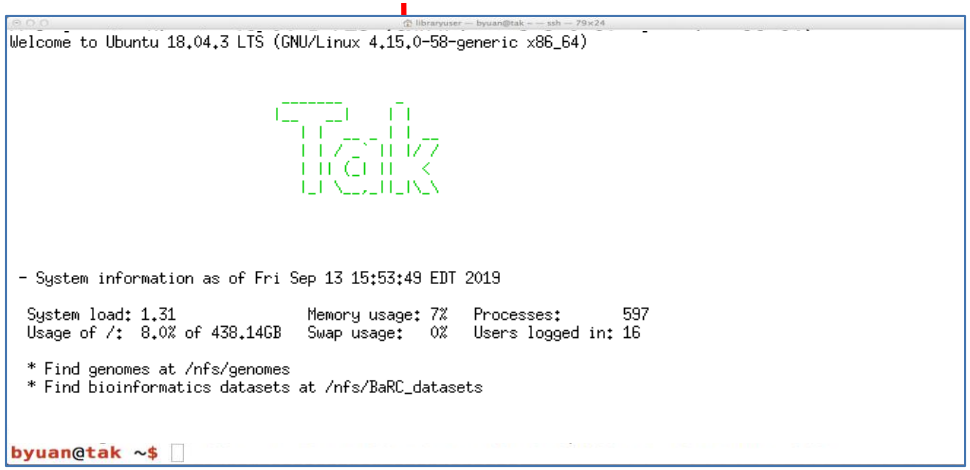
Log in to tak for Mac





ssh –Y username@tak.wi.mit.edu

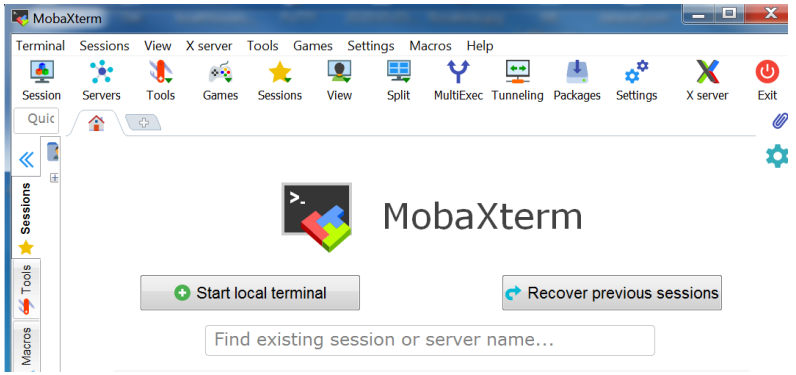




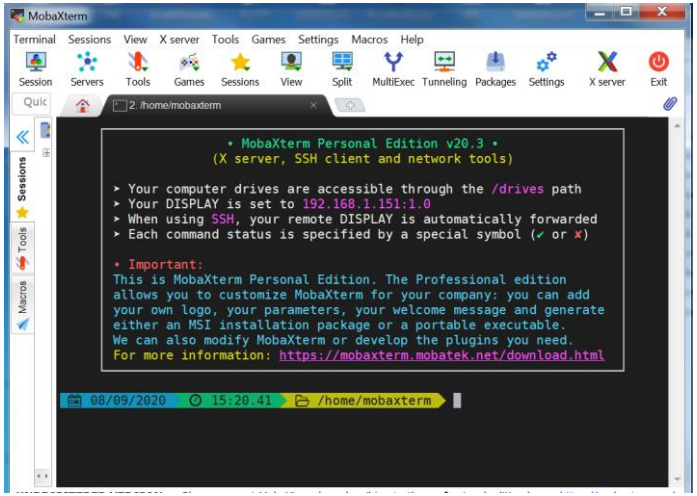
Connecting to tak from Windows

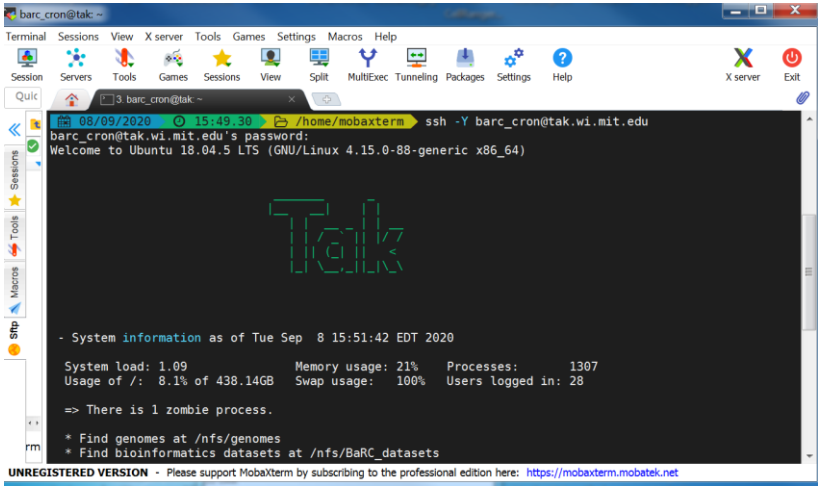
MobaXterm

1) Open MobaXterm



2) Click on the ”Start local terminal” button





3) Type:

`ssh -Y username@tak.wi.mit.edu`

Note:
When you write the password you won’t see any characters being typed.

Hot Topics website: http://barc.wi.mit.edu/education/hot_topics/

- After you login to tak, create a directory for the exercises within your home directory, and use it as your working directory

  $ mkdir Hot_Topics

  $ cd Hot_Topics

- Copy all files into your working directory

  $ cp -r /nfs/BaRC_training/Linux_Beyond/data_files/* .

- You should have the files below in your working directory:
  - foo.txt, sample1.txt, exercise.txt, Ensembl_info.txt, Gene_exp.txt, HumanGenesPlusMinus3kb.bed, peaks.bed, datasets folder
  - You can check they're there with the 'ls' command

Linux Review: Commands

- **`command [arg1 arg2 … ] [input1 input2 … ]`**

$ sort -k2,3nr foo.tab

start end

-n or **-g**: -n is recommended, except for scientific notation or a leading '+'

-r: reverse order

$ cut -f1,5 foo.tab

$ cut -f1-5 foo.tab

-f: select only these fields
-f1,5: select 1st and 5th fields
-f1-5: select 1st, 2nd, 3rd, 4th, and 5th fields

$ wc -l foo.txt

How many lines are in this file?

Linux Review: Common Mistakes

- Case sensitive

cd /nfs/Barc_Public is different from **cd /nfs/BaRC_Public**

```
-bash: cd: /nfs/Barc_Public: No such file or directory
```

- Spaces may matter!

rm –f myFiles* vs rm –f myFiles *

- Office applications can convert text to special characters that Linux won't understand
  - Smart quotes, dashes
  - Carriage return from DOS
    - Use fromdos to remove carriage return

Linux Review: Pipes

- Stream output of one command/program as input for another
  - Avoid intermediate file(s)
  - Merge multiple commands in to one long command

➢ $ cut -f1 myFile.txt | sort | uniq -c > uniqCounts.txt

↗ pipes ↗

What we will discuss today

- Aliases (to reduce typing)
- sed (for file manipulation)
- awk(to filter by column)
- join (merge files)
- groupBy and intersect from bedtools (not typical Linux)
- loops (one-line and with shell scripts)
- scripting (to streamline commands)

Aliases

- Add a one-word link to a longer command
- To get current aliases (from ~/.bashrc)

```
alias
```

- Create a new alias (two examples)

```
alias sp='cd /lab/solexa_public/Reddien'
alias CollectRnaSeqMetrics='java -jar
/usr/local/share/picard-tools/CollectRnaSeqMetrics.jar'
```

- Make an alias permanent
  - Paste command(s) in ~/.bashrc

sed:

stream editor for filtering and transforming text

- Print lines 10 - 15:

```
$ sed -n '10,15p' bigFile > selectedLines.txt
```

- Delete 5 header lines at the beginning of a file:

```
$ sed '1,5d' file > fileNoHeader
```

- Remove all version numbers (eg: '.1') from the end of a list of sequence accessions: eg. NM_000035.2

```
$ sed 's/\.[0-9]\+//g' accsWithVersion > accsOnly
```

s: substitute *g: global modifier (change all)*

- Get good examples from “sed cheat sheet”:
  - https://www.pement.org/sed/sed1line.txt

Join files together

With Linux join

`$ join -1 1 -2 2 --nocheck-order -t $'\t' sorted_File1 sortedFile2`

Join files on the 1st field of FILE1 with the 2nd field of FILE2,

only showing the common lines.

FILE1 and FILE2 must be sorted on the join fields before running join

-t $'\t' : Both input and output use tab as separator

--nocheck-order: good for sorted files with header line.

Sorted sample tables to join:

Symbol	Heart	Skeletal Muscle	Skin	Smooth Muscle	Spinal cord
HHAT	8.15	7.7	5	6.55	6.4
INPP5D	19.65	5.95	4.55	5.25	14.5
NDUFA10	441.8	160.2	24.9	188.85	158.75
RPS6KA1	85.2	47.75	46.45	35.85	44.55
RYBP	20.45	13.05	11.95	20.7	17.75
SLC16A1	15.45	20.45	12.2	248.35	27.15

Ensembl Gene ID	Symbol
ENSG00000280680	HHAT
ENSG00000280820	LCN1P1
ENSG00000280584	OBP2B
ENSG00000280775	RNA5SP136
ENSG00000252303	RNU6-280P
ENSG00000280963	SERTAD4-AS1

Symbol	Heart	Skeletal Muscle	Skin	Smooth Muscle	Spinal cord	Ensembl Gene ID
HHAT	8.15	7.7	5	6.55	6.4	ENSG00000280680

Regular Expressions

- A sequence of characters defining a search pattern
- Powerful, but syntax is often non-intuitive
- Examples

  List all txt files: **`ls *.txt`**

  Replace CHR with Chr at the beginning of each line:

  `$ sed 's/^CHR/Chr/' myFile.txt`

  Delete a dot followed by one or more numbers

  `$ sed 's/\.[0-9]\+//g' myFile.txt`

	Matches
.	All characters
*	Zero or more; wildcard
+	One or more
?	One
^	Beginning of a line
$	End of a line
[ab]	Any character in brackets

- Note: regular expression syntax may slightly differ between sed, awk, Linux shell, and Perl
  - Ex: \+ in sed is equivalent to + in Perl

awk

- A simple programing language to process files
- Good for filtering and manipulating multiple-column files
- “awk” comes from the original authors:

  Alfred V. Aho, Peter J. Weinberger, Brian W. Kernighan

awk

- By default, awk splits each line by spaces

- Print the 2nd and 1st fields of the file:

$ awk ' { print $2"\t"$1 } ' foo.tab

- Convert sequences from tab delimited format to fasta format:

```
$ head -1 foo.tab
Seq1   ACTGCATCAC
$ awk ' { print ">" $1 "\n" $2 }' foo.tab  >  foo.fa
$ head -2 foo.fa
>Seq1
ACGCATCAC
```

awk: field separator

- Issues with default separator (white space)
  - one field is gene description with multiple words
  - consecutive empty cells
- To use tab as the separator:

```
$ awk -F "\t" '{ print NF }' foo.txt
or
$ awk 'BEGIN {FS="\t"} { print NF }' foo.txt
```

BEGIN: action before read input
NF: number of fields in the current record
FS: input field separator
OFS: output field separator
END: action after read input

Character	Description
\n	newline
\r	carriage return
\t	horizontal tab

awk: arithmetic operations

Add average values of 4th and 5th fields to the file:
$ awk '{ print $0 "\t" ($4+$5)/2 }' foo.tab

$0: all fields

Operator	Description
+	Addition
-	Subtraction
*	Multiplication
/	Division
%	Modulo
^	Exponentiation
**	Exponentiation

awk: making comparisons

Print out records if values in 4th or 5th field are above 4:
$ awk '{ if($4>4 || $5>4) print $0 } ' foo.tab

Sequence	Description
>	Greater than
<	Less than
<=	Less than or equal to
>=	Greater than or equal to
==	Equal to
!=	Not equal to
~	Matches
!~	Does not match
||	Logical OR
&&	Logical AND

More awk examples

- Conditional statements:

Display expression levels for the gene NANOG:
$ awk '{ if(/NANOG/) print $0 }' foo.txt
or
$ awk '/NANOG/ { print $0 } ' foo.txt
or
$ awk '/NANOG/' foo.txt

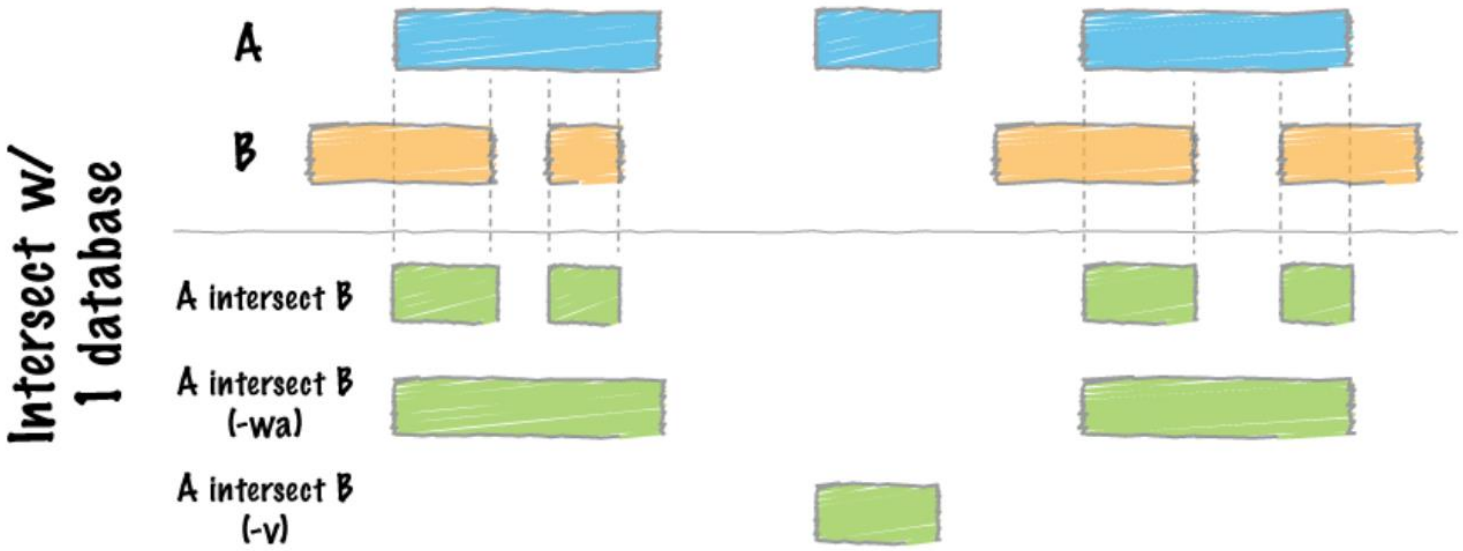
Add line number to the above output:
$ awk '/NANOG/ { print NR"\t"$0 }' foo.txt
NR: line number of the current row

- Looping:

Calculate the average expression (4th, 5th and 6th fields in this case) for each transcript
$ awk '{ total= $4 + $5 + $6; avg=total/3; print $0"\t"avg}' foo.txt
or
$ awk '{ total=0; for (i=4; i<=6; i++) total=total+$i; avg=total/3; print $0"\t"avg }' foo.txt

intersect from bedtools (intersectBed)

- Find overlaps between two sets of genomic features



https://bedtools.readthedocs.io/en/latest/content/tools/intersect.html

intersectBed: Examples

$ head -2 HumanGenesPlusMinus3kb.bed

chr1	50899700	50905978	ENSG00000271782_RP5-850O15.4
chr1	103814769	103831355	ENSG00000232753_RP11-347K2.1

$ head -2 peaks.bed

chr1	19921	20016	MACS_peak_1	50
chr1	20025	20890	MACS_peak_2	568

$ intersectBed -wa -wb -a HumanGenesPlusMinus3kb.bed -b peaks.bed | head -2

chr1	45956538	45968751	ENSG00000236624_CCDC163P	chr1	45955389	45956863	MACS_peak_3385	1192.24
chr1	45956538	45968751	ENSG00000236624_CCDC163P	chr1	45957202	45957380	MACS_peak_3386	121.87

$ intersectBed -a HumanGenesPlusMinus3kb.bed -b peaks.bed | head -2

chr1	45956538	45956863	ENSG00000236624_CCDC163P
chr1	45957202	45957380	ENSG00000236624_CCDC163P

$ intersectBed -a HumanGenesPlusMinus3kb.bed -b peaks.bed | cut -f4 |sort -u |head -2

ENSG00000000003_TSPAN6
ENSG00000000419_DPM1

Summarize by Columns: groupBy (from bedtools)

Input file must be pre-sorted by grouping column(s)!

input

Ensembl Gene ID	Ensembl Transcript ID	Symbol
ENSG00000281518	ENST00000627423	FOXO6
ENSG00000281518	ENST00000630406	FOXO6
ENSG00000280680	ENST00000625523	HHAT
ENSG00000280680	ENST00000627903	HHAT
ENSG00000280680	ENST00000626327	HHAT
ENSG00000281614	ENST00000629761	INPP5D
ENSG00000281614	ENST00000630338	INPP5D

-g grpCols	column(s) for grouping
-c -opCols	column(s) to be summarized
-o	Operation(s) applied to opCol: sum, count, min, max, mean, median, stdev, collapse (comma-sep list) distinct (non-redundant comma-sep list)

Print the gene ID (1st column), the gene symbol , and a list of transcript IDs (2nd field)

```
$ sort -k1,1 Ensembl_info.txt | groupBy -g 1 -c 3,2 -o distinct,collapse
```

Partial output

```
!Ensembl Gene ID   !Symbol   !Ensembl Transcript ID
ENSG00000281518 FOXO6     ENST00000627423,ENST00000630406
ENSG00000280680 HHAT      ENST00000625523,ENST00000626327,ENST00000627903
```

Shell Flavors

- Syntax (for scripting) depends the shell

```
echo $SHELL          # /bin/bash (on tak)
```

- bash is common and the default on tak.
- Some Linux shells (incomplete listing):

Shell	Name
sh	Bourne
bash	**Bourne-Again**
ksh	Korn shell
csh	C shell

Shell script advantages

- Automation: avoid having to retype the same commands many times
- Ease of use and more efficient
- Outline of a script:

#!/bin/bash ⟵ shebang: interprets how to run the script

commands… ⟵ *set of* commands used in the script

#comments ⟵ write comments using "#"

- Commonly used extension for script is .sh (eg. foo.sh), file must have executable permission

Bash Shell: ‘for’ loop

- Process multiple files with one command
- Reduce computational time with many cluster nodes

```
for mySam in `/bin/ls *.sam`
do
        bsub  wc -l $mySam
done
```

When referring to a variable, $ is needed before the variable name ($mySam), but $ is not needed when defining it (mySam).

Identical one-line command:
for samFile in `/bin/ls *.sam`; do bsub wc -l $samFile; done

Shell script example

```
#!/bin/bash

# 1.  Take two arguments: the first one is a directory with all the datasets, the second one is for output
# 2. For each file, calculate average gene expression, and save the results in a file in the output directory

inDir=$1                    # 1st argument
outDir=$2                   # 2nd argument; outDir must already exist
                            # Define variables: no spaces on either side of the equal sign
for i in `/bin/ls $inDir `  # refer to variable with $
do
        # output file name
        outFileName="${i}_avg.txt"          # {}: $i_avg is not valid;
        # calculate average gene expression
        # NM_001039201    Hdhd2   5.0306    5.3309   5.4998
        bsub "sort -k2,2 $inDir/$i | groupBy -g 2 -c 3,4,5 -o mean,mean,mean >| $outDir/$name"
done
```

 # You can use editors such as nedit, gedit, emacs, or pico to create shell scripts

Accessing Shared Resources at Whitehead

- Linux
  - **/nfs/BaRC_Public**
  - **/nfs/BaRC_training**
  - **/lab/solexa_public**
- Windows (access using *Start Menu → Search*)
  - **\\wi-files1\BaRC_Public**
  - **\\wi-files1\BaRC_training**
  - **\\wi-bigdata\solexa_public**
- Macs (access using *Go → Connect to Server…*)
  - **smb://wi-files1/BaRC_Public**
  - **smb://wi-files1/BaRC_training**
  - **smb://wi-bigdata/solexa_public**

Where's my lab's share?

- http://it.wi.mit.edu/systems/file-storage/lab-share-paths

Further Reading

- BaRC one liners:
  - http://bioinfo.wi.mit.edu/bio/bioinfo/scripts/#unix
- Linux Info for Bioinfo:
  - http://bioinfo.wi.mit.edu/bio/education/unix_intro.php
- Online books via MIT:
  - http://proquest.safaribooksonline.com/
  - *Bioinformatics Data Skills* by V.Buffalo (2015)
- Bash Guide for Beginners:
  - http://tldp.org/LDP/Bash-Beginners-Guide/html/

Upcoming Hot Topics

- An Introduction to R - October
- An Introduction to R Graphics - October
- Python: An Introduction - November
- Python: Advanced Topics – November

http://barc.wi.mit.edu/education/hot_topics/upcoming/