How to examine the health effect of chemicals

Current status of testing methods development by METI and MHLW

Dr. Masahiro Takeyoshi

Chemicals Evaluation and Research Institute (CERI), Japan

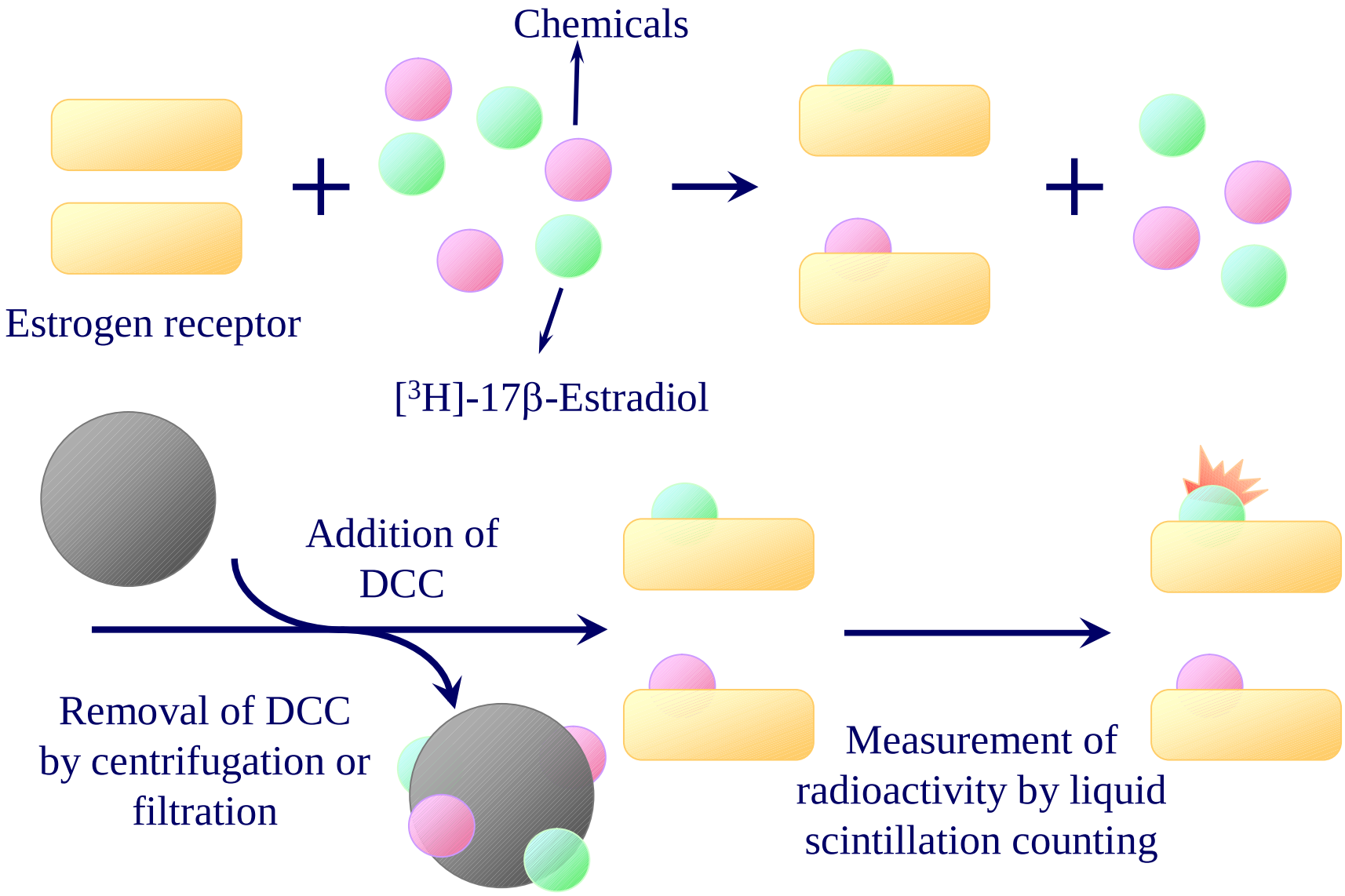
OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals

Level 1 **Sorting & prioritization based upon existing information**	- physical & chemical properties/fate (MW, reactivity, volatility, persistence and bioacumulation, pH, Po/w - exposure information/models (production volume, release and use pattern, human and environ-mental monitoring data, etc.) - hazard information (e.g. QSAR, human data, available toxicological data)	
Level 2 ***In vitro* assays providing mechanistic data**	- ER, AR, TR receptor binding affinity - transcriptional activation - aromatase and steroidogenesis inhibition *in vitro* - Aryl hydrocarbon receptor recognition/binding - QSARs	- High Through Put Prescreens - Thyroid function - Fish hepatocyte VTG assay - Others (as appropriate)
Level 3 ***In vivo* assays providing data about single endocrine mechanisms**	- Uterotrophic assay (estrogenic related) - Hershberger assay (androgenic related) - Non –receptor mediated hormone function - Others (e.g. thyroid)	- Fish VTG (vitellogenin) assay (estrogenic related)
Level 4 ***In vivo* assays providing data about multiple endocrine mechanisms**	- enhanced OECD 407 (endpoints based on endocrine mechanisms) - male and female pubertal assays - adult intact male assay	- Fish gonadal histopathology assay - Frog metamorphosis assay
Level 5 ***In vivo* assays providing adverse effects data from endocrine & other mechanisms for RA**	- 1-generation assay (TG405 enhanced)[1] - 2-generation assay (TG416 enhanced)[1] - reproductive screening test (TG421 enhanced) [1] - combined 28 day/reproduction screening test (TG 422 enhanced) [1] 1 Potential enhancements will be considered by VMG mamm	- Partial and full life cycle assays in fish, birds, amphibians & invertebrates (developmental and reproduction)

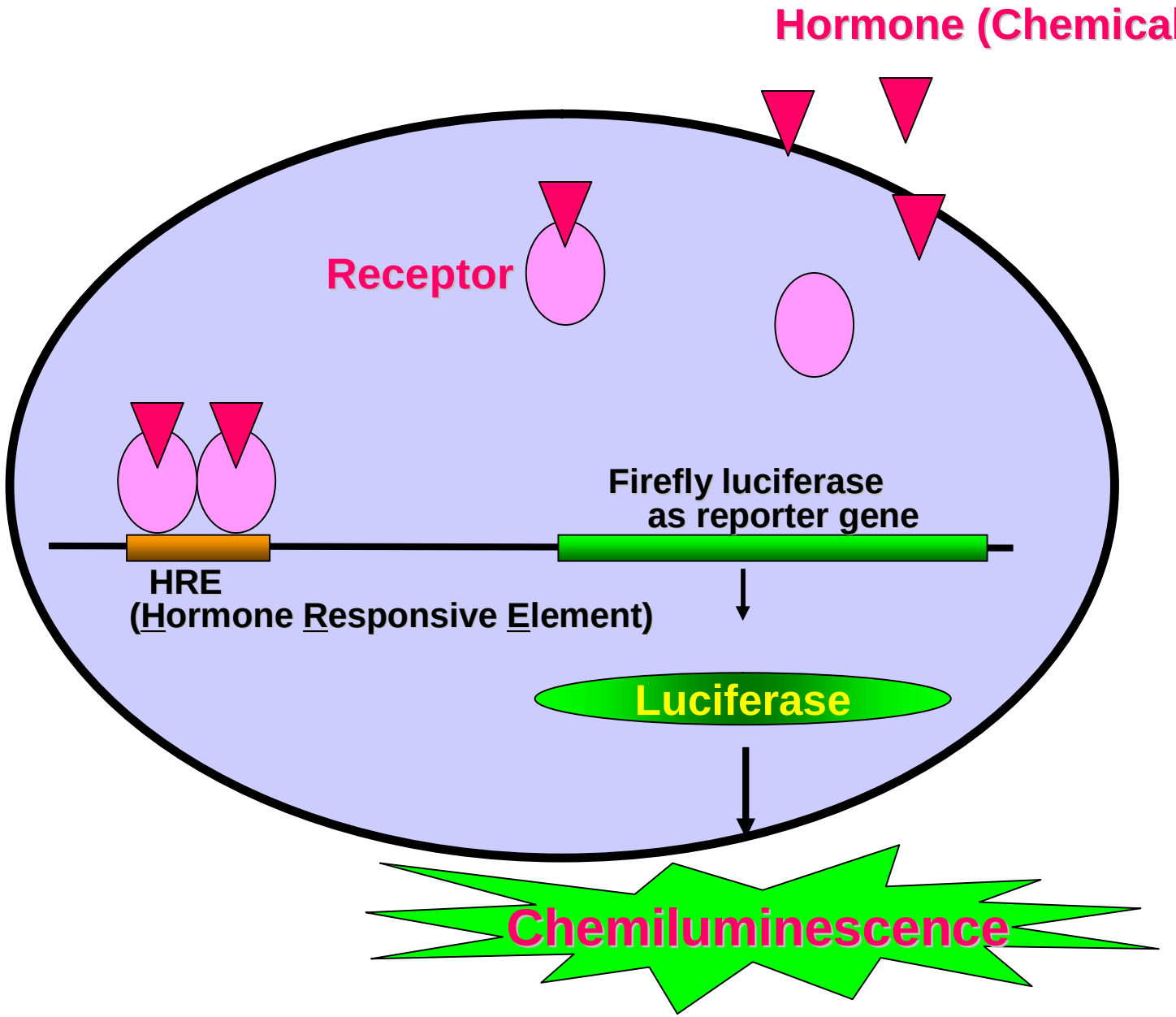
Level 2 testing

- ER, AR, TR receptor binding affinity
- Transcriptional activation
- Thyroid function
- Aromatase and steroidogenesis inhibition
- Aryl hydrocarbon receptor recognition/binding
- QSARs

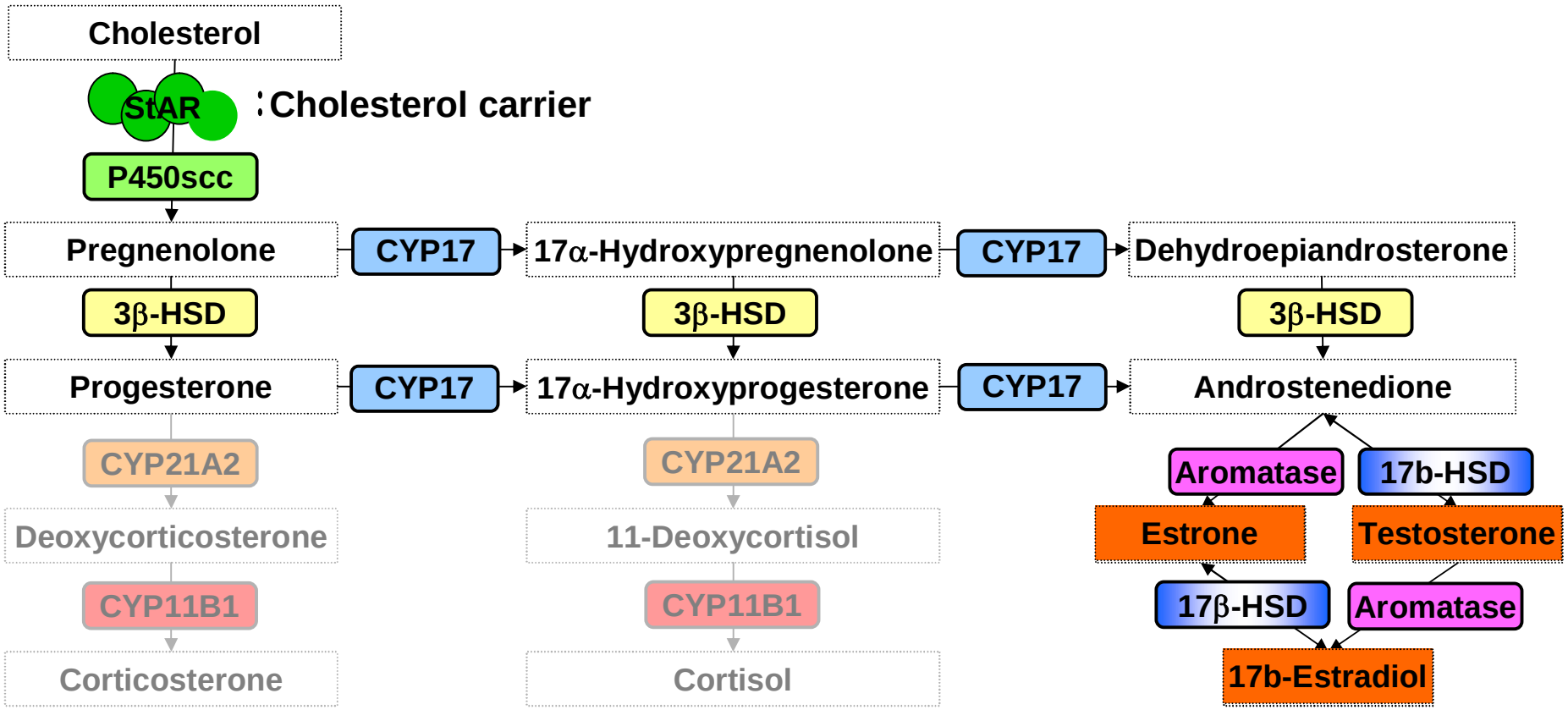
Principle of Receptor-Binding Assay



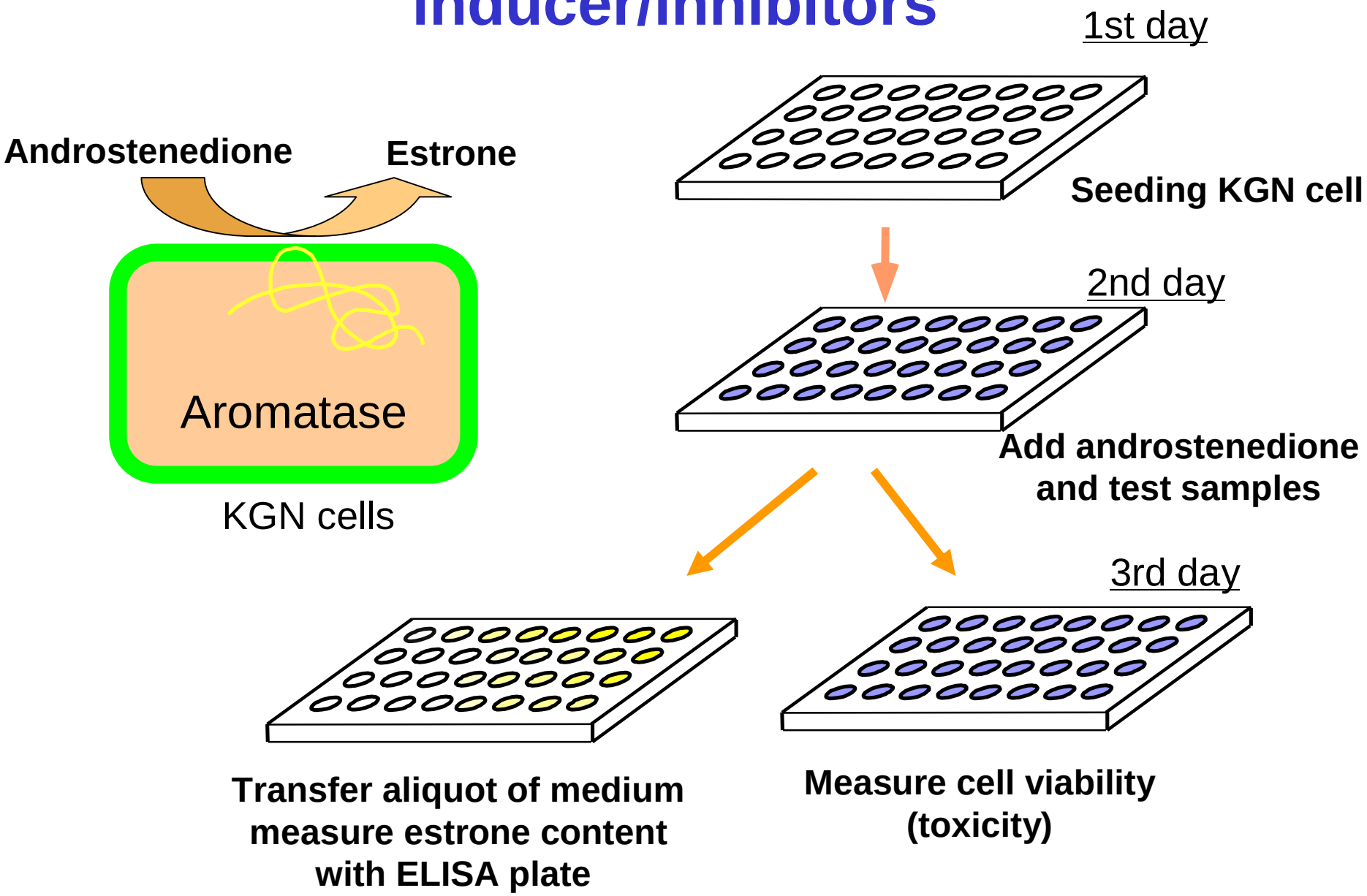
Principle of Reporter gene assay



Steroidgenesis Pathway



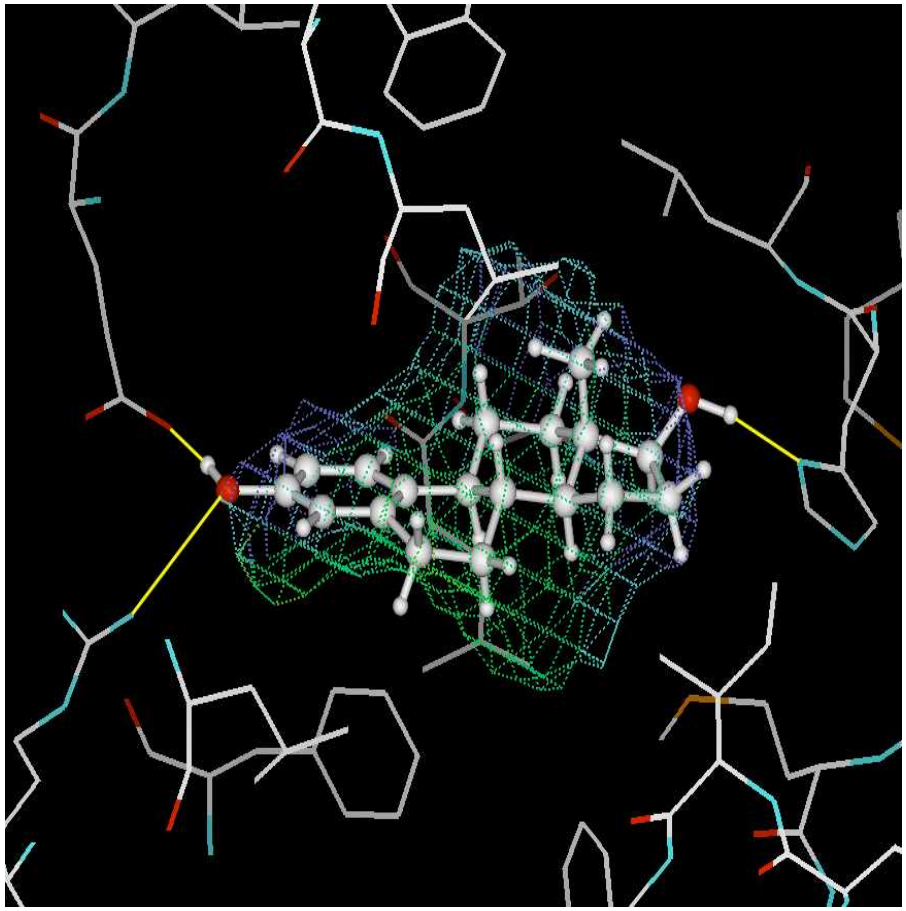
High throughput screening for aromatase inducer/inhibitors



In silico virtual screening for binding activity (QSAR)

- Docking Model

  (Drs. Akiko Itai, Nobuo Tomioka)



Estradiol molecules in the ER alpha LBD pocket