Application of Next Generation Sequencing (NGS) in Food Authenticity

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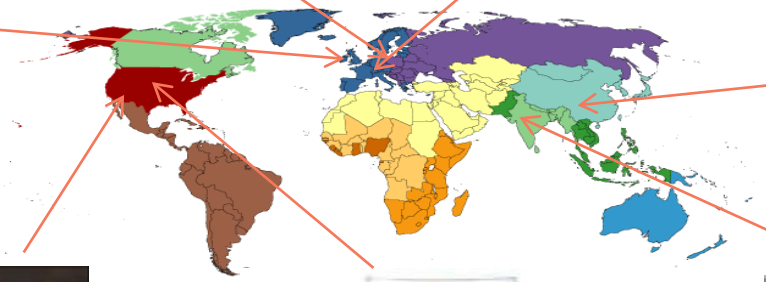
Current Authenticity Issues and Challenges

Species Substitution

Horsemeat (EU)

Olive oil (EU)

Chili powder (Britain)



Oil (China)

Maple syrup (USA)

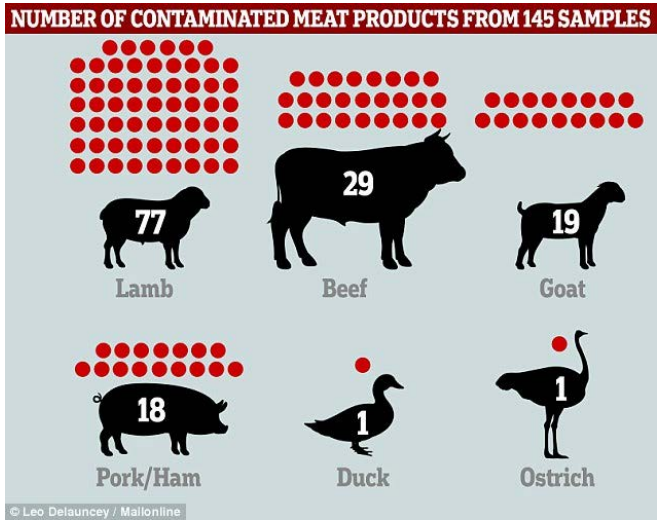
Apple juice (USA)

Milk (India)

Food fraud has been a global issue

Current Authenticity Issues and Challenges

UK Food Standards Agency Says 1/5 of Meat It Tested Contained Mystery DNA



- One in five of 665 tested samples “were partly or wholly made up of unspecified meat.”

https://www.bbc.co.uk/news/uk-45371852

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Analytical Methods

DNA barcoding and mini-barcoding in authenticating processed animal-derived food: A case study involving the Chinese market

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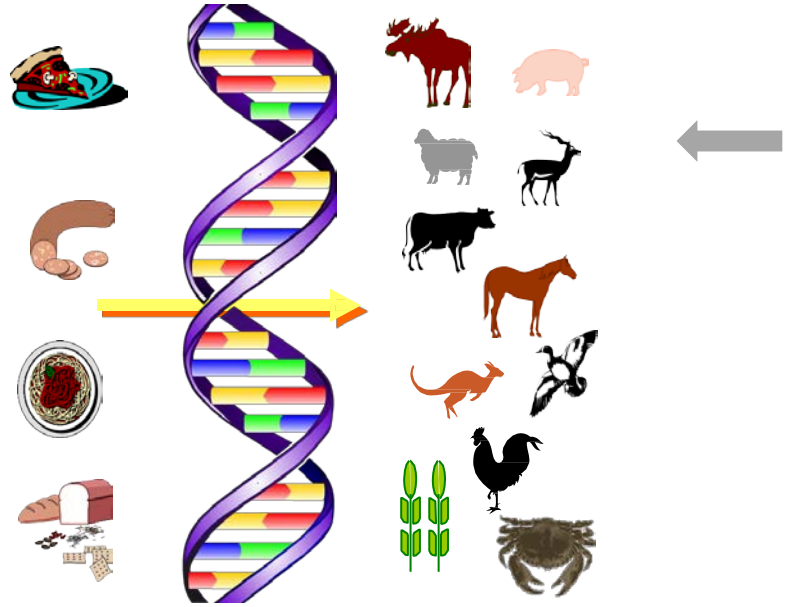
- Approximately 23% of selected commercial samples were determined to be mislabeled.

Xing, Ran-Ran, et al. Food Chemistry (2019): 125653.

Current Authenticity Issues and Challenges



- DNA techniques are now routinely and officially used for species identification in food products.

Advanced methods used to test for authenticity

Current Authenticity Issues and Challenges

Current Issues

- The vast majority of existing DNA typing methods are targeted methods that can only detect either one or a small number of ingredients at a time.

- For example: To detect a claimed “100% beef” product

- Traditional DNA testing with PCR technique :
  - Check the presence or not of some selected specific target(s)

Test (PCR) of a beef product: *presence of Beef* *« Positive » result* √
presence of Horse : *« Negative » result* ×
presence of Pork : *« Negative » result* ×
. ……..

  - duck ? chicken ? dog ? ……

We won’t know until we check for them.

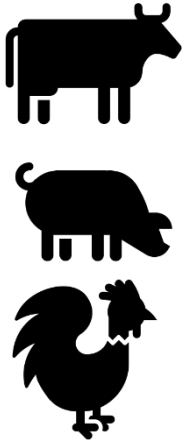
Why NGS ?

Next Generation Sequencing (NGS)

- Allows simultaneous detection of multi-species
- Untargeted screening approach

Can identify all animal species contained in the beef product.

NGS



What is Next Generation Sequencing?

- **1st Generation = Sanger Sequencing**
  - –~ 1000 bps
  - –Low throughput
  - –High sequencing quality

Golden standard

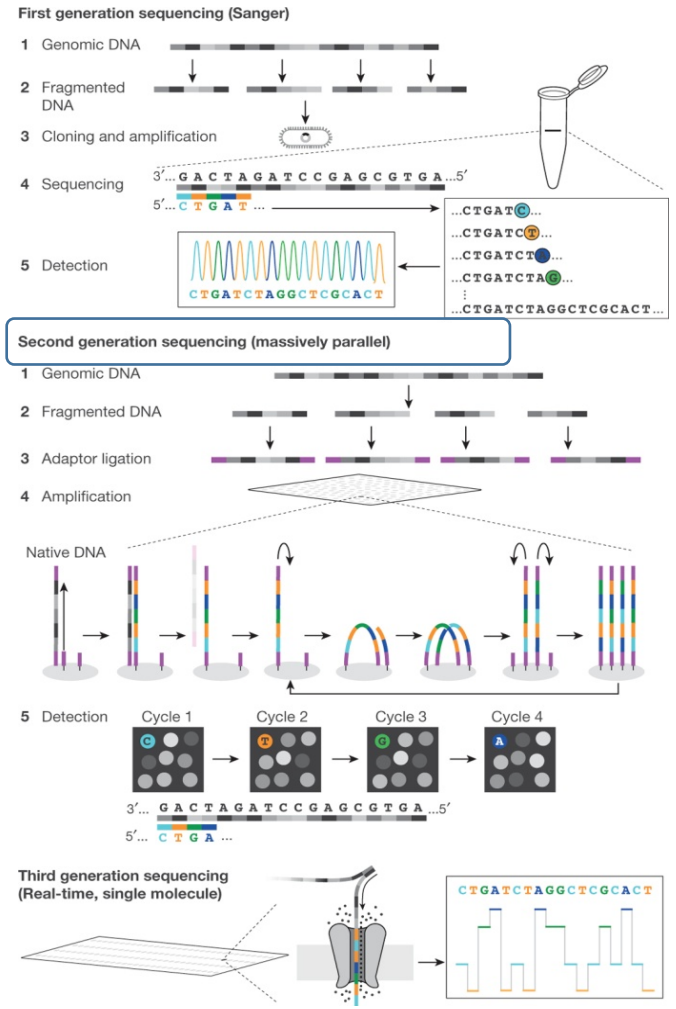
- **2nd Generation = Next Generation Sequencing**
  - –~ 600 bps
  - –High throughput
  - –High sequencing quality

- **3rd Generation = Single Molecule Sequencing**
  - –~ 10K – 1 M bps
  - –Medium throughput
  - –Acceptable sequencing quality

NGS also known as

High throughput sequencing

Ultra-deep sequencing



J Shendure *et al. Nature* 1–9 (2017)
doi:10.1038/nature24286

nature

What NGS Can Do?

Human Genetics Research

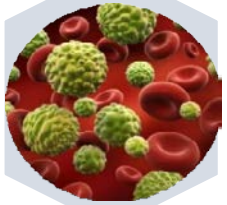
Forensic Genomics

Metagenomics Research

Microbial Genomics
Microbiota

Complex Disease Genomics

Oncology



Agri-genomics Research

Applying agricultural genomics to improve the food supply

Reproductive Health

Noninvasive Prenatal Testing (NIPT)

Drug Development

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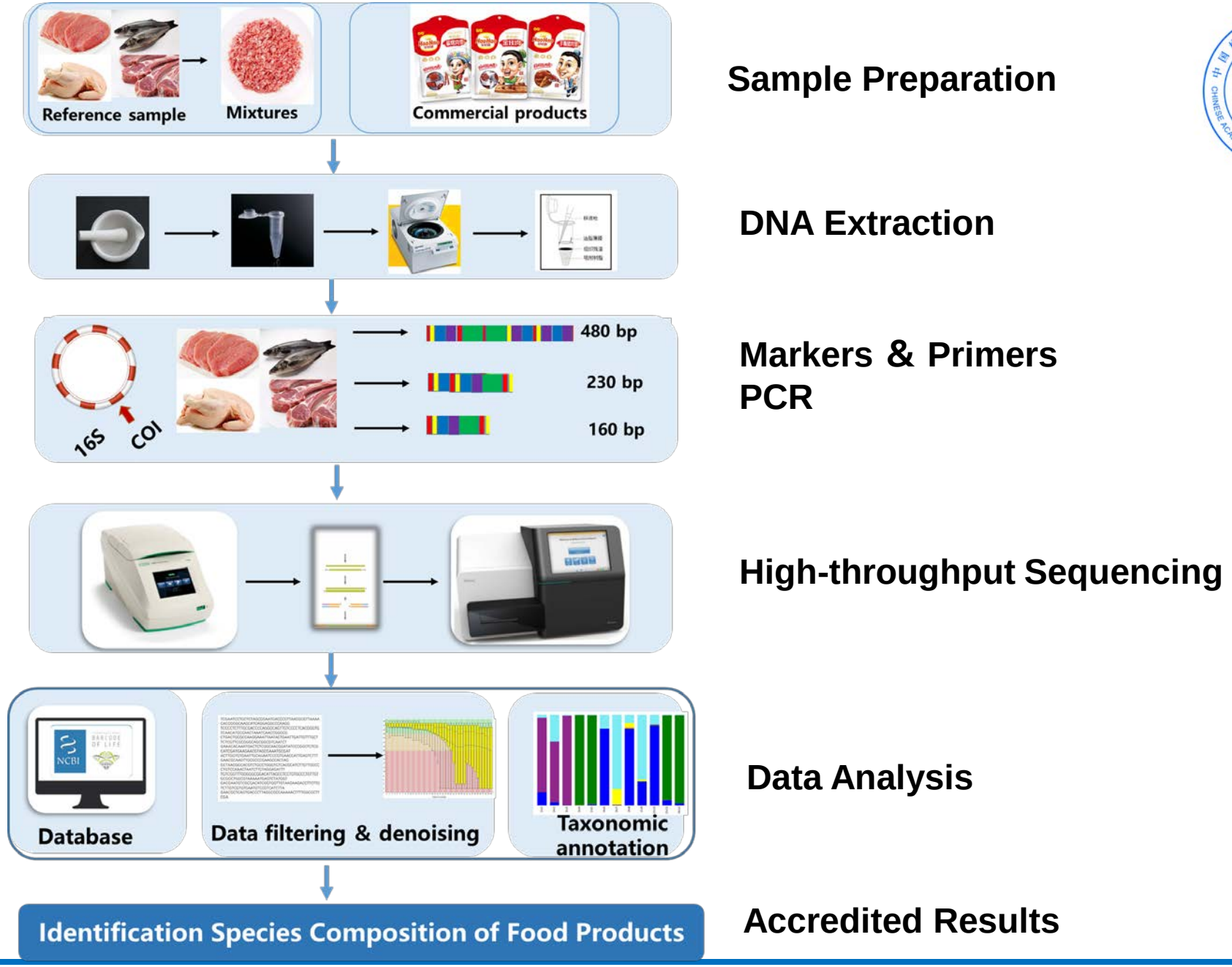
Food Authenticity

NGS Application

Animal Species Identification in Food Products

Workflow



Sample Preparation

DNA Extraction

Markers & Primers PCR

High-throughput Sequencing

Data Analysis

Accredited Results

Sample Preparation

Artificially mixed meat species

No.	Raw Material	Species components in prepared mixture
S1-1	meat	50% pig + 50% chicken
S1-2	meat	50% pig + 50% chicken
S2-1	meat	90% pig + 10% chicken
S2-2	meat	90% pig + 10% chicken
S3-1	DNA	90% pig DNA + 10% chicken DNA
S3-2	DNA	90% pig DNA + 10% chicken DNA

Assess the quantitative ability

Effect of DNA extraction

Artificially mixed meat and fish species

No.	Raw Material	Species components in prepared mixture
H1	meat	20% duck+20% salmon+59% Atlantic salmon+1% chicken
H2	meat	20% duck+20% salmon+50% Atlantic salmon+10% chicken
H3	meat	20% duck+20% salmon+30% Atlantic salmon+30% chicken
H4	meat	20% duck+20% salmon+ 0 Atlantic salmon +60% chicken
H5	meat	12.5% duck+12.5% salmon+12.5% Atlantic salmon +12.5% chicken+12.5% rainbow trout+12.5% pig+12.5% pink salmon+12.5% tilapia

Sensitivity

Accuracy

Throughput

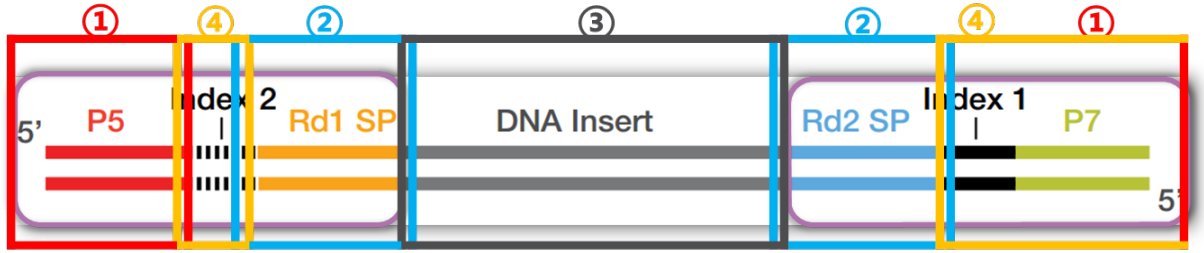
Library Construction

PCR amplification of a marker gene

Marker choice：

- Variable region for classification
- DNA from processed food samples can be degraded, mini-barcodes (100–300 bp) may be more suitable.
- The read length of the second generation sequencing is much shorter (100–600 bp).

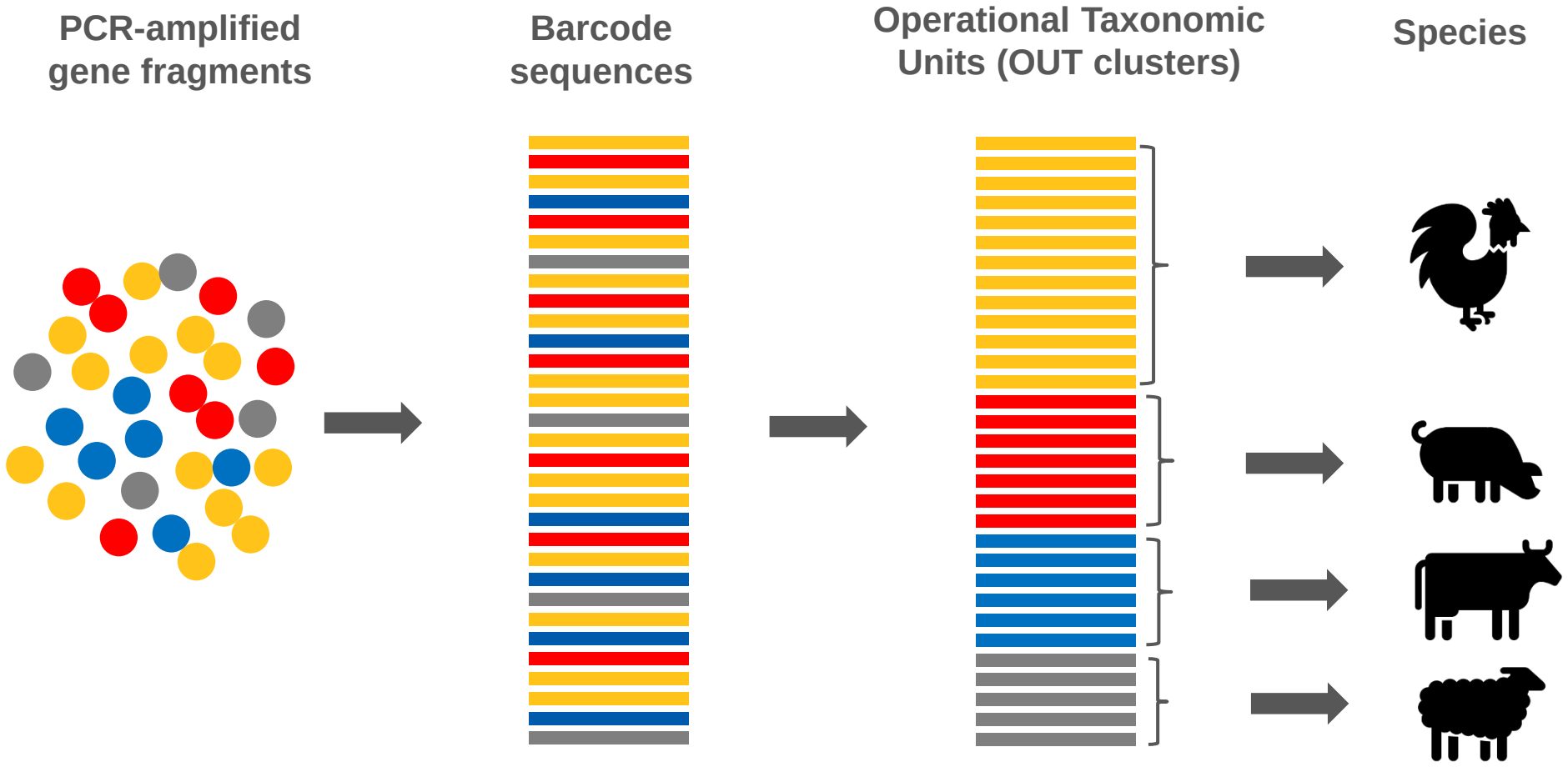
16S rRNA ~220 bp



A DNA library is a population of DNA fragments ready for sequencing.

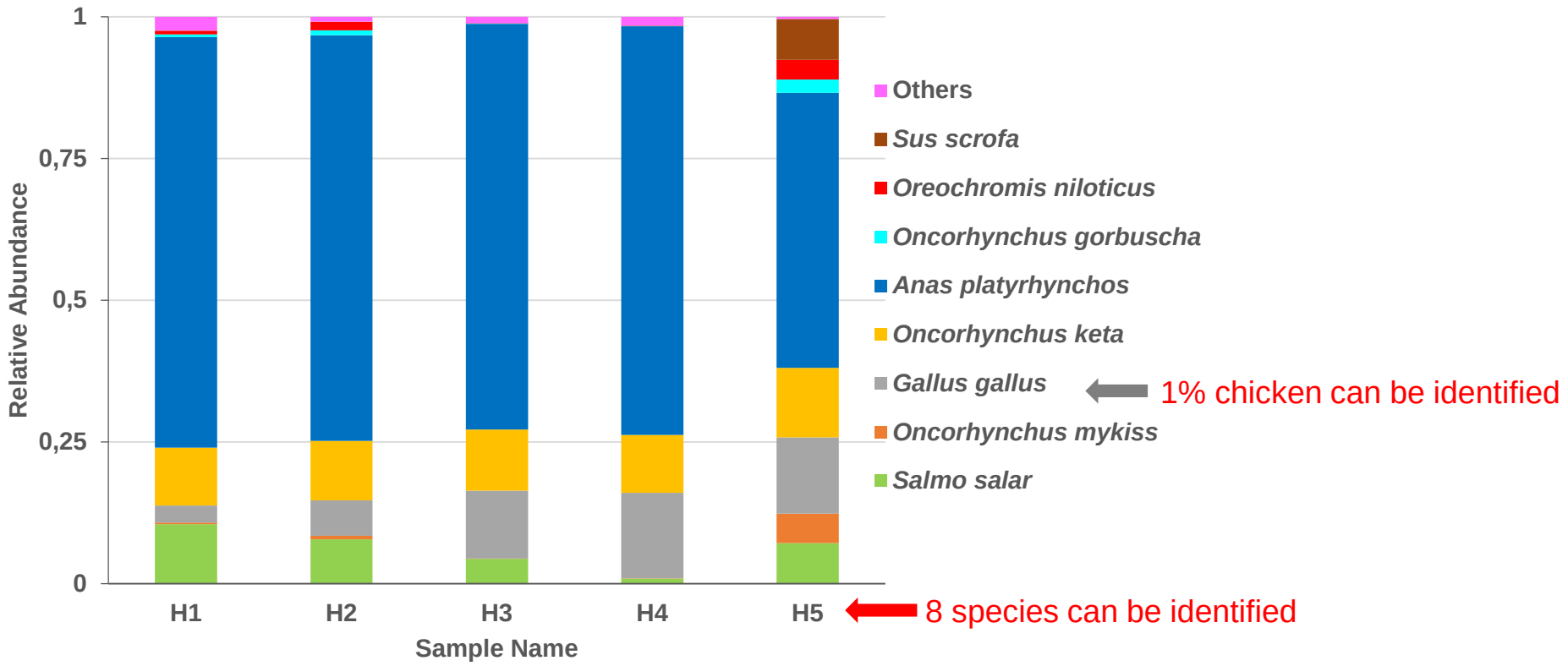
LIBRARY CONSTRUCTION

Cluster Generation



Species Identification

The method can identify different species in complex sample

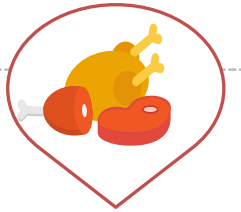


Relative abundance of the animal species in the artificially mixed samples

NGS Food Screening for Species Identification

Different commercial animal-derived food representing a variety of product types and species were obtained from supermarket, local market, restaurants and three online retail sources in China.

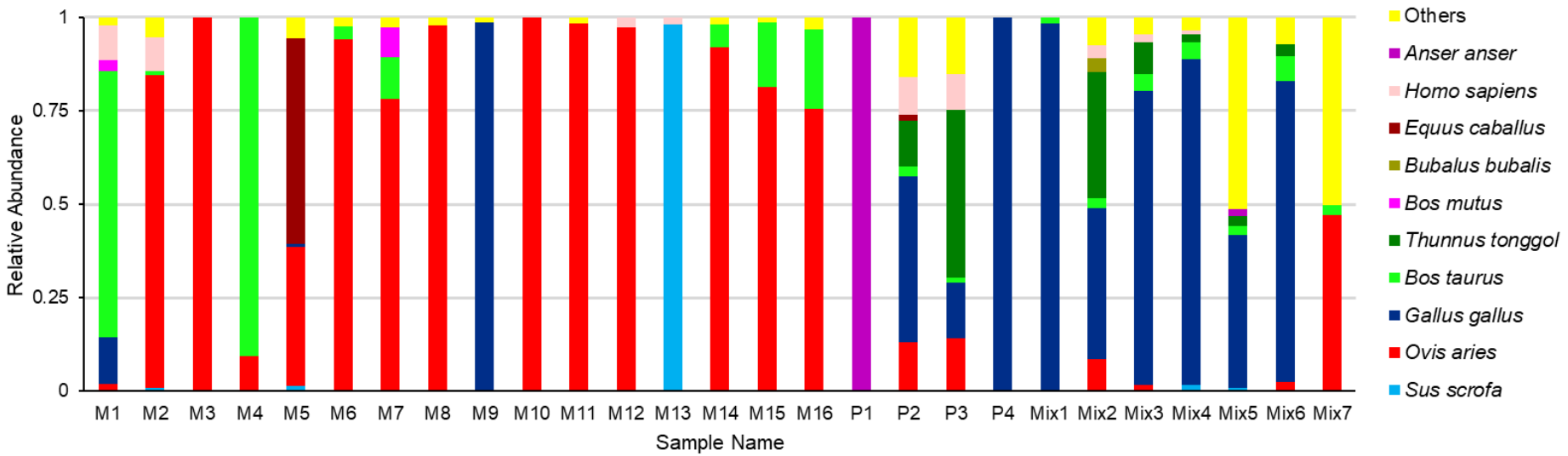
Listed one or several animal species on the label.



Twenty-seven commercial meat and poultry products, 11 fish products were analyzed.

Commercial Food Products

NGS Food Screening for Species Identification

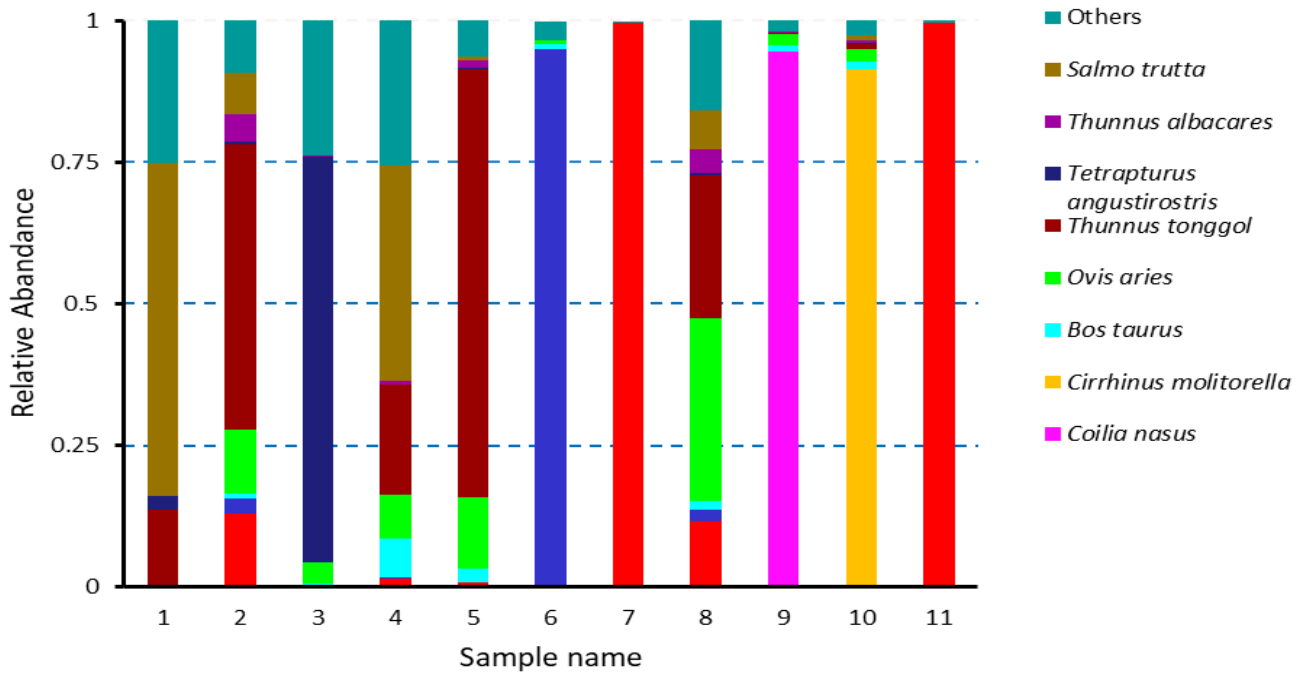


Relative species composition in meat samples based on sequence read counts

Classification	Example	Product	Species listed	Species identification	Yes/No?
Nine samples sequenced were consisted with the label	M3	Mutton	Sheep	Sheep	√
Ten samples listed a single animal species were detected to contain a mixture of two or more species	M1	Beef Stuffing	Cattle	Cattle, Chicken	×
Six samples listed more than one animal species were detected not consisted with the label	Mix1	Dog Food	Duck, Chicken, Cattle, Sheep, Deer	Chicken, Cattle	×
Two samples were entirety mislabeled that contained completely different animal species with the label	M4	Roasted Camel Meat	Camel	Cattle, Chicken	×

Xing, Ran-Ran, et al. Food Control 101 (2019): 173-179.

NGS Food Screening for Species Identification



Relative species composition in fish products based on sequence read counts

Sample Name	Product	Species listed	Species identification	Yes/No?
1	Fried Salmon Floss	Salmon	*Salmo trutta*, *Thunnus albacares*	×
2	Tuna Floss	Tuna	Tuna, Chicken, Sheep	×
11	**Fish balls**	**No label**	**Chicken**	**×**

Strengths and Limitations of NGS

KEY ADVANTAGES OF NGS

- High throughput with sample multiplexing
- High sensitivity to detect low-frequency variants
- Universal database，database continuously growing
- High specificity

SIGNIFICANT CHALLENGES

- NGS infrastructures must consist of appropriate expertise
- Quantification is hard for NGS in food authenticity testing
- For food samples, the price is relatively high

The Future of NGS in Food Authenticity

The cost of NGS platforms are decreasing

2001

Human Genome Project

20-year

$

$3billion

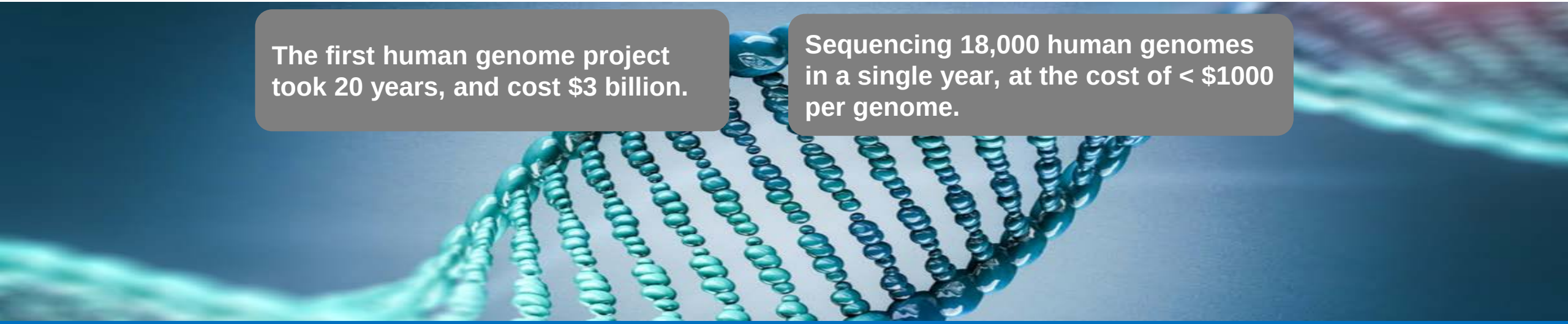
NOW

Human Genome Sequencing

< 1 day

$

< $1000



The first human genome project took 20 years, and cost $3 billion.

Sequencing 18,000 human genomes in a single year, at the cost of < $1000 per genome.

The Future of NGS in Food Authenticity

The development of NGS

2nd Generation
High throughput

3rd Generation
Long read-length
Single molecule, PCR-free

NANOPORE MinION
Portable, Real-time

Thanks for your attention

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