

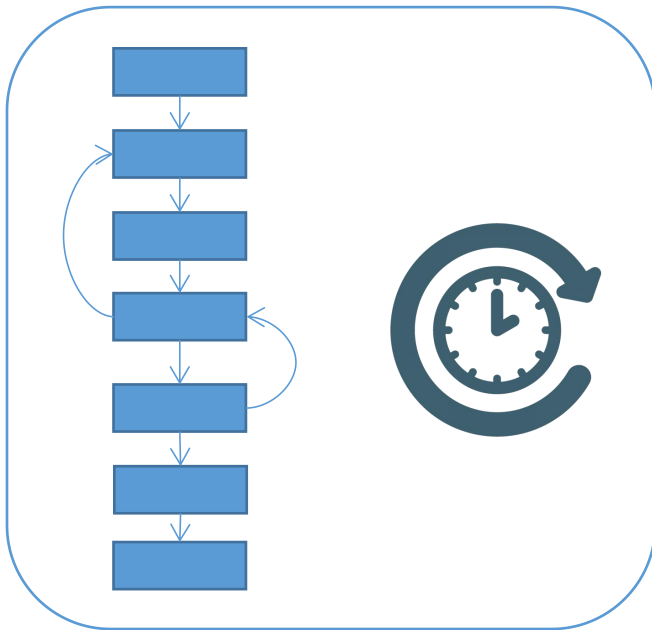
Seurat scRNA pipeline

Mingbo Cheng

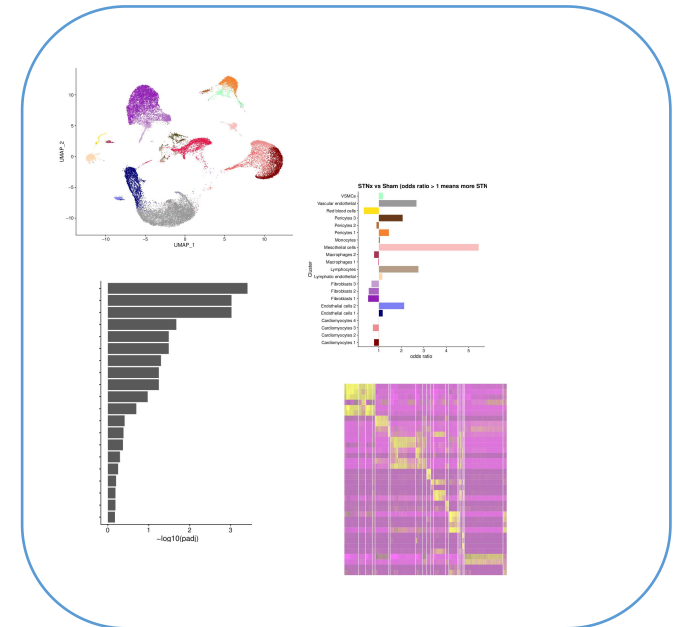
13/12/2020

Idea

Data Producing

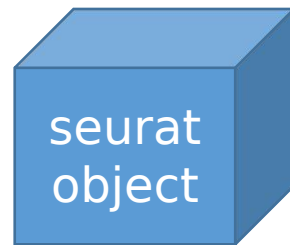
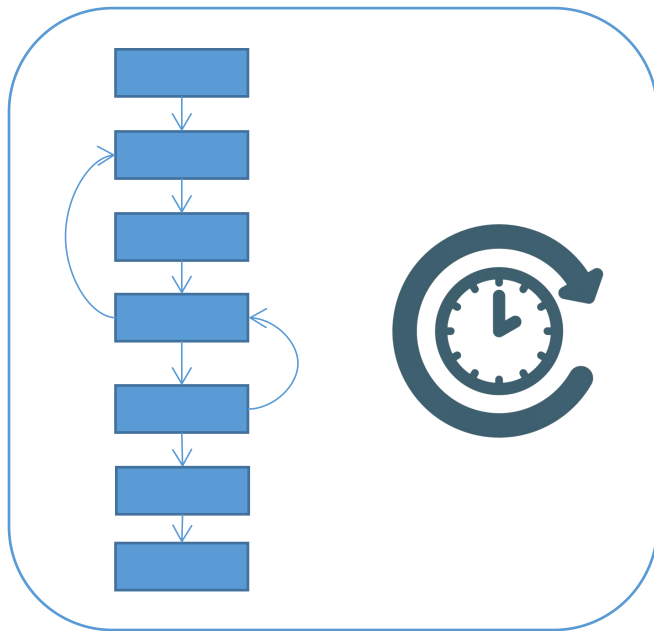


Visualization



How it works

Data Producing



meta.data

clustering results
annotation
.....

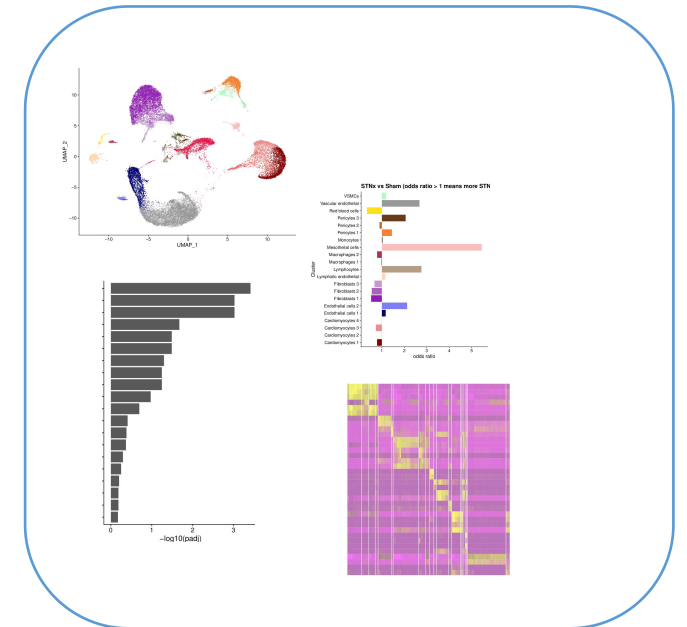
reductions

PCA
UMAP
.....

tools

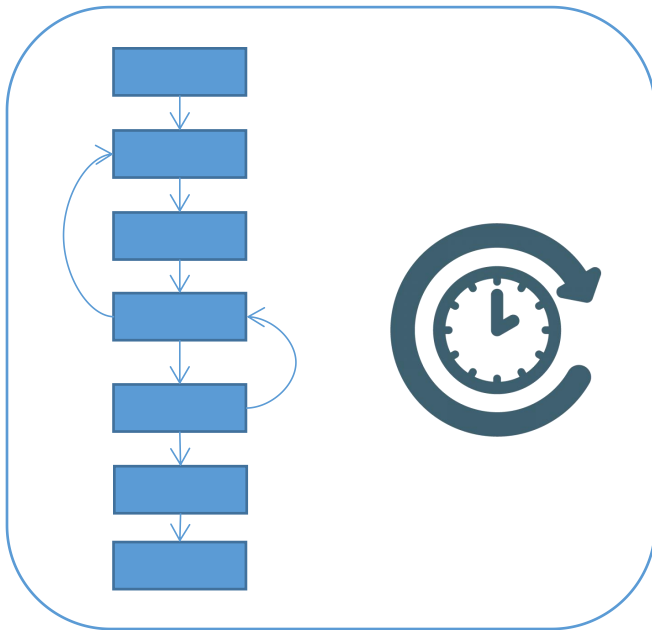
DE analysis
GO analysis
Pathways analysis
.....

Visualization

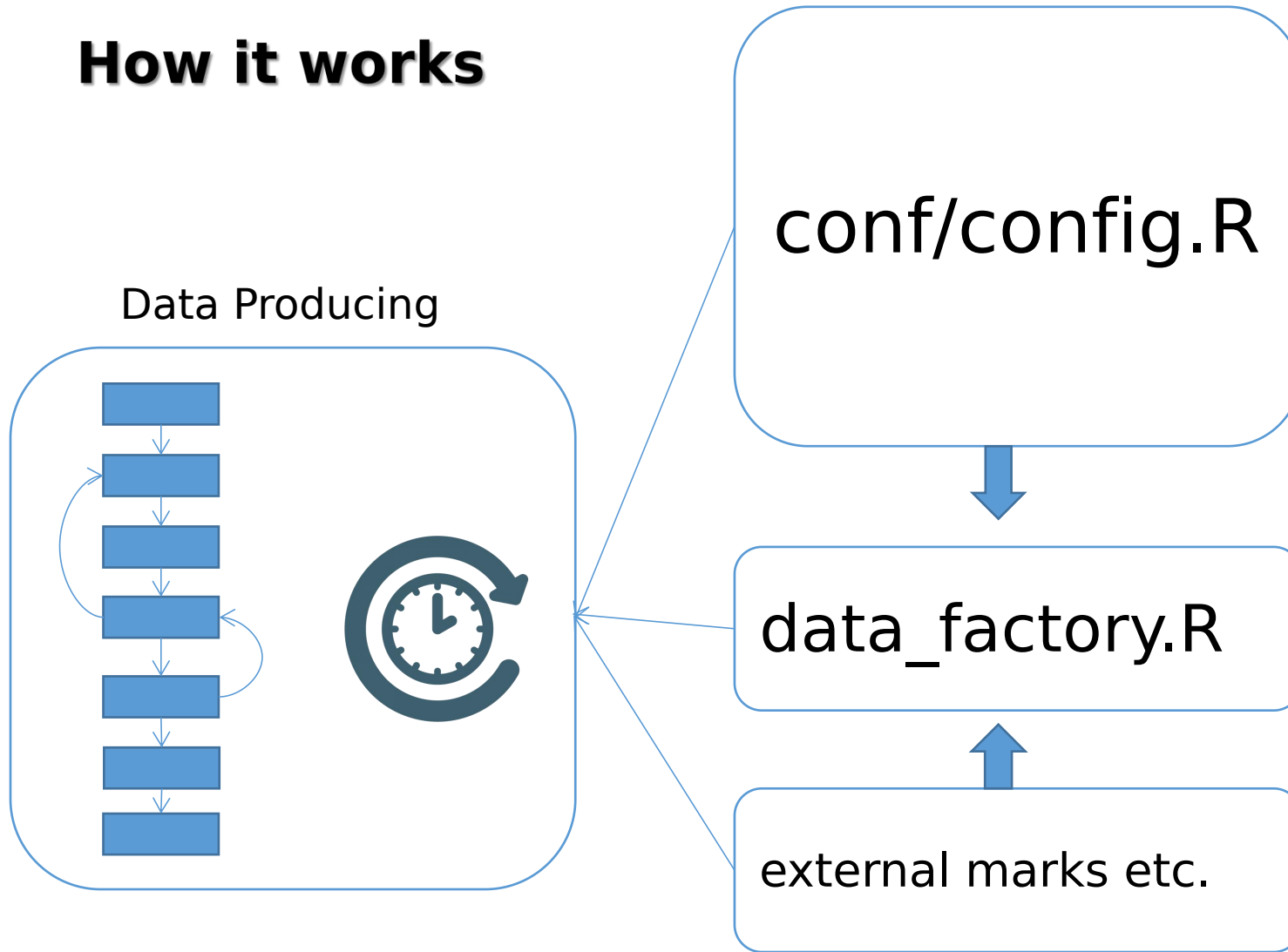


How it works

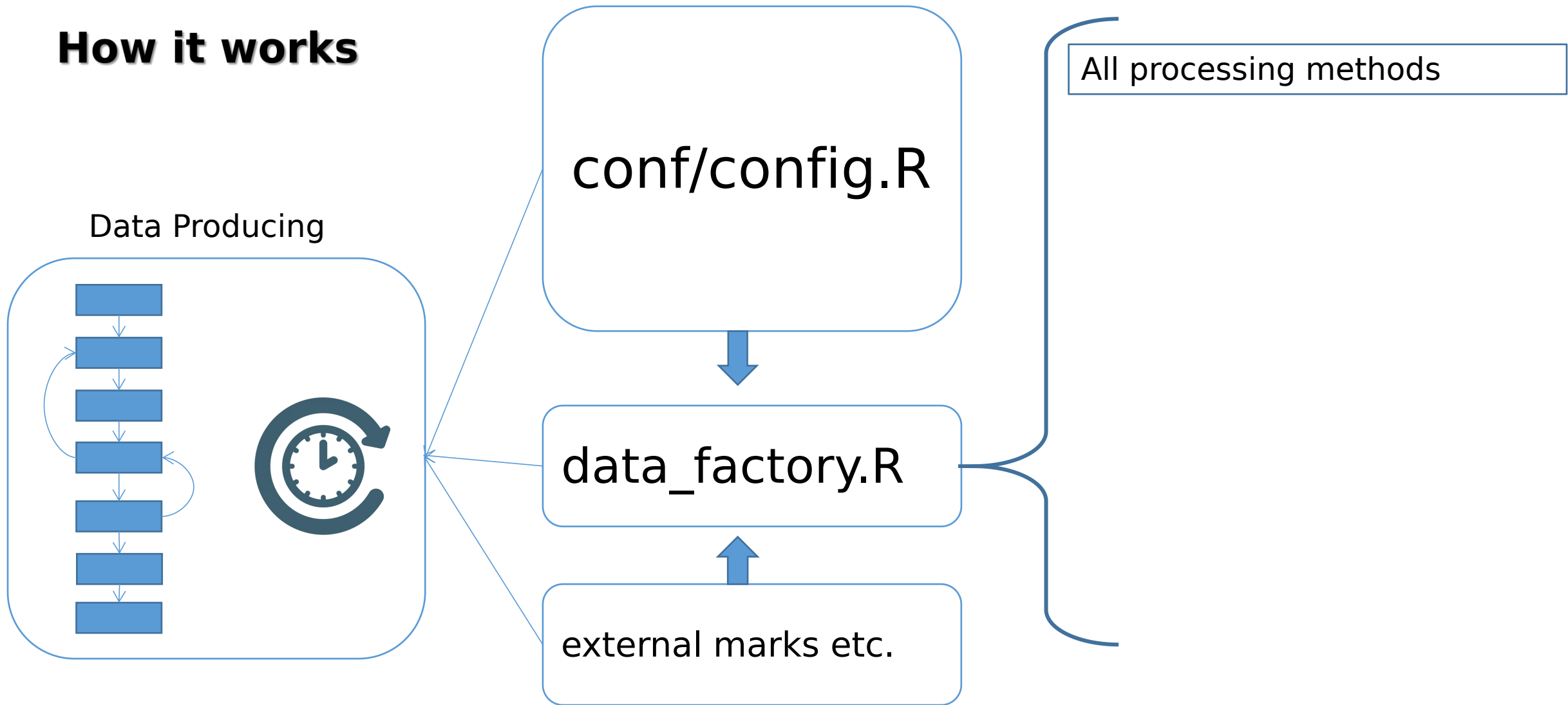
Data Producing



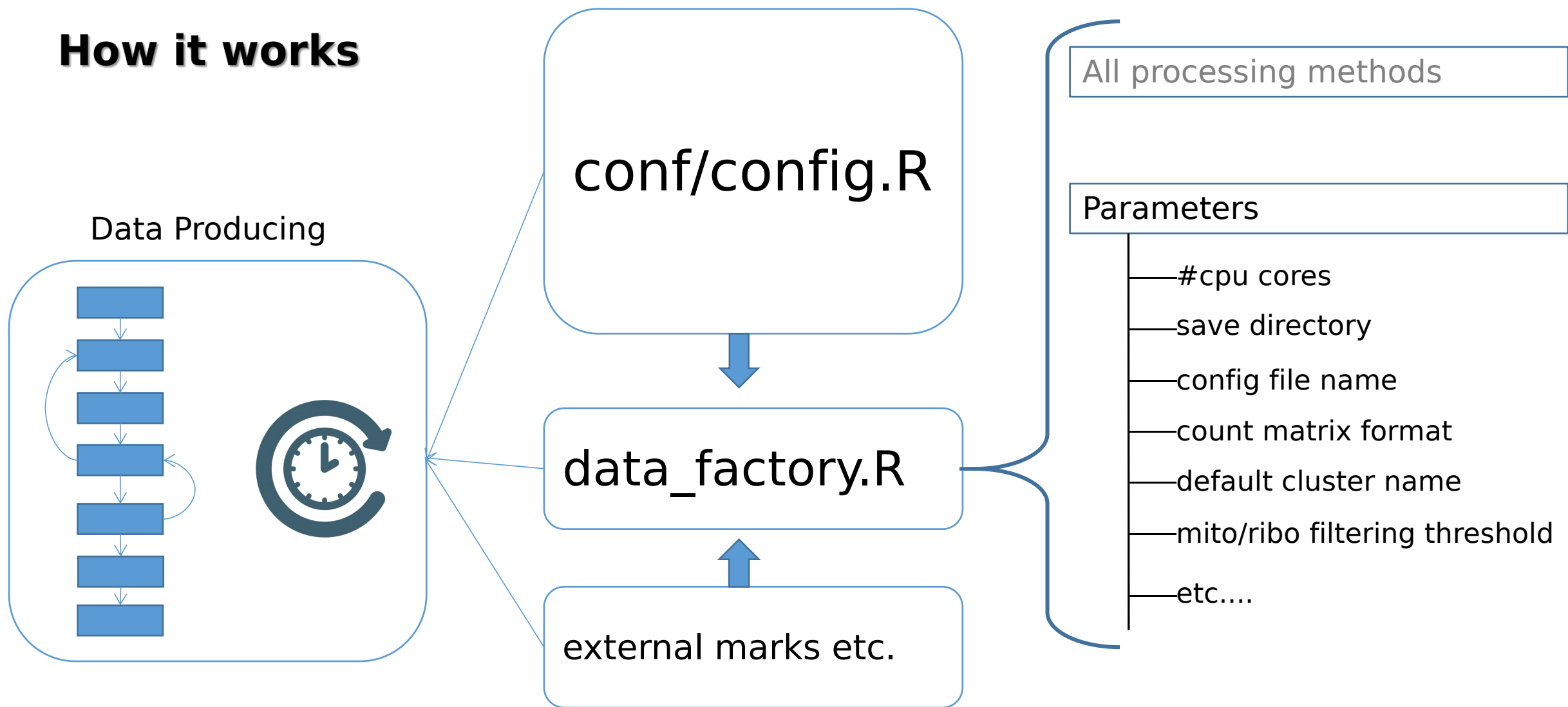
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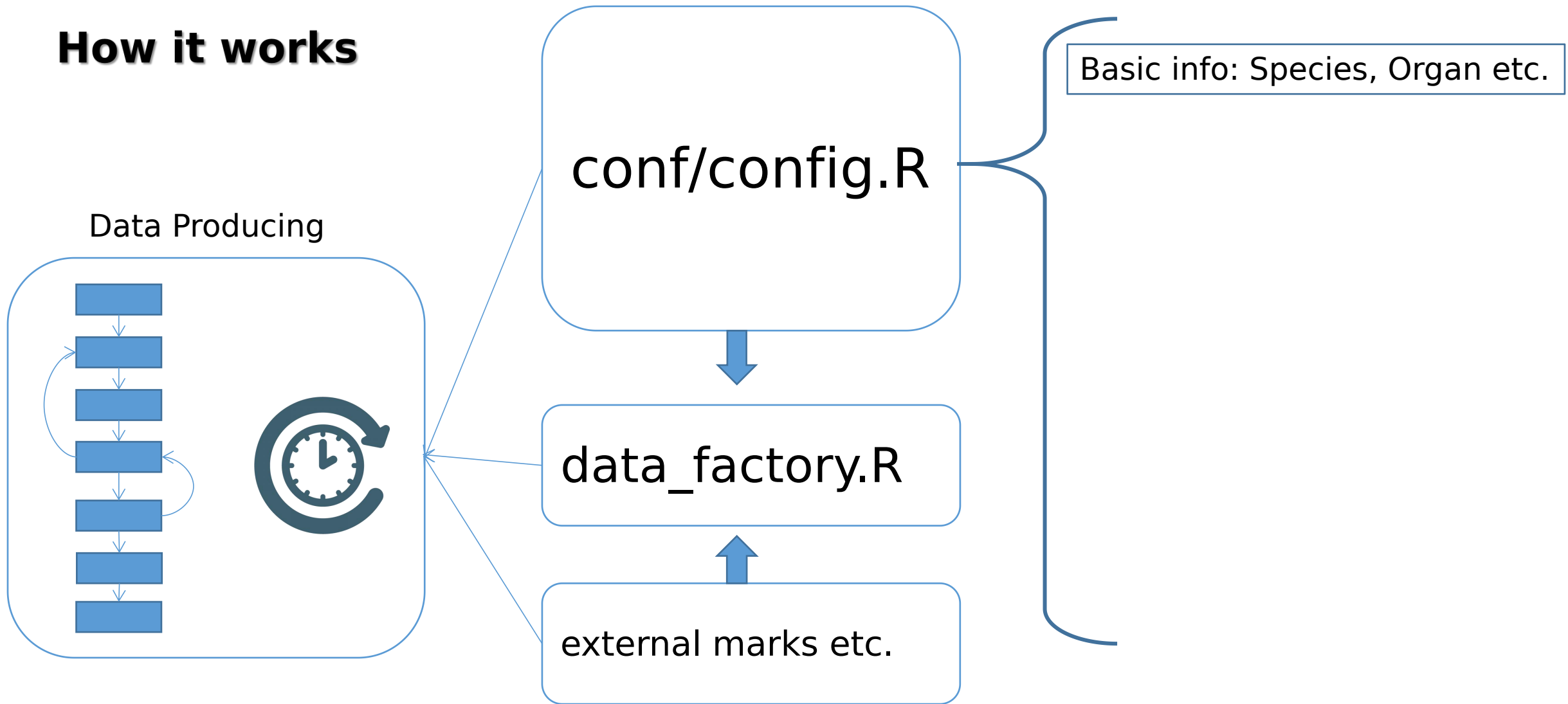
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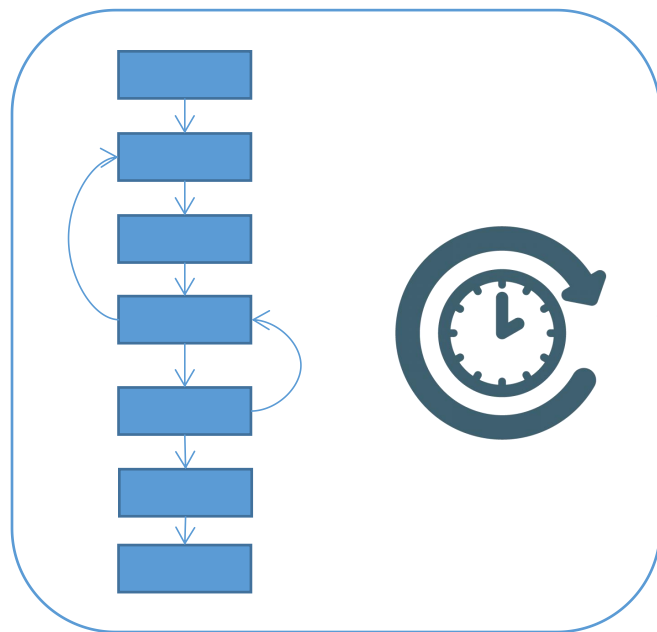


How it works



How it works

Data Producing



conf/config.R

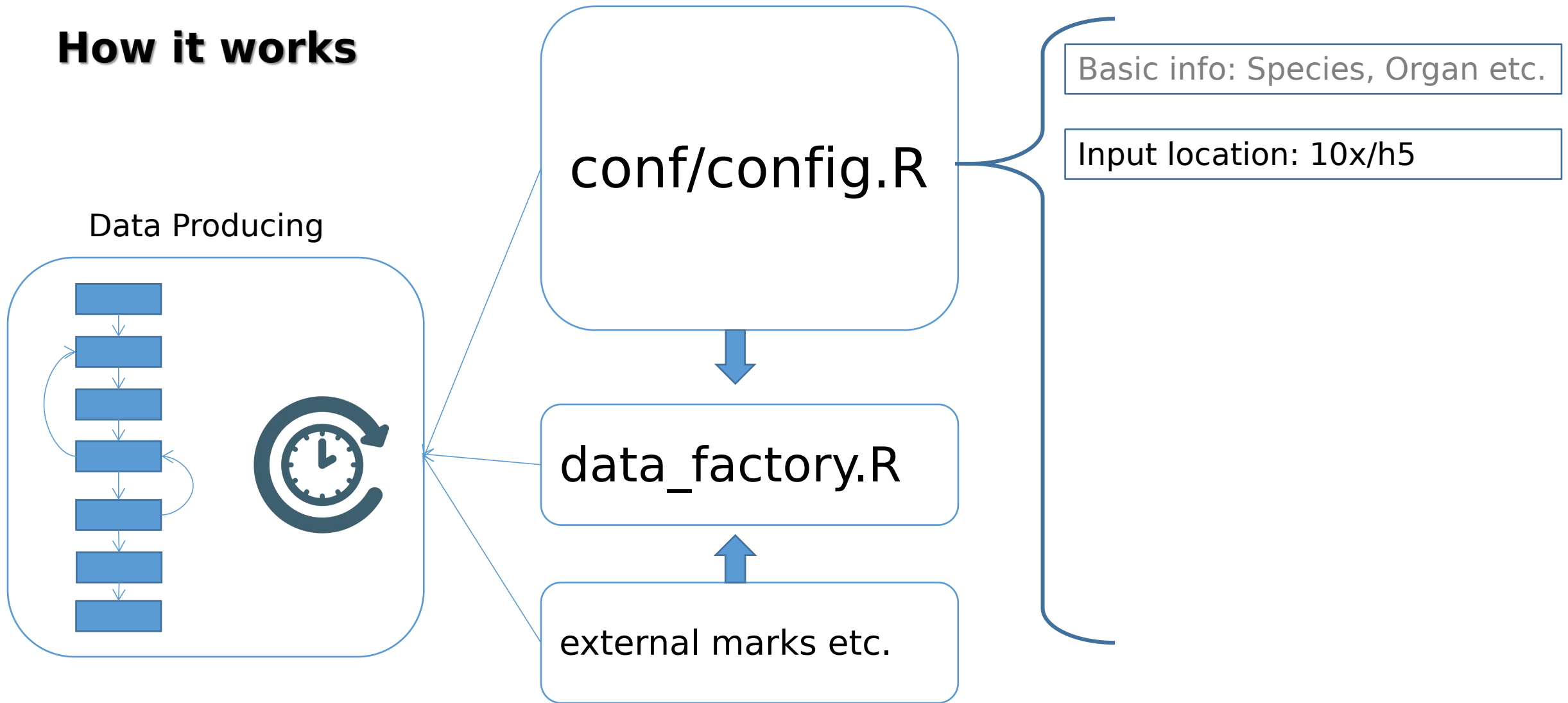
data_factory.R

external marks etc.

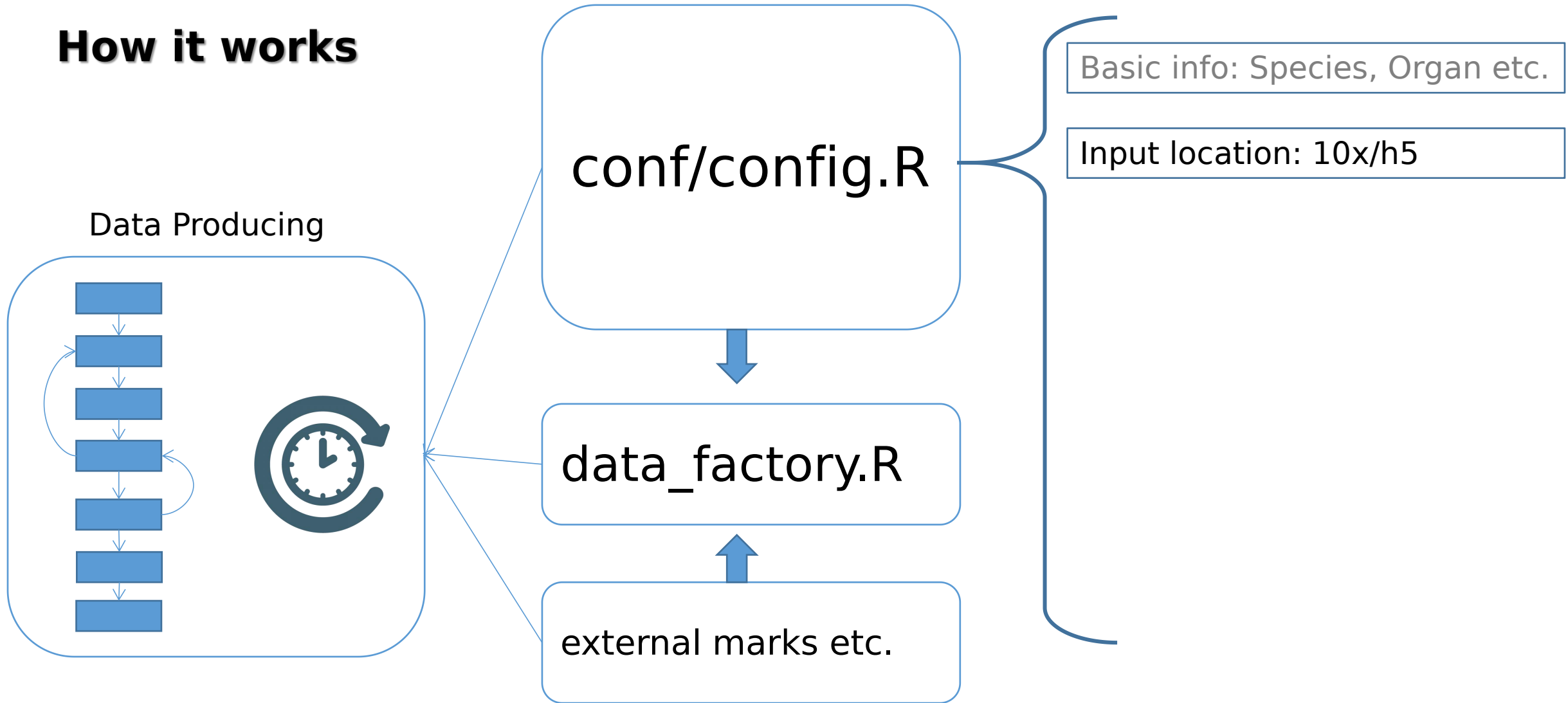
Basic info: Species, Organ etc.

```
### ----- Initail info -----  
PROJECT = "Mouse Blood project" ## set project name  
ORGAN = 'Blood' #For external annotation. Options: Blood, Heart, Intestine, Kidney  
SPECIES = "Mouse" #For external annotation. Options: Human, Mouse  
MCA_NAME = "Bone-Marrow" #For MCA annotation. Options: check http://bis.zju.edu.cn/MCA/  
  
# filtering params when create seurat object  
MINCELLS = 5  
MINGENES = 50  
  
### ----- Data SRC -----  
ANNOTATION_EXTERNAL_FILE = "external/Human_and_mouse_cell_markers-Markers.tsv"  
  
data_src = c(  
  A_MxCre = "data/A_MxCre",  
  B_MxCre = "data/B_MxCre",  
  C_Csnk = "data/C_Csnk",  
  D_Csnk = "data/D_Csnk"  
)  
  
## ----- SET REPLICATE GROUP -----  
stage_lst = c(  
  A_MxCre = "MxCre",  
  B_MxCre = "MxCre",  
  C_Csnk = "Csnk",  
  D_Csnk = "Csnk"
```

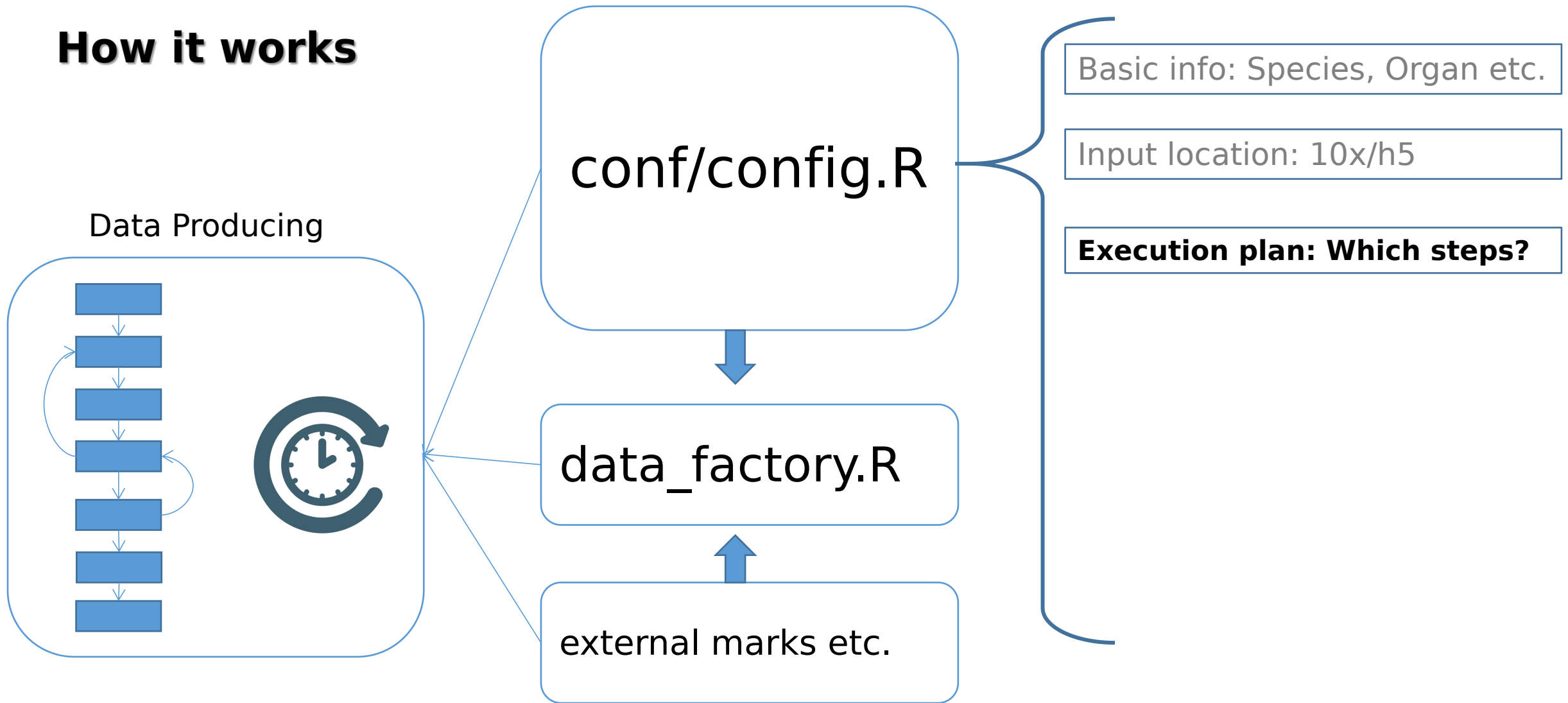
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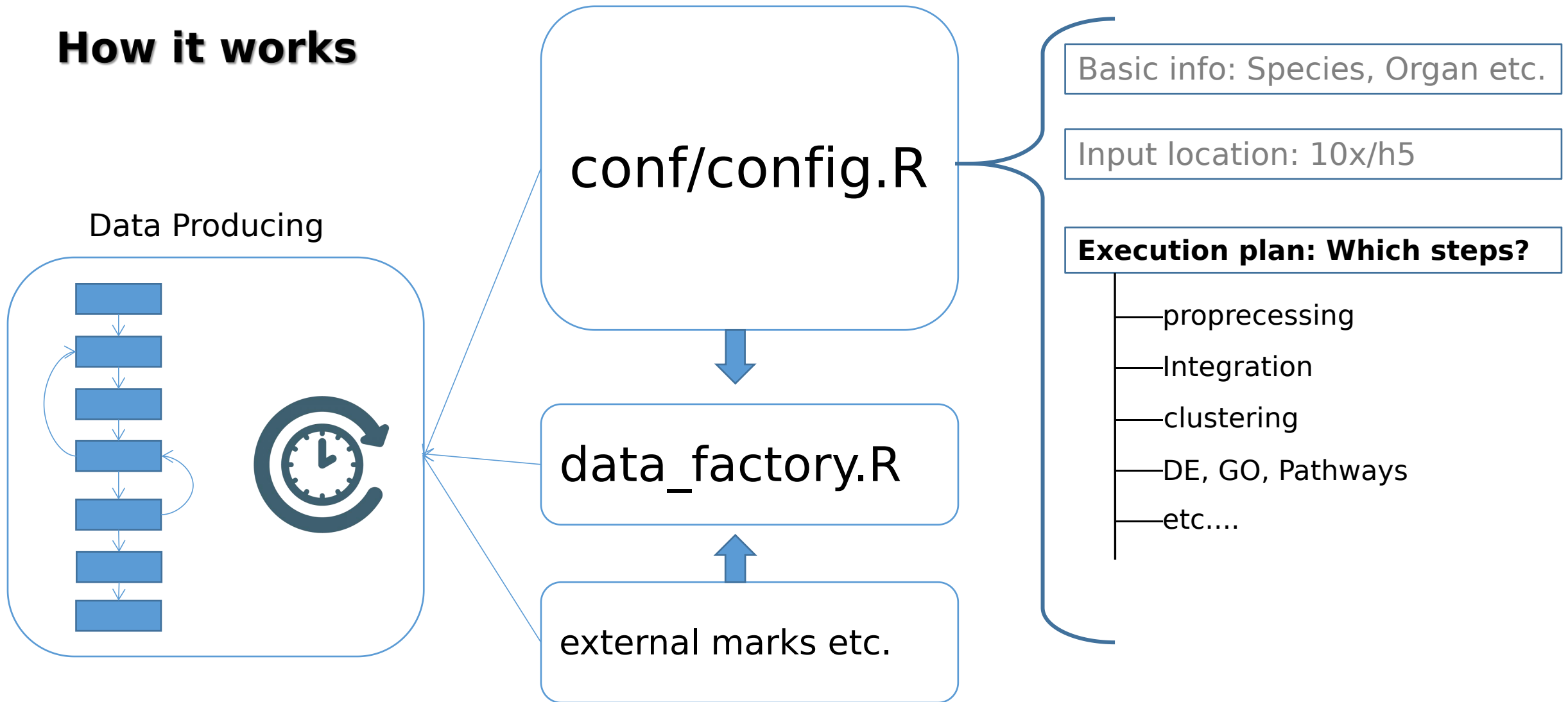
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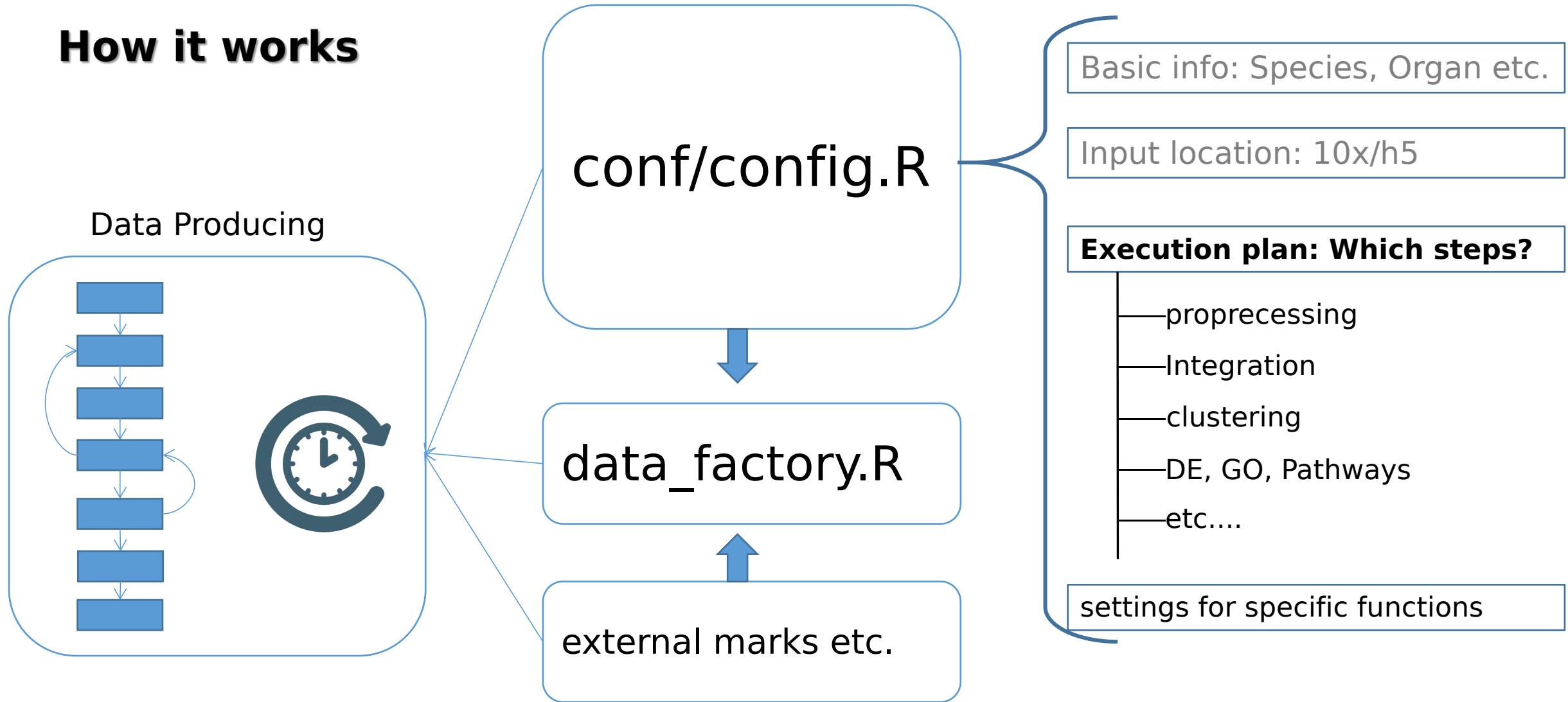
How it works



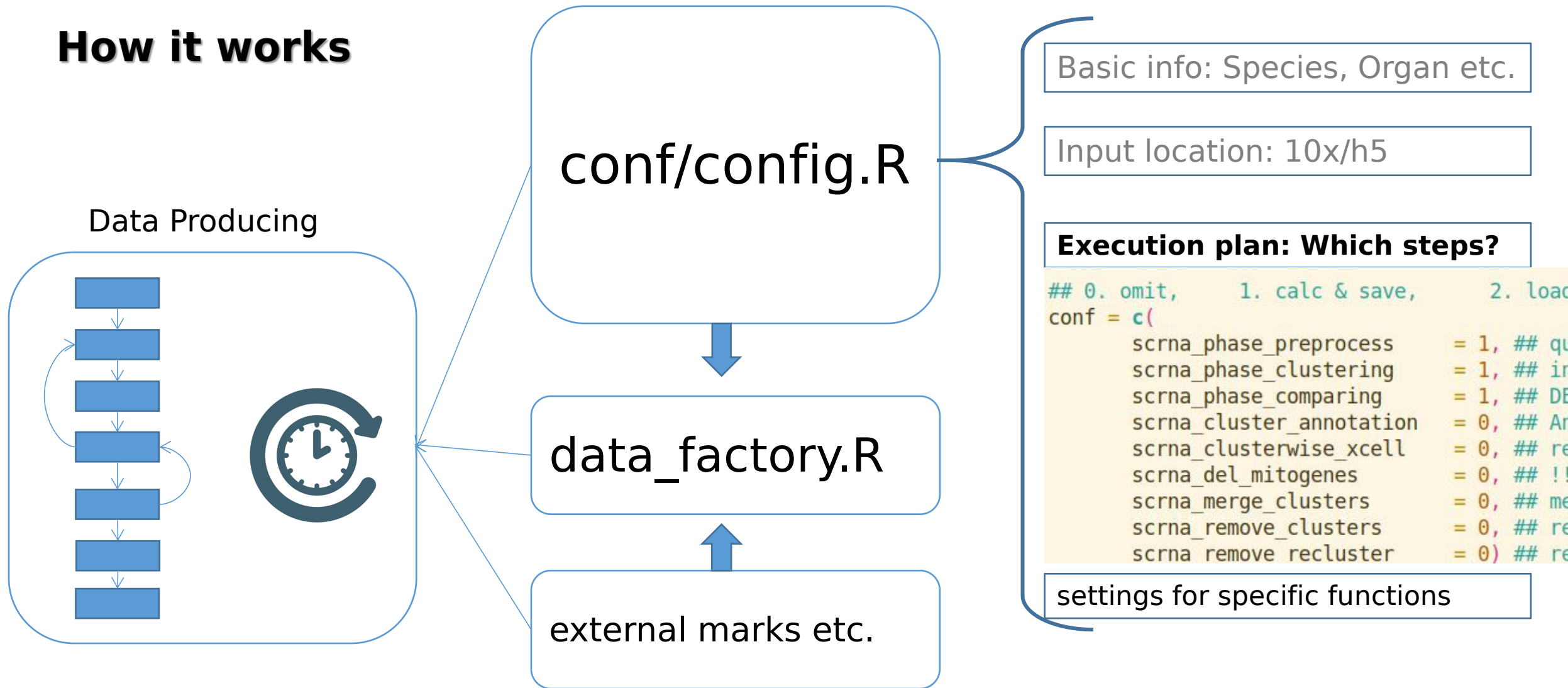
How it works



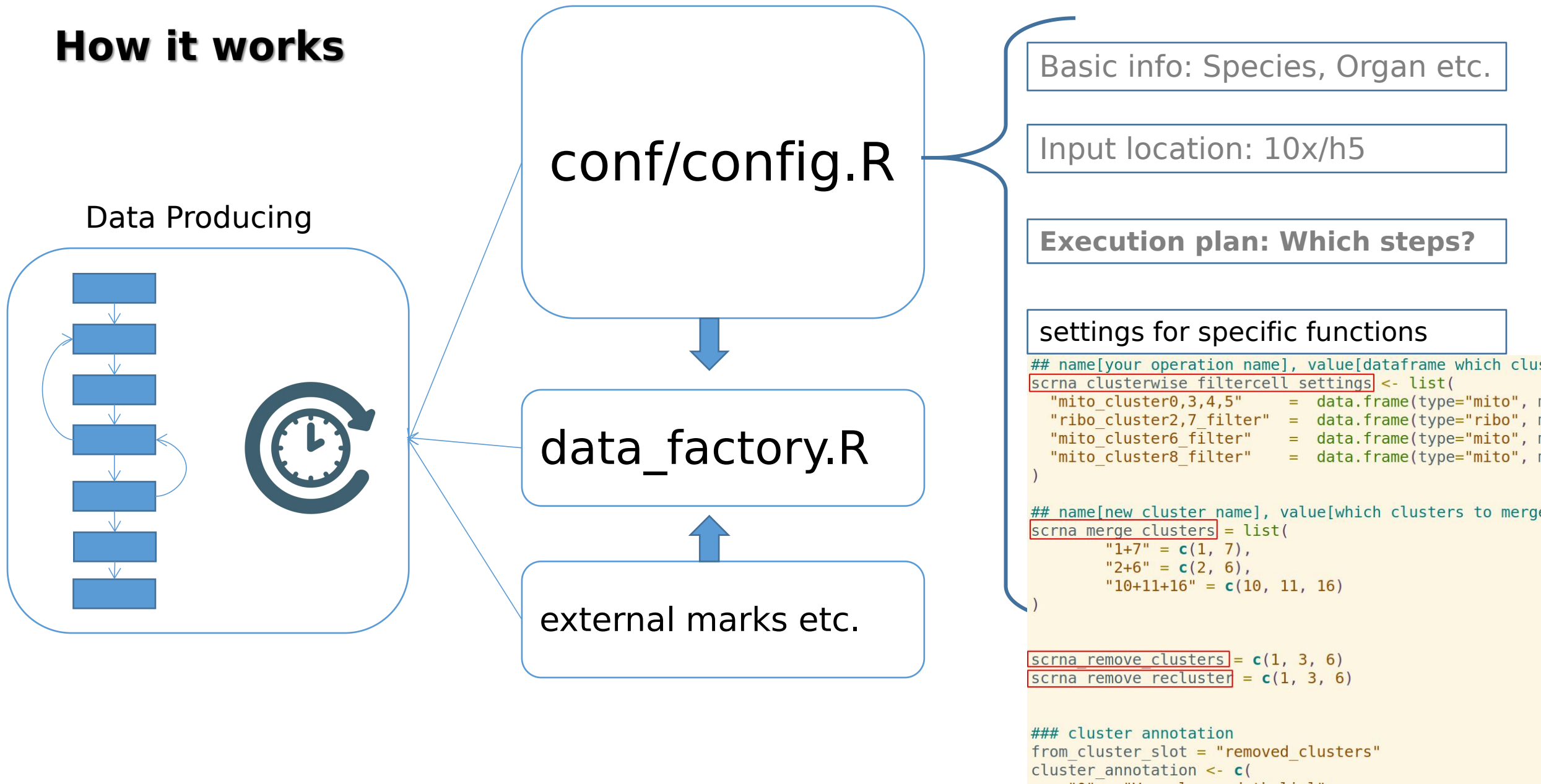
How it works



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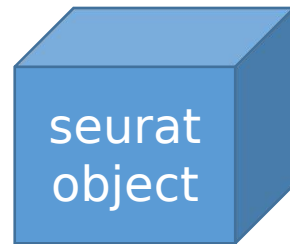
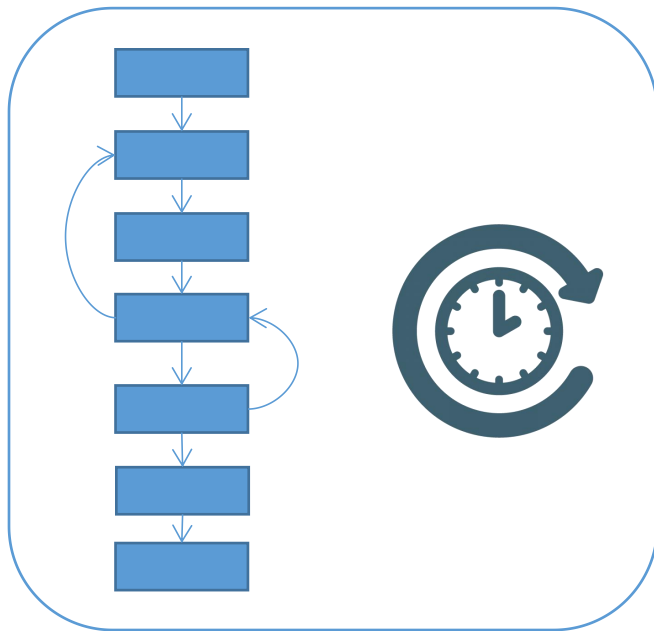


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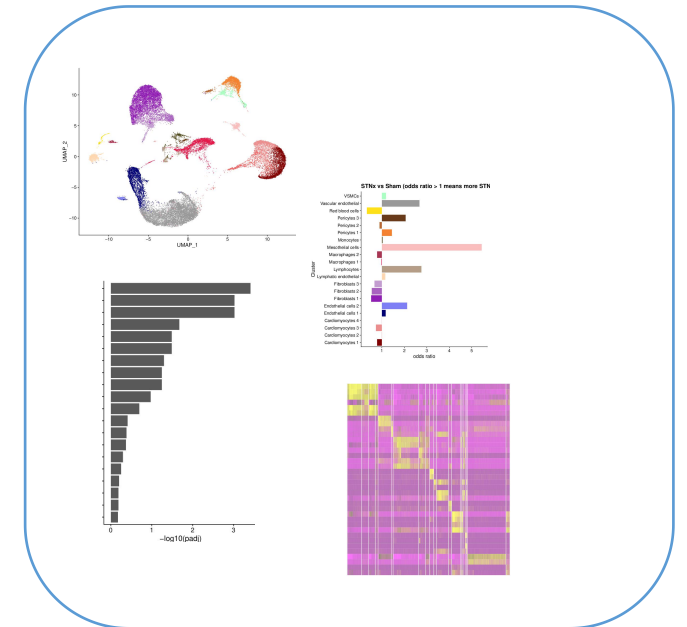
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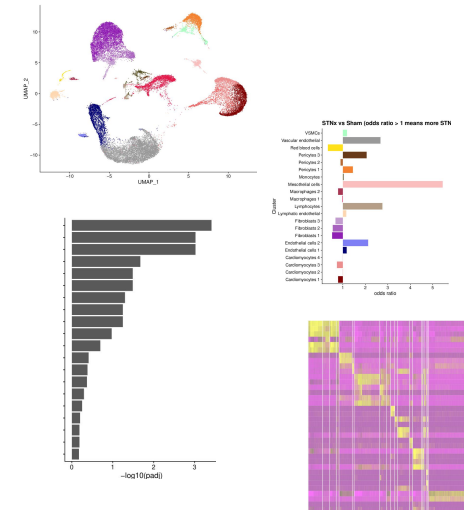


Visualization

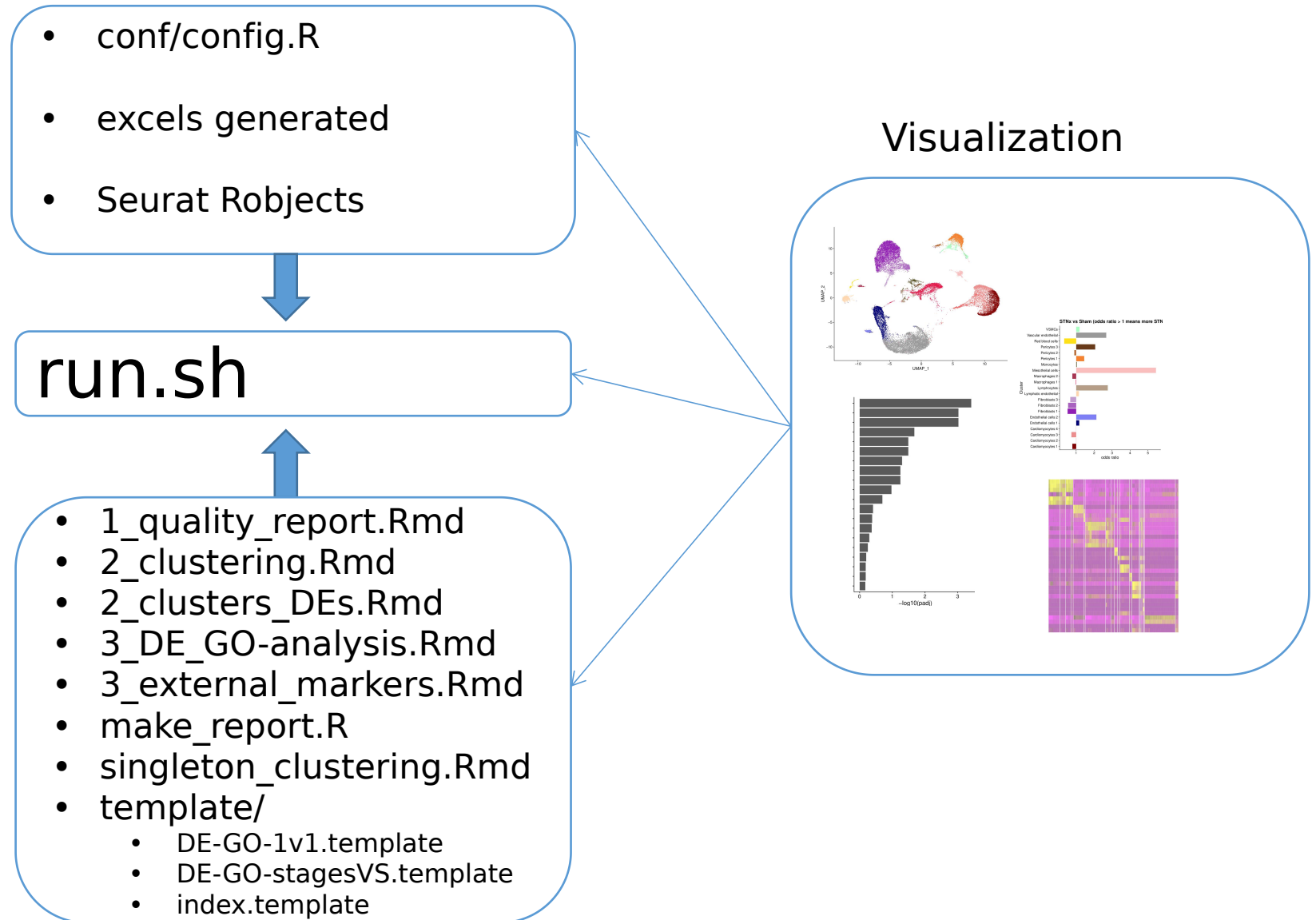


How it works

Visualization



How it works



How it works

```
#!/bin/bash
RED='\033[0;31m'
NC='\033[0m' # No Color
```

```
FUNCS=(
    QC
    DEs
    Clusters
    # DEGO
    ## EXT_MARKERS
    DEGO_1v1
    DEGO_stage
)
```

```
#!!!!!!!!!!!!-----clusters to choose-----
# In general, we choose seurat_clusters,
# If you are using removed or merged clusters,
# choose the following:
# seurat_clusters
# merged_clusters
# removed_clusters
# remove_recluster
```

```
#cluster="removed_clusters"
#cluster="remove_recluster"
#cluster="merged_clusters"
#cluster="annotation"
#cluster="singleton"
```

```
cluster="seurat_clusters"
```

```
#!!!!!!!!!!!!-----
```

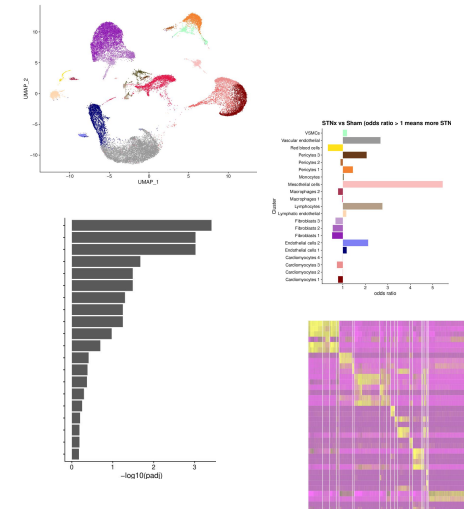
```
echo -e "Use cluster slot ${RED} $cluster ${NC}"
mkdir -p report/data
```

- conf/config.R
- excels generated
- Seurat Robjects

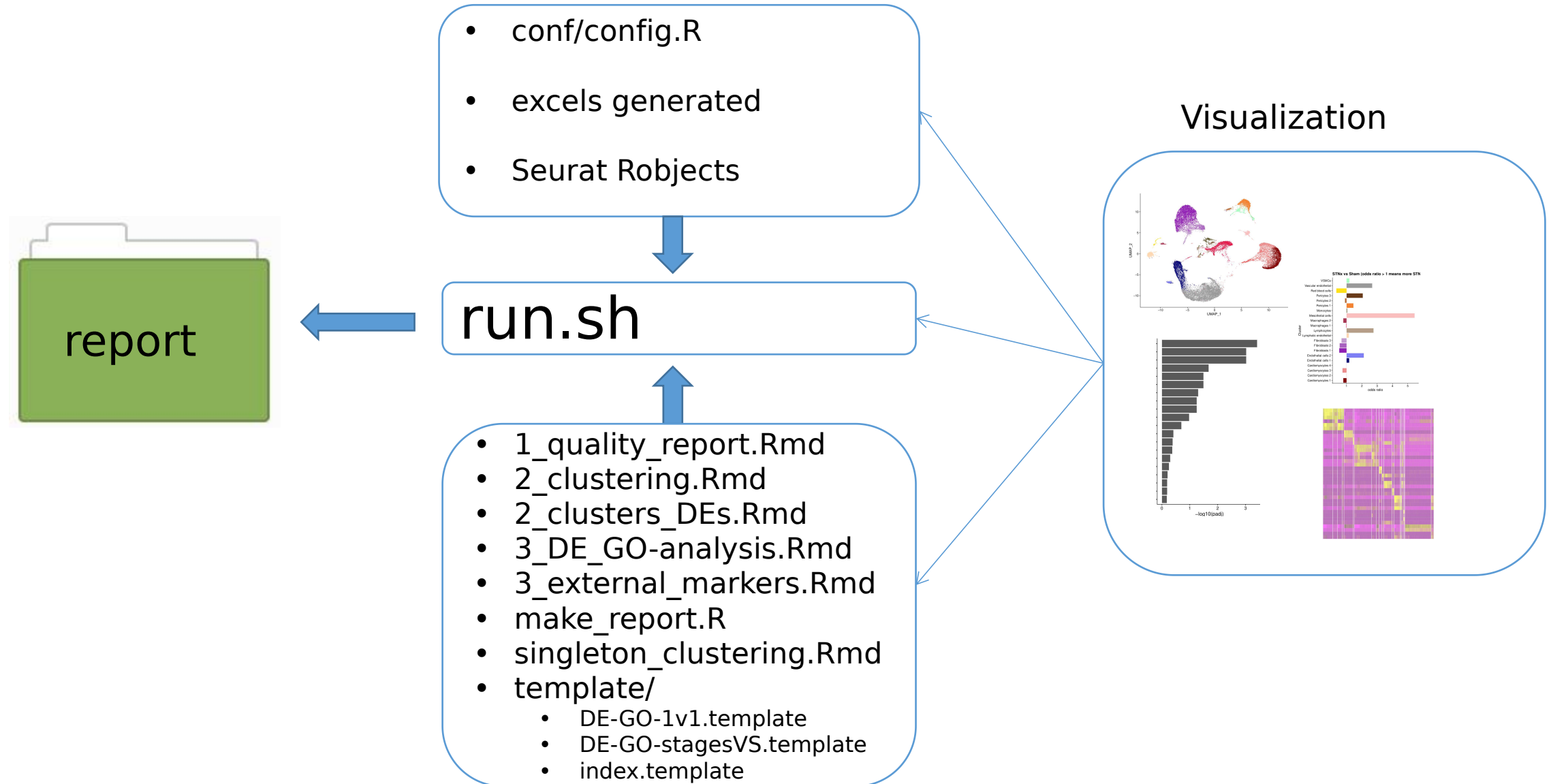
run.sh

- 1_quality_report.Rmd
- 2_clustering.Rmd
- 2_clusters_DEs.Rmd
- 3_DE_GO-analysis.Rmd
- 3_external_markers.Rmd
- make_report.R
- singleton_clustering.Rmd
- template/
 - DE-GO-1v1.template
 - DE-GO-stagesVS.template
 - index.template

Visualization



How it works

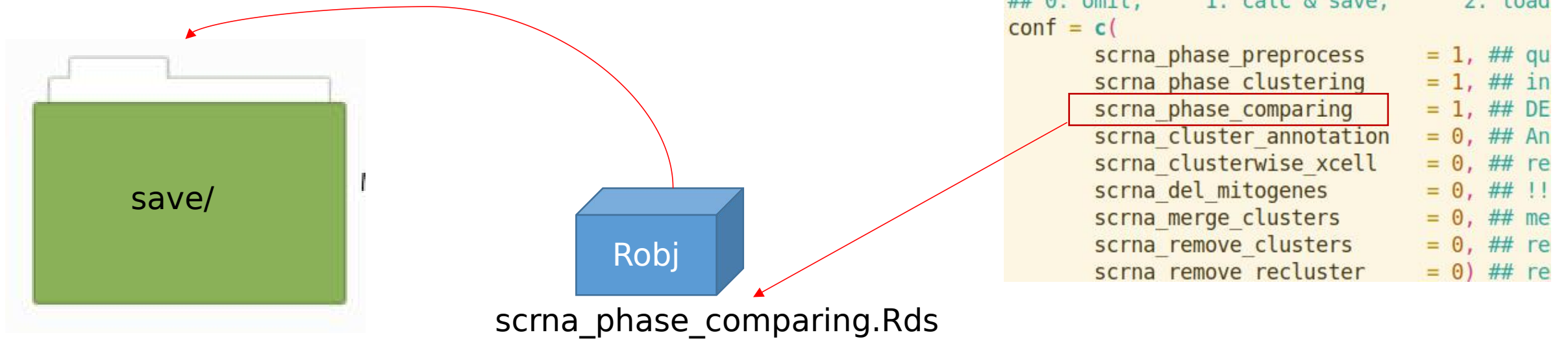


Most Frequent questions

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```
## 0. omit,      1. calc & save,      2. load
conf = c(
  scrna_phase_preprocess = 0, ## qu
  scrna_phase_clustering = 0, ## in
  scrna_phase_comparing = 2, ## DE
  scrna_cluster_annotation = 0, ## An
  scrna_clusterwise_xcell = 0, ## re
  scrna_del_mitogenes = 0, ## !!
  scrna_merge_clusters = 0, ## me
  scrna_remove_clusters = 0, ## re
  scrna_remove_recluster = 0) ## re
```


Most Frequent questions

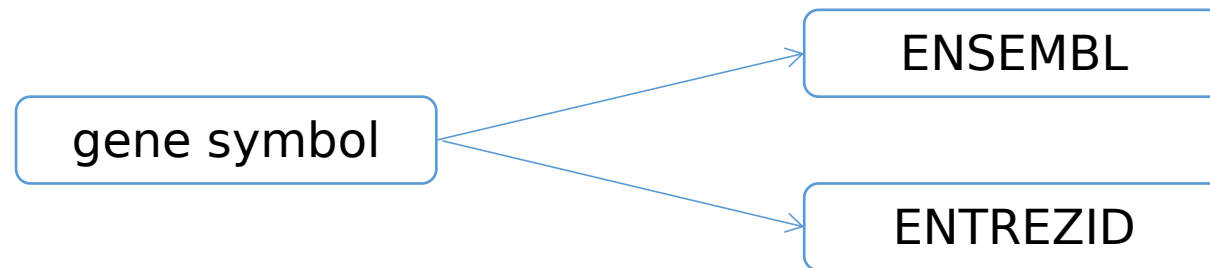
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!!! umap reduction name

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## 0. omit,      1. calc & save,      2. load
conf = c(
  scrna_phase_preprocess = 0, ## qu
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- Why there are so many warnings especially for GO & pathway analysis?
- Failed to run visualization part: cannot find 'INTE_UMAP'
- Why there are so many Robjects generated?

```
378M Dec 31 13:22 scrna_for_debug.Rds  
25M Jan 4 10:29 scrna_rawdata.Rds  
298M Jan 4 10:30 scrna_phase_preprocess.Rds  
378M Jan 4 10:49 scrna_phase_clustering.Rds  
659M Jan 4 11:34 scrna_phase_comparing.Rds
```

TODO

- [] Merge code from Tiago
- [x] Add harmony integration
- [] NABA geneset score
- [x] Add KEGG/Reactome/hallmark visualization
- [] scHCL for human cell annotation
- [] Integrated with ligand receptor analysis?

Dummy example

- our hpc : 134.130.18.27
 - module add scRNA/1.0.3
 - /data/scRNA/scrna_seurat_pipeline_demo

Thanks

Q&A